PROCEEDINGS OF II. INTERNATIONAL AGRICULTURAL, BIOLOGICAL & LIFE SCIENCE CONFERENCE

SEPTEMBER 1-3, 2020

EDIRNE, TURKEY

PROCEEDINGS OF II. INTERNATIONAL AGRICULTURAL, BIOLOGICAL & LIFE SCIENCE CONFERENCE

1 – 3 SEPTEMBER, 2020

Organized by

Trakya University

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WELCOME NOTES

You are welcome to our II. AGBIOL Conference that is organized by Trakya University. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal was to bring three communities, namely science, research and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to benefit from the interaction with each other but we have to organize as online due to Covid_19 stiuation. I hope next one we could host you in Edirne.

In September 2018, we organized the first AGBIOL Conference with more than 700 scientists and researchers from all over the world with over 800 scientific papers. Therefore, this great interest gave ambition to organizers to make it a periodical event then we decided to organize 2nd one in this year.

The Organizing Committee of AGBIOL 2020 considers the health, safety, and security of its conference attendees and community as its top priority. Due to uncertain COVID-19 situation, which results in a very difficult travel restriction for most countries and the fact that there is no definite end in sight, with a careful consideration in all aspects**,** then AGBIOL 2020 has decided to move towards the organization of a fully on-line AGBIOL 2020 as digital event, in lieu of an in-person event**.** Despite all limitations, our e-conference will be probably one of the biggest online conferences in recent years in the world with 480 papers and over 1100 authors with 333 oral and 147 e-poster presentations from 55 countries.

The participants with paid conference fee will be able to access all the virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided participant ID and codes. The selected abstracts will be published in the Conference Abstract and Proceedings Book. Participants might send us their full papers, which based on their preferences will be published either in our Conference Abstract and Proceedings Book or in selected International Indexed Scientific Journals.

Conference Topics:

Agriculture, Forestry, Life Sciences, Agricultural Engineering, Aquaculture and Biosystems, Animal Science, Biomedical science, Biochemistry and Molecular Biology, Biology, Bioengineering, Biomaterials, Biomechanics, Biophysics, Bioscience, Biotechnology, Botany, Chemistry, Chemical Engineering, Earth Sciences, Environmental Science, Food Science, Genetics and Human Genetics, Medical Science, Machinery, Pharmaceutical Sciences, Physics, Soil Science.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA

Head of the Organizing Committee

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EDITOR OF THE PROCEEDING BOOK

Prof Dr Yalcin KAYA

E-AGBIOL 2020 CONFERENCE PROGRAM

PLENARY SESSION

TUESDAY, SEPTEMBER 1ST, 2020		
10 00 –10 30	Opening Ceremony and Talks	
10 30 - 12 00	**OPENING SESSION I:** Session Chair: ***PROF. DR. YILMAZ ÇAN,*** Trakya University, Edirne, Turkey	
10 30 –11 00	**PROF DR LARA HANNA WAKIM,** *Holy Spirit University, Lebanon* - "Potential use of origanum essential oils as alternative to antibioticgGrowth promoters: Effect on resistant *E. coli* strains isolated from Lebanese raw c **Identification of Fungal** hicken"	
11 00 –11 30	**DR YUNUS BAYRAM,** *Ministry of Agriculture and Forestry, Turkey* "Plant health, quarantine and pest management in Turkey"	
11 30 –12 00	**PROF DR SEZEN ARAT,** *Tekirdag Namik Kemal University, Turkey* - "Transgenic and clone animals from past to present"	
12 00 – 1300	***LUNCH***	
13 00 - 15 00	**OPENING SESSION II:** Session Chair: ***PROF. DR. MUSTAFA TAN,*** Trakya University, Edirne, Turkey	**OPENING SESSION III:** Session Chair: ***PROF. DR. METIN AYDOGDU,*** Trakya University, Edirne, Turkey
13 00 –13 30	**PROF DR MEHMET EMIN CALISKAN,** *Niğde Ömer Halisdemir University, Turkey* - "Breeding climate resilient potato cultivars using conventional and next generation breeding techniques"	**PROF DR didar,** *Inst. of Animal Science, Bulgaria* - "Black soldier fly (*Hermetia illucens*) in poultry nutrition- effect on the performance, carcass composition and meat quality in broilers"
13 30 –14 00	**PROF DR IOANNIS TOKATLIDIS,** *Democritus University of Thrace, Greece* - "Crop adaptation to spacing to promote resilience to environmental diversity"	**PROF DR AHMET ULUDAG,** *Canakkale Onsekizmart University, Turkey* -"The Need for Integrated Approach for Fleabanes Management"
14 00 –14 30	**PROF DR YAROSLAV BLUME,** *Inst. of Food Technology and Genomics* **-***NAS, Ukraine* - "Molecular genetics background for breeding of wheat varieties with resistance to highly virulent Asian patotypes of yellow rust"	**ASSOC PROF DR OZLEM TOKUSOGLU,** *Manisa Celal Bayar University, Turkey* - "AgriFood and plant based effervescent tablets: Research data on chemical and nutritional quality"
14 30 –15 00	**DISCUSSION**	**PROF. DR. NURHAN TURGUT DUNFORD,** *Oklahoma State University, USA* - "Opportunities and challenges for recovering phospholipids from oilseeds"
15 00 –15 30	***COFFEE BREAK***	***COFFEE BREAK***

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	2.9.20 WEDNESDAY	**2.9.20 WEDNESDAY**	**2.9.20 WEDNESDAY**	**2.9.20 WEDNESDAY**	**2.9.20 WEDNESDAY**	**2.9.20 WEDNESDAY**	**2.9.20 WEDNESDAY**
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AGRIFOOD AND PLANT BASED EFFERVESCENT TABLETS: RESEARCH DATA ON CHEMICAL AND NUTRITIONAL QUALITY

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ABSTRACT

Recently, the potential efficacy of the bioactive phenolics from natural sources has been the focus of great attention owing to their health benefits to human health for reduced risk of coronary heart problems and selected cancers. Plant based food tablets as dietary supplements, and/or fortificated foods, food byproduct based food powders may be great value-added products for getting healthy bioactive constituents. Activated phenolic antioxidants, bioactives and probiotics that have been naturally extracted to be highly potent and easily absorbed by using food tablets. For safety manufacturing; unit dose, temper evident, solid preparations of active ingredient and ingredient mix, powder quality should be monitored. Through the powder forming, the flexible design of dosage forms as technical manufacturing parameters has been considered. Due to supplement manufacturing, consumer demand, routes of drug delivery, oral utilization capacity should be examined. In this context, the bulk density, the tapped density as pre-compression parameters have been confirmed while thickness, hardness, % weight variation, % friability, % invitro drug release as post-compression parameters have been carried out as physiochemical properties. Not only chemical profiles, functional properties, but also detailed clinical nutrition data of expressed effervescents. In this presentation, agrifood and plant based supplement manufacturing strategies and research data on mulberry-based, citrus-based, tomato-based effervescent chemistry and nutritional quality have been carried out.

Keywords: Plant foods, effervescent, strategy, quality, property, supplement

INTRODUCTION

Recently, the potential efficacy of the bioactive phenolics from natural sources has been the focus of great attention owing to their health benefits to human health for reduced risk of coronary heart problems and selected cancers. Food tablets as dietary supplements, and/or fortificated foods, food by-product based food powders may be great value-added products for getting healthy bioactive components. The functional constituents of the foods, some preferable functional foods or some functional plant/fruits/ vegetables/spice foods has been standardized as the nutraceutical product and generate under good manufacturing practices (GMPs) (Table 1). Meanwhile these plants have been used in effervescent food supplements such as effervescent food tablets (Tokusoglu 2018; Tokusoglu and Swanson, 2015; Tokusoglu and Hall, 2011).

Nutraceutical food tablets has been prepared by direct compression method through selected tablet machines and has been manufactured according to established prescription methods. The functional constituents of the foods, some preferable functional foods or some

functional plant/fruits/ vegetables/spice foods has been standardized as the nutraceutical product and generate under good manufacturing practices (GMPs). Primarily, a nutraceutical or selected food must be detected for “non-toxic food constituent strategy" by advanced toxicity analyses, then it must be detected and analyzed in terms of health benefits including disease treatment and/or prevention (Tokusoglu 2018; Pham,2015; Prabhakar and Krishna, 2011; Stahl, 2003).

Table 1. Plant Parts and Their Main Constituents Utilized as Food Products

Plant Parts	Main Constituents	Uses as Food Products
Fruit	Sugars	Fresh Fruits
	Vitamin C	Marmelades
	Betalains	Juices
	Polyphenols	Candys
	Fibre	Liquors
		Syrup
Flowers	Flavonoids	Infusions
	Betalains	Salad Vegetable
Seed	PUFA, MUFA and Sterols	Cosmetic Products
	Polysaccharides	
Cladodes	Fibre	Salad Vegetable
	Mucilage	Pickles
	Minerals	Flour and Additives
	Chlorophyl Derivatives	
Root	Flavonoids	Infusions

Activated phenolic antioxidants are derived from the healthiest of plants,fruit, vegetable and/or spices. A wide range of free radicals are neutralized by absorbtion of antioxidant phenolics through effervescents; thence body cells are protected from damage and inflammation.

Uniquely containing activated phenolic antioxidants that have been naturally extracted to be highly potent and easily absorbed by your body in food tablets. Activated phenolic antioxidants are derived from the healthiest of plants, fruit, vegetable and /or spices. A wide range of free radicals are neutralised by absorption of antioxidant phenolics through effervescents; thence body cells are protected from damage and inflammation. Effervescent tablet has been proved its utility as an oral delivery system in the pharmaceutical and dietary industries for decades

Food Effervescent Tablet Properties

In effervescent nutraceutical technology; a balanced ratio of acids and carbonates are used for forming a buffer and it has optimal compatibility with the stomach. Gas bubbles occur from the liquid after chemical reaction by adding water; alkali metal bicarbonates and acids (majorly citric or tartaric acids) are utilized to produce effervescence. In effervescent system, when organic acid and bicarbonate get together in the water, CO_2 is released; the solving process is performed in 17–20°C water. The foam of them helps to kill the local bacteria.

Food tablet is described as unit dose, temper evident, solid preparations including one or more active ingredients or whole food powder. Patient and/or consumer demand, routes of drug delivery, oral utilization capacity, the flexible design of dosage forms as technical manufacturing parameters has been considered; also the bulk density (g/ml), the tapped density (g/ml) as pre-compression parameters have been confirmed while thickness (mm), hardness (kg/cm^2), % weight variation, % friability, % *in- vitro* drug release as post-compression parameters have been carried out as physiochemical properties. Among the alkali sources, sodium carbonate is preferred due to its low cost, high solubility and intensity of reaction than potassium carbonate and bicarbonate.

Polyvinylpyrrolidone (PVP) is used as binder in effervescent; its form is as dry powder or wet forms of aqueous or hydroalcoholic solutions Water-soluble lubricants, colors, flavorings and sweeteners are also added as other ingredients. Mannitol and PEG 6000 are other utilized effective binders. At production stage in tablet machine, relative humidity should be low (25%) and ambient temperature should be at room temperature (24±1°C). Tablet forming quality parameters (weight, hadrness, pH, solution time and friability) are inspected.

Dyes or lake color pigments have been added to manufacture colored solutions or products. In effervescent tablets, color stability is also significant. These should be chosen as anhydrous materials. Generally dried flower bud, herbs, chamomile extract may be utilized for this purpose, their percentage may be lower than 1–2%. The remain part of the 0.1–2% in effervescent should be consisted of vitamin E, squalene, almond oil and cosmetic esters. Besides, PEG-30 castor oil, laureth 4, polysorbate 80 or 85 can be used as emulsifiers.

Probiotic Using in Effervescent Manufacturing

Probiotics are living microorganisms that confer benefits on the recipient health when administered in appropriate amounts. Probiotics are mainly utilised in nutritional supplements owing to their positive impacts on health (Do Espirito et.al,2011) An adequate selection among different probiotic strains has to be performed to allow manufacturing probiotic-supplemented food and plant consuming products. The aim in administering probiotics is to induce a balanced enteric microbiota, that will have a favorable effect on consumer health. Table 2 shows main probiotic species applied commercially in food and supplements (Anadón et.al,2010) (Table 2).

Table 2. Main Probiotic Species Applied Commercially in Foods and Food Supplements

Lactobacillus	*pseudocatenulatus*	*Leuconostoc*
acidophilus	*catenulatus*	*mesenteroides*
johnsonii	*bifidus*	*Pediococcus*
plantarum	*infantis*	*acidilactici*
rhamnosus	*longum*	*Enterococcus*
delbruecki	*thermophilus*	*faesium*
reuteri	*adolescentis*	*Lactococcus*
fermentum	*Streptococcus*	*lactis*
Brevis	*intermedius*	*Saccharomyces*
lactis	*salivarius*	*boulardii*
cellobiosus	*cremoris*	*Propionibacterium*
paracasei	*lactis*	*freudenreichii*
helveticus	*Aspergillus*	
Bifidobacterium	*niger*	
lactis	*oryzae*	

The strain selection process guarantees the survival of the probiotic microorganisms in the demanded products during its manufacturing and storage, in addition to provide that the product will confer sufficient technological properties (Bansal and Garg, 2008).

The food effervescent matrix selected for incorporating probiotic strains should be carefully fortified so that an adequate selection of the probiotic strain-food pair is attained. The compatibility and adaptability among the selected strains and matrixes is principal. Varied lactic probiotic products, chiefly the fermented as well as some non-lactic products, havebeen improved as fruit effervescent containing probiotics and have been achieving interest in the global marketplace. To define the composition of effervescent probiotics in tablet form, an assessment of the component`s effects on the viability of the microorganisms are performed. Table 3 shows the advantages of probiotic food supplements from different perspectives, in a comparative criterion (Table 3).

Table 3: The advantages of probiotic food supplements from different perspectives

	Description	
	Supplement	Food
Efficiency (in the product and in GIT)	Transfer of considerably higher numbers of viable probiotics into GIT without considerable loss during the storage. Therefore, they are preferred when specific therapeutic functions are required.	Good to excellent matrix protection for probiotic cells during delivery in GIT.
Hedonism	-	Being consumed for hedonism and comprise sensory attributes.
Product and market development	-	Have higher potential of variation and therefore, product and market development
Simplicity of production (probiotic stability in product during the storage and sensory properties)	Simpler design and formulation from production point of view; namely, the stability of probiotics during the process and storage as well as not having negative effects of added probiotics on sensory properties of final product.	-
Regulatory administration and legislation		Foods are subjected to more difficulties

Prebiotic Using in Effervescent Manufacturing

Prebiotics are food ingredients that induce the growth or activity of beneficial microorganisms (e.g., bacteria and fungi). Approximately 47% of chicory root fiber contain the prebiotic fiber inulin; inulin nourishes the gut bacteria, improves digestion and helps relieve constipation. Tablets including prebiotics have metabolic properties and improves the intestinal ecosystem and colon cells, stimulating the peristalsis, improves lipids and reducing the cholesterol and triglyceride serum levels and also facilitates the mineral salt absorption (Brunser and Gotteland, 2010).

The powder blend has been thoroughly mixed with talc and magnesium stearate and compressed into 300-400 mg tablet using single rotatory punching machine based on tablet processing strategy. Among the trial /serial tablet formulations; "mesir effervescent tablet" could be more efficacious owing to majorly cinnamaldehyde (as v/v) whereas "black mulberry

effervescent tablet" could be more beneficial due to the presence of morusin and apigenin phenolic anticarcinogenics and also "mandarin peel effervescent tablet" could be salutary because of its naringenin and hesperidin flavonone phenolic bioactives, also ‘'tomato peel effervescent tablet" could be important due to its lycopene and anthocyanidin phenolic antioxidants.

CONCLUSION

In this context, the innovative and conventional food tablet processing strategies has been focused to chemical characterization, functional properties, their unique bioactive features, antioxidative, anticarcinogenic reports of above-mentioned developed tablets. General usage, bioactive materials from plants and probiotic /prebiotic fortification are so important than ordinary supplement materials. Specific applications are more efficient and are required to meticulous manufacturing.

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THERMAL CAMERA USE IN ANIMAL PRODUCTION

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ABSTRACT

Thermal cameras are devices which temperature measurement is made by using a method of imaging from a certain distance without contacting the surface with infrared rays. These cameras (depending on the specification of the camera) can detect very small temperature differences. The detected temperatures are shown as photographs that different temperatures are shown in different colors. Thus, it gives information about the surface temperatures, or afterwards, detailed analysis can be made on the images thanks to the camera software program. Due to its accurate, fast and easy evaluation, it finds uses in many different sectors as well as in the livestock sector. There are researches that thermal camera imaging can be used in the detection and control of ovulation, stress factors such as pain, disease, and inflammation, also in evaluating the deterioration processes of feed and silage. The aim of the study is to present a review about thermal camera technology and its usage possibilities in animal production.

Keywords: Thermal camera, Infrared thermography, Silage, Aerobic stability.

INTRODUCTION

Imaging with thermal camera (TC) is a two-dimensional, objective, non-invasive diagnostic method for measuring temperature of surfaces. This analytical technique is suitable for the animal management, health control and animal welfare. While the device and method were originally developed for military purposes, it has recently emerged in other industries (Hovinen et al., 2008; Gowen et al., 2010).

By converting the infrared radiation emitted by a heat source into pixel intensity, the infrared thermal cameras provide a signature image of temperatures (also called "thermogram") of the skin surface (Weschenfelder et al., 2013).

Thermal camera imaging is an important non-destructive quality evaluation tool to assess inflammatory reactions, early detections of mastitis, ovarium cycles, foot pathologies in ruminants, equines and poultry, grain quality, deterioration of feed and silage (Addah et al. 2012; Talukder et al. 2013; Alsaaod et al., 2015).

The temperature of body parts (wing, leg etc.) and skin generally influenced by metabolism rate and underlying blood flow (Berry et al., 2003). Due to changes in circulation will change the amount of radiated heat that thermal cameras could identified and cause variation in thermograms. This variation might be related to inflammation of tissues underlying that point or to changes in metabolic activity (Alsaaod and Büscher, 2012).

In past studies , thermal camera imaging technique has been used to assess of hoof disorders of cows (Alsaaod and Büscher, 2012), lameness in equines (Alsaaod and Büscher, 2012), for

cattle infected with foot-and-mouth disease (Alsaaod et al., 2015), and for evaluating the milking process (Kunc et al., 2007), viral diarrhea of calves (Schaefer et al., 2004) and prediction of ovulation in dairy cows (Talukder et al., 2013).

This paper provides an overview of thermal camera technology and its usage possibilities in animal production.

How does infrared cameras work?

Heat energy is transferred by three ways: Conduction, Convection and Radiation. Only radiated heat energy can be detected by infrared cameras, or thermal imagers.

All objects emit electromagnetic energy at the speed of light. Electromagnetic energy is radiated in waves, that has electric and magnetic properties. Human eye perceives visible light wavelengths, while infrared cameras perceive the infrared radiation wavelengths (Anonymous, 2020a).

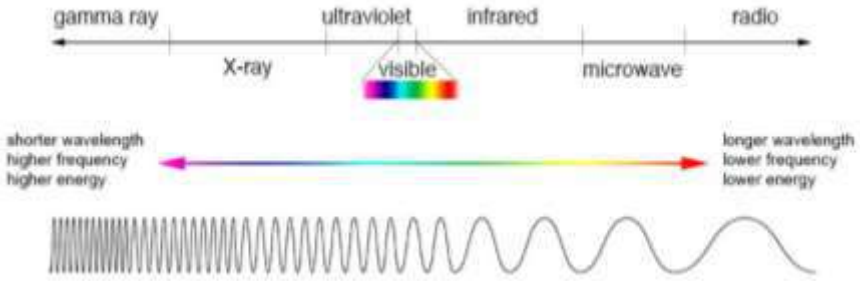

Figure 1. Electromagnetic spectrum (Anonymous, 2020b)

Light is a type of radiation and does not only contain visible light. The range of light includes the entire electromagnetic spectrum that consists of visible and invisible light (such as X-rays, gamma rays, radio waves, microwaves and ultraviolet light). Wavelength makes the difference between each of light types from one another (Figure 1).

By understanding infrared, we can use thermal imaging cameras to identify the heat signature of almost any object. Almost all materials (or items or objects), even ice, emit at least some heat. For example; if the object is at absolute zero (-273.15°C), its atoms moves from side to side with small rapid movements, bouncing, bumping and generating heat (Anonymous, 2020c).

Thermal cameras are today's high-tech devices. But infrared light was discovered a long time ago, in 1800. Sir William Herschel (a German-born British astronomer) discovered infrared radiation by using a prism to split sunlight into different wavelengths and then holding a thermometer next to each color of light. He noticed that the thermometer detects heat even where there is no visible light (infrared) (Meola, 2012; Anonymous, 2020c).

All objects emit infrared energy that can be identified as a heat signature. Thermal cameras detect, measure objects' infrared energy and create a detailed electronic image (thermogram) (Meola, 2012; Anonymous, 2020c).

An infrared camera contains an optical system that focuses infrared energy on the object's surface onto a special detector chip (sensor array) containing thousands of pixels arranged in a grid.

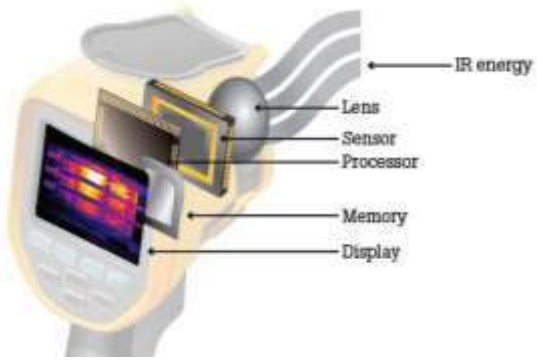

Figure 2. Therma camera process (Anonymous, 2020d)

Each pixel in the sensor array generates an electronic signal in response to infrared energy focused on it. The camera processor receives the signal from each pixel and applies a mathematical calculation to it to create a color map of the object's apparent temperature. A different color is assigned to each temperature value. The resulting color matrix is sent to the camera's screen as a thermal image (thermogram) of the object (Alsaaod et al., 2015; Anonymous, 2020d; Figure 2).

Animal Welfare

Meaning of animal welfare can be summarized as "the quality of life" (Fraser et al., 1997). Animal welfare include different components, mainly could be can be grouped under three main headings such as below;
Basic health and functioning,
Affective states (happy, free from stress, pain, and fear),
Natural living (to carry out natural behaviors).

However, meeting a single parameter does not guarantee welfare of high level and a good life for animals. It means not meeting the needs of the animal as desired. These all three headings should be overlapped (Fraser et al., 1997; Fraser, 2008; Figure 3).

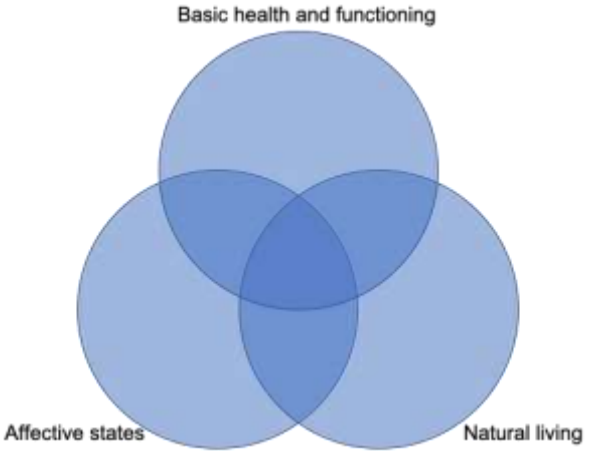

Figure 3. Main headings of animal welfare (Fraser et al., 1997; Fraser, 2008)

Karakuş and Karakuş, (2017) used infrared thermography to determine the effects of ear tag application (metal, plastic, and electronic) on causing infection and to indicate the effect on welfare by monitoring behaviors of lambs. The infrared images of ears of the lambs were taken with thermal camera and recorded, before the ear tag the applications. Also, measurements

were repeated 1th, 4th, 10th, and 30th days after application. Temperatures on days 1, 4 and 10 were found different in the control and treatment groups (P<0.001). This difference has been associated with fewer problems. Infrared thermography images showed that the electronic ear tags caused more inflammatory reactions compared to metal or plastic ear tags (Figure 4).

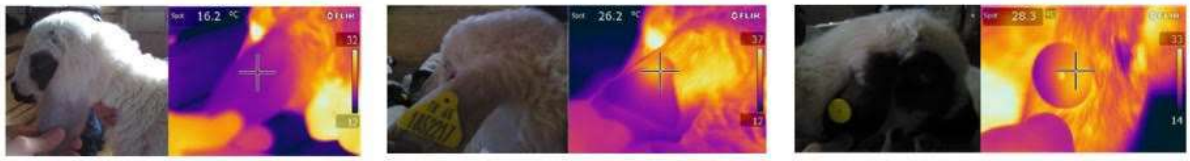

Figure 4. Infrared images of ear tissue in lambs (Karakuş and Karakuş, 2017)

The researchers (Sassi et al. 2016; Karakuş and Karakuş 2017) suggested that infrared thermography might be used as an early diagnosis tool for the inflammatory reactions, infections and metabolism changes to allow a better assessment of animal welfare, health and management problems.

Poultry farm conditions and heat stress

For poultry production, controlling ambient conditions (temperature and relative humidity) have important role to minimize welfare problems. For example, Heat stress is an important problem in poultry production and caused by high ambient temperature, relative humidity, and bad air ventilation. Due to heat stress, birds' performance and thus farm profitability might be affected adversely. Infrared thermal imaging (IRT) technology uses body surface temperature to create thermograms. The most accurate body temperature can be measured from the eye area of birds comparing to the other parts. This finding is related to absence of feather around the broiler eye (Sassi et al., 2016; Bloch et al., 2019).

When checking a poultry house, it is not always possible to identify the source of the problem since human eye can't see temperature like thermal cameras. While strategically placed thermometers can be an indicator of the temperatures at those areas, farmers still would not estimate the full picture of the thermal environment inside the house. So, usage of thermal camera imaging in poultry houses might be important to monitor temperature inside (University of Kentucky, 2009a).

IRT systems (infrared thermal camera imaging) in poultry houses is used to measure thermal energy emitted from walls, ceilings, doors, curtains etc. Identification of air leakage, bad insulation and other problematic places in poultry houses might be determined with this method. Also having quality ceiling insulation provides significant benefits to poultry producers year-round. If the ceiling is not insulated properly, heat loss will occur through it, resulting in higher heating costs in the winter. On the other hand, in the summer season ceiling insulation also minimizes the amount of heat entering the house through the ceiling (University of Kentucky, 2009a; 2009b).

Zaninelli et al. (2016) aimed to develop and test a sensor that would start disinfectant applications without any harmful effect to animal's health and welfare. Obtained results have shown that sensitivity of 97.9% and a specificity of 94.9%. Similarly, Zaninelli et al. (2017) used thermal cameras and a sensor as a monitoring system to detect the presence of animals in the housing system before the ozone treatment starts (to reduce bacterial load and atmospheric ammonia levels), in order to not expose the laying hens to ozone (O_3). While the birds are outside of the housing system, ozone treatment could be applied efficiently and without any harm to the hens and employers. When ozone concentration decreased inside the room, animals

would be led to the in. The obtained results indicated an improvement of the sensor detection accuracy that reached 95.1% sensitivity and 98.7% specificity. The results showed that infrared technology, and an advanced elaboration of acquired images, might allow to detect a hen in a closed room of a commercial farm that produces organic eggs. In addition, the range of time need to detect a hen, or classify a case, was reduced to 2 seconds (Zaninelli et al., 2017).

Cândido et al. (2018) studied with Lohmann pullets raised in four different environment conditions (20, 25, 30 and 35ºC) and pictures were taken using two cameras (low cost and high cost thermal cameras from same company) in equal time and distance.

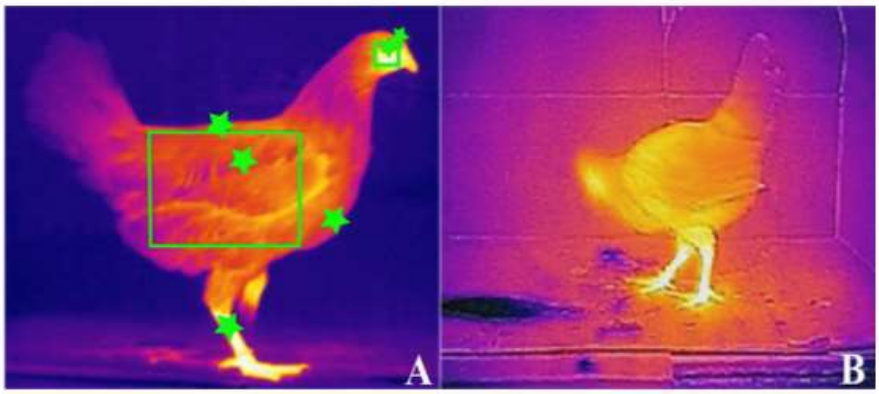

Figure 5. Side view of birds with HC (A) and LC (B) cameras (stars show the body parts; Cândido et al., 2018)

Cândido et al. (2018) concluded that the tested low-cost thermal camera (LC) can replace the high-cost thermal camera (HC) if appropriate corrections are made via calibration. The differences in absolute readings between the two cameras are large, varying from 7.45 to 4.11°C. However, using the regression equation subtraction it is possible to use the LC regardless of the difference from the HC camera. In a qualitative way, the images from LC were unable to be used in some cases, especially in the highest temperature environment tested (35°C; Figure 5).

Zao et al. (2013) used infrared thermal imaging method to find out its potential to evaluate feather coverage. They reported that IRT (taking into account feather coverage area and surface temperature measurement) has potential that might advance a more objective and quantitative measurement of laying-hen feather conditions, compared to the traditional feather coring (FS) method (Figure 6). The IRT method also provides surface temperature profiles of the birds, which help clarify their sensible heat loss or relative comparison between hens with different feather coverage. The disadvantage of IRT method compared to the traditional FS method is the higher cost of equipment (infrared thermal camera) and additional labor in data analysis. Due to feather condition of birds affected by age, it is a good indicator for poultry producers. Elder hens have fewer areas of excellent feather coverage, but more areas without feather or fair feather coverage as compared with younger hens. For this reason, older chickens have a higher surface temperature, which makes them more susceptible to heat loss in cold weather and may result in higher feed conversion ratios.

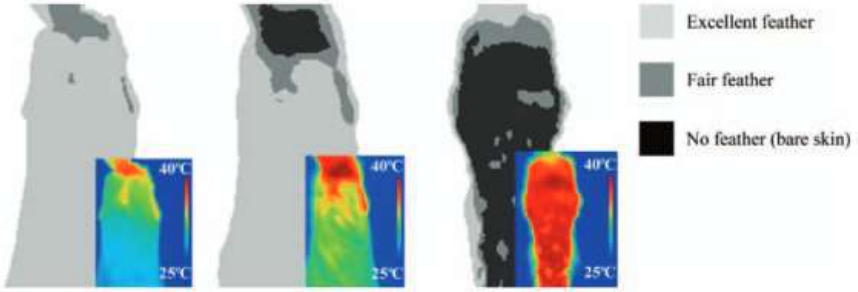

Figure 6. Hen head feather coverage image samples (Zao et al., 2013)

Animal Health

To assess the diseases at early stages is important for enhancing animal welfare and preventing economic losses. One of the major diseases is mastitis and it is very common in dairy cows. Clinical mastitis leads to systemic (lack of appetite, fever) and local (swelling, pain, and heat) signs in the quarter of udder. Several techniques exist to analyze deviations in milk (Rasmussen, 2004; Hovinen et al., 2006). However, there is a need for a diagnostic method that is not related to milking and to identify mastitis during the dry period. Infrared thermal camera imaging method used for measuring radiated heat emitted by the skin, reflecting subcutaneous circulation and metabolism (Jones and Plassmann, 2002). Berry et al. (2003) presented a predictive model for the udder surface temperature based on healthy cows and ambient temperature measurements. They concluded that IRT is promising for early detection of mastitis.

Hovinen et al. (2008) stated that the thermal camera was found successful to detect temperature changes (1 to 1.5°C) on udder skin associated with clinical mastitis. Local signs on the udder were seen before the rise in udder skin and body temperature. Because udder is a sensitive site for detection of any disease with fever by using a non-invasive method. (Figure 7). They suggested to mount a thermal camera in a feeding or milking section of the farm could be used to detect temperature changes associated with clinical mastitis or other diseases in a dairy herd.

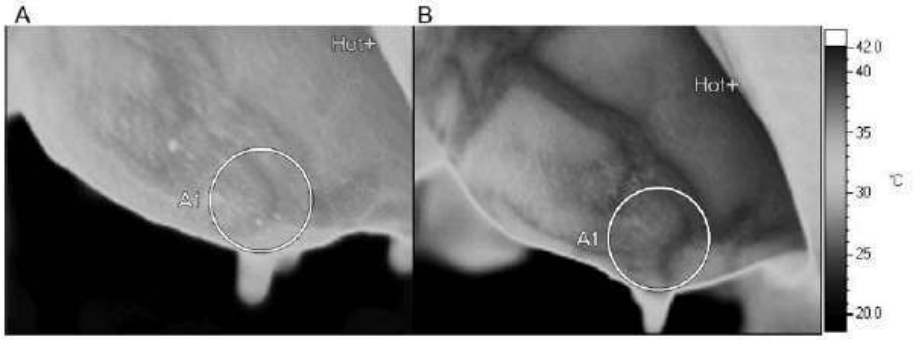

Figure 7. Thermal images changes of udder tissue (A1= 40x40 pixel area; Hovinen et al., 2008)

Lameness is another major health problem with devastating impact on animal welfare, performance and economics in the dairy industry. The visual observation is the most common recognition method at claw pathologies. However, this method is dependent on observer's

skills and not sensitive in detection of all claw and foot lesions. Besides, good management practices such as routine claw trimming is useful to detect, prevent claw lesions and diseases. Alsaaod and Büscher (2012) suggested that thermal camera imaging might be a reliable alternative method for early evaluation of claw disorders. Early evaluation can be important o reduce negative effects of lameness, to increase the treatment success (Alsaaod et al., 2015).

Alsaaod and Büscher (2012) found that coronary band temperature, measured by IRT, increases the detection of abnormalities of the hoof before routine hoof trimming (Figure 8). A combination of thermography image analysis with clinical examination would be a helpful technique for preventing lameness (Alsaaod and Büscher, 2012; Alsaaod et al., 2015).

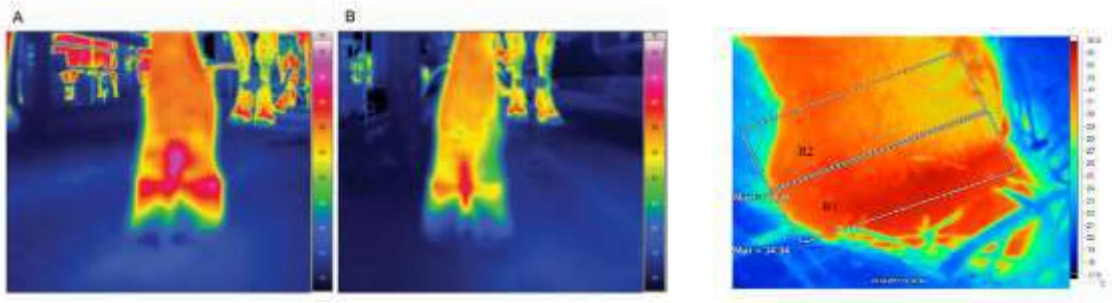

Figure 8. Claw and Coronary band thermograms of hoof (Alsaaod and Büscher, 2012; Alsaaod et al., 2015)

Wilcox et al. (2009) used thermographic imaging technique to diagnose of subclinical bumblefoot disease, which is one of the major health problems in birds. Bumblefoot (pododermatitis or foot pad dermatitis) is a chronic inflammation that causes pain, impedes perching and walking, and may limit access to food and water In the poultry industry, bumblefoot causes economic losses due to field rejection, carcass rejection, and poor growth due to lameness (Hester, 1994;Wilcox et al., 2009; Figure 9).

Increased incidence of bumblefoot is associated with wet litter, usually found in floor-housed chickens. The etiology of bumblefoot involves bacterial components. Staphylococcus aureus has been isolated from 90% of bumblefoot case (Wilcox et al., 2009).

Early diagnosis of bumblefoot is essential for the prevention of economical loss and the improvement of animal well-being. Wilcox et al. (2009) found correlation between thermal images and the visual score of clinical for bumblefoot was 86.7% ($P < 0.001$). However, the correlation between the thermal images classified as mild (subclinical) and a visual score of mild was only 26.7%. The authors suggested thermogram technique is more sensitive than visual scoring for subclinical infections (Figure 10).

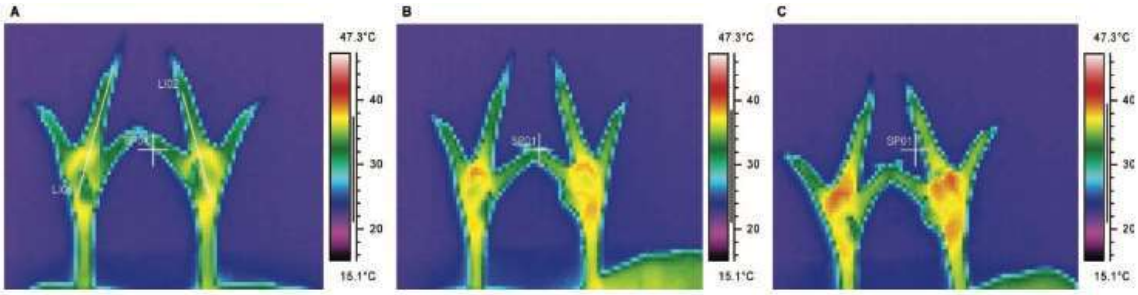

Figure 9. Thermogram samples of hen feets. A) typical thermal image of feet, B) mildly clinical bumblefoot or suspect, C) clinical bumblefoot (Wilcox et al., 2009)

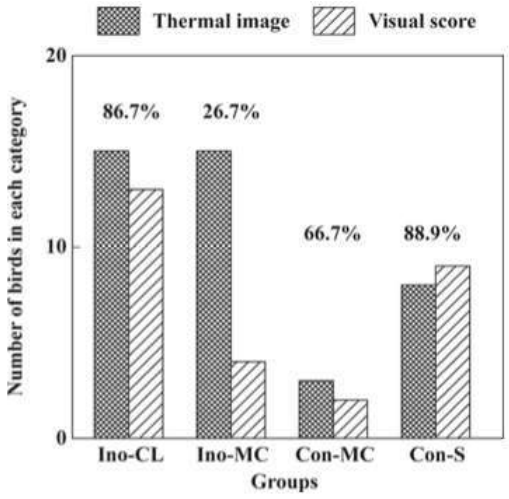

Figure 10. Thermal image and visual scores (Ino-CL: clinical infected; Ino-MC: mild clinical infected; Con-MC: mild clinical cases in the control group; Con-S: sound cases in the control group; (Wilcox et al., 2009).

Interpretation of thermograms of the avian foot not only revealed subclinical bumblefoot but also helped to evaluate its severity and to monitor the progression of the disease. Early detection and easy monitoring may improve prognosis and decrease associated pain, which improves animal well- being. Thanks to the early stage of the disease, animal welfare and recovery percentages will improve (Wilcox et al., 2009). However, all advantages of thermal image technique, the equipment cost is still high, and this might be an important obstacle in front of its commonly usage (Cândido et al., 2018).

Silage control

The silage process might be divided in four stages: (1) the aerobic stage in the silo immediately after harvest, (2) the fermentation stage, (3) the stable storage stage in the silo, and (4) the feed-out stage when the silo feed face is opened and exposed to air. Producing high-quality silage, besides avoiding dry matter losses as much as possible, is a challenge and can occur during entire silage process (Borreani et al., 2018).

The primary cause of silage quality deterioration is respiration. Temperature of silage and oxygen concentration are also important to control respiration and silage process. Silage temperature is initially increased due to fermentation process. When the fermentation period is over, silage temperature should be lower and stable for good silage quality, and researchers reported that any significant increases in silage temperature would be sign of aerobic deterioration. Even if ensiled material is not exposed to oxygen during the production, fermentation and stable period, however when the silo is opened, it is unavoidable that conditions turn to the aerobic stage with air entering the silo (Filya ve ark. 2004; Junga and Trávníček, 2015).

During the decomposition process, the dry matter breaks down into H_2O and CO_2 with a release of heat (McDonald, et al. 1991). Aerobic deterioration resulted from activity of aerobic microorganisms such as yeasts and molds, using water-soluble carbohydrates and fermentation products, resulting with pH value and heat rising, dry matter loss, decrease in protein and

cellulose digestibilities and energy release (Muck, et al. 1991; Filya ve ark. 2004). In addition, the growth of molds may produce mycotoxins, which threaten the health of humans and animals (Pahlow et al. 2003; Santos et al. 2014). It would be useful to monitor entire silage process and detect abnormalities in its early stages. Due to that an effective conservation process might be achieved (Junga and Trávníček, 2015).

Kaya and Polat (2010) emphasized that the difference between the ambient and silage temperature greater than 2°C may indicate deterioration. And this difference can be simply and correctly identified by Thermographic images.

The proof of good silage management and subsequent handling is its temperature. It should never exceed 20°C. If temperature exceeds 20°C, it is not suitable for dairy cattle (even in summer). Literature sources indicate that the self-heating of maize silage to 30°C increases the losses caused by anaerobic fermentation of silage by 1.7% of dry matter per day.

The main observed problems are residues after milling and residues of air containing silage pushed back to the silage wall. Thus, layered material begins to warm up, which means nutrient losses. These losses are 3% for each 10°C temperature increase per day.

The thermal camera is used for detecting the temperature increase, silage quality and problems of silage wall, which at first sight upon arrival in the silo is not visible. According to researchers, an infrared thermometer is more affordable (Galik et al., 2016).

Koç et al. (2020) studied the effect of sodium diacetate and sodium benzoate addition on the aerobic stability characteristics of high moisture corn grain silage at different storage temperatures (27-28°C and 36-37°C). They carried out chemical and microbiological analyzes, in addition thermal camera imaging were recorded at day 0 and 12 (Figure 11). During aerobic stability period, temperature changes in silage samples and ambient temperatures were recorded every 30 minutes for 12 days. They found similarity in thermal camera footages and heat sensors' data. Especially, in 12th day of the aerobic stability there was a significant heat increase and it could be clearly observed by the infrared thermography. This heat differences are major signs of deterioration.

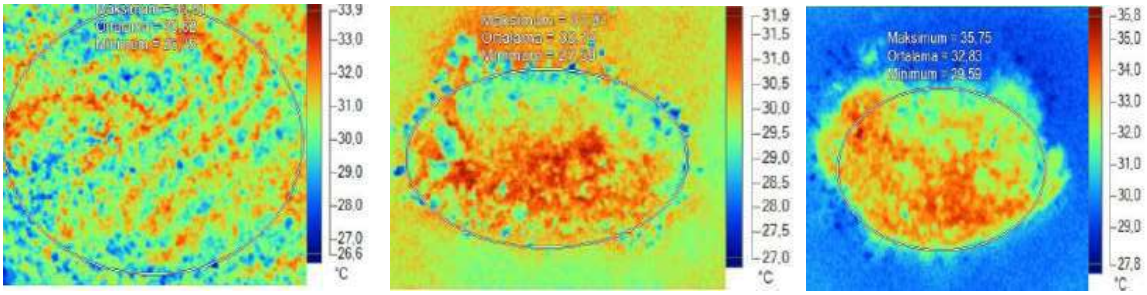

Figure 11. a) Day 0 b) Day 12 (27-28°C) c) Day 12 (36-37°C)
Thermal camera sample images from high moisture corn grain silage (Koç et al., 2020).

Erdoğan and Koç (2020) recorded temperature values and ambient temperature of the three regions (right, middle and left region) of the bunker silo at the 0th, 7th, 14th, 21st, 30th and 40th days of the study (carried out between 15 April- 24 May). Analysis regarding chemical and microbiological parameters was carried out on silage samples taken from 3 different fixed points of the silo. At the same time, thermal imaging camera was used at a distance of 1 meter to record imaging from constant points in the silo surface. As a result, the values regarding thermal camera imaging technique and temperature sensors were found parallel. Koc et al. (2018) found similar results in their research about corn silage at silage bunker from 5 different farms. The results show that thermal camera imaging technique offers prospects as a practical method for assessing the aerobic stability of silages on farm.

Günler	Termografi	Silo İçi (data logger)	Çevre Sıcaklığı
0.	18,20	20,90	17,09
7.	17,51	18,58	17,94
14.	28,06	31,59	22,52
21.	30,53	29,90	17,71
30.	29,07	35,18	18,95
40.	30,03	30,91	19,67
Ortalama	**24,70**	**27,20**	**18,80**

Figure 12. Recorded temperatures changes from data logger and thermogram (Erdoğan and Koc, 2020).

Junga and Trávníček (2015) used infrared thermography technique to evaluate the maize silage conditions in four monolithic steel concrete bunker silos from different locations at winter conditions (air temperatures -3.5, 2, 8, 9°C). Thermograms of exposed silo face have been recorded (Figure 13). They have found a connection between infrared thermography images and with chemical and microbial analyze results. Samples from surfaces with higher temperature have resulted with increase in pH values, mold and yeast counts. Santos et al. (2014) indicated that feeding silages with high concentrations of yeasts from aerobic spoilage is often implicated as a cause of poor animal performance on dairies.

Junga and Trávníček (2015) suggested that infrared thermography techniques might be used for quick temperature detection of silo surface, of silage layers and areas to evaluate intensive decomposition process.

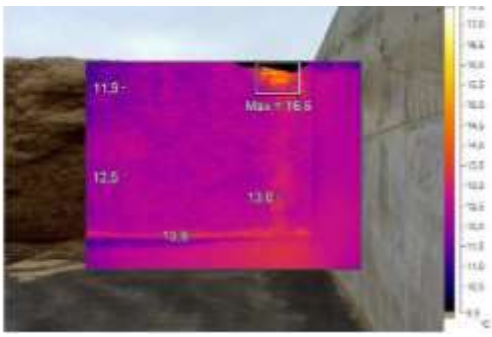

Figure 13. Thermogram of maize silage layer profile with 8.5°C air temperature (Junga and Trávníček, 2015)

Ünal et al. (2018) studied corn and wheat bale silages after 75-days fermentation period and stored at different environment temperatures (7-8°C, 24-25°C and 32-33°C). Analyzes of chemical and microbiological parameters was carried out in silage samples at days 0, 1, 4, 6, 12 and 15 of aerobic stability. They also recommended that the thermal camera imaging technique might be used as a practical method to evaluate the aerobic stability of silages in laboratory conditions.

Addah et al. (2012) used IRT technique to asses the heating of barley silages stored in large cylindrical bag silos could serve as a simple and rapid method of directly measuring and visualizing heat distribution over the feed-out face of silos in real-time. Thermal imaging offers prospects as a practical method for assessing the aerobic stability of silages on-farm.

Borreani and Tabacco (2010) evaluated the temperature at 11 locations on 54 silos and correlated the temperature with chemical composition and microbial count. They concluded that temperature is linked to microbial activity and might be an important indicator of the early stages of aerobic degradation.

Mycotoxins are secondary metabolites of certain fungal genera and cause several undesirable effects in humans and animals. Schmidt et al. (2015) evaluated mycotoxin

incidence in whole-crop corn silages from totally 109 farms at five different dairy-producing regions in Brazil. On each dairy farm, an infrared thermography camera was used to identify 3 sampling sites that exhibited the highest temperature, a moderate temperature, and the lowest temperature on the silo face, and 1 sample was collected from each site. Also, concentrations of mycotoxins were evaluated, including the levels of aflatoxins B1, B2, G1, and G2; zearalenone; ochratoxin A; deoxynivalenol; and fumonisins B1 and B2. They found no correlation between silage temperature and the presence of mycotoxins. Similarly, differences were not observed in the concentration or incidence of mycotoxins across silage locations with different temperatures. they emphasized that pre-harvest phase of the ensiling process might be the main source of mycotoxins, even in well-managed silage without heat sites. They concluded that infrared thermography was useful for identifying heat spots. However, temperature alone was not an appropriate indicator of mycotoxin incidence or concentration (Schmidt et al., 2015).

DISCUSSION

Advantages of infrared thermal camera imaging are
1) to be a rapid method
2) not need to contact with measured surface,
3) more sensitive than vision and palpation,
4) early diagnostic method (improving animal welfare and health),
5) able to combine with other techniques and technologies.
6) low labour need

Disadvantages of infrared thermal camera imaging;
1) equipment costs are high, however, decrease every year.
2) should be performed under controlled environmental conditions (Alsaaod et al., 2015; Sassi et al., 2016).

Infrared camera imaging technique should be performed under controlled conditions by trained labor. Alsaaod et al. (2015) reported that environmental factors such as airflow, environment temperature, humidity, sunlight and motion could be affected the thermograms. Environment conditions preferred to be in the neutral zone temperature, without direct sunlight and detectable airflow. In addition, infrared thermal imaging technique does not need light or shadow to capture frame accurately (Zaninelli et al., 2016). However, presence of dirt, hair or water on the skin would influence the level of emitted radiation and alter the values (Weschenfelder et al., 2013; Alsaaod et al., 2015).

Environmental conditions such as ambient temperature affect the reliability of thermal images. For such investigations, the cows should be in their thermal neutral zone and the ideal ambient temperature for imaging is in the range of 20°C. With increasing ambient temperature, the claw temperature increases from 12 to 20°C (Alsaaod and Büscher, 2012).

Calibration is another important factor that affect the accuracy, sensitivity and image resolution (Alsaaod et al., 2015). Zaninelli et al. (2016) emphasized the thermographic camera lenses were checked daily and cleaned by a researcher to ensure that high quality images were acquired during the experiment. This cleaning process might be thought as a limiting factor. Furthermore, a dedicated device that automatically clean lenses of the thermographic camera could be adopted. Otherwise, a commercial pneumatic system for shielding thermographic cameras, able to open a small door only when the camera has to acquire images, could be always adopted.

Combining different technique and technologies with infrared thermal camera imaging would have positive effects on diagnosing and early detecting the animal welfare and health problems (Alsaaod et al., 2015).

CONCLUSIONS

Infrared thermography is a useful, practical and accurate technique for identifying heat differences without invasing the surface. However, thermal camera images might be affected by environment temperatures and the presence of dirt or water on the surface might be blocked the emitted radiation.

Researches support that the infrared thermography method may be used in field conditions as a promising tool in the early detection of farm animals' health, welfare and management problems. Despite all advantages, cost of the equipment is still high, and this factor is a barrier for common usage.

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CHEMICAL AND ANTIBACTERIAL INVESTIGATIONS OF *ROSMARINUS OFFICINALIS*

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ABSTRACT

Rosmarinus officinalis was extracted using Soxhlet extraction method, hexane extract and acetone extract were obtained. Four phenolic compounds were diagnosed with HPLC after acid hydrolases of acetone extract (Kemferol, Gallic acid, Catechine and Qurcetine) and nine fatty acids were diagnosed with GLC after the saponification of hexane extract (Hexanoate, Myristate, Myristoleate, Palmatic, Stearic, Arachidate, Araghidatente and Lignocerate). The inhibitory effect of the active compounds separated against two types of Gram negative bacteria *E. coli* and *Proteus vulgaris* was tested using the disc diffusion method and there was a noticeable and differentiated effect compared to the antibiotic used.

Key words: *Rosmarinus officinalis*, GLC analysis, HPLC analysis, Phenolic compound, Antibacterial activity

INTRODUCTION

Many people, including many researchers, have turned to the active ingredients of plant origin because they are very safe and have no significant side effects and due to the indiscriminate use of antibiotics and the resulting dangerous side effects (Nieto, *et al*., 2011). The medicinal plant was used in the past and is still used to the present time and the medicinal plant is important if it has a vital effect or if it is used in the treatment of common diseases, especially bacterial infections (Raškovic, *et al*., 2014). Its use has increased because it contains medically effective chemical compounds such as alkaloids, resins, cyclosides, tannins, volatile oils, etc. More than 85% of people use the medicinal plant for treatment, whether it uses the entire plant or part of it or plant extract (Habtemariam, 2016).

Rosmarinus officinalis is an evergreen and small tree and from the family Lamiaceae. *Rosmarinus officinalis* contains multiple active substances with a medicinal therapeutic effect, among which it is used in the treatment of poisoning cases because it contains Rosmarinic acid, as well as antioxidants and other insect repellents (Nieto, *et al*., 2012). Many researchers confirmed the role of the rosemary plant in treating many positive and negative Gram such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermis* (Teixeira *et al*., 2013 and Vegara, *et al*., 2011). NMR, GC / MS, HPLC, ESI-MSn, LC / DAD and many diagnostic methods that represent the quality of the active compound and its concentration within each medicinal plant can know the effect of these compounds and their quantities and that in treating many diseases and this is confirmed by many researchers (Khalel and Sultan, 2019 and Sultan, *et al*., 2020a).

MATERIALS AND METHODS

The preparation of plant extracts

Rosemary leaves were used and Prepared hexane and IMS extracts consisting of mixing 95% ethanol with 5% methanol, depending on the method by the researcher (Sultan, *et al*., 2020b) and using the Soxhlet system, where 250 ml of the solvent used with the 40g plant was mixed for 10 hours per day until the color of the first solvent, which is hexane, disappeared, and then the second solvent was applied. It is the acetone and the process is repeated until the solvent color disappears and becomes colorless, and then the solvent from the extract evaporates at a temperature of 45°C with a rotary evaporator device (Harborne, 1984).

Separate the active chemical compounds from the *Rosmarinus officinalis*

Fatty acids were separated according to the method of the researcher Arthur, 1972 by the process of saponification, where he mixes 2g of hexane extract to the leaves of rosemary plant with 25 ml of potassium hydroxide and then refluxed for an hour and at a temperature of 100°C. We add distilled water and start separating the fat by adding ether and separating it from the aqueous layer by separating the separation then we increase the acidity of the separated solution using H_2SO_4 until reaching the acidity degree 2 and after obtaining the fatty acids with ether the result is esterified so that the sample is ready for diagnosis by GLC device (Loury, 1967 and Sultan, *et al*., 2020b).
Also in this study, a number of free phenolic compounds were separated according to the researcher's method (Sultan, 2018) in a process acid hydrolysis, as 2g of acetone extract is mixed with 25 ml of hydrochloric acid and refluxed for an hour at a temperature of 100°C, then the phenols are separated in the separation funnel by ethyl acetate and then evaporated with a rotary evaporator to obtain free phenols and then kept in the refrigerator until diagnosed with a device HPLC (Harborne, 1998).

Diagnosis of separated active compounds under study

Two different techniques were used in the diagnosis of active compounds separated from the leaves of the rosemary plant. Fatty acids were diagnosed with the GLC device, as well as the free phenols separated by the HPLC device were diagnosed, depending on the retention time, each compound inside the apparatus in the extract is compared to standard compounds and was measured at the Ministry of Technology / Baghdad.

Inhibitory effect for separated chemical compounds against human pathogenic bacteria

In this study, two types of Gram negative bacteria were selected *E. coli* and *Proteus vulgaris*. Ready isolates were taken from the College of Science, University of Mosul, and three replications of each isolate were preserved in Nutrient broth until they were used in the biological activity experiment (Al-Dulayymi, 2014).

Experiment of antimicrobial active

The bacterial trap after preparation was compared with the standard MacFarland solution, then the bacteria were spread with a glass rod in the center of Hinton agar after dilution by normal slain, then the 6 mm diameter filter paper tablets saturated with the active compounds of rosemary leaves at a rate of 0.1 cm^3, The extracts were dissolved by DMSO and in four different

concentrations (100, 75, 50, 25) mg/cm^3, then placed in the incubator at 37°C and after 16 hours the diameter of the inhibition zone is measured Antibiotic Floroxin 5µg/ml was used as a control sample to compare the inhibitory effect of extracts on bacteria, The Duncan test was used to analyze the data statistically and with a significant difference of $p \leq 0.01$ using the SAS program (Miladinovic and Miladinovic, 2000; Djipa, *et al*., 2000 and Sultan, *et al*., 2020a).

RESULTS AND DISCUSSION

Diagnosis of fatty acids

Fatty acids from the hexane extract of *Rosmarinus officinalis* were diagnosed with Gas-liquid chromatography GLC. Standard compounds of fatty acids were compared with the retention time values of the extract present in the GLC-chromatograms within the apparatus and through the GLC apparatus the concentrations of each fatty acid present inside the extract were known. The following fatty acids have been diagnosed: (Hexanoate, Myristate, Myristoleate, Palmatic, Stearic, Arachidate, Araghidatente and Lignocerate) and as shown in Table 1 and Figure 1 which takes time and proportions for each of the extracted and diagnosed fatty acids with a GLC device which It was separated from the hexane extract after conducting the soaponification process from the lowest to the highest concentration (Lignocerate 0.0045, Araghidatente 0.0059, Myristoleate 0.008, Hexanoate 0.100, Palmatic 0.828, Arachidate 4.037, Stearic 0.0035, Myristate 0.0037). Also, the detection time and concentration of each fatty acid separated from rosemary was detected.

Table (1): The Conc. and Ret. time of standard and fatty acid extract by GLC analysis.

Standard	Fatty acid							
	Stearic		Myristate		Lignocerate		Arachidatenate	
	R_t min 20.216	Conc.	R_t min 15.578	Conc.	R_t min 29.456	Conc.	R_t min 24.407	Conc.
Extract	20.212	0.0035	15.574	0.0037	29.441	0.0045	24.470	0.059
Standard	Myristoleate		Hexanoate		Palmatic		Arachidate	
	R_t min 16.098	Conc.	R_t min 6.015	Conc.	R_t min 17.785	Conc.	R_t min 23.755	Conc.
Extract	16.094	0.008	6.010	0.100	17.739	0.828	23.784	4.037

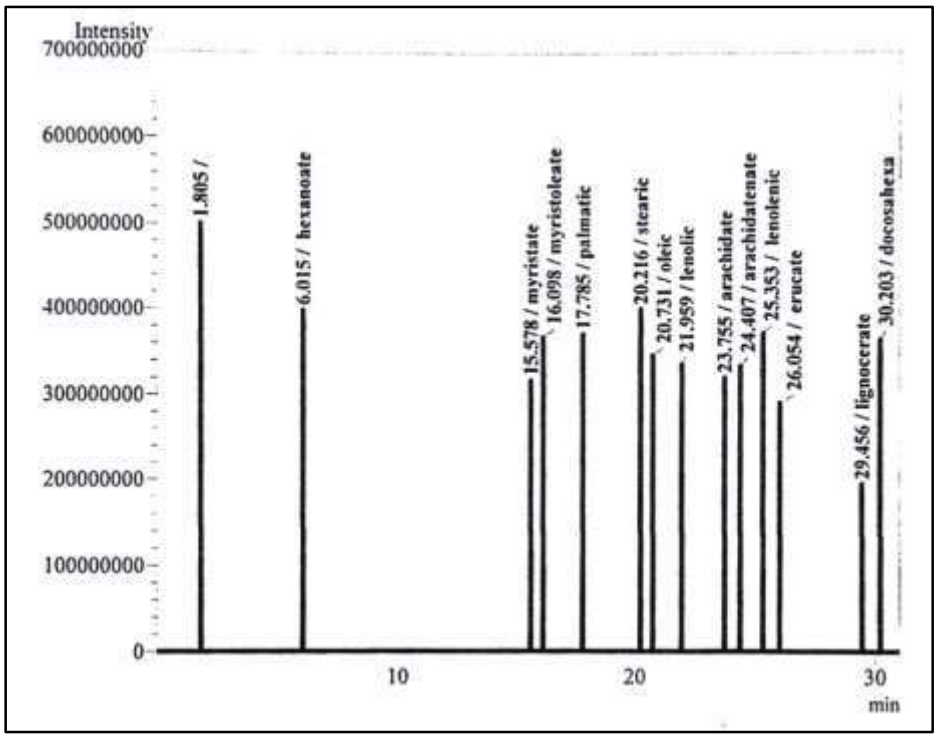


Figure (1): GLC analyses of standard fatty acid

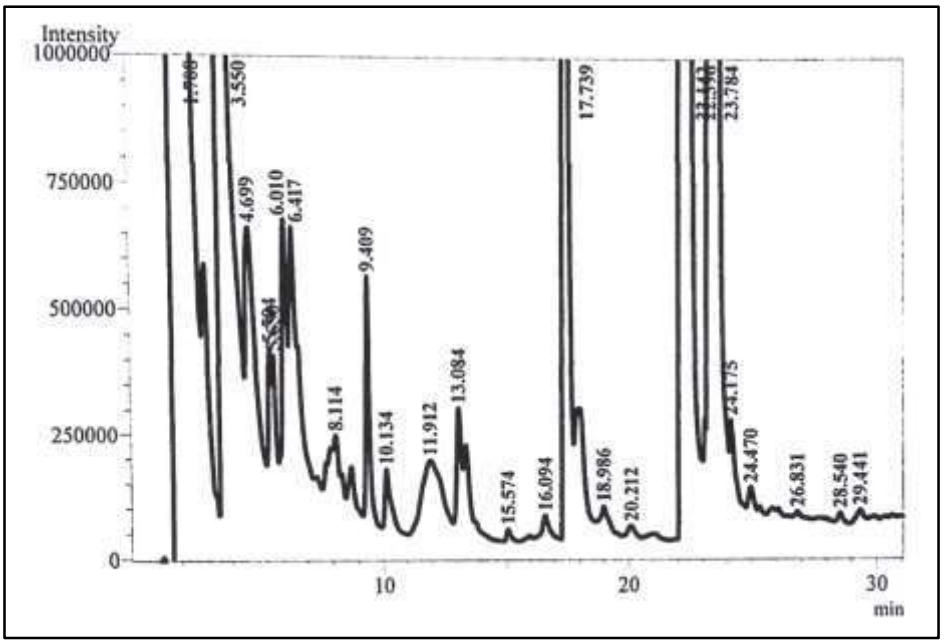


Figure (2): GLC analyses of extract fatty acid

The results showed that Arachidate is present with the highest percentage of 4.037 and that Stearic is present with the lowest percentage of 0.0035 and lies between them the rest of the fatty acids and both according to the percentage of his presence in the laboratory and as suggested in Table 1, successive separation of the separated fatty acids between them (Mohamed, *et al*., 2017).

The researchers confirmed that rosemary has many active compounds, which include fatty acids, as well as alkaline and other flavonoids, turbines and many of the compounds that have effectiveness against bacteria, anti-oxidant, anticancer, and the treatment of many other diseases and other compounds. The major role in increasing the immunity of the human body.

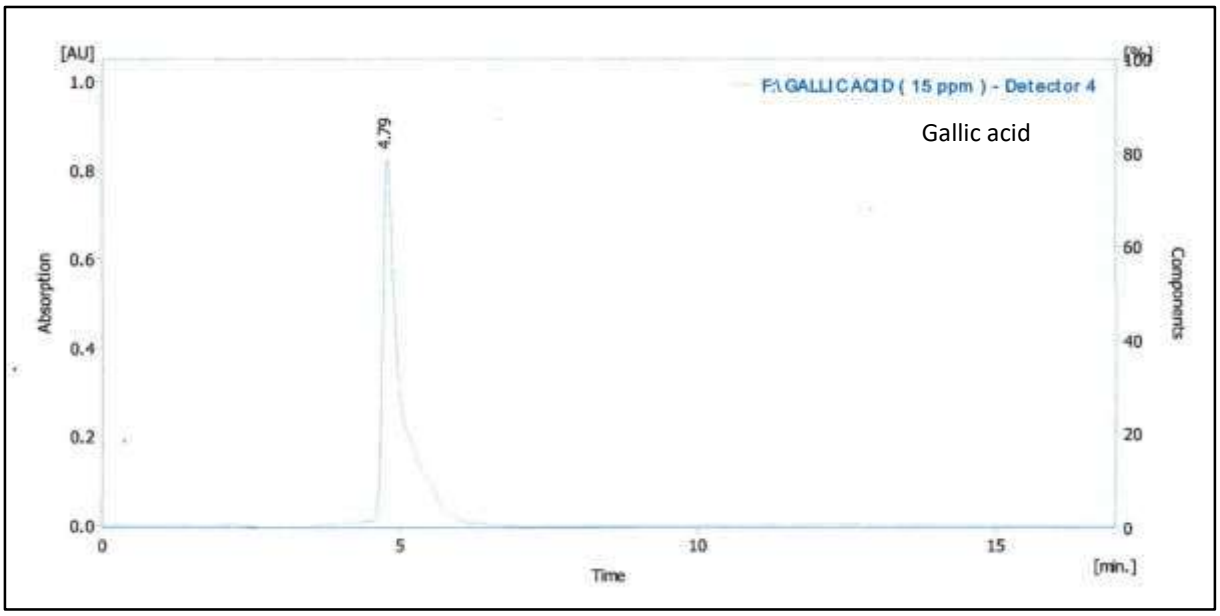
The important compounds are Arachidate, Oleanolic acid, Rosmanol isomer, Carnosic acid (Kesatebrhan and Tesema, 2014 and Kontogiann, *et al*., 2013).

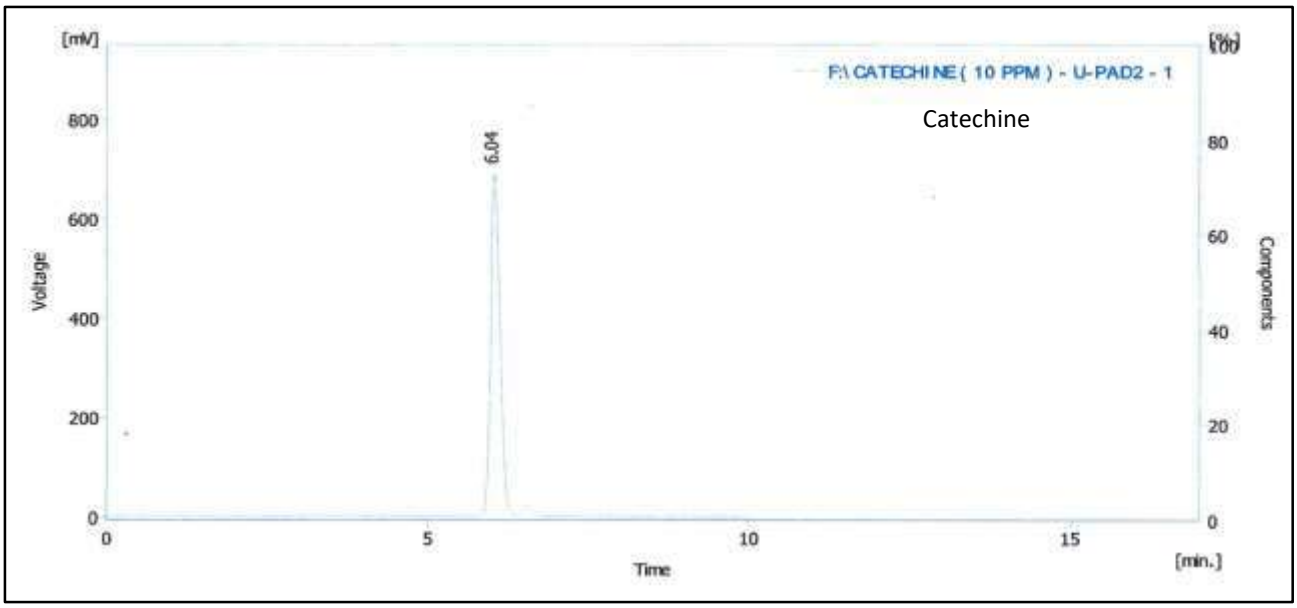
Diagnosis of phenols compound

Table 2 and Figures 3 and 4 show the separated phenolic compounds as free phenolic compounds were obtained from the rosemary plant by conducting the acid hydrolysis process on the IMS extract for rosemary leaves. These phenols were diagnosed using the HPLC device and it was found that rosemary contains many phenolic compounds And with different concentrations and with a different detention time from one compound to another, including it (Gallic acid 4.833, Kemferol 13.150, Catechine 6.367, Qurcetine 11.570).
The results of the study showed that the highest concentration of phenolic compounds separated from rosemary is Catechine, at a ratio of 11.1, while the lowest concentration of compounds was Kemferol, at a percentage of 2.0. This is in agreement with researcher (Kesatebrhan and Tesema, 2014), who has proven, through his research, that rosemary contains many free and diagnosed phenols with the latest chromatographic diagnostic methods such as GC and GC / MS like 1,8-cineole, Camphor, Linalool, Qurcetine, Catechine.

Table (2): The Ret. time of standard and extract phenolic compounds by HPLC analysis.

Standard	Phenolic							
	Gallic acid		Catechine		Qurcetine		Kemferol	
	R_t min 4.79	Conc.	R_t min 6.04	Conc.	R_t min 11.90	Conc.	R_t min 13.75	Conc.
Extract	4.73	3.5	6.06	11.1	11.97	6.3	13.65	2.0

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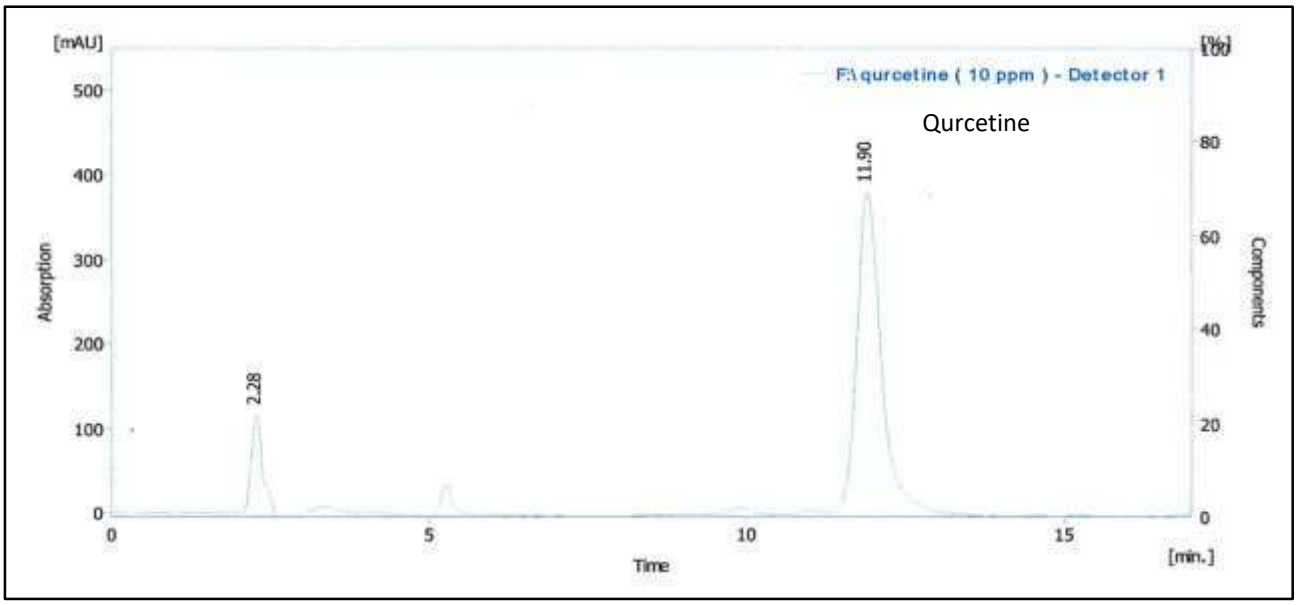
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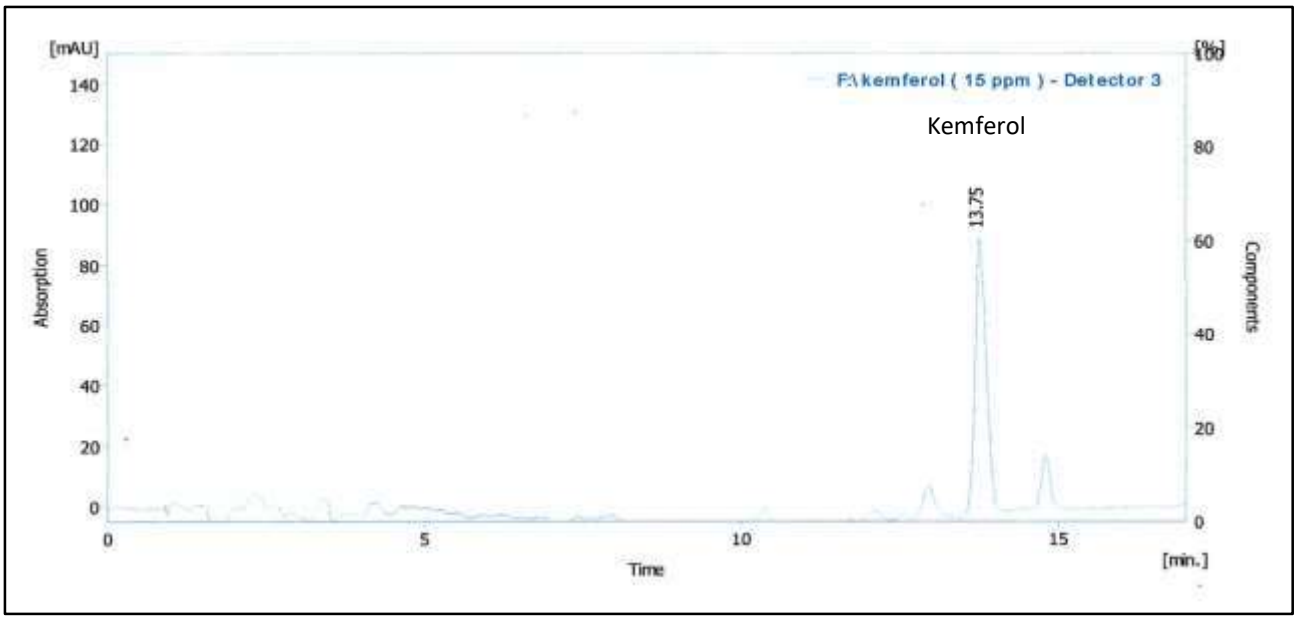


Figure (3): HPLC analyses of compound standard phenolic

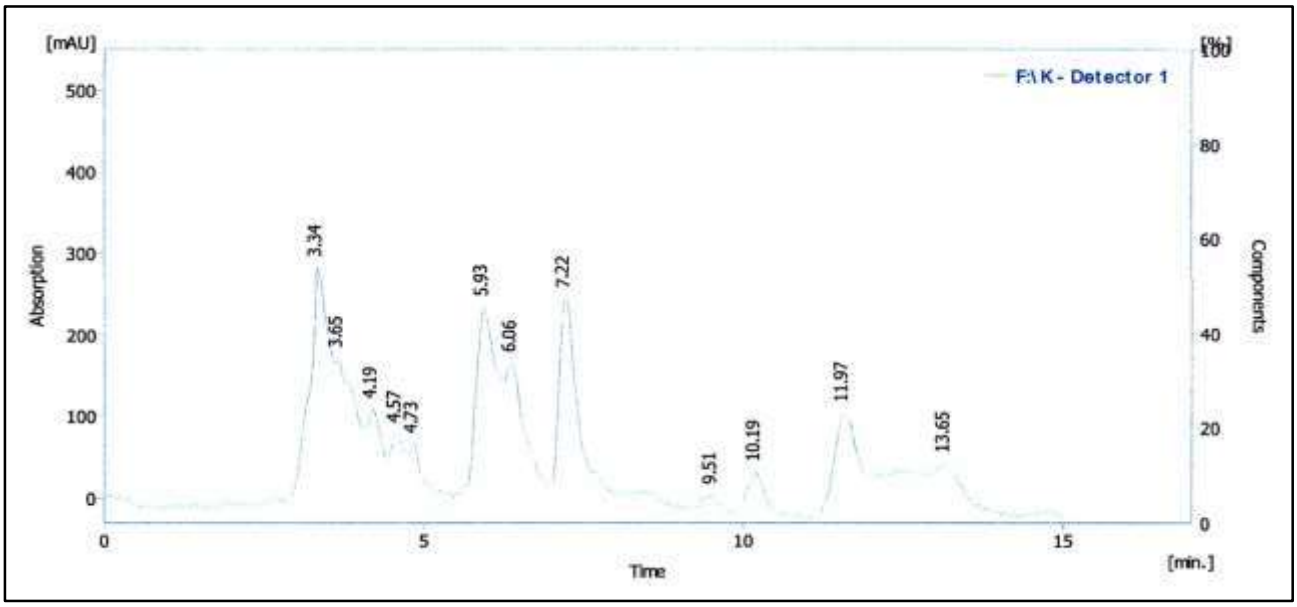


Figure (4): HPLC analyses of extract phenolic compound

The effect of the fatty acid and phenolic compound separated from Rosmarinus on pathogenic bacteria. The biological activity process was carried out on two types of Gram negative bacteria *E. coli, Proteus vulgaris* The test method is a method disc diffusion. To know the effect of fatty acids and free phenols separated from rosemary, as the effect of fatty acids was less than the effect of phenolic compounds separated from rosemary compared to the standard antibiotic used under study, Table 3 and Pictures 1+2.

The separated fatty acids affected bacteria *E. Coli* as much as an antibiotic. Ciprofloxacin as the highest effect was with a Zone inhibition of 15 mm at a concentration of 100 mg/C^3 and a minimum Zone inhibition of 8 mm at a concentration of 25 as fatty acids less than the antibiotic under study affected bacteria *Proteus vulgaris*. It affected 12 mm in Zone inhibition with a concentration of 100 mg/C^3. As for the effect of phenolic compounds on the bacteria used under

study, it was higher than that of the antibiotic used, it was the highest effect of phenolic compounds with a Zone inhibition of 22 mm at a concentration of 100 mg/C^3 in bacteria. *E. Coli* The Zone inhibition of the inhibition diameter of 18 mm with the same concentration against bacteria *Proteus vulgaris*.

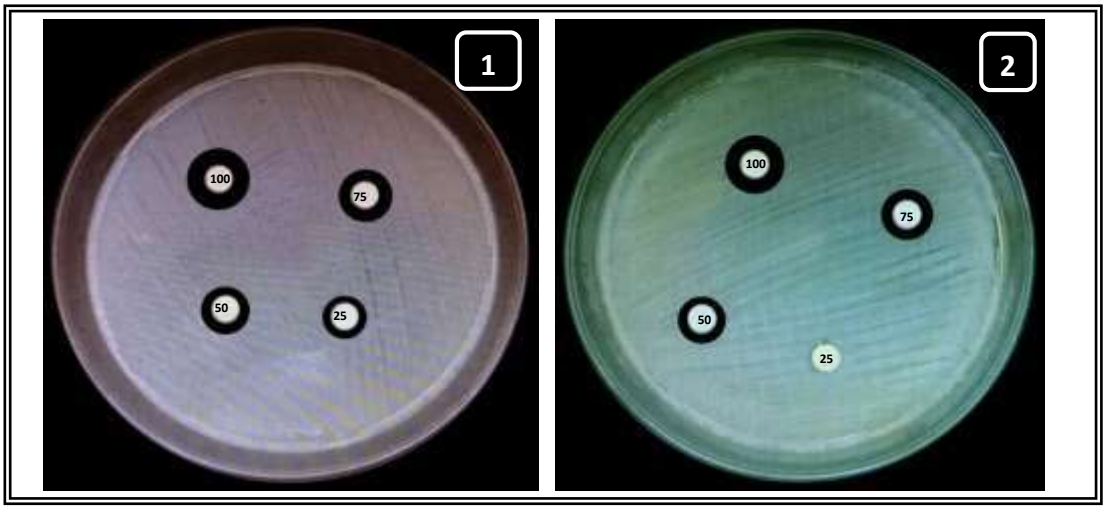
Many researchers emphasized the effect of fatty acids on treatment of many diseases, including bacterial infections, due to the role of fatty acids in their effect on the oxygen pathway and energy complex of the bacterial cell and thus its death (Ahmed *et al*., 2015; Yuan *et al*., 2006 and Abd El-Mageid, *et al*., 2009). Phenols affect bacteria by denaturation protein (Williams and Lewis, 2011).

The results of the current study agreed with (Kesatebrhan and Tesema, 2014 and Mohamed, *et al*., 2017) on possessing rosemary plant on many phenolic compounds that have an inhibitory effect against many positive and negative Gram. As the researcher separated the oil from the rosemary plant by water distillation and separated and diagnosed 43 phenolic compounds by the GC / MS apparatus, and these compounds showed their inhibitory effect against many positive and negative Gram, and this is consistent with the current study (Kesatebrhan and Tesema, 2014). The researcher also confirmed (Nieto, *et al*., 2018) rosemary plant containing many phenols. It has proven anti-bacterial, anti-fungal, anti-insect and anti-oxidant effect (Sahalie, *et al*., 2018).

Table (3): Antimicrobial efficacy of chemical Composition of *Boswellia serrate* extract (mm).

Microorganism	Chemical Composition	Concentration (mg/C^3)/ Zone inhibition in (mm)				
		100	75	50	25	Ciprofloxacin (5µg/ml)
E. coli	Fat	15	13	10	8	15
	PH	22	18	15	12	
Proteus vulgaris	Fat	12	10	8	-	10
	PH	18	15	10	8	

Fat = Fatty acid, PH = Phenolic Compound.



Photos (1+2): Antibacterial activity effect of 1. fatty acids agents *E. coli*, 2. fatty acids agents *Proteus vulgaris*.

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EFFECT OF TILLAGE PRACTICES ON YIELD AND PHOSPHORUS UPTAKE OF A DURUM WHEAT CROP UNDER SEMI-ARID CONDITIONS IN NORTHERN ALGERIA

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ABSTRACT

The aim of this study is to compare the effect of three tillage practices: conventional tillage (CT), reduced tillage (RT), and no-tillage (NT), on yield and phosphorus uptake of a durum wheat crop (*Triticum durum* var Simeto) in semi-arid conditions of northern Algeria. The study was carried out in 2017-2018 growing season in a trial established since the year 2015; the experiment is conducted in a randomized complete block design with three replicates. Grain, straw and biomass yields were assessed, phosphorus concentration in grain and straw was analyzed, and the total phosphorus uptake was determined by multiplying the grain and straw dry matters by their respective P concentrations. There were no effects of tillage on durum wheat production in this particularly rainy growing season. The favorable climatic conditions had a positive impact on durum wheat grain and biomass production, grain yields of 4.06 Mg ha^{-1}, 4.04 Mg ha^{-1} and 3.65 Mg ha^{-1} were obtained by RT, CT and NT systems respectively, which is superior to the average yields obtained usually in the region. A biomass production of 14.9 Mg ha^{-1}, 14.6 Mg ha^{-1} was recorded by RT and CT respectively and 10.9 Mg ha^{-1} by NT system. Phosphorus concentration in both grain and straw and the total P uptake by plants were not significantly affected by tillage systems, statistically similar P concentrations were obtained by the three treatments. P content values of: 5.1 g kg^{-1}, 5.2 g kg^{-1} and 4.8 g kg^{-1} were found in grain harvested from NT, CT and RT plots respectively, these values are above those reported by several authors, which indicate a fairly good phosphorus nutrition.

Key words: No-tillage, conservation tillage, phosphorus, durum wheat, semi-arid regions.

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the most important cereal crop in Algeria and in the Mediterranean basin, it occupies more than 2Mha of the Algerian agricultural land with an annual production of 2Mt which is far from covering the demand of the growing population. The cereal production in the country is concentrated in the interior high plains characterized by a Mediterranean climate, with variable rainfall and frequent droughts. The severe natural conditions associated with the use of cropping systems based on monoculture of cereals, fallow and deep and intensive tillage have exposed the soils of the region to degradation and loss of production potential. The adoption of the conservation agriculture techniques, especially,

conservation tillage has proved its advantages in the Mediterranean region. Conservation tillage has considerable potential for stabilizing production in the semi-arid zones. Many authors have reported a greater yield production under no-tillage compared to the conventional tillage in conditions of water shortage (López-Bellido *et al*., 2000; De vita *et al*., 2007; Mazzoncini *et al*., 2008). Devita *et al*., (2007) estimated that under 300 mm rainfall a superior wheat yield is occurred under no-tillage system.

Phosphorus is one of the major nutrients that plants need for their growth, it is considered as a limiting factor for biomass production in many types of areas. The phosphorus availability can be improved by the conservation tillage techniques. Many researchers noticed a stratification of available P in the soil surface layers under reduced tillage and no-tillage systems (Piegholdt *et al*., 2013 and Peigné *et al.*, 2018), this may be attributed to a greater microbial activity under undisturbed soil. A higher P concentration in durum wheat grain under no-tillage system has also been reported by Mozafar *et al*. (2000), Mazzoncini *et al.* (2008) and Woźniak *et al.* (2014).

The aim of this study is to compare the effect of three tillage practices: conventional tillage, reduced tillage and no-tillage on yield and phosphorus uptake of a durum wheat crop under rain-fed conditions in a semi-arid region of northern Algeria.

MATERIAL AND METHODS

Experiment site

The experiment is conducted on a heavy soil (54% of clay) in a farm located in the region of El-Hachimia in northern Algeria (36° 14′ 21″ N, 3° 50′ 23″ E in an altitude of 713 m above the sea level), the region is characterized by a Mediterranean climate with a great variation of the rainfall in the year and between years, the mean annual rainfall in the region in the last 30 years is 479 mm.

Experimental design and crop management

This study is a part of an experiment started in the year 2015. The trial consists on a comparison between three tillage practices in a continuous durum wheat crop: (i) conventional tillage (CT), consisting of moldboard ploughing to 30 cm depth followed by a disk harrowing for bed seed preparation, (ii) reduced tillage (RT) using a cultivator to 7 cm depth, and (iii) no-tillage (NT) with crop residue retained in surface. The different tillage practices were arranged in plots of 60 m long and 10 m wide in a randomized complete block design with three replications.

No-tillage plots were sawn using a no-tillage seed drill, the seeding rate of all treatments was 180 kg ha^{-1}, Semito cultivar being the most cultivated in the region is chosen for the study. NT weed control was accomplished by applying glyphosate (2.5 l ha^{-1}), a fertilization as mono-ammonium phosphate (12% N and 52% P) in the rate of 150 kg ha^{-1} was applied before sowing, and 100 kg ha^{-1} urea fertilizer (46% N) was applied in the tillering stage of the plant, fungicides, pesticides and herbicides were also applied at the time of appearance of fungal diseases, pests or weeds.

Measurements and analysis

The harvest was mechanically done and grain yield was directly determined in each plot. In order to obtain the biomass yield, three samples of two linear meters have been harvested and weighted after they had been oven-dried at 75°C for 48 hours, the average of the dry matter weight of the three samples is reported to square meter and then per hectare; the straw yield

was then obtained by subtraction. Grain and straw sub-samples were taken from each of the three samples, and P was determined by the colorimetric method and expressed in grams per dry matter kilogram (g kg^{-1}). The plant total P uptake was determined by multiplying the grain and straw dry matters by their respective P concentrations.

Statistical analysis

Data were evaluated by two-way ANOVA. The F test was applied at 5% probability level to check the significant differences between the three tillage practices.

RESULTS

Climatic conditions

The climatic conditions of the growing season 2017-2018 (table 1) are particular compared to the long term data in the region. The quantity of rainfall observed during the vegetative cycle of the crop (December to June) is 22 % higher than the cumulative rainfall obtained in the same period in the last 30 years. This significant amount of rainfall was characterized by a good distribution in the year which was beneficial for the vegetation development. The important quantity of precipitations obtained in the months of April and May which coincides with flowering and grain filling stages induced a good average yields in all the experiment treatments as well as in the region.

Temperatures of this season have followed the long term data, except for the month of May (grain filling stage) which was less hot than the long term average.

Table 1. Monthly rainfall and mean maximum and minimum temperature in the vegetative cycle of the durum wheat crop for the growing season 2017-2018.

Month	Rainfall (mm)	T min (c°)	T max (c°)
November	87	7.3	18.6
December	55	5.2	13.3
January	23	5.0	13.9
February	33	2.8	11.6
March	99	6.8	14.8
April	131	9.0	19.2
may	60	10.5	21.2
June	4	14.6	29.1
Total	492		

Yields and Harvest Index

The grain, biomass and straw yields, as well as the harvest index were not significantly affected by tillage systems (table 2). Grain yields of all treatments (4.06 Mg ha^{-1}, 4.04 Mg ha^{-1} and 3.65 Mg ha^{-1} obtained by RT, CT and NT systems respectively) were about 50 % higher than the average obtained in the country in the last decade which is equivalent to 2 Mg ha^{-1} (statistics of agriculture ministry), this is due to the exceptional climatic conditions, in particular, the great amount of rainfall observed in the grain filling period. The means of grain, biomass and straw yields and the harvest index obtained by the different treatments are summarized in the table 3.

Phosphorus uptake

There were no significant effects of soil tillage on P concentration in grain and straw as well as on the total P uptake by durum wheat plants (table 2). The P content in the grain harvested from the different plots was ranged from 4.31 g kg^{-1} to 6.62 g kg^{-1}, which indicates fairly good phosphorus nutrition. Means are summarized in table 3.

Table 2. Mean square and significance of traits studied

Traits	Mean square			
	Treatments (df = 2)	Blocks (df = 2)	Error (df = 4)	CV %
Grain yield (Mg ha^{-1})	16.29 ns	118.81*	12.23	8.9
Biomass yield (Mg ha^{-1})	1482.2 ns	242.4 ns	476.8	16.19
Straw yield (Mg ha^{-1})	1101.61 ns	643.96 ns	356.94	21
Harvest Index	0.003 ns	0.014*	0.002	13.45
Grain [P] g kg^{-1}	0.147 ns	1.461 ns	0.429	13
Straw [P] g kg^{-1}	0.308 ns	0.071 ns	0.429	58.5
Total P uptake (kg ha^{-1})	6.66 ns	11.82 ns	30.65	19.53

NB: ns = not significant at $p<0.05$, * significant at $p<0.05$.

Table 3. Means of all traits studied during the growing season 2017-2018

Traits	Means		
	NT	RT	CT
Grain yield Mg ha^{-1}	3.65	4.06	4.04
Biomass yield Mg ha^{-1}	10.92	14.90	14.61
Straw yield Mg ha^{-1}	6.79	10.19	10.01
Harvest Index	0.35	0.29	0.30
Grain [P] g kg^{-1}	5.12	4.77	5.18
Straw [P] g kg^{-1}	1.49	0. 92	0.95
Total P uptake (kg ha^{-1})	29.35	26.64	29.07

NB: NT: no-tillage, RT: reduced tillage, CT: conventional tillage

DISCUSSION

The grain yields obtained by all the tillage systems were statistically similar. These results are in disagreement with those obtained by several authors in similar environment. López-Bellido *et al*. (2000), De vita *et al*. (2007), Mazzoncini *et al*. (2008), Amato *et al.* (2013) and others affirm that durum wheat yield obtained by CT system is significantly higher than NT in conditions of a good water supply. De vita *et al.* (2007) estimate that in the Mediterranean conditions, a superiority of CT is occurred when rainfall recorded during the vegetative cycle is more than 300 mm.

Even the rainfall recorded in this season was about 492 mm, the CT grain and biomass production was similar to the conservation tillage systems. The reason why the CT could not express its superiority in this favorable climatic conditions may be attributed to its poorly made seed bed. Because of the wet conditions which characterized the period of soil preparation (87 mm rainfall in November) and the clay texture of the soil, a non-homogeneous seed bed has been performed in CT plots which had negatively affected the seed germination and plant density.

Our results show that no reduction of grain and biomass production results from the adoption of the conservation tillage systems (RT and NT), with their proven advantages of time, fuel and labor savings, added to their superiority to CT in the driest seasons; the conservation tillage techniques may be the perfect alternative for farmers in the region who, in addition to grain production, are also envisaging the straw since the agricultural system is based on association of cereal production with livestock.

Phosphorus concentration in the grain was not significantly affected by tillage, similar P content was found in both conservation tillage systems and CT, this is in contradiction with the findings of Mozafar *et al*. (2000), Mazzoncini *et al.* (2008) and Woźniak *et al.* (2014) which have reported a significantly higher P content in durum wheat grain under NT. In the same manner, P content in straw did not significantly vary with tillage, which is in disagreement with the results of Mazzoncini *et al*. (2008) who find a higher P content in the straw under NT in nine out of ten years of experimentation. The total P uptake has not been affected by tillage systems; this can be attributed to the insufficient duration of the experiment to see the stratification of available P in the upper soil layers as reported in literature.

The grain P concentrations in the different treatments are above those reported by Mazzoncini *et al.* (2008) who find average values of 3.5 g kg^{-1} and 3.84 g kg^{-1} in CT and NT respectively, in a ten-year experiment conducted in southern Italy, similarly Woźniak *et al.* (2014) obtain values of 3.11 g kg^{-1} in CT and 3.47 g kg^{-1} and 3.68 g kg^{-1} in RT and NT respectively in more wet conditions. The grain obtained from all the plots in our experiment seems to be sufficiently rich in P, according to Bilal *et al*. (2019), a high P content in wheat grain can be a good strategy to overcome a P deficiency in the plant in its first growing stages.

CONCLUSION

Our results show a similar grain and biomass production under the three tillage techniques in this particularly wet season. A reduction or even a total removal of soil tillage did not affect the durum wheat production; several studies in similar environment have found an advantage of the NT and RT in the driest years. Since the NT adoption in the region still needs time to obtain the necessary equipment, the reduced tillage without soil returning seems to be the best alternative in the region, added to its time and labor saving compared to the CT, it allows a good seed bed preparation, a good drainage and a better seed germination.

The P content in grain and straw as well as its total uptake by the plant seems to be not affected by tillage, at least, after three years of the adoption of no-tillage and reduced tillage.

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CONSUMER PERCEPTION OF FRESH MEAT QUALITY IN TIRANA

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ABSTRACT

The quality of meat products differs from intrinsic and extrinsic indications so that it can be establish in accordance with preferences of consumer. This survey examines the consumer confidence, attitude and behavior towards the quality of fresh meat in Tirana. The source of information and data were collected from a detailed questionnaire using random interviews 367 persons in different areas in the city of Tirana. Chi squared or Fisher's exact tests was utilized to analyzed relationships between two categorical variables. A significant relationship between education, income and frequency of meat consummation was observed. Place of origin (51.6%) was the most extrinsic factor that consumer prefer more while colour (49.2%) was the most important intrinsic cues.

Keywords: *meat quality; Tirana consumers' perception; survey*

INTRODUCTION

Meat is an important component in the daily diet of a large proportion of society and is regarded as a valuable food from a nutritional perspective (Fayemi & Muchenje 2012). Chemically meat is composed of four major components including water, protein, lipid, carbohydrate and many other minor components such as vitamins, enzymes, pigments and flavour compounds (Lamber et al., 1991). The present demand of consumers for receiving information on food quality and safety, determines in great measure the characteristics of the food chains and the strategies to develop by the industry (Garcia et al., 2004). Nowadays, food safety is an issue that regards credence and trust on food products, which is becoming more and more important for consumer (Brunso et al., 2002). As result of the recent food crises, consumer feels more concerned with food quality and safety, demanding more transparency in the food-chain and more information on the diverse qualitative characteristics of foods (nutritious value, origin, way of production, etc.) (Ventura-Lucas, 2004). The latest food scandals such as BSE, Foot and mouth disease, the Dioxin crisis in Belgium, Horse meat (2013) and Listeriosis in Spain (2019) have significantly increased the consumer incredulity and suspicion in meat products. Food safety perception not only differs between countries, but also may appear dissimilar within a single country.

Patterns in meat consumption are unpredictable and it is clear that changes are occurring in the way consumers behave towards food (Grunert, 2006). Food choice is a complex phenomenon affected by changing preferences and many variables. Especially meat represents an unpredictable task which is influenced by many factors such as knowledge, previous experience, extrinsic and intrinsic cues. Despite our knowledge on the type and importance of

meat quality attributes, consumers still have difficulty in accurately predicting experienced quality by perception at the point of purchase (Grunert et al., 2004). When a consumer evaluates a product alternative that may satisfy the same need, desire or want, he integrate the perceptions of the alternatives into an overall judgement, or attitude, about the attractiveness of each product alternative (Steenkamp and Trijp, 1989).

Consumption of meat in Albania has almost tripled compared to the 1990s, passing from 20 kg to nearly 60 kg per capita (Instat 2017). Nevertheless, these level still remains lower compared to the EU countries. This research intent to investigate deeply perceptions, attitude, that mostly affect consumer behavior when they choose meat products in the city of Tirana. The main focus was understanding consumer opinion, knowledge and demands above quality of meat products. The paper presentation includes a comprehensive information on the research methodology and final results. At the same time these publication intents to discover findings and recommendations for meat industry to develop products in accordance with consumer expectations and preferences.

MATERIALS AND METHODS

Study Site

The study was carried out in the capital of Albania (Tirana), which is located in the center of Albania. Tirana is the biggest city in Albania with a population of 906,166 as of 2018, makes for 31.84% of Albania's population (Instat 2018).

Selection of Respondents

The proposed format was considered in order to understand the habits, trends and attitudes towards food safety and meat quality before purchase. A random sampling strategy of 367 consumers (48.8% Male and 51.2% Female) from the city of Tirana was utilized in the study to fulfill the objectives of the study. The survey data were collected through face-to-face interview methods by the first author using survey instruments which they prepared reviewing the earlier studies (Grunert 2006). The purpose of the questionnaire was clearly explained to the consumer before the interview were addressed.

Data collection

In order to get detailed information the survey questionnaire compromise 11 questions, divided in three different parts. The questionnaire paid a special attention on consumer profile, consumption habits, knowledge of certification and differentiation, beef purchase process, knowledge of beef traceability, beef consumption, and a general question. The first part was related with the gender, age, education and incoming, while on second part information on, type of meat they prefer most, frequency on meat consume and place of purchase. The third part of the questionnaire was focused in the evaluation of the extrinsic and intrinsic quality cues.

Extrinsic and intrinsic quality cues

To evaluate extrinsic and intrinsic quality cues was utilized a scaling method with five alternatives in different scores range from 1-“very important” to 5-“totally unimportant”. Extrinsic cues were focused on place of origin, price, transability and packaging while intrinsic were focused on colour, flavour, tenderness and marbling.

Statistical analysis

First, descriptive statistics was used to present date as mean, standard deviation (SD), range or number and percentage. Then, Chi-square Goodness was used to compare the observed distribution with the expected probability distribution (assuming all categories equal) while the relationships between two categorical variables were analyzed by Chi squared or Fisher's exact tests. In addition, z-tests was used to compare column proportion. A P-value < 0.05 was considered statistically significant All analyses were performed using SPSS version 25.0 statistical analysis software (IBM Inc., Chicago, IL, USA).

Results

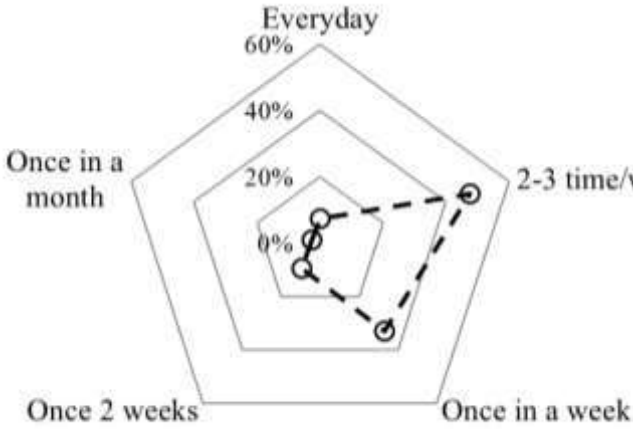
Table 1 represent demographic data of participants. A total of 367 people participated in the study. There was no difference in the proportion of males and females: 48.8% were males (n=179) and 51.2% were females (n=188; P>0.1). The ages of the participants ranged from 19 to 74 years with a mean±SD age of 44 ± 14 years. Most were 29-48 years old (P<0.001), with high school (39.2%) or university education (58.9%; P<0.001). Most of the participants had a (87.9%, n = 323) had monthly income between 17 and 2000 mije lek.

Table 1. Demographic data of participants

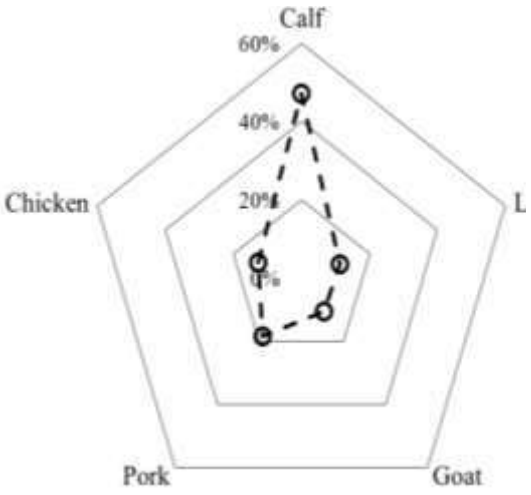
Parameter		**Count (N=367)**	**N %**	**P value**
Gender	**Male**	179	48.8%	0.639
	Female	188	51.2%	
Age	**19-28 ys**	61	16.6%	**0.017**
	29-38 ys	89*	24.3%	
	39-48 ys	89*	24.3%	
	49-58 ys	58	15.8%	
	>58 ys	70*	19.1%	
Education	**Primary school**	7	1.9%	**<0.001**
	High school	144*	39.2%	
	University	216*	58.9%	
Month income	**Until 17 mije lek**	9	2.5%	**<0.001**
	From 17-50 mije lek	97*	26.4%	
	From 50-100 mije	140*	38.1%	
	From 100-2000 mije	86*	23.4%	
	Up to 200 mije	35	9.5%	

* higher observed number respect to expected (all categories equal)

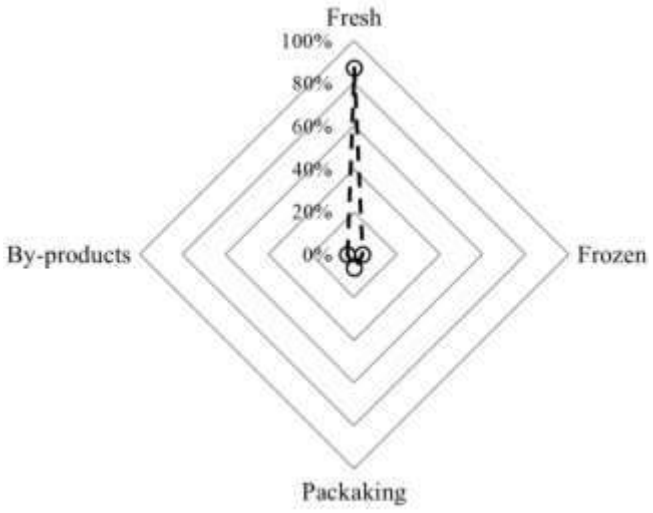
Most of the participants consumed meat 2-3 times per week (P<0.001) and almost half preferred calf meat (47.1%; P<0.001; Table 2/Figure 1). Over 80% consumed fresh meat (P<0.001), purchased at the butcher (43.1%) or in the village (45.0%; P<0.001).



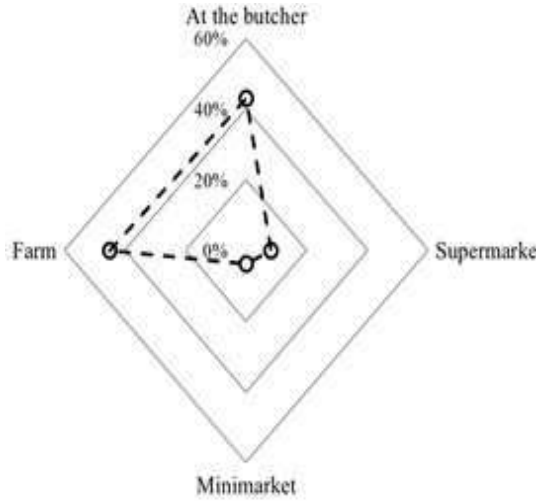
Consumption per week



Consumption per animal species



Consumption per kind of product



Where consumers prefer to buy meat

In particular, a greater proportion of men than female claimed to eat meat every day. For simplicity, age was classified into 3 levels (young adult: 18-34 ys; middle-aged adult: 35-54 ys; senior adult: >54). Participant aged between 35 and 54 have eaten more often meat (every day, 10.9%, or 2-3 time/week, 53.8%) compared to senior adult. Conversely, a greater proportion of senior adults ate meat once per weeks (18.9%) compared to younger participants.

Table 2. Associations between gender of participants and dietary patterns associated with meat intake.

Eating practices		Sex		P value
		Male	**Female**	
Consumption per week	**Everyday**	21_a (11.7%)	6_b (3.2%)	**0.006**
	2-3 time/week	82_a (45.8%)	93_a (49.5%)	
	Once in a week	52_a (29.1%)	69_a (36.7%)	
	Once 2 weeks	17_a (9.5%)	18_a (9.6%)	
	Once in a month	7_a (3.9%)	2_a (1.1%)	
Consumption per animal species	**Calf**	84_a (46.9%)	89_a (47.3%)	0.924
	Lamb	21_a (11.7%)	20_a (10.6%)	
	Goat	17_a (9.5%)	22_a (11.7%)	
	Pork	35_a (19.6%)	32_a (17.0%)	
	Chicken	22_a (12.3%)	25_a (13.3%)	
Consumption per kind of product	**Fresh**	155_a (86.6%)	164_a (87.2%)	0.931
	Frozen	6_a (3.4%)	8_a (4.3%)	
	Packaging	12_a (6.7%)	11_a (5.9%)	
	By-products	6_a (3.4%)	5_a (2.7%)	
Where consumers prefer to buy meat	**At the butcher**	75_a (41.9%)	83_a (44.1%)	0.802
	Supermarket	13_a (7.3%)	17_a (9.0%)	
	Minimarket	8_a (4.5%)	6_a (3.2%)	
	In village	83_a (46.4%)	82_a (43.6%)	

Table 3. Associations between age of participants and dietary patterns associated with meat intake

Eating practices		Age (years) 18-34	35-54	>54	P value
Consumption per week	Everyday	3_{a} (2.5%)	17_{b} (10.9%)	$7_{a, b}$ (7.8%)	**<0.001**
	2-3 time/week	63_{a} (52.1%)	84_{a} (53.8%)	28_{b} (31.1%)	
	Once in a week	44_{a} (36.4%)	43_{a} (27.6%)	34_{a} (37.8%)	
	Once 2 weeks	9_{a} (7.4%)	9_{a} (5.8%)	17_{b} (18.9%)	
	Once in a month	2_{a} (1.7%)	3_{a} (1.9%)	4_{a} (4.4%)	
Consumption per animal species	Calf	58_{a} (47.9%)	76_{a} (48.7%)	39_{a} (43.3%)	0.092
	Lamb	13_{a} (10.7%)	18_{a} (11.5%)	10_{a} (11.1%)	
	Goat	8_{a} (6.6%)	23_{b} (14.7%)	$8_{a, b}$ (8.9%)	
	Pork	19_{a} (15.7%)	27_{a} (17.3%)	21_{a} (23.3%)	
	Chicken	23_{a} (19.0%)	12_{b} (7.7%)	$12_{a, b}$ (13.3%)	
Consumption per kind of product	Fresh	100_{a} (82.6%)	143_{b} (91.7%)	$76_{a, b}$ (84.4%)	0.242
	Frozen	7_{a} (5.8%)	2_{b} (1.3%)	$5_{a, b}$ (5.6%)	
	Packaging	10_{a} (8.3%)	7_{a} (4.5%)	6_{a} (6.7%)	
	By-products	4_{a} (3.3%)	4_{a} (2.6%)	3_{a} (3.3%)	
Where consumers prefer to buy meat	At the butcher	49_{a} (40.5%)	69_{a} (44.2%)	40_{a} (44.4%)	0.184
	Supermarket	14_{a} (11.6%)	7_{b} (4.5%)	$9_{a, b}$ (10.0%)	
	Minimarket	4_{a} (3.3%)	4_{a} (2.6%)	6_{a} (6.7%)	
	In village	54_{a} (44.6%)	76_{a} (48.7%)	35_{a} (38.9%)	

Instead, the monthly salary affects several aspects associated with meat intake (Table 5). Participants with the highest monthly salary ate meat more often (everyday: 17.4%, 2-3 time/week: 65.3%; P<0.001), more fresh (92.6%) and less frozen (0.8%) products, ate less

chicken (8.3%; P<0.05), and preferred to buy at the butcher (46.3%) or in the village (49.6%) than in the supermarket or minimarket (P<0.001).

Tble 4. Associations between monthly salary and dietary patterns associated with meat intake

Eating practices		Month income (miji lek) <50	50-100	>100	P value
Consumption per week	**Everyday**	2a (1.9%)	4_a (2.9%)	21_b (17.4%)	**<0.001**
	2-3 time/week	14_a (13.2%)	82_b (58.6%)	79_b (65.3%)	
	Once in a week	62_a (58.5%)	43_b (30.7%)	16_c (13.2%)	
	Once 2 weeks	23_a (21.7%)	10_b (7.1%)	2_c (1.7%)	
	Once in a month	5_a (4.7%)	1_b (0.7%)	$3_{a, b}$ (2.5%)	
Consumption per animal species	**Calf**	47_a (44.3%)	68_a (48.6%)	58_a (47.9%)	0.372
	Lamb	11_a (10.4%)	14_a (10.0%)	16_a (13.2%)	
	Goat	7_a (6.6%)	16_a (11.4%)	16_a (13.2%)	
	Pork	21_a (19.8%)	25_a (17.9%)	21_a (17.4%)	
	Chicken	20_a (18.9%)	$17_{a, b}$ (12.1%)	10_b (8.3%)	
Consumption per kind of product	**Fresh**	82_a (77.4%)	125_b (89.3%)	112_b (92.6%)	**0.030**
	Frozen	8_a (7.5%)	$5_{a, b}$ (3.6%)	1_b (0.8%)	
	Packaging	11_a (10.4%)	7_a (5.0%)	5_a (4.1%)	
	By-products	5_a (4.7%)	3_a (2.1%)	3_a (2.5%)	
Where consumers prefer to buy meat	**At the butcher**	45_a (42.5%)	57_a (40.7%)	56_a (46.3%)	**0.001**
	Supermarket	16_a (15.1%)	10_b (7.1%)	4_b (3.3%)	
	Minimarket	9_a (8.5%	$4_{a, b}$ (2.9%)	1_b (0.8%)	
	In village	36_a (34.0%)	69_b (49.3%)	60_b (49.6%)	

Table 6 reports the frequency with which participants rated the importance of statements about their dietary patterns associated with meat intake. All the factors listed in the table were rated

as important or very important for many participants (P<0.001). Place of origin (51.6%) is most important cues for consumers in Tirana follow by a narrow difference in meat colour (49.1%). However, there was also more than 20% of the participants judging price, packaging, aroma, tenderness, presence of fat as Neither important nor unimportant. On the other side meat packaging and labelling have the higher percentage (12.6%) as extremely unimportant.

Table 5. The frequency (number and percentage) with which participants rated the importance of statements about their dietary patterns associated with meat intake.

Statement	Very important	Important	Neither important nor unimportant	Unimportant	Extremely unimportant
How do you evaluate the origin of the meat (national vs imported meat)	196* (51.6%)	78* (20.5%)	41 (10.8%)	46 (12.1%)	19 (5.0%)
How do you evaluate the price of the meat	62 (16.3%)	139* (36.6%)	113* (29.7%)	48 (12.6%)	18 (4.7%)
How do you evaluate the transability of the meat	168* (37.8%)	139* (31.2%)	89 (20.0%)	26 (5.8%)	23 (5.2%)
How do you evaluate the packaging of the meat	118* (31.1%)	97* (25.5%)	89* (23.4%)	28 (7.4%)	48 (12.6%)
How do you evaluate the colour of the meat	187* (49.2%)	102* (26.8%)	71 (18.7%)	14 (3.7%)	6 (1.6%)
How do you evaluate the flavour of the meat	147* (38.7%)	73 (19.2%)	101* (26.6%)	41 (10.8%)	18 (4.7%)
How do you evaluate the tenderness of the meat	97* (25.5%)	114* (30.0%)	89* (23.4%)	44 (11.6%)	36 (9.5%)
How do you evaluate the presence of marbling in meat	79* (20.7%)	93* (24.4%)	104* (27.3%)	71 (18.6%)	34 (8.9%)

* higher observed number respect to expected (all categories equal).

DISCUSSION

This survey analyzed the behavior, frequency, preference, knowledge and attitudes of consumers towards meat quality. Based on surveyed consumer gender have a strong influence where male consume often meat than female. The majority of the people in these questionnaire (52.1%) prefer to consume meat at least 2-3 time in a week. Beef meat was the first preference choose for (43.3%) the consumer from Tirana follow by pork 23,3% and chicken 13,3%. Pork and poultry meat is cheaper compered to beef meat and price can strongly affect consumer's decision during meat purchase. High correlation in incoming levels and types of meat types were noticed were people with higher income consume more fresh meat in front of people with lower income who consume more frozen meat. Nevertheless, the income level doesn't influence the selection of the type of meat.

This survey show that the majority of consumers prefer to bay their meat at village 45.0% and butcher shop 43.1 %, while only 8.2% prefer to buy their meat in supermarkets. Often consummators in Tirana prefers to bay meat products directly households in village or to their relatives in order to ensure quality, taste and safety. Buy meat directly at local vendors is part of traditional behavior not only for meat but also for other products. Especially consumers believe that the quality of meat is higher from the mountainous clean environment where animals are left free in green pastures. Specialized local butchery was the second preferred place to buy meat. This may be related with the fact that consumers prefer to buy meat from the trusted place where they usually can develop relationship with the seller. Butchers and independent retailers are by far the most trusted source for information on the safety of meat (Becker et al., 2000).

Country of origin was the most important extrinsic attribute, while these studies point out the strong importance for the consumers to buy domestic meat. In fact, 72.1 % of the consumer's think that country of origin is either very important or important. A research from (Imami et al 2011 and Wongprawmas, et al 2018) found same results for the place of the origin in a study for the lamb meat in Central and Southwest urban Albania and Kosovo. Origin was the most important extrinsic attributes for five European regions (Bernues, 2003). Origin of meat has also been pointed out as an indicator of meat safety (Becker, 1999; Cowan, 1998; Latouche et al., 1998; De Andrade 2016). These study display that people with higher incomes prefer to bay meat directly on farms. Here, the high regard for this attribute seemed to be linked to the value of 'locality', or the 'consumer sense of belonging', as expressed by (De Cicco, Van der Lans, and Loseby 2001). The high request of consumers to bay domestic meat shows the opportunity for meat industry to become even more important, especially beef market shows great potential to expand even more.

Meat traceability was the second most valued extrinsic cues for 69% of the consumers in the city of Tirana. With respect to traceability characteristics, there is a distinction between functional and process attributes, the former referring to the intrinsic opportunities of the systems, i.e. the ability to organize the chain more efficiently, monitor the chain, and assess individual responsibilities (Gellynck, Xavier, and Wim Verbeke 2001). With the rapid development of information technology, the traceability systems increase important being integral part of labelling schemes. However, only 11 % of the consumers considered not important or extremely not important the traceability at the moment of the meat purchase. These percentages can be associated with insufficient knowledge or incredulity over the application of this system in the country. Almost 30% of the persons think that price was neither important nor unimportant at the moment of the purchase. The elevated percentage of the people with high income and education level of the interview persons can be the main factor

in benefiting from these results. Actually, Tirana represent the most developed and rich city in Albania composed from comprehensive terms of population in incoming, education and religion. This phenomenon suggests that economic variables are becoming less suitable indicators in describing segments of consumers, whereas nationality, cultural level, age, place of residence, lifestyle, etc. are increasingly important (Dagevos & van Gaasbeek, 2001; Issanchou, 1996; Wandel & Bugge, 1996). Color was the most important intrinsic visual cues for the consumers in the city of Tirana where 49.2% think it is very important and 26.8% considered an important factor. The color of the fresh meat is of the utmost importance in meat marketing since it is the first quality attribute seen by the consumers who it is as indication of freshness and wholesomeness (Troy et al 2010). Consumers relate red–purple color with freshness and brown color with lack of freshness (Carpenter, Cornforth, & Whittier, 2001; Faustman & Cassens, 1990; Issanchou, 1996). For consumers colour is equal to meat quality, nevertheless these merit is not well related with meat quality. Colour preferences differentiate among consumers within the same country and is affected from the intensity and tonality of the color.

Food label is an important source of relevant information for consumers' meat buyer when evaluating different alternatives. Using label information can be considered as an important and effective instrument that influence consumer purchase behaviors and judge, before buy it However, 43.4% show mistrust in the meat labeling as a process, while many persons think that meat labeling is easy to remove or adulterated. Falsified meat products represent a serious problem for many country and the scandal of horse meat in beef burgers is a clear indication. A lack of trust among consumers remains a significant issue for meat industry in Albania and impressive action will be necessary to increase the credibility in the minds of consumers. Meat flavor stems from lipids and water-soluble components. Although hundreds of volatile compounds have been identified in cooked meat, only a small subset of molecules have been proven to be of sensory significance (Cerny and Grosch, 1992,1993; Rochat et al., 2007). Flavour depends on intrinsic and extrinsic factors (i.e., species, genetics, sex, feeding regimen, and management practices) (Maughan & Martini, 2012; Melton, 1990). In these study 57,9 of the consumers classified flavor as very important or important attributes while 26.6% of consumers considered it as neither important nor unimportant. These high percentages may be associated with the fact consumer sensory attributes frequently are considered simultaneously without a completely segment regarding juiciness, tenderness, and flavor.

Only 45.1 % of the consumer prefer the presents of the fat in meat and these data indicate that consumers from Tirana have a poor preference for marbling. It must be acknowledged that consumers constantly don't have the accurate information regarding marbling which is often confused with subcutaneous and visceral fat. Marbling is the visible fat present in the interfascicular spaces of a muscle and the architecture of the muscle influences the pattern of fat deposition such that looseness of the fascicular organization generally parallels the quantity of interfascicular lipid present (Kauffman & Marsh, 1987). Tenderness, juiciness, mouthfeel, flavor development, and delivery are highly dependent on the level of marbling in meat (Corbin et al., 2015; Lorido et al., 2015; Frank et al., 2016a). Marbling fat contains more oleic acid and less stearic acid than subcutaneous adipose which effects not only the palatability of beef but also has a positive health aspect (Troy et al 2016).

CONCLUSIONS

The study defines red meat as a significant component for a balanced diet and beef meat was the first choice the most of the consumers. Individual meat consumption is a reflection of incoming level, education and physiological factors. The results of the survey determined that

country of origin and color were the most important cues for consumers in the city of Tirana, while shopping meat. In this context, the national brand of meat has a great potential to increase rapidly in future. Therefore, these results should be considered from policy makers for incentive helping the development of the meat industry as an important source of profit.

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STUDY OF THE EFFECT OF *CITRUS SINENSIS* AND *CITRUS AURANTIUM* ESSENTIAL OILS ON *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

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ABSTRACT

Two essential oils were extracted from the peel of Citrus sinensis and Citrus aurantium to evaluate their acaricidal effect on the adults, larvae and eggs of the pest mite Tetranychus urticae Koch (Acari: Tetranychidae). The repellent effect and the residual activity were also assessed. The chemical composition of two essential oils was assessed by gas chromatography/mass spectrometry. Limonene was obtained as the majority compound in the essential oil of C. sinensis (96.11%) and C. aurantium (96.10%). The efficacy of both essential oils was studied by means of toxicity tests. Five increasing concentrations of each product were tested: 0.5, 1, 2, 4 and 8% with contact treatment. The toxicity tests demonstrated a good efficacy of the two essentials oils studied against the various stages of development of T. urticae. At a concentration of 8% of C. sinensis and C. aurantium, the mortality was 63.33% and 68.74% on adults, 100% and 75% on larvae and 85.41% and 95% on eggs, respectively. These oils had a repellent effect on T. urticae adults and they were not very persistent on the leaves. These results indicated that both essential oils have the potential to be developed as botanical acaricides for eco-friendly management of T. urticae.

Key words: *Essential oil, toxicity, Citrus aurantium, Citrus sinensis, Tetranychus urticae*

INTRODUCTION

The two-spotted spider mite Tetranychus Urticae Koch (Acari: Tetranychidae) is one of the most polyphagous herbivores that feed on more than 1500 plant species, including over 150 cultivated species (Migeon & Dorkeld, 2016). T. urticae is worldwide an economic pest both in the field and in the greenhouse. The perimeter of the Loukkos (North of Morocco) confirmed its dominance in the production of strawberry. Indirect effects of feeding may result in a yellow to white discoloration of the leaf often referred to as bronzing. The burning of leaves leads to defoliation which results in the death of the host plant. These symptoms are due to a decrease in photosynthesis and transpiration. These effects cause a loss of quality and yield. Indeed, T. urticae mites can cause yield losses of up to 70% in strawberries (Park & Lee, 2002). For several years, the control of this mite has traditionally relied on sprays of pesticides. Their high reproductive potential and short life cycle, combined with frequent applications of synthetic acaricides, result in even faster resistance to many active ingredients (Nicastro et al., 2013; Rahayu & Mairawita, 2018). On the other hand, the unwise use of synthetic pesticides leads to growing environmental problems and risks to human health (Cavalcanti et al., 2010). In addition, this resulted in the elimination of many natural enemies of the mites by reducing egg

production and their reproductive capacity which resulted in a reduction of predation pressure on the mite (Prischmann et al., 2005; Kumral et al., 2009) thus affecting biodiversity. Among these natural enemies, the Athias-Henroit mite Phytoseiulus persimilis (Acari: Phytoseiidae), a specific predator for T. urticae, has been well affected by the unreasonable use of synthetic pesticides (Kaplan et al., 2012) For all these findings, it is necessary to find more selective and safe materials to use to control mites.

Plants provide an alternative to currently used pesticides for pest control, as they are a rich source of bioactive chemicals (Tong & Coats, 2010) that are active against specific target species, are not toxic products, and are potentially suitable for use in integrated pest management (Tare et al., 2004; Prabakar & Jebanesan, 2004). On the other hand, most of these products are non-persistent and non-toxic to humans (Miresmailli & Isman, 2006). Therefore, the use of essential oils (EOs), in pest management programmes has recently attracted the attention of many scientists (Abdel-Tawab, 2016; Waked, 2016; Walia et al., 2017). These products provide their direct toxic effects on pests through contact, ingestion and fumigation. Plant extracts can affect pest behaviour, including repelling the pest or prohibiting feeding activity, pest physiology, including moulting, respiratory inhibition, growth, fecundity reduction, and also cuticle disruption (Enan 2001; Gokce et al., 2011). Among the species that are characterised by the production of EOs, those which stand out are the ones that constitute the genus Citrus (Sapindales: Rutaceae). Citrus plants are the most popular and important food in the world (Qiao et al., 2008). Over that last few decades, following advances in analytical chemical methodologies, the toxic properties of Citrus essential oils against many pests have been further explored. Mahmoudvan et al. (2011) tested the fumigant effect of EOs of several plant species on stored-grain pests and found that Citrus sinensis had a good fumigant effect against Tribolium castaneum (Coleoptera: Tenebrionidae), Sitophilus granarius (Coleoptera: Curculionidae), and Callosobruchus maculatus (Coleoptera: Chrysomelidae). In this sense, Dutra et al. (2016) tested the toxicity by contact and fumigation of the essential oils of two species of Citrus genus: C. latifolia and C. sinensis on the cowpea weevil, C. maculatus which is a major pest of cowpea Vigna unguiculata (Fabales: Fabaceae) in storage units. The study showed that the number of eggs and newly emerged adults was inversely proportional to essential oil concentration increase. In addition, the toxic activity of EOs of the Citrus genus has been well demonstrated on larvae of flies and mosquitoes. Research carried out by Kumar et al. (2012) indicated, using contact toxicity and fumigation bioassays, the insecticidal activity of the essential oil of C. sinensis against the larvae and pupae of Musca domestica (Diptera: Muscidae). In fact, significant mortality of housefly larvae and inhibition of pupae was observed in different bioassays. Toxic properties of the Citrus rind on medfly larvae Ceratitis capitata (Diptera: Tephritidae) have also been demonstrated by Salvatore et al. (2004) and by Papachristos et al. (2009). The toxicity of Citrus oils against medfly larvae is related to quantitative and qualitative aspects of their principal components (monoterpenes and sesquiterpenes). Citrus essential oils contain mainly limonene (> 90% in some species), which causes moderate toxicity against medfly larvae compared to other components of Citrus oils. Furthermore, Palazzolo et al. (2013) proved the toxicity of EOs from fruit peel of C. sinensis, C. limon and C. aurantium, containing high proportion of limonene against the mosquito larvae of Culex pipiens (Diptera: Culicidae). A strong toxicity was exhibited by the Citrus essential oils against larvae with the LC (50) values ranging from 30.1 (lemon) to 51.5 mg/l (orange) depending on the genotype and on the oil composition

In Morocco, the Citrus sector is considered one of the country's flagship sectors. Industries look for additional sources of income from the material they only partially use. The solution lies in the transformation of waste into marketable products. In this respect, the essential oils extracted from Citrus zests are a good solution from an economic and ecological point of view.

These oils are good candidates for management of spider mites, as they are readily available in abundant quantities in Morocco and elsewhere.

The objective of this work is to evaluate the effect of the essential oils extract from fresh peel of two species of Citrus, C. sinensis (sweet orange) and C. aurantium (bitter orange) on T. urticae under laboratory condition. The first objective is to determine the toxicity of the two Eos on T. urticae at the egg, larval and adult stages as well as their residual toxicity on the treated leaves. The second objective was to test the repellent effect of essential oils on adults of T. urticae.

MATERIALS AND METHODS

Plant material and isolation of essential oils

Sweet orange (C. sinensis) and bitter orange (C. aurantium) are cleaned then their bark is grated. The peel of each fruit was subjected to hydrodistillation for 3 h, using a Clevenger type apparatus. The hydrodistillation was carried out in the laboratory of the National Institute of Agronomic Research of Tangier during the month of March in 2017. The essential oils collected by decantation at the end of the distillation were filtered on filter paper to remove residual water traces. The oil collected was stored in an amber bottle at 4 °C until used.

GC–MS analysis of essential oils

The analysis of the chemical composition of the essential oils extracted from the plants was carried out on gas chromatography coupled with mass spectrometry in the laboratory of the National Institute of Agronomic Research of Tangier. The mass spectrometer provided the mass spectrum of each of the constituents and often identifying them. The SCAN lode was used to achieve the spectra. The device was equipped with a capillary column TR-5MS (300 × 0.25 μm) with a film thickness of 0.25 μm. The temperature of the column is programmed from 50 °C to 240 °C at a rate of 5 °C/min. A volume (1 μl) of the essential oil injected with 10 μl of the solvent hexane. The major components of essential oils were identified on the basis of comparison of their retention indices and mass spectra with those of authentic samples or published data (Adams, 2007). The GC-MS was linked to a computer system managing a mass spectrum library. The retention indices (RIs) were calculated for all volatile constituents using a homologous series of n-alkanes C8-C20.

Mite rearing

Five strains of T. urticae were collected from commercial strawberry fields in the Loukkos region of Morocco during the month of April in 2017. After collection, the mites were reared mixed continuously on bean plants, under laboratory conditions at 25±1°C, 70±5% relative humidity and a 14L: 10D photoperiod. Bioassays were conducted during 2017 at Department of Biology, Faculty of Science and Technology, Abdelmalek Essaadi University, Tangier, Morocco.

Contact toxicity of essential oils on *Tetranychus urticae*

Biological tests were based on the method described by Knight et al. (1990). The strain of *T. urticae* was placed on a bean leaf disc (4 cm diameter) on water-soaked cotton in a Petri dish (9 cm diameter). The principle was to treat the mites during the three stages (adult, larvae and egg) with preparations of essential oils. For each stage, five individuals were deposited with a brush on the underside of each disc of bean leaves. The application of preparations was carried out with a hand-held sprayer (Butt & Goettel, 2000). The quantity applied was 9.6±0.83 μl/cm^2.

The five increasing concentrations were used: 0.5, 1, 2, 4 and 8%. The various preparations were obtained by dilution of the essential oils in 0.1% Triton X-100 (Sigma-Aldrich, France). Control Petri-dish was treated with distilled water containing 0.1% Triton X-100 only. Four replications were conducted for each concentration of both essential oils tested. The percentage mortality rate was calculated after 24, 48 and 72 h for adults while for larvae and egg stage, the count was made after 24 h. For treatment of eggs, five *T. urticae* females were allowed to oviposit for 24 h on 2 cm diameter of bean leaf discs. Then, adults were removed and 20 eggs were kept on each disc. Egg mortality was recorded when the hatched mites in the control treatment had reached the larval stage.

Residual activity of essential oils

The effect of both essential oils against *T. urticae* was evaluated at 1, 7 and 14 days after treatment. Bean plants were sprayed with each of essential oils concentrations. On a whole bean leaf, a quantity of 10 µl of the essential oil was sprayed per cm² of leaf area. The leaves were treated taking care not to detach them from the plants. One day after foliar application of each essential oil, the sprayed leaves were removed from the plants. Five adults of *T. urticae* were carefully placed on a bean leaf disc (4 cm diameter) on water soaked cotton in a Petri dish. Four replicates per treatment were used. The same procedure was done on day 7 and day 14 after treating the plants with each essential oil. For control leaves 0.1% of Triton-X100 in distilled water alone was added.

Repellent effect of essential oils

The repellency tests were performed according to method described by Kogan & Goeden, (1970). Leaf discs of bean of 3cm diameter were used. Half of the disk was infected with solution of both oils in five concentrations and the other half of the disk was immersed in distilled water containing 0.1% Triton-X100 that was used as control. Both treated and untreated leaf disks were placed on water soaked cotton in a Petri dish. There were four replicates for each treatment. Five pairs of *T. urticae* adults were transferred in the middle of treated and untreated leaf discs. At the end of half an hour, mites are counted on each part of the disc. According to McDonald et al. (1970), the repulsion percentage is provided by the following formula:

$$PR\ (\%) = [(NC - NT) / (NC + NT)] \times 100$$

With: NC: number of spider mites present on the part not processed

NT: number of spider mites present on the part treated by the essential oil.

As per the ratios obtained, there are five repellency classes. A substance is considered as repellent is the ratios are between 60% and 80%.

Statistical Analysis

Mortality was corrected using Abbott formula (Abbott, 1925). All data in each experiment were evaluated using analysis of variance (ANOVA). Each ANOVA is followed by a Tukey test to compare the averages two to two (Dagnelie, 1975). Significant differences among means were detected by Tukey test $P < 0.05$. All statistical analyses were made using the Statistical Package for Social Scientists (SPSS) version 13.0.

RESULTS

Chemical composition of essential oils

The analysis of *C. sinensis* and *C. aurantium* essential oil composition were carried out using GC and GC-MS according to the methodology described in Methods and Materials **(Figures 1 & 2).**

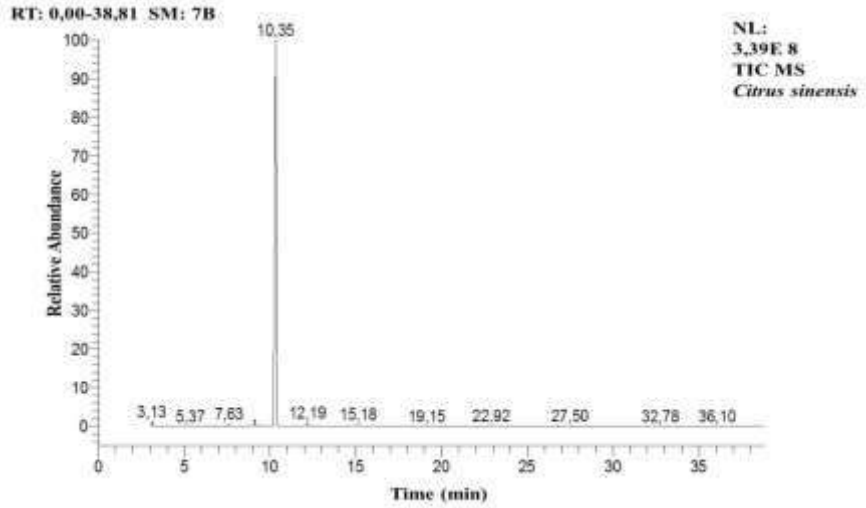

Figure1. GC-MS spectrum of *C. sinensis* essential oil.

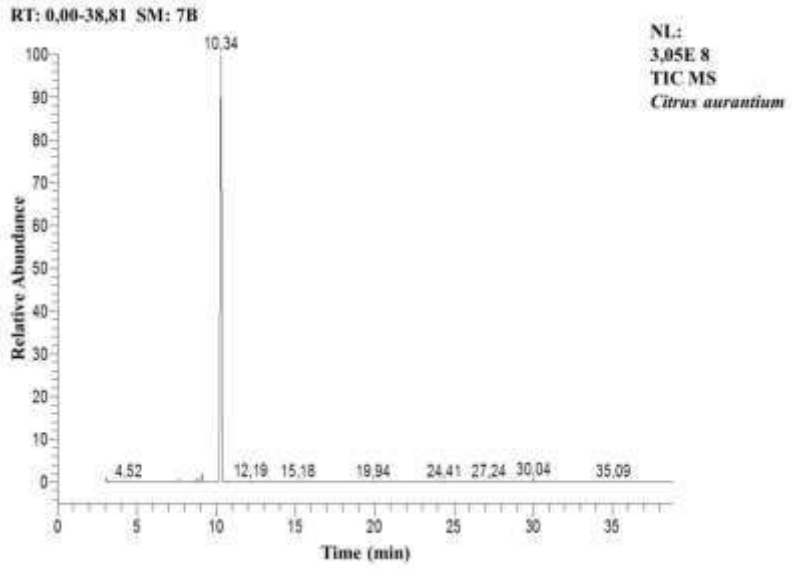

Figure 2. GC-MS spectrum of *C. aurantium* essential oil.

Limonene, a monoterpene hydrocarbon, is unquestionably the most abundant compound in the essential oil of *C. sinensis* (96.11%) and *C. aurantium* (96.10%). Other compounds were also present but at very low levels **(Table 1).**

Table 1. The main constituents of *C. sinensis* and *C. aurantium* essential oils and their percentages, identified by GC-MS

		C. sinensis	*C. aurantium*
Limonene	1050	96.11	96,10
α-Pinene	935	0.30	0,88
Myrcene	993	0.84	-
α -Humulene	1444	-	0,34
Linalol	1101	0.48	-
ϒ-Terpineol	1146	-	0,1
Total		97.73%	97,42

[a] The calculated retention index;

[b] The distribution air of each compound in each essential oil;

The dash indicates that the compound was not found. The values in bold indicate the most abundant compounds of the oils.

Acaricidal effect of essential oils on *Tetranychus urticae*

Contact test

There was no mortality with adult control. Whereas the treatment (0.5 to 8%) showed a significant increase on adult mortality. For *C. sinensis*, the Abbott mortality of adults varied following the product concentration from 26.11% to 63.33%. Adults treated by *C. aurantium* oil, mortality increased from 39.16% to 68.74% **(Figure 3).**
On the other hand, the statistical analysis revealed that the mortality rates of *T. urticae* during the 72 h following the treatment with both essential oils were very significant ($F = 7.064$, $df = 2$, $P < 0.01$ for *C. sinensis* and $F = 9.016$, $df = 2$, $P < 0.001$ for *C. aurantium*).

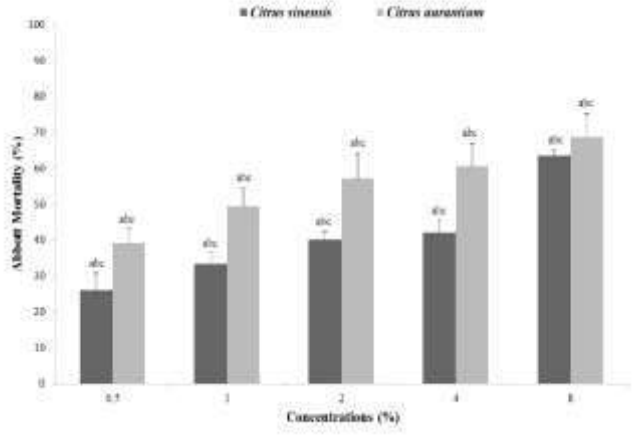

Figure 3. Effect of different concentrations of essential oils on adult mortality of *T. urticae*. Bars represent means ± SE. Different letters among treatments indicate significant differences according to Tukey's test ($^{abc}P < 0.001$).

Analysis of single-factor variance (concentration) shows that direct application of increasing concentrations for each essential oil on larvae results in significantly different mortality rates. On average, mortality rates varied from 27.50% to 100% in the larvae groups treated by 0.5 to 8% of *C. sinensis* oil. Larvae treated with *C. aurantium* oil, mortality increased at 75.0% at concentration 8% **(Figure 4).**

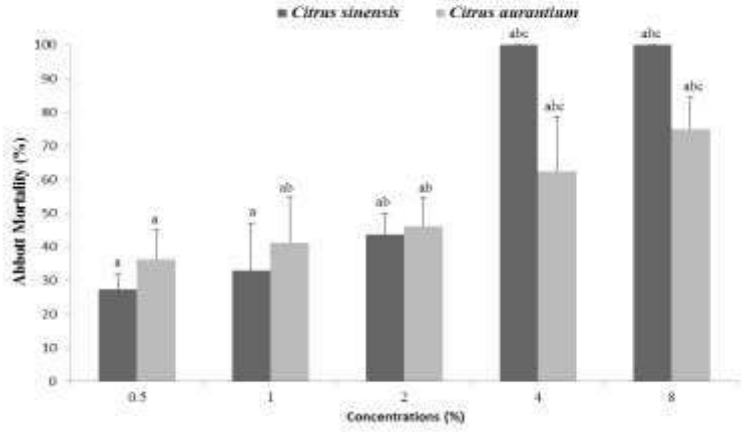

Figure 4. Effect of different concentrations of essential oils on larvae mortality of *T. urticae*. Bars represent means ± SE. Different letters among treatments indicate significant differences according to Tukey's test ($^{a}P < 0.05$, $^{ab}P < 0.01$ and $^{abc}P < 0.001$).

Statistical analysis of single-factor variance (concentration) revealed that the concentrations tested for the two essential oils had a significant effect on egg mortality. Both essential oils are highly toxic to *T. urticae* eggs at a concentration of 8% (85.41% for *C. sinensis* and 95.0% for *C. aurantium*) **(Figure 5).**

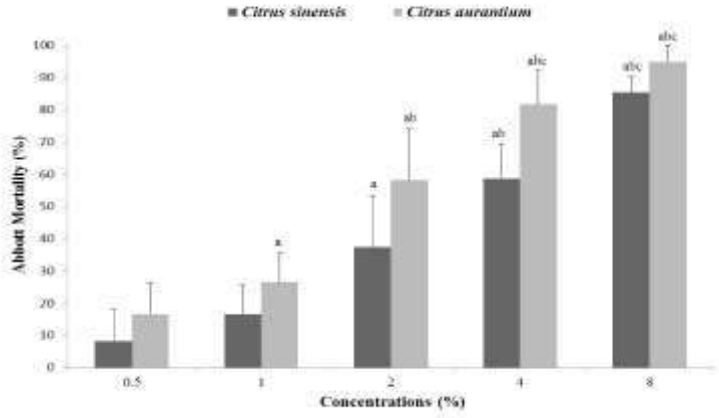

Figure 5. Effect of different concentrations of essential oils on eggs mortality of *T. urticae*. Bars represent means ± SE. Different letters among treatments indicate significant differences according to Tukey's test ($^{a}P < 0.05$, $^{ab}P < 0.01$ and $^{abc}P < 0.001$).

Residual essential oils activity

The residual activity of the two essential oils is evaluated by determining the mortality of *T. urticae* adults placed for 24 h on leaves beforehand treated with increasing concentrations of one of these oils 1, 7 or 14 days previously. A two-factor variance analysis (concentrations and Post-Treatment Time) and Tukey tests were conducted on *T. urticae* adult mortality.

The statistical analysis revealed a significant reduction in the residual activity of both essential oils during two weeks following the treatment of the plants ($F = 8.10$, $df = 2$, $P < 0.01$ for *C. sinensis* and $F = 7.50$, $df = 2$, $P < 0.01$ for *C. aurantium*). At the end of experiment (14 days), *C. sinensis* oil residues were able to eliminate only 4.72% of the mites (17.08% at day 1). The residues of *C. aurantium* oil are capable of killing about 16.87% of the mites on day 1 against only 3.54% on day 14 **(Figure 6).**

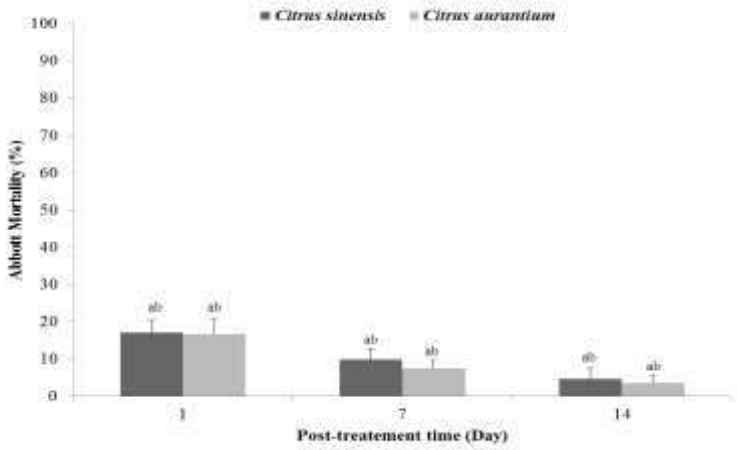

Figure 6. Percentage mortality of T. urticae, recorded after 1, 7 and 14 days of bean leaf treatment with essential oils. Bars represent means ± SE. Different letters among treatments indicate significant differences according to Tukey's test ($^{ab}P < 0.01$).

Repellency tests

The study showed that the numbers of mites counted in the parts treated with *C. sinensis* oil are significantly reduced when increasing the concentration of the oil used. Indeed, the repulsion rate increases from 47.37% when the mites have the choice between 0 and 0.5% of *C. sinensis* oil and 100% when they have to choose between 0 and 8% of the same oil. While for *C. aurantium* essential oil, the rate of repellency increases from 33.33% and 88.24% respectively at 0.5% and 8% of this oil. These findings indicated that when concentration increased the repellency percent also increased. According to McDonald's classification the *C. sinensis* oil showed in general more repellent than the *C. aurantium* oil **(Table 2).**

Table 2. Classification of the concentrations of *C. sinensis* (C.s) and *C. aurantium* (C.a) essential oils according to their repellent properties.

MR: Moderately repellent / WR: Weakly repellent / R: Repellent/ VR: Very repellent

essential oil	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a	C.s	C.a
Repellency %	47.37	33.33	52.94	44.44	76.47	76.47	88.89	78.95	100	88.24
Repulsive Class	III	II	III		IV		V	IV	V	
Effect	MR	WR	MR		R		VR	R	VR	

DISCUSSION

GC-MS analysis revealed that limonene is the major constituent of the essential oil of *C. sinensis* and *C. aurantium*. The chemical composition of the isolated essential oils is in accordance with those previously reported (Sarrou et al., 2013; EL-Akhal et al., 2014; Da Camara et al., 2015). In general the contact miticide activity increases with the increase in the concentration of both oils studied. On the other hand, lethality of these EOs on adults increases after 72 h of treatment**.** Many studies have confirmed the toxicity of EOs on insect pests. However, little research has been done on the acaricidal effect of these oils on pest mites such as *T. urticae*. The study conducted by Araujo et al. (2010) approved a fumigant toxicity of the peel essential oils of *C. sinensis* and *C. aurantium* cultivated in northeast Brazil against *T. urticae.* In fact, the most potent fumigant toxicity was found with *C. aurantium* oil followed by the *C. sinensis* oil. In the same context, Roh et al. (2011) tested the acaricidal effect of the peel essential oil of *C. sinensis* in the laboratory using a leaf-dip bioassay, this study confirmed an acaricidal activity of this essential oil on *T. urticae* adult females at 0.1% concentration 24h after treatment with 45.6% of mortality and decreased significantly the total eggs number. In the literature, the contact toxicity of the essential oil of *C. sinensis* and *C. aurantium* has been observed by several authors for other species of mites or insects. In this sense, Karamaouna et al. (2013) showed the insecticidal effect of *C. sinensis* essential oil on adults of the flour mealybug of *Planococcus ficus* vine (Hemiptera: Pseudococcidae) (LC_{50} of 5.4 mg/ml). Tarelli et al. (2009) showed an insecticidal effect of *C. sinensis* essential oil by the fumigant activity and the topical application on *Musca domestica* (Diptera: Muscidae) adults. In the same sense, Kumar et al. (2011) confirmed the larvicidal effect by contact of *C. sinensis* essential oil on *M. domestica*. In addition, through the larvicidal and knockdown effects, the study conducted by Manimaran et al. (2012) clearly demonstrated that *C. sinensis* oil had high potency to control three species of vector mosquitoes (*Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*) (Diptera: Culicidae). Also, Saad Mona (2013) confirmed a remarkable toxic effect against the fourth instar larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae) of *C. sinensis* essential oil with LC_{50} value of 6.88 µl/l. Zarubova et al. (2014) have observed significant mortality rates in larvae of *Oulema melanopus* (Coleoptera: Chrysomelidae) after topical application with *C. sinensis* essential oil, these rates can wait 85.0% after 48 h of exposure to this essential oil. The efficacy of *C. sinensis* essential oil on larvae was also

confirmed by Araujo et al., (2016) who reported that this oil exhibited strong larvicidal activity on different *Aedes aegypti* populations. Such comments also join those of Michaelakis et al. (2009) who tested the effect of essential oils of the genus *Citrus* on larvae of the *Culex pipiens* (Diptera: Culicidae). According to these authors, among those essential oils studied, that *C. sinensis* and *C. aurantium* have a very high larvicidal power with respective LC_{50} of 51.5 and 39.81 mg/l. For their part, Oulebsir-Mohandkaci & Ait kaki (2016) studied the insecticidal effect of the polyphenols of the essential oil of *C. aurantium* on false moths of *Galleria mellonela wax* (Lepidoptera: Pyralidae). These authors recorded mortality percentages of 100%, 24h after treatment at a concentration of 30 µl/ml. On the other hand, the ovicidal effect of *C. sinensis* essential oil on two species of insects of stored commodities *Callosobruchus maculatus* F (Coleoptera: Chrysomelidae) and *Dermestes maculatus* Deg (Coleoptera: Dermestidae) was reported by Don-Pedro (1996) with respective LC_{50} of 7.8 and 21.5 µl/l after 24h of treatment. In addition, Ramar et al. (2014) demonstrated an 81.25% eradication of the eggs mosquito of *Culex quinquefasciatus* Say, administered at 200 ppm. Nevertheless, studies by Sinthusiri & Soonwera (2014) indicate that the mortality rate does not exceed 3.33% when the eggs of the housefly *Musca domestica* (Diptera: Muscidae) are treated with 10% of the orange oil.

One of the objectives set in the present work is to evaluate the residual activity of both essential oils mentioned above on *T. urticae* pest mites. It has been found that the residual activity of both EOs decreases during the first 14 days after the treatment of the plants. These results are comparable to those of Zarubova et al. (2014) who showed that the essential oil of *C. sinensis* evaporates very quickly on treated wheat plants, rising from 0.018µl/g after one minute of treatment to 0.0028µl/g after 60 min. The search for new pesticides should consider products that do not affect non-target organisms and have low persistence in the environment compared to pre-existing pesticides (Miresmailli & Isman, 2006; Hany & El-Zahi, 2011). However the low residual activity can constitute certain disadvantage regarding to the pesticide efficiency. In fact, the instability of the product will reduce the time of protection against pests (Dekeyser, 2005).

In the current study it was found that toxic effects of *C. sinensis* and *C. aurantium* oil not only have contact toxicity against *T. urticae* but also repellent effect. These EOs have a good repellent effect on *T. urticae* adults when given at a concentration greater than or equal to 4% for *C. sinensis* oil and 8% for *C. aurantium* oil. These results are in agreement with Araújo et al. (2010) and Da Camara et al. (2015). According to these studies sweet orange and bitter orange demonstrated proved in repellency to spider mite *T. urticae*. These authors emphasize that the difference between the repellent effects of these two EOs is due to the presence of certain minority compounds which can act synergistically with the major compound, in this case limonene. Amer et al. (1993) found a repellent effect of *C. sinensis* oil on both *T. urticae* and *Eutetranychus orientalis* pests. The repellent property of essential oils of the *Citrus* genus has also been highlighted against insects. Yoon et al. (2009) found that *C. sinensis* and *C. aurantium* oils were the most repellent among the *Citrus* species against *Blattella germanica, Periplaneta americana* Linnaeus and *Periplaneta fuliginosa* Serville (Blattodea: Blattellidae). Recently, Murugan et al. (2012) demonstrated the repellent activity of *C. sinensis* oil against the mosquitoes *A. stephensi, A. aegypti* L. and *C. quinquefasciatus* Say (Diptera: Culicidae). For their part, Dehghani & Ahmadi (2013) recorded a 55% repellency rate of this oil against *Trialeurodes vaporarioru* (Homoptera: Aleyrodidae) and this after 6 days of treatment at a concentration of 40 µl/ml .In matter of fact, over the last 50 years, thousands of plants and their essential oils have been screened as potential sources of repellents and insecticides/acaricides (Nerio et al., 2009 ; Waked, 2016).

Some previous studies reported that the acaricidal and/or insecticidal effects of plant essential oils were related to their chemical compositions (Pascual-Villalobus & Ballesta-Acosta, 2003). The monoterpenes that characterize the chemical composition of essential oils such as thymol, carvacrol, α-terpinene, 1.8-cineole, limonene, α-pinene and camphor have proven their toxicity and significant repulsive activity against various pests (Roh et al., 2013; Abdelgaleil et al., 2019).

The major constituents of essential oils have been effective in controlling various pests including *T. urticae*. In this regard, Lee et al. (1997) showed that limonene has a significant miticide effect on *T. urticae.* According to these authors, the application of this compound causes a total mortality (100%) in treated mites and this from the first 24h after treatment at 10000ppm. Moreover, these authors have confirmed in their study the toxicity of this monoterpene on adults of *Musca domestica* (Diptera: Muscidae) and the larvae of *Diahrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Hummelbrunner & Isman (2001) also demonstrated the efficacy of limonene on insects such as the tobacco worm *Spodoptera litura* (Lepidoptera: Noctuidae) with a mortality rate of 90% of treated insects. Karr & Coats (1988) reported that d-limonene exhibited slight toxicity, and the appearance of dead western corn rootworm *Diahrotica virgifera virgifera* larvae was quite unusual: the cuticle of the larvae was very soft and darkened and the body seemed to be partly liquefied.

Several studies have confirmed that EOs has neurotoxic effects, evident from their rapid action against some insects and mites. Thus, there are pieces of evidence that support the fact that plant essential oils affect octopamine pathways and GABA-Gated chloride ion channels (Isman, 2006; Sertkaya, 2010). In this sense, Enan et al. (1998) reported that several essential oil compounds have been demonstrated to block octopamine, a neurotransmitter unique to insects that functions similarly to epinephrine (Adrenaline) and norepinephrine found in vertebrates. Because it is unique to insects, the octopaminergic system is of considerable interest as a target site for control agents. This neurotransmitter can have profound adverse effects on insect behavior, with symptoms including knockdown, agitation, hyperactivity, tremors, forced diuresis, convulsions, and death (Nathanson et al., 1993). On the other hand, because of the multiple sites of action through which the plant materials can act, the probability of developing a resistant population is very low.

CONCLUSIONS

Much effort has been focused on plant essential oils as potential sources of pest control agents. Based on the present findings, peel oils of *C. sinensis* and *C. aurantium* are promising for the control of *T. urticae*. The associated toxicity by contact and repellent properties of these essential oils make these oils a strong candidate as the active ingredient in a plant-based acaricide. On other hand, considering its very low residual activity in treated plants and thus low negative impact on the environment (Miresmailli & Isman, 2006 ; Hany & El-Zahi, 2011), these oils are very promising alternative to synthetic pesticides, suitable for implementation to integrated pest management and organic farming. However, this study only tested the acaricidal effect of these essential oils in the laboratory. Further studies are needed to evaluate the acaricidal effects of *C. sinensis* and *C. aurantium* oils in the field. Lastly, formulations (e.g., spray, fumigant, smoking agent, or tablet) for improving acaricidal potency and stability need to be developed.

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TEST OF CONTAMINATION OF A LICHENIC SPECIES "*XANTHORIA PARIETINA*" AND A MUSCICOLE SPECIES "*HYLOCOMUIM SPLENDENS*" BY LEAD/EFFECTS ON SOME PHYSIOLOGICAL PARAMETERS

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ABSTRACT

Air pollution, especially that caused by lead has constantly evolved over time, due to the considerable increase in the number of vehicles on the market. Lead, which is one of the first used metals by mankind, represents a major risk to human health but also to the ecosystem. Living beings are a reflection of the environment in which they evolve and their observations at various levels of the biological organization can provide guidance on the quality and characteristics of this environment. The use of lichens and mosses can provide very precise indications and show as early as possible the natural or the induced changes. That's why, our work is essentially based on the characterization of the accumulating power as well as the impact of the different concentrations of lead on two species, a lichenic species "*Xanthoria parietina*" and muscicole species "*hylocomium splendens*" collected in the region of Bir El Djir "Oran". In order to determine the ability of these plants to accumulate lead and its impact on some physiological parameters (ph, chlorophyll and proline), a contamination in vitro by different concentrations of Pb (NO3) was carried out during a period of 45 days. The obtained results show the presence of lead in the thalli of both species, at levels increasing in parallel with the concentrations to which they were exposed, As well as a disturbance of the cellular metabolism which is interpreted by an increase in ph. Also we have noticed variations of measured physiological parameters; Chlorophyll and proline content that can result from stress, degradation of the fresh material, and disturbance of the photosynthetic process. It is obvious to mention that the two studied species are proven good bio accumulators of lead which makes them excellent bio-indicators for the estimation of pollution especially by metals.

Key words: *lichens, mosses, Lead, bioaccumulation, bio-indication, physiological parameters*

INTRODUCTION

Air pollution and the transfer of harmful quantities of natural or synthetic materials in the atmosphere, the direct or indirect consequence of human activity, is one of the important manifestations of environmental degradation, as it threatens directly the most necessary element for life ‹air›. This type of pollution can have a dramatic effect, if the main problem concerns the health of the populations, the impact on the ecosystem must also be taken in consideration.

The use of bio indicators is based on their integrator character in respect of environments factors. In effects, the livings beings are the reflection of the environment in which they evolves and their observation in various levels of biological organizations can offer indications about

the quality and the characteristics of that environment. In bio-monitoring of heavy metal pollution, bio indication techniques occupies an interesting place. The use of lichens and mosses can offer so precise indication and make obvious as early as possible the natural or caused modifications (Alioua, 2001).

MATERIAL AND METHODS

Presentation of the sampling region

The city of Bir-el Djir (Fig 1) our sampling region is the main city in the immediate suburbs to the east of Oran outside the borough baptized agricultural in colonial times, the municipality is located 8km from the city center. It is a city that remained essentially agricultural until the end of the years 80, it now hosts a population of 118,000 inhabitants and becomes a major pole of the Oranean agglomeration. It houses several corporate offices with modernist architecture, the new University Hospital 1st November, University education Institutes which USTO is the biggest.

The territory of district of Oran is characterized by a relief consisting mainly of mountains, hills and plains, we distinguish in particular the mountain of the Aidour (429m). According to the National Meteorological Organization 2011 The Oran region has a classical mediterranean climate marked by a summer drought, mild winters, and a bright and clear sky. During the summer months, precipitation becomes rare or even non-existent. The subtropical anticyclone covers the Oranean region for nearly four months. On the other hand, the area is well watered during the winter. The weak precipitations (420 mm of precipitations) and their frequency (72.9 day by year) are also characteristic of this climate: Minimum annual average temperature is about 12 C°. Maximum annual maximum temperature is about 22 C°

Justification of the choice of the sampling site

After prospecting different region in Oran, we noticed that the lichenic and muscicole species are limited, only the two species Xanthoria parietina and Hylocomium splendens have taken our attention. Our sampling takes place at the sciences and technology university of Oran ‹‹Mohamed Boudiaf ›› for its proximity and also for the abundance of the species.

Fig 1: *localization of Bir-el Djir city in the district of Oran.*

Experimental protocol

Sampled biological material

Our study concerned a nitrophile foliated specie of lichens with a lush color developing on different phorophytes, especially on «*Acacia albida*» from where we have chosen it for our experimentation, it is «*Xanthoria parietina»*.

For the mosses our choice concerned the most present specie in the samling site, it is *«Hylocomium splendens».*

Sampling techniques

For lichen thalli, we detached them from their phorophyte using a knife, as for the muscicoles thalli, they were carefully detached from the soil. The samples taken are placed in a well labelled bag, transported to the laboratory for identification.

The essays

Preparation of lead solutions using lead nitrates

We have prepared three solutions with different concentrations of lead, 50, 100 and 350 µg/l, compared to the world reference, the concentration of 50µg/L is the admissible dose in water (Durfor et Baker, 1964).

Contamination essays of two species by lead solution

Once the solutions are prepared, we will proceed to the contamination of the samples in order to test the power of the lichens and the mosses to accumulate the lead

Analytical techniques

pH measurement

Using a pH meter, we followed the temporal evolution (each week) of the pH of all samples in solutions treated at different concentrations

Chlorophyll dosage

In order to evaluate the effect of the pollution on the photosynthesis and especially on the chlorophyll of lichen talli and mosses, we opted for the dosage of chlorophyll a and b using the method proposed by (Rao and Leblanc, 1965).

Proline dosage

In order to evaluate the effect of the pollution on the proline content in lichens and mosses the calculation of the proline content is determined following the formula proposed by (Mon neveux and Nemmar, 1986).

Lead dosage

The technique of spectrophotometry of atomic absorption (S. A. A) is the most used for the dosage of heavy metals, after mineralization lead measurements were done with solutions of 20 ml of nitric acid of 2%.

Before proceeding lead dosage in samples, first, a calibration curve must be established from the solutions of known concentrations of lead.
The results are directly read on the device if it is preset according to the manufacturer's indications or the calibration curve in lead
The device used is a spectrophotometer (PERKIN- AIMER model 400)

Results

Variations of pH related to the contamination test of the two species

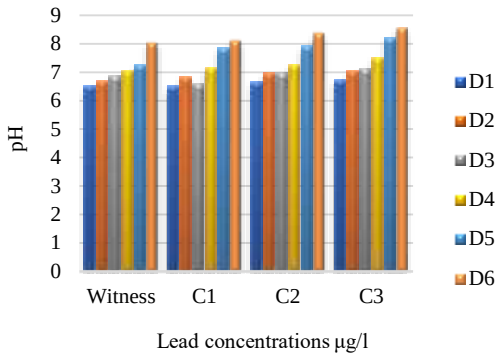

Figure 02: Flucturation of the pH solutions
In time for *Xanthoria Parietina.*

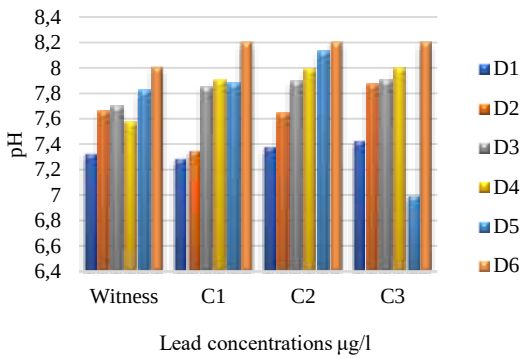

Figure 03: Flucturation of the pH solutions in time dor *Hylocomium splendens.*

For both species whatever the lead concentration, the pH of the solutions has increased, due to the absorption of some elements resulting from the dissolution of lead nitrates which implies the dissociation of its constituent ions.

Variation of Proline content

Date of mesurement	D1 (day of First Collection) S (collected from its natural habitat)	D2 (day of second collection		
		T	C1	C2
Proline content in *lichens*	0,0004	0,0001	0,0005	0,0007

Figure 04 : Variation in Proline content in *Xanthoria parietina* and *Hylocomium splendens.*

The proline content of the sample (S) was very high in both species, tended to stabilize for the concentration C1, and increased respectively With C2 and C3 indicating acute stress during the sudden disturbance of plan.

Variation of chlorophyll a, b and a+b content

In a chlorophylien plant as we can see in the witness, the content of Chl a is superior than Chl b, but chlorophyll a, b, and a+b is conversely proportional to accumulated lead doses both in lichens and mosses, and decreases as a result of the reduction in photosynthetic intensity.

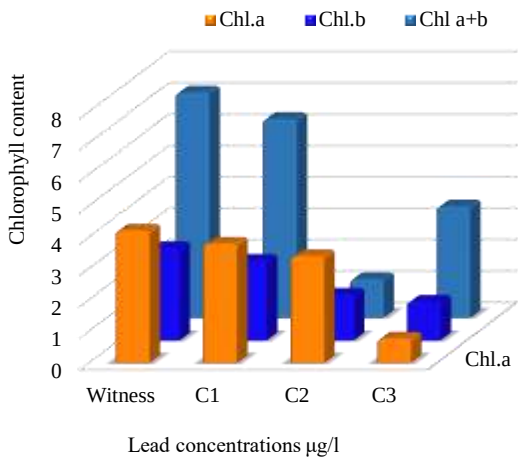

Figure 05 : Variation in chlorophyll content in *Xanthoria parientina.*

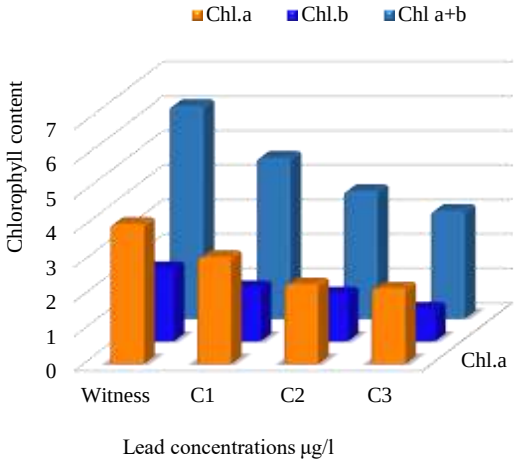

Figure 06 : Variation in chlorophyll content in *Hylocomium splendens.*

The content of lead related to the contamination test

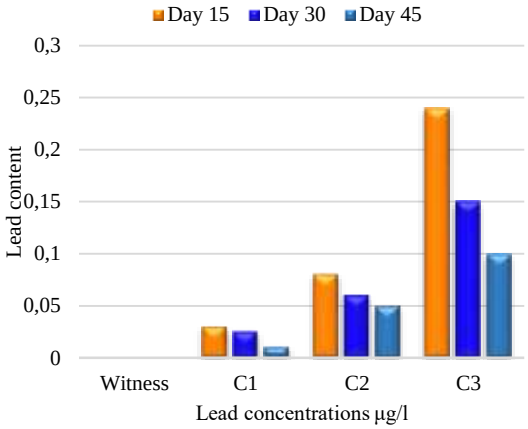

Figure 07 : Temporal variation of the lead content of sollution where *Xanthoria parietina* has stayed.

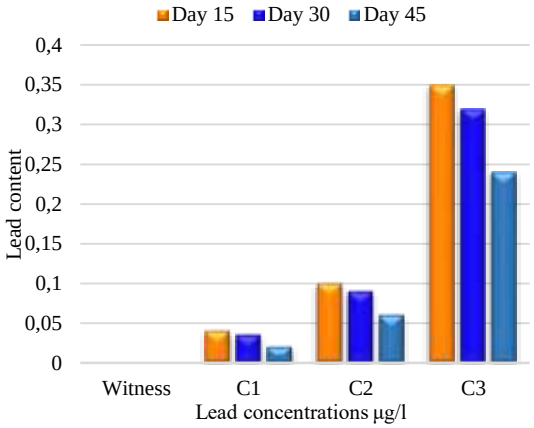

Figure 08: Temporal variation of the lead content of sollution where *Hylocomium splendens* has stayed.

For both species, the lead content in the three solutions is reduced depending on the exposure time with lower content in solutions where lichen has stayed.

Solutions at different concentrations	**Accumulated lead Mass in lichens**	**Accumulated lead Mass in mosses**
Witness	**0,03**	**0,01**
C1	**1,6**	**1,2**
C2	**2**	**1,6**
C3	**10**	**3,6**

Figure 09: content of accumulated lead µg/g by the thallus of the two species.

The lead levels increased in the thallus of the two plants regardless of the concentrations of lead in the solutions but with a higher content in the lichen thallus. It appears globally for both species that their ability to accumulate lead in time is important.

DISCUSSION

The deterioration of the air quality and the evolution of the technologies have aroused an awareness of the public authorities and the citizen. The development of sensors capable of quantifying certain types of pollutants has contributed positively to the improvement of the quality of our environment, however, the use of indirect methods such as bio-indication proved to be very useful quickly. By analyzing the results the set of values clearly indicates that both lichens and mosses accumulate and concentrate lead in their tissues. Both species have proven to be very good bio hyperaccumulator of lead which makes them excellent bio accumulators for the estimation of pollution especially by metals (case of lead) Nevertheless the lichen species *Xanthoria parietina* has a higher accumulator power than that of *Hylocomium splendens*. The variation in concentrations remains effectively depending on the nature of the species and the time of exposure to pollution. As regards to variations in measured physiological parameters, chlorophyll content, proline, they can testify to the effect of lead on the two species studied, the chlorophyll content tends to decrease due to the disturbance of the photosynthetic process as well as the degradation of the fresh material, while proline content tends to increase with exposure time due to undergoes stress

CONCLUSIONS

The deterioration of air quality and the evolution of technology have aroused an awareness of the public authorities and of the citizen. However, the use of indirect methods such as bio-indication were quickly very useful in analyzing the results, the set of values clearly indicates that both lichens and mosses accumulate and concentrate the lead in the tissues, which in its turn is responsible of many physiological disturbances.

It is obvious to mention that the two studied species are proven good bio accumulators of lead which makes them excellent bio-indicators for the estimation of air pollution especially by metals, nevertheless the lichen species *Xanthoria parietna* presents a higher accumulator power than *Hylocomium splendens*, that returns to the symbiosis that makes lichens a complex and fantastic biological material to study, whose operating mechanisms, still little known compared to the superior plants and offer the possibility of numerous research.

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INVESTIGATION OF BIOLOGY TEACHERS' ATTITUDES TOWARDS USING LABORATORY MATERIALS

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ABSTRACT

In order for biology lessons with important topics related to daily life to be loved by students, to increase their success and to train potential scientists who want to develop themselves in this field, teachers should use student-centred contemporary teaching methods. Laboratory studies improve students' critical thinking, understanding science, operation skills, manual skills. In addition, laboratories are a part of education because they enable them to use information, develop a general concept, define a new problem, explain an observation, and make a decision. However, most studies conducted in our country reveal that teachers do not use laboratories effectively in their lessons and that biology laboratories are not made at a sufficient level. The practical achievements in the curriculum are closely related to the available facilities of schools and the attitudes of teachers. When the data obtained as a result of the studies conducted are investigated, it is seen that there are many results such as improving laboratory facilities, eliminating the lack of equipment and participation of biology teachers in in-service training seminars for laboratory use. In this study, it is aimed to determine the attitudes of biology teachers towards laboratory studies in biology lessons and to suggest solutions to the problems encountered.

Keywords: *Biology teacher, Laboratory lesson, Microscope, Biology education*

INTRODUCTION

Biology, which has become a part of our new life, has gained a quality that keeps pace with today's changes and affects humanity rapidly. With these positive aspects, biology will have an important function in solving the problems that concern the person and her environment.

Today, societies aim to raise individuals who can produce modern and scientific contents that can ensure the development of the country and present them for the benefit of humanity. The field of biology is a science aimed at achieving these goals. Erectly affects human life, the need for education on these issues increases in the society and therefore, biology education is gaining importance day by day.

In the use of educational methods and tools for teaching purposes, attention is paid to address five sensory organs, to be appropriate for the level of students and the quality of the subject to be taught (Fidan and Erden, 1993). In the studies carried out in biology education, it is aimed to use modern teaching methods that allow them to learn by making concrete experiences through first-hand experience. Universities and the Ministry of National Education have important responsibilities to train teachers who can use all these methods and to train them during service (Altunoğlu and Atav, 2005; Chuang and Cheng, 2003).

In order for biology education to be carried out in a qualified and successful manner in laboratory environments, it is essential that teachers and students involved in the learning-teaching process are aware of their responsibilities and participate in this process with this awareness. Since the learning-teaching process is a two-way communication process, focusing

only on the teacher dimension in this process and doing research in this direction means to leave it unresolved by approaching possible problems unilaterally.

For these reasons, it is a fact that the quality of biology education in high schools and universities is not limited to biology teachers working in those schools. In biology teaching, there are many factors that affect the ability of teachers to make their practice lessons more efficient and high quality. The responsibilities and behaviours that students take in laboratory classes may be one of the most important of these.

The importance of laboratory courses in biology teaching

The importance of laboratory lessons, which started to enter school programs since the middle of the 19th century, came to the agenda in various ways in the teaching of biology, as in other sciences, and was often adopted as one of the most effective methods in biology teaching (Ekici, 2003). The scientific method, which was developed as a way of obtaining scientific information, has been adopted as one of the most effective methods in teaching biology, as the science of biology started to be included in the teaching methods in the field of science. The teaching method is the way to be followed by the student to reach the goal in the best way. This method allows students to use scientific process skills in order to gain skills, knowledge and attitudes. Environments with the possibility of application such as a laboratory are all sources of these expressions. The purpose of laboratory studies in biology education is to help students learn science by acquiring conceptual and theoretical knowledge and to provide them with information about science fiction development methods. Studies also enable students to understand science, nature, and obtain information using scientific inquiry protocols. Support for purposeful learning defines scientific theories and how to apply them. In addition, laboratory studies should encourage the development of analytical and critical thinking skills and children's interest in science.

The applied curriculum states that laboratory studies should include research skills: identify problems, create research questions, plan, and carry out research, formulate, communicate, and defend explanations (Hofstein and Lunetta, 2004). However, studies show that most laboratory studies do not meet this goal. Therefore, laboratory studies at schools require more research, including planning and design (Tweedy and Hoese, 2005).

Laboratory studies form the basis of biological research today. It requires to know the morphological and anatomical structures before starting to research plants and animals on the laboratory. Therefore, it is useful to know the plant and animal material, morphology, anatomy and taxonomy as laboratory study objects. However, morphology and anatomy of plants and animals cannot be learned only from theoretical lessons or from books and written sources. For this, students must examine the plant and animal materials themselves and take their sections (if necessary). That's why the biology teacher, while organizing the education program, argues that students should pay attention to laboratory studies as well as their theoretical knowledge (Bozkurt, 1960; Erten, 1993). In many countries with advanced technology, modern learning methods include theoretical knowledge as well as laboratory studies in all kinds of teaching fields.

All teachers agree that laboratory work is important in biology lessons. In addition, they stated that the main goals of laboratory studies are to encourage students by accustoming students to scientific objects and events, triggering their feelings of curiosity and attracting their attention to the specified topic. According to educators, laboratory studies also allow students to resist misconceptions and challenge them (Bozkurt, 1960; Erten, 1993).

General objectives of laboratory studies

All science teachers graduate from the universities they are affiliated to, using a variety of teaching methods and techniques, depending on the variety and content of the subjects. The

most preferred method, which provides critical thinking and permanent learning, is a method that gives importance to mental activities and allows students to work individually and in groups. Biology laboratories are the most meaningful environments where education can be made by living and learning (Tamir, 1978).

Laboratories are a controllable environment where certain concepts or subjects are taught by having students taught, tested, or demonstrated and their limits are determined. These environments are environments where information is used, the problem is defined, hand skills and computing capabilities are developed. Laboratories are environments where it is understood that the questions formed in the mind because of many observations or abstract perceptions are made concrete and that the information obtained has a vital value (Figure 1) (Güneş et al., 2013).

Theoretically, information given in biology should be transferred to students through experiments. In this way, students' feelings of curiosity are triggered and their interest in biology lessons increases.

Figure 1. Examples of biology materials in laboratory cabinets

Status of biology laboratories in schools where teachers work

According to the results of Akaydın and Soran (1998), more than half of biology teachers stated that biology laboratories in their high schools are not fully equipped. A few of the teachers stated that the biology laboratories in their schools are full-fledged (Figure 2).

According to the research, half of the biology teachers can provide the opportunity to work with a microscope for 5-6 students, while less than half have revealed that they can provide the whole class with only one microscope. There is no school without a microscope and no school that can provide a microscope for each student has been found. Less than half of the teachers who participated in the study stated that they were able to work with a microscope to 15 students (Akaydın and Soran, 1998).

In the study of Ensari (2008), most of the teachers who graduated from the faculty of science and education stated that the missing course materials for the biology course were not sent by the National Education. It is seen that one third of the teachers are undecided. This shows that some of the teachers are not aware of the missing course materials by the National Education. This shows that teachers use the materials available in their hands and do not look for other alternatives.

Figure 2. Microscope and glassware in the laboratory

Frequency of Use of Laboratories by Teachers

Some teachers refrain from using even very simple materials, as they do not know how to use existing tools. The teacher, who knows how to use the tools in her laboratories, the acquisition and limitations of the tool to the student, will look for different ways of using the tools (Köseoğlu and Soran, 2004; Küçükahmet, 2002).

They stated that very few of the teachers did not have a biology laboratory in their schools and that they used the laboratory only once during the school year. Some teachers, on the other hand, stated that they did not use the biology laboratory in their schools even though they existed.

In many studies, the main reason that teachers do not use tools and equipment is that their knowledge and skills are not sufficient in using tools (Özdemir, 2000; Teker, 2002; Tabar, 2002; Tüy, 2002). Teachers cannot perform biology lessons without using tools.

For this reason, ensuring the competence of the use of tools and equipment is an important factor in achieving the goals of biology courses. The ability of teachers to use tools and equipment is directly related to the use of tools and materials taken during their undergraduate education.

In the study of Köseoğlu and Soran (2005), the frequency and cognitive competencies of biology teachers using tools and equipment differ according to their seniority. The cognitive competency test scores of the teachers with 0-10 years and 11-20 years of seniority were higher than the teachers with seniority of 21 years or more.

Teacher competencies in terms of use of tools and materials in biology lessons

In biology education, the education-teaching process cannot be carried out in a meaningful way without tools. Biology teachers' use of tools while explaining the topics will provide more proven data and will provide meaningful and permanent learning. In many studies, teachers state the reason for not using tools and equipment in the form of not having knowledge and skills and needing in-service training (Özer, 1996; Uzunahmet, 1996; Özdemir, 2000; Teker, 2002; Tüy, 2002). Biology teachers' level of using tools and equipment in their lessons can be achieved by having equipment, knowledge and skills related to the tools.

It has been determined that there is a difference in teachers' ability to use tools and equipment according to the schools they graduated from. It has been determined that the teachers who graduated from the faculty of education and science have higher cognitive

competency test scores from the education institute and education associate degree graduates. This may be due to the fact that teachers who graduate from education and science faculties are more exposed to technology. According to the results of the researches, it has been determined that teachers' proficiency in using tools and equipment is positive (Köseoğlu and Soran, 2005). In parallel with this research, according to Namlu (1998), teachers' attitudes towards course tools are generally above average.

The cornerstone of the education system is teachers. The equipment and qualifications that teachers have are directly related to the success of our education system. In order to train equipped teachers, first of all, the competencies of the teachers should be determined and solutions should be sought in line with the deficiencies. In the achievement test applied to determine teachers' cognitive competencies, their competencies related to the use of tools and equipment were found to be 57.3%. In parallel with this result, Korkmaz (2000) stated that the cognitive competencies of biology teachers regarding the use of tools and laboratory practices are at a medium level.

Biology teachers' ability to use tool and equipment and cognitive competence in laboratories differ depending on whether they attend in-service training courses. In most of the researches, it has been determined that in-service training is positive in improving teachers' knowledge and abilities, and teachers are separated from the program with positive emotions (Seferoğlu, 2001; Uçar and İpek, 2006; Demirtaş, 2010). On the other hand, it has been determined in some studies that in-service training courses are not effective in developing teachers' knowledge and skills and teachers are not satisfied with the applied program (Kanlı and Yağbasan, 2002).

In a parallel study, it was determined that there was a significant positive relationship between educational technology and self-efficacy, and there was a low relationship between teacher competence and interest (Archambault, Kulikowich and Brown, 2002). According to the results of all these, it has been revealed that the positive attitudes of teachers' using tools and equipment are not enough to increase their cognitive competencies alone, and that teachers with positive attitudes must constantly change and follow the developing technology closely.

Frequency of material use in the lessons of biology teachers

In today's rapidly changing and developing world, it is aimed to educate individuals who know the ways of accessing information instead of getting information from one source and memorizing them, and who can apply solution methods by using information in the face of problems. Individuals' ability to acquire these skills depends on teachers designing effective and interactive learning environments, and using teaching materials prepared in accordance with the principles of educational technologies and instructional Technologies (Doğdu and Arslan, 1993; Dursun, 2006; Yanpar, 2007; Yanpar Yelken, 2011).

According to the studies, the theoretical and laboratory studies applied in biology show that students have difficulty in understanding. The fact that the topics included in the scope of biology are complex and abstract, the teaching process is meaningful and the process should be supported with various materials in order to ensure permanent learning. One of the most frequently used materials in biology laboratories is light microscopes. Since the biological materials are usually below the eyesight of the human eye, most of the experiments performed in the laboratory can only be carried out with the help of a microscope (Dikmenli et al., 2002).

According to the study by Yeşilyurt (2004), the teachers who participated in the research had difficulty in understanding because of the low frequency of using microscopes in their university education, and biology students were more successful due to the characteristics of the departments. The difficulties in understanding the microscope associated with high school students may be due to the lack of laboratory conditions in their schools, lack of materials,

biology teachers prefer a traditional way while performing the teaching process and teachers do not receive the necessary in-service training.

According to the study of Ensari (2008), when teachers need to make sacrifices from their private lives in order to prepare various materials that can be used in biology lessons, teachers of education faculty have come to the fore more than teachers of science and literature. This situation is stated by the teachers of science and literature origin, who stated that preparing material is a part of their job, and teachers from education faculty think that it is easier to prepare course material and does not take much time.

The use of materials provides meaningful and permanent learning by increasing efficiency in the educational process. It contributes to academic success by increasing students' interest in the lesson. In this context, the degree of readiness in preparing, using, and evaluating the materials that teachers can use in their lessons should be improved (Ensari, 2008).

In addition to the lack of vocational training in teachers, poor laboratory conditions, especially the lack of technological equipment, it can be interpreted that laboratory practices cannot be performed efficiently (Akaydın et al., 2000; Akaydın and Soran, 1998). Although the suitability of the conditions is important here, in most of the researches, teachers and school administrators complain about the insufficiency of the physical conditions of the laboratories; but showed that they do not use it effectively even though there is a laboratory in schools (Figure 3) (Akaydın and Soran, 1992; Erten, 1993).

Figure 3. Unsuitable and unused laboratory sample

The uneasiness and unwillingness of teachers is also an important obstacle in laboratory practices. In addition to the problems such as class size and textbooks not supporting laboratory applications, it has been observed that teachers are not willing to make laboratories. In addition, it was observed that they did not show the teaching method behaviours expected from them during laboratory applications, and laboratory applications had significant deficiencies in preparation, presentation, application, and evaluation stages (Ekici, 2003). The reluctance and anxiety of teachers is largely due to their inability to gain laboratory practice behaviour during their education, their inability to master the subjects and experiments, and their inability to use materials and equipment. Also, a significant number of teachers are not aware of the importance and necessity of laboratory applications (Böyük and Erol, 2008; Erten, 1993).

Ekici, Ekici and Taşkın (2004), on the other hand, conducted their research in schools including elementary schools and high schools in Denizli and its districts as biology laboratories, where they examine the presence of existing environments. In the study, it was

determined that while the courses of Physics, Chemistry, Biology and Science were being used, laboratory and laboratory materials were not used, and the existing laboratories were in a revised position. Based on their observations, they found that in most existing schools, laboratories were not used for experimental applications, but were prepared as images.

It was stated by the teachers that it was difficult to perform the experiments in the program due to the conditions in the schools, and that the schedule was intense and time limited, restricting teachers to use various teaching methods. In the research carried out by Yaman and Soran (2000), the lack of laboratories or the limited use due to the common use with other science lessons and the lack of laboratory equipment and equipment are shown as the reasons for the very few experiments in biology lessons. In another study, it is stated that among the reasons for not conducting experiments, there is not enough time to train the program and the classes are crowded (Erten, 1991). In a study by Scott (1994), besides various problems such as insufficient time and schools having infrastructural problems, it was stated that exams and students' indifference to the lesson were among the factors limiting teachers.

Problems encountered in biology laboratory applications

The success of education systems is related to the qualifications of teachers. Studies have reported that there is a relationship between motivation and attitude. Therefore, motivation of teachers affects student success. Teachers' attitudes and behaviours were also found to be effective on students (Altınok, 2004; Çeliköz and Çetin, 2004; Oktar and Bulduk, 2000; Yenice et al., 2008).

According to Erten (1993) and Akaydın et al. (2000), 20% of teachers saw laboratory practices in biology education as an effective teaching method. As a result of the studies carried out on teachers, they found that especially expensive and difficult-to-supply equipment was insufficient in biology laboratories. In this, they stated that the teacher would decrease the efficiency in the application of the laboratory method sufficiently.

Although laboratory applications in biology education are necessary for attracting attention to the lesson and learning the subjects more effectively and meaningfully, it is understood that the laboratory applications could not be done sufficiently and effectively due to the lack of laboratory use proficiency, material and lesson hours, and laboratory safety. (Karaca, Uluçınar and Cansaran, 2006; Saka, 2002). In biology lessons, teachers mentioned that the activities could not be done to the required extent due to the above-normal number of students in laboratories, lack of equipment and auxiliary staff in laboratories (Alpaut, 1993; Ayas et al., 1994; Ekici, 1996; Erten, 1993; Gürdal, 1991; Özbaş and Soran, 1993).

CONCLUSIONS

Biology teachers generally do not find the laboratory facilities in their schools adequate. In many studies conducted in our country, it was emphasized that insufficient physical conditions are the leading reasons that prevent laboratory use (Alpaut, 1993; Ayas et al., 1994; Ekici, 1996; Erten, 1993; Gürdal, 1991; Özbaş and Soran, 1993; Özkan and Öztürk, 2018). However, regardless of the theoretical benefit of tools, their use requires a certain level of knowledge and skills. If the teacher does not have the ability to use the necessary tools or does not believe in the benefit of these, she will not want to use them significantly (Yalın, 1997).

Our teachers should be informed about the methods of preparing and developing simple materials through in-service courses. With in-service training programs for the use of technology in education, our teachers should be informed about educational technology and technological equipment and skills should be developed with various applications to be carried out. Teachers should have knowledge of the institutions from which they can obtain teaching materials that are not available in their school.

In addition to enabling students to develop positive attitudes towards science subjects, correct and effective laboratory practices will contribute significantly to the development of students' problem solving and psychomotor skills, and to create their own knowledge (Azizoğlu and Uzuntiryaki, 2006; Hofstein, 2004; Singer, Hilton and Schweingruber, 2005).

According to the data obtained, teachers prefer to conduct their lessons either without demonstration or experimenting, rather than conducting individual or group experiments. They claim that there are not enough tools in laboratory cabinets as a reason for this. It is important to review and renew the biology laboratory cabinets that exist in schools from time to time and to complete the deficiencies in terms of ensuring permanent learning. In today's conditions, equipping school laboratories with technological materials and informing teachers about the use of these materials will increase the level of education (Dindar and Yaman, 2003; Seniş, 1993).

Biology topics are based on observation, investigation, and research. The places where these activities will be carried out in schools are undoubtedly complete and well-organized laboratory environments. A biology lab with these features allows students to see something that is said or defined in a concrete way. Since learning in the laboratory will be by doing and living, the permanence of what is learned is ensured (Akaydın et al., 2000). For this reason, laboratory facilities should be increased and renewed throughout the country. By establishing material development laboratories in the Faculties of Education, prospective teachers can be enabled to produce and use their own materials.

Despite very carefully prepared training programs and rich resources, what is important is what the teacher can do in the classroom. To create a realistic training program in terms of applicability, it is necessary to analyse the education-training environment well, and to determine the characteristics and needs of the field.

As a result, it was determined that a significant part of the teachers did not benefit from the laboratories and even the experiments that could be done with the materials used in daily life were passed without applying. On the other hand, performing biology education experimentally based on laboratory will facilitate the transformation of knowledge from theory to practice and daily life. Moreover, students who learn in the laboratory by learning by mistake or by mistake will have the chance to benefit from what they have learned in real life. As Ensari (2008) puts it, "a school is only as good as the teachers working there" reveals the role of teachers in the quality of education in our schools. The teachers, who are the main and irreplaceable element for the country, are the people who operate and train the human power.

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BIOCLIMATES OF THE LUMBARDH VALLEY OF PRIZREN, KOSOVO

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ABSTRACT

The climate of a region is the main determining factor in the distribution of vegetation in that region. The study of the average multi-year performance of the climate and its two main determining parameters, the regime of temperatures and precipitation, as well as their analysis through bioclimatic indicators, allows to judge the distribution and type of vegetation in that region. For proper explanation of the relationships between climate and vegetation, for the purpose of phyto-climatic characterization, based on thermal and pluviometry data, in this study are used the bioclimatic indicators proposed by Rivas Martinez, the pluvial-factor indicator of Lang, the drought indicator of De Martonn and the ombro-thermal diagrams of Walter and Leith, as the most suitable methods for this purpose. They are valuable indicators in orderto determine its climatic features in relation to vegetationfor the Mediterranean environment, where also the Prizren region in Kosovo is part of it too. Calculating the values of bioclimatic indicators and analyzing them enables us to compare between different types of bio climates and the bio climates between different areas (thermotypes and ombrotypes) and to evaluate the extent and type of vegetation for each bioclimatic type. Precisely, this study, located in the Lumbardh valley of Prizren in Kosovo, serves this purpose.

Keywords: *climate, bioclimatic indices, precipitation, temperature, Mediterranean vegetation*

INTRODUCTION

The study of climate and its impact on the distribution of vegetation and plant species, requires knowledge of the concept of climate and the study of its average multi-year progress, ie the climatic characterization of a given area. While to determine the impact it has on vegetation should be studied its climatic behavior and the relationships it creates with plants. This is achieved through the bioclimatic characterization of the area and the use of bioclimatic and phytoclimatic indicators, which highlight the link between climatic performance and plant growth. To determine the climate of a region should be recorded the performance of its main constituent meteorological parameters such as air temperature, precipitation, air humidity, wind speed, for a period of over 30 years (WMO - World Meteorological Organization). Climate is a dynamic system, the result of the interaction of its constituent elements and that determine the climatic course of different areas.In particular, the climate of the Mediterranean basin is represented by a great variability (Mitrakos, 1980) which is due to the interaction of different factors in this area. This climate variability is most evident at local levels where very different climate subtypes are created (Grove & Rackham 2001). Particularly in the mountainous areas are created significant climatic differences from the lowlands, influenced

by the degree of continentally of the area. Warm Mediterranean currents often penetrate into the valleys of mountainous areas creating differentiated climatic subtypes (Krasniqi S., 2017).These climatic subtypes in narrow areas have their impact on the distribution of vegetation. Climatic characterization of different ecological areas is with study interest for many disciplines but especially for studies of biological nature, which highlight the relationships between climate and vegetation. Through them we can argue the geographical distribution of plants. In phytoclimatic studies, bioclimatic characterization, which is based on the relationships between biological parameters of plants and climatic parameters, is quite useful from a practical point of view.Unlike the climatic classification which are based only on meteorological parameters, which makes the classification only from the climatic point of view of different areas, the bioclimatic characterization is important because it points out the connection between vegetation and climatic factors. The different types of vegetation that we distinguish in an area, seen both in terms of space and time, or in relation to altimetry (altitude), are the result of changing climatic parameters, the identification of which in floristic and vegetation studies is a necessity. The distribution of plant communities is closely related to the type of climate (Blasi, 1996).The study of physical-geographical characteristics, altitude from level sea, distance from the sea and other factors are important in the study of climate as part of climate-forming factors. The study of the vegetation features of the areas under study is important as the vegetation itself is an influential factor in the formation of zonal climates and microclimates. This is due to the fact that: *"... vegetation regions are climatic regions"*(Giacobbe, 1948,1949) and *"... the comparable climatic conditions correspond to similar aspects of vegetation"*(Braun-Blanquet,1932).In different climatic zones are formed many bioclimatic types and subtypes which determine the distribution of plant species. The study and determination of bioclimatic types and subtypes of a region is made possible through the use of bioclimatic indicators. In many countries in the Mediterranean basin and in Europe, the Rivas- Martinez indicator (1995,1996,1999, 2004) are more appropriate for this purpose, giving a clearer picture of the relationship between climate and vegetation; pluviofactor indicator of Lang (1915); drought indicator of the De Martonn (1926); ohmothermic diagrams of Bagnouls e Gaussen (1957) modified by Walter e Lieth (1960); which are among the most used indicators and efficient for this purpose.Looking at the types of climate in relation to the distribution of vegetation, we can distinguish in the Mediterranean space bioclimatic types such as infra-Mediterranean, thermo-Mediterranean, eu-Mediterranean, super-Mediterranean, mountain-Mediterranean and oro-Mediterranean (Quézel, 1976).This bioclimatic classification takes into account the distribution of plant and vegetation species which have been studied early (Von Humboldt & Bonpland, 1807). The physico-chemical characteristics of the soil also play an important role in the distribution of vegetation.Therefore, when we study the floristic and vegetative aspects of an area or their dynamics, in fact we should study the pedo-climatic characteristics of the crtain area. In the study area, which lies in the valley along the river Lumbardhi of Prizren, based on the physical-geographical position, altitude above the level sea and the influence of climatic, hydrological and terrestrial factors, there are four phytoclimatic belts with more or less clearly between them and which are: *(1) shrub and oak forest belt; 2) belt of deciduous forests (beeches); (3) belt of beech and coniferous forests (beech and fir) and, (4) belt of alpine pasture;* where in each belt we find a typical vegetation representative of the Mediterranean regions.These phytoclimatic belts, with a more or less clear difference between them, are an expression of the interaction of the vegetation of this arearelated with the conditions of the physical environment, ie of the habitats and in particular of the altitude above

sea level. In the interior of each belt we find a floristic wealth with defined features, represented by herbaceous, shrubby and woody plant species.

1) Shrub and oak forest belt. In this forest belt dominate the species: *Quercus petreae (Mat) Liebl., Fraxinus ornus L., Ostrya carpinifolia Scop., Quercus cerris L., Quercus frainetto Ten., Carpinus betulus L., Quercus pubescens Willd., Carpinus orientalis Miller., Robinia pseudoacacia L., Cornus mas L., Corylus avellana L., Pyrus pyraster Burgsd., Prunus spinosa L., Juniperus communis L., etc.*

2) Mesophilic belt of deciduous forests (beeches). In this forest belt dominate the species: *Fagus moesiaca (K.Maly)., Carpinus betulus L., Carpinus orientalis Miller., Quercus petreae (Mat) Liebl., Quercus pubescens Willd., Acer plantanoides L., Acer obtusatum Waldst.et, Kit., Acer campestre L., Prunus avium L., Populus tremula L., Viburnum lantana L.*

3) Belt of beech and coniferous forests (beech and fir). In this forest belt dominate the species: *Fagus moesiaca (K.Maly)., Pinus heldreichii Christ., Pinus peuce Griseb., Abies alba Mill., Acer pseudoplantanus L., Acer plantanoides L., where are represented some shrubs such as: Juniperus nana Willd., Vacinium myrtillus L., Robus idaeus L., Rosa pendulina L., Bruckenthalia spiculifolia (Spike Heath).*

4) Alpine pasture belt. This belt extends over the area of beech and conifers to the highest peaks. Represented by plant associations *Agrostis rupestris All., Alopecurus gerardii Vill., Alchemilla alpina L., Bromus racemosus L., Crocus scardicus Koš., Crocus velchensis Herb., Caltha palustris L., Dianthus scardicus Wedst., Festuca panciculata (L.),Schinz&Thell., Gentiana verna L., Gentiana punctata L., Geum montanum L., Geum reptans L., Lilium albanicum Griseb., Nardus stricta L., Narthecium scardicum Koš., Potentilla dorfleri Wettst., Ranunculus crenatus Waldest.et.Kit., Ranunculus montanus Willd., Primula veris L., Veratrum album L., Salix reticulata L., Sesleria coerulans Friv., Viola elegantula Schott.*

In alpine pastures, in addition to alpine vegetation, many shrubs or bushes grow, among which: *Juniperus nana Willd.,* blueberry associations *Vaccinium myrtillus L.*

MATERIAL AND METHODS

The bioclimatic study was conducted in the Prizren region of Kosovo and analyzed the bioclimatic and phytodiversity of the Lumbardh valley of Prizren. This valley is characterized by a dense vegetation with a variety in its composition, which is differentiated in relation to the altitude and which is a consequence of climatic, hydrographic and pedological conditions. Bioclimatic analysis of this valley will enable the differentiation of its bioclimatic types and plant diversity for each bioclimatic type. Bioclimatic indicators were used to realize this study, among which those of Rivas- Martinez (1996, 1999, 2004), have found application in many countries in the Mediterranean basin and in Europe, as they give a clearer picture of the connection of climate with vegetation.The Rivas-Martinez method provides the opportunity to make a more detailed classification of phytoclimatic generations. Other indicators such as pluviofactor of Lang (1915) and drought indicator of De Martonn (1926) further serve this purpose. The calculation of indicators is done through formulas, while graphic methods (ombrothermal diagrams), record the progress of different climates and their impact on vegetation. The othermotherm diagrams of Bagnouls e Gaussen (1957) modified by Walter e Lieth (1960) are among the most widely used and efficient for this purpose. On the basic of temperature and precipitation databasesare constructed the ombrothermal diagrams, following

the methodology of thermodograms (Walter e Lieth, 1960). For their realizationare taken into account the average values of temperature and monthly precipitation, as the most significant meteorological factors, which are reflected in a Cartesian graph in which the temperature values are presented with a scale twice of the precipitation values (1 °C = 2 mm). The overlap of the thermal and pluviometric curves (ombrothermal zone, shaded), which occurs when the amount of precipitation is less than twice of the temperature (P <2T), represents the climatic situation in relation to drought (which in this period of the year is considered aride or dry).Evidence of the climatic situation, through the use of this graphic indicator is important especially in areas with little rainfall, because it highlights the conditions of hydric stress for plants. For the bioclimatic inclusion of the study area, the data of the thermopluviometric station of Prizren (1948-1978) for a period of 30 years have been taken into account (Jarosllav *et al.*,1983). The performance of these data and their climatic variability has been compared with other subsequent data for the Prizren station for the years 2017 and 2018 (IHMK, 2001-2018). Bioclimatic classification in the study area was done through the use of the above bioclimatic indicators, as the most appropriate methods for this purpose. For the bioclimatic interpretation of the study space, the network www.globalbioclimatics.org was consulted to determine its features and climatic behavior.

RESULTS AND DISCUSSION

Climatic characterization. Kosovo's climate is mostly continental, it is characterized by harsh and cold winters, with more rain and snow, while the summer season is hot. It is influenced by both Mediterranean and Alpine currents. The climate of the Prizren region is characterized by a somewhat modified climate, milder than what would be expected from its latitude. The climate is continental alpine, harsh climate, but also penetrates the Mediterranean Adriatic currents through the "*Drini i Bardhe*" canyon which have favored the adaptation and development of various plant forms which appear through its morphological diversity. Average annual temperatures in the Prizren area are around 11.9^0C; the coldest month is January and December with perennial minimum average temperatures going to -1.6^0C and 0.19^0C respectively and the hottest month is July with a maximum average temperature going up to 26.8^0C followed by August at 26.3^0C. This area is included in the Dukagjini Plain which is one of the hottest in Kosovo.There are about 229 days without frost. The average date of the first frost is November 11th, while the average date of the last frost is March 27th. The average value of precipitation in this area varies from 670 to 1200 mm/year. The largest amount of precipitation recorded is about 79 mm in October and the average annual amount is about 747 mm. The largest amount of precipitation falls during the autumn-winter period from October to April by about 455 mm (IHMK, 2001-2018).

Average air humidity varies, depending on the seasons and climatic conditions. It reaches its highest level during the winter period (December-January) where it reaches the values 82–83%, while in the summer months, the air humidity decreases, reaching the lowest values during the month of August, by about 59%. The average value of relative air humidity is about 60-70%. There are 220 to 280 sunny days a year in this region. In Kosovo, on average there are about 2066 hours of sunshine (IHMK, 2013), this value varies according to the seasons of the year, terrain topography, altitude and degree of cloud cover. Solar radiation in the vegetation period goes 458 kcal/cm^2/day. The average value of wind speed is about 2.8 m/sec. The wind ranges from 1 to 30 m/sec where the wind directions from the south, southwest and south-east dominate and the lowest average wind speed occurs in August and September

(IHMK, 2001-2018). This climate has created the conditions for a very rich natural phytodiversity.For the bioclimatic characterization of the study area are taken the values of thermo-pluviometrical indicators of average temperature, maximum average temperature, minimum average temperature and the amount of precipitation at the Prizren station for a period of 30 years (1948-1978) (I.H., Jarosllav Çerni, 1983).

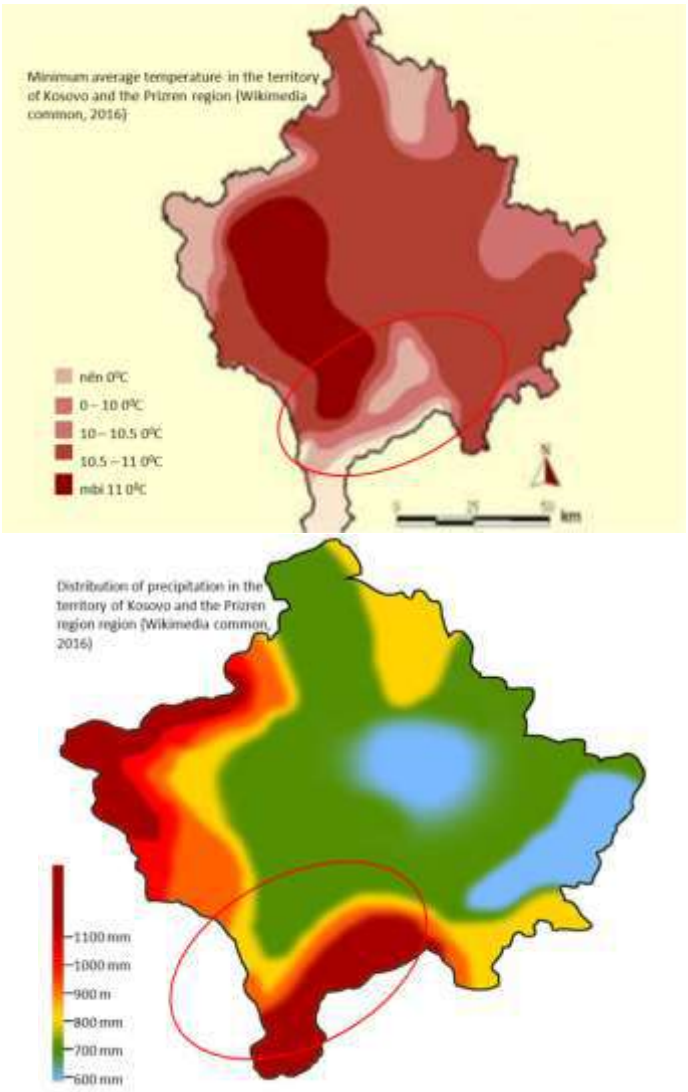

Figure 1. Temperatures and rainfall in Kosovo and the Prizren region

Table 1. Average temperature, maximum average, minimum average and amount of precipitation at Prizren station (1948-1978).

Indicators	January	February	March	April	May	June	July	August	September	October	November	December	Annual average
Average temperature	0.18	2.49	6.46	11.87	16.67	20.23	22.27	22.09	17.99	12.08	7.36	2.46	11.87
Maximum temperature	1.27	4.81	9.83	16.03	20.9	24.73	26.83	26.31	21.92	15.38	10.02	4.73	15.99
Minimum temperature	-1.63	0.17	3.09	7.7.1	12.44	15.73	17.71	17.87	14.06	8.78	4.70	0.19	7.75
The amount of precipitation	65	56	59	61	72	59	58	38	65	62	79	73	747

Bioclimatic characterization. For the bioclimatic characterization of the study area, the indicators were taken into analysis and calculated according to the method of Rivas-Martinez (1996,1999) such as the simple continentality indicator (Ic), the thermal index (It), the compensated thermal index (Itc), the indicator, annual ohmbrothermic (Io) and four-month summer ohmothermic indicator (Ios4). The definition of the thermotype and the low or high horizon is determined by the simple continentality indicator: *Ic = tw - tc*; where: tw - average temperature of the hottest month, tc- average temperature of the coldest month), where from the calculations it results that: *Ic* = 22.27 - 0.18 = 22.09. For values of this indicator> 21 the climate is continental and in the range 21-28 it is considered semi-continental (Rivas-Martinez classification, 1995).

Table 2. Bioclimatic thermotype horizons and related indicators (Rivas–Martinez, 1999).

Bioclimatic thermotype horizons and related indicators (Rivas–Martinez, 1999).			
Thermotype horizons	**Acronyms**	**It, Itc**	**Tp**
Inframesdhetare inferiore	Lime	515-580	>2650
Inframesdhetare superiore	Uime	450-515	2450-2650
Termomesdhetare inferiore	Ltme	400-450	2300-2450
Termomesdhetare superiore	Utme	350-400	2150-2300
Mesomesdhetare inferiore	Lmme	280-350	1825-2150
Mesomesdhetare superiore	Umme	210-280	1500-1825
Supramesdhetare inferiore	Lsme	145-210	1200-1500
Supramesdhetare superiore	Usme	80-145	900-1200
Oromesdhetare inferiore	Lome	-	675-900
Oromesdhetare superiore	Uome	-	450-675
Kriomesdhetare inferiore	Lcme	-	150-450
Kriomesdhetare superiore	Ucme	-	1-50
Mesdhetare atermike	Mea	-	0
Infratemperato inferiore	Lite	445-480	>2450
Infratemperato superiore	Uite	410-445	2350-2450
Termotemperato inferiore	Ltte	355-410	2175-2350
Termotemperato superiore	Utte	300-355	2000-2175
Mesotemperato inferiore	Lmte	240-300	1700-2000
Mesotemperato superiore	Umte	180-240	1400-1700
Supratemperato inferiore	Lste	100-180	1100-1400
Supratemperato superiore	Uste	(20-100)	800-1100
Orotemperato inferiore	Lote	-	590-800
Orotemperto superiore	Uote	-	380-590
Kriotemperato inferiore	Lcte	-	80-380
Kriotemperato superiore	Ucte	-	1-80
Atermik temperato	Tea	-	0

Since the value of this indicator is> 21it is calculated the offset thermal index (Itc). Its calculation requires the calculation of the thermal index: *It = (T + M + m) x 10*; where: T - is the average annual temperature; M - maximum average temperature in the coldest month and m - minimum average temperature in the coldest month.*Itc = It + Ci*; where: It - the thermal index which It = (T + M + m) × 10 and Ci has a compensation value equal to Ci = 5 x (Ic - 18) .It = (11.87 + 1.27 + (-1.63)) x 10 = 115.1; Ci = 20.45; Itc = 115.1 + 20.45 = 135.55. For values of the compensated thermal index Itc = 135 the climate is considered mountain tempered (Rivas-Martinez, 1995, modified). For values of the It thermal index, included in the range 200 <It <70; according to the definitions of the Rivas-Martinez method (1999), where respectively the values are 115.1 and 104 the climate is considered Supratemperato inferior. Based on the

values of the calculated indicators (It and Itc) the thermotype and the horizon are determined according to the classes defined by Rivas Martinez (1999).

To determine the ombrotype, calculate the annual ohmothermic indicator (Io). *Io = 10 x Pp / Tp*; where: Pp - the amount of monthly precipitation of months with average temperatures greater than 0^0C and Tp - the sum of average temperatures of the same months. *Io* = 10 x 747/2100.65 = 3.5. The relatively low value of Io highlights the lack of rainfall in the region making it relatively dry, especially in low-lying areas. Based on the value of Io the ombrotype is defined referring to the classes defined by Rivas-Martinez (1999).

Table 3. Horizons of bioclimatic ombrotypes and relevant indicators (Rivas-Matinez, 1999)

Horizons of bioclimatic ombrotypes and relevant indicators (Rivas-Matinez, 1999)		
Horizons of ombrotypes	**Acronyms**	**Io**
Ultraperiaride	Uha	<0.1
Hiperaride inferiore	Lhar	0.1-0-2
Hiperaride superiore	Uhar	0.2-0.3
Aride inferiore	Lari	0.3-0.6
Aride superiore	Uari	0.6-1.0
Semiaride inferiore	Lsar	1.0-1.5
Semiaride superiore	Usar	1.5-2.0
E thate inferiroe	Ldry	2.0-2.8
E thate superiore	Udry	2.8-3.6
Subumide inferiore	Lshu	3.6-4.8
Subumide superiore	Ushu	4.8-6.0
Umide inferiore	Lhum	6.0-9.0
Umide superiore	Uhum	9.0-12.0
Hiperumide inferiore	Lhhu	12.0-18.0
Hiperumide superiore	Uhhu	12.0-24.0
Ultrahiperumide	Uhh	>24.0

For annual precipitation values from 500-700 mm in tempered regions determines the subumido ombrotype, the inferior horizon (Blasi, 1994), with a tendency towards dry ombrotype. The four-month summer was also calculated also the ohmbrothermic index (Ios4). This indicator serves to distinguish the regions with Mediterranean climate from those with temperate climate. If its value is> 2 the region is tempered and if it is <2 the region has a Mediterranean climate.

$Ios4 = (P_{May} + P_{June} + P_{July} + P_{August}) / (T_{midMay} + T_{midJune} + T_{midJuly} + T\mathit{midAugust})$.

Ios4 = (72 + 59 + 58 + 38) / (16.67 + 20.23 + 22.27 + 22.09) = 227 / 81.26 = 2.79.

The value of Ios4 = 2.79 means that the climate of the region is temperate and that the amount of precipitation in the period considered is twice the sum of average temperatures (P> 2T).

Table 4. Bioclimatic indicators for Prizren station (Rivaz-Martinez,1999)

Bioclimatic indicators for Prizren station (Rivaz-Martinez,1999)									
tw	**tc**	**Ic**	**Tp**	**It**	**Itc**	**Io**	**Ios4**	**Thermotypes**	**Ombrotypes**
22.27	0.18	22.09	2100.65	115.1	135.55	3.55	2.79	Lste	Lshu

From the processing of thermometric and pluviometric data the studied area is determined: Macroclimate: *Temperato*; Variants: *Submediterranea*; Bioclimate: *Continental temperature*; Thermotype: *Inferiored supratemperato (Lste)*; *Ombrotype*: Superior dry (Udry) in high areas and Inferior subumide (Lshu) in low areas. Based on the obtained values of the indicators according to the Rivas-Martinez method (1999), the bioclimate is defined as *temperate* and the thermotype*supratemperato inferior (Lste)*. According to the definitions, this bioclimatic type (thermotype) includes the lower mountain part located at an altitude between 950-1000 m and 1350-1450 m. It is characterized by an average annual temperature of 9-11^{0}C, minimum average temperature below 0^{0}C in December, January and February; average annual rainfall between 1100-1300 mm and summer drought.

Heavy frosts are verified from period of November to March, accompanied by snowfall. The vegetative period is about 150-180 days. Forest vegetation is composed of semi-mesophilic and mesophilic deciduous plants (mainly beech) accompanied by hilly floristic elements and mountain species. Here are found plant species such as: *Fagus moesiaca (K.Maly)., Pinus heldreichii Christ., Pinus peuce Griseb., Acer pseudoplantanus L., Acer plantanoides L., Acer obtusatum Waldst.et.Kit., Populus tremula L., where are represented and some shrubs such as: Juniperus nana Willd., Vacinium myrtillus L., Robus idaeus L., Rosa pendulina L., Bruckenthalia speciduifolia (Spike Heath).* On the basis of interpolation of values are also classified other boundary zones based and on altitude based on the definitions given in the Rivas-Martinez method.

In the lower area (low and hilly) the limiting bioclimatic type is lower *Inferior mesotemperato (Lmte)*, which lies at an altitude of 300-450 m above sea level and is characterized by an average temperature of 13-15^{0}C, annual rainfall including between 750 and 850 mm, summer drought for about a month (during July), most pronounced in southern areas. The vegetative period varies between 210-240 days. In the higher areas lies the bioclimatic type of *Mesotemperato superior (Umte)* (high hills) between 450-1 000 m above sea level with an average annual temperature around 11-13^{0}C, precipitation between 850 and 1 100 mm, lack of summer drought period, with minimum winter temperatures in January and February, with frosts from November to March. The vegetative period is about 180-210 days. Here we find plant species such as: *Quercus petreae (Mat) Liebl., Fraxinus ornus (K. Maly)., Ostrya carpinifolia Scop., Quercus cerris L., Quercus frainetto Ten., Carpinus betulus L., Quercus pubescens Willd., Carpinus orientalis Miller., Robinia pseudoacacia L., Alnus incana L.(Moench)., Betula pendula Roth., Ulmus minor Miller., Salix alba L., Alnus glutinosa L., Cornus sanguinea L., Cornus mas L., Corylus avellana L., Pyrus pyraster (L.)Burgsd., Juniperus communis L., Rosa canina L., Eupotarium canabium L., Sambucus ebulus L., Juglans regia L., Rubus ulmifolius Schot.*

In the upper part of the bioclimatic type of the study area is the bioclimatic type *Supertemperato superior(Uste)* (high mountains) located at an altitude of 1400-1450 and 1850-1900 m. It is

characterized by an average annual temperature of 7-9^{0}C and an average annual rainfall of 1300-1500 mm, without summer drought, with a minimum temperature below 0^{0}C during the months of December, January, February and March. Frosts are verified from October to April, with prolonged winter cold. In this area snow can cover the ground for weeks. The vegetative period is 120-150 days. Forest vegetation is composed of mesophilic deciduous (beech) and typical mountain species such as: *Fagus moesiaca (K.Maly)., Pinus heldreichii Christ., Pinus peuce Griseb., Acer pseudoplantanus L., Salix reticulata L.,where they are represented and some shrubs such as Juniperus nana Willd., Vacinium myrtillus L., Rododendron ferrugineum L., Robus idaeus L., Rosa pendulina L., Bruckenthalia speculifolia (Spike Heath)., Sambucus racemosa L.*

Above this belt is the bioclimatic type *Orotemperato (subalpino)* which is located at an altitude between 1850-1900 and 2300 m. It is characterized by an average annual temperature of 5-7^{0}C, rainfall included between 1300-1500 mm/year, minimum temperature below 0^{0}C for more than 5 months. In this area snow can cover the ground from December up to April. The vegetative period is 90-120 days. Forest vegetation is missing. It is represented by plant associations such as: *Agrostis rupestris All., Alopecurus gerardii Vill., Achillea multifida DC.(Boiss.)., Barbarea longirostris Vel., Bromus racemosus L., Crocus scardicus Koš., Crocus velchensis Herb., Caltha palustris L., Dryas octoptala L., Dianthus scardicus Wedst., Festuca panciculata L., Festuca halleri Schinz&Thell., Gentiana verna L., Gentiana punctata L., Geum montanum L., Geum reptans L., Geum coccineum Sibth.et Sm., Lilium albanicum Griseb., Narthecium scardicum Koš., Nardus stricta L., Primula veris L., Potentilla dorfleri Wettst., Ranunculus montanus Willd., Ranunculus crenatus Waldest.et.Kit., Rumex alpinus L., Salix reticulata L., Sesleria coerulans Friv., Sorbus aria L., Sedum alpestre Vill., Trolius europaeus L., Trifolium badium Schreb.,Viola elegantula Schott. In alpine pastures, in addition to alpine vegetation, many shrubs or bushes grow, among which: Juniperus nana Willd.*, blueberry associations *Vaccinium myrtillus L*.

In the upper area it is the bioclimatic type *Criotemperato (alpine)* which is located above 2300 m above sea level, where we find: *Alchemilla alpina L., Asperulla doerfleri Wettst., Crocus scardicus Koš., Crocus velchensis Herb., Campanula alpina L., Carex paniceae L., Carex leporine L., Dianthus scardicus Wedst., Dianthus crenatus Griseb., Draba korabensis Kum.et.Deg., Empetrum nigrum L., Galanthus nivalis L., Gentiana verna L., Narthecium scardicum Koš., Nardus stricta L., Lilium albanicum Griseb., Ligusticum albanicum Jav., Linaria alpina L., Ranunculus crenatus Walt.et. Kit., Ranunculus incomparabilis Janka., Rumex alpinus L., Nardus stricta L., Juncus alpines Vill., Potentilla dorfleri Wettst., Poa annua L., Poa cenisia All., Salix herbaceae L., Scilla bifolia L., Silene asterias Griseb., Silene pusilla W.K., Saxifraga scardica Gris., Saxifraga glabella Bertol., Scabiosa crenata Cyr., Trifolium norcium L., Viola elegantula Schott., Viola gracilis L.*

From a previous floristic study, conducted in this area (Krasniqi S., 2017) it results that have been found 735 plant species,which are distributed in the bioclimatic type *Supratemperato inferior (Lste)*; in the bioclimatic type of *Mesotemperato inferior (Lmte)*; in the bioclimatic type *Supertemperato superior (Uste)*; in the bioclimatic type *Orotemperato (subalpino)* in the bioclimatic type *Criotemperato (alpine)*.

The calculated bioclimatic indicators highlight the bioclimatic characteristics of the study area, but it should be noted that the bioclimatic study has a limited character, especially in terms of

altitude, as the lack of meteorological stations and data available in narrow areas, does not create the possibility of a complete bioclimatic characterization of the space under consideration. The comparison between different types of bioclimates (ombrotypes) in bioclimatic analysis is done by constructing ombrothermal diagrams according to the method of Walter and Lieth (1960). From the analysis of the graphic model and based on the classification of Walter and Lieth (1960), it results that the bioclimate of the region it is of the *submediterrane type*, with drought periods included in the interval of 1 up to 2 months.

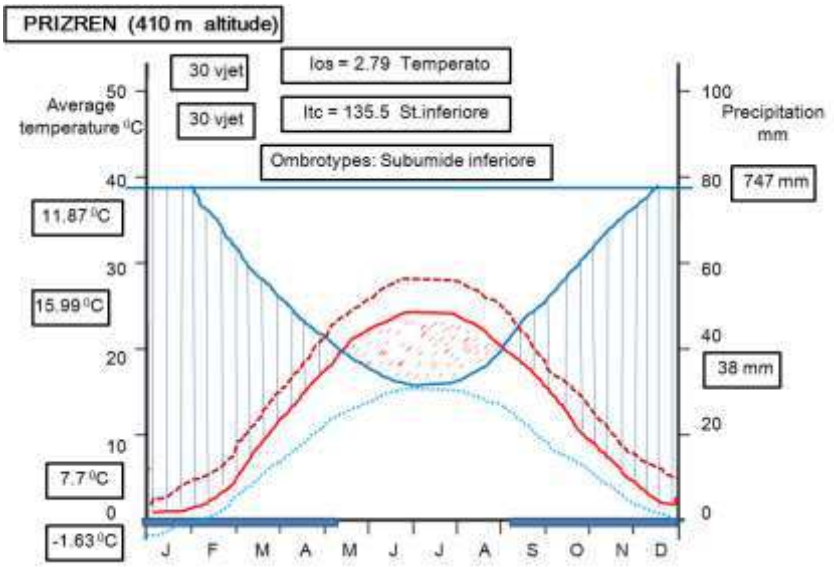

Figure 2. Bioclimate of the Prizren region (Walter e Lieth (1960)

To further explain of the relationships between climate and vegetation, for the purpose of phytoclimatic characterization itwas calculatedon the basis of thermal and pluviometric data, the pluviofactor indicator of Lang (1915), which is valid for Mediterranean environments in relation to drought. This indicator relates the average annual rainfall P (mm) to the average annual temperature T (°C), giving a ratio called the *Lang pluviofactor* (PI). *PI = P / T*: where: P = average annual rainfall in (mm) and T = average annual temperature (°C). This indicator gives the humidity level of an area within certain defined temperature limits. *PI* = 747/11.87 = 62.93. Based on the definitions of Lang (1915), for this indicator this value included in the range 100 - 60 corresponds to a hot tempered climate. Based on Lang's definitions, in cases where P/T> 1 –the vegetation is of wood type.

The De Martonn (1926) indicator is used to further specification of the bioclimatic features on the study area. It is given by: *IA = P/(T + 10)*; where: P - average annual rainfall and T - average annual temperature. Specifically: *IA* = 747 / (11.87 + 10) = 34.1. Based on the definitions of De Martonn (1926), when I> 20 - the vegetation is of the forest type. Based on the classification done for the degrees of drought, the climate of the area can be defined as *subumide* (relatively humid), which is dominated by a vegetation of Mediterranean shrubs. This definition, based on the indicator of De Martonn (1926), is valid from the point of view of the assessment of vegetation and special floristic elements to determine their geographical distribution in this area. Regarding the above, we can say that this bioclimatic study in the area of the Lumbardh valley of Prizren gives us the opportunity to judge and explain the way of growth of plant species and their distribution in this area estimating them closely related with the performance of climatic parameters.

CONCLUSIONS

From the bioclimatic analysis of the studied area, based on the Rivas-Martinez method (1999), and the values of the indicators, it results that the macroclimate is *"temperate"*, of the *"sub-Mediterranean"* climatic variant; with *"continental tempered"* bioclimates; the thermotype *"supratemperato inferior (Lste)"* and the ombrotype *"dry superior (Udry)"* in the high areas and the *"inferior subumide (Lshu)"* in the lowlands. Based on the classification made for the degree of drought, the climate of the area can be defined as *"subumide"* (relatively humid), which is dominated by a vegetation of Mediterranean shrubs. This bioclimatic study is valuable from the point of view of evaluating specific floristic and vegetation elementsto determine the relationship between climate and plants and their geographical distribution in this area.

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OVERVIEW OF THE FAUNA BIODIVERSITY IN TURKEY

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ABSTRACT

Biodiversity means the diversity of species in an area. Greater numbers of animal and plant species mean the richness of biodiversity on a particular plot of area. Conservation of biodiversity is very important for mankind. Topics such as natural balances, food sources, industry and economy are directly related to biodiversity. For this reason, the extinction of the species means that human beings will disappear in the future. There are many factors that threaten biodiversity today. The extinction of wild animal and plant species has been one of the most remarkable environmental problems for a long time. A species loss occurring elsewhere in the World can directly have negative consequences for the life of all living things. Integrated approaches of appropriate conservation and sustainable development strategies provide important opportunities for conservation of biodiversity. High species diversity is very important for natural sustainability and conservation and monitoring of diversity of life is essential for human survival. Biodiversity is tried to be protected by various protection methods such as conservation areas, combat with invasive species, biosafety studies, and gene conservation. The fact that protection concerns society raises the concept of public interest. In this study, the importance of biodiversity, the overall situation in Turkey and fauna biodiversity impact factors, destruction and threats to biological diversity are investigated. In addition, information's on biodiversity conservation and sustainability has been discussed.

Keywords: *Biodiversity, Fauna, Sustainable development, Environment*

INTRODUCTION

Biological diversity is accepted as a whole formed by genes, species, ecosystems and ecological events in a region (Özçelik, 2006). In other words, biological diversity refers to the richness of living things in a region in terms of species and number (Çepel, 2003). The high number of animal and plant species in a region means rich biological diversity (Çakmak, 2008).

Conservation of biological diversity is extremely important for human beings. Biodiversity is the basis of living resources, which have an indispensable place in meeting the basic needs of people, especially food. Issues such as natural balances, our food resources, industry, and economy are directly related to biological diversity. For this reason, extinction of species means the extinction of human beings in the future. There are many factors threatening biological diversity (Çakmak, 2008).

Turkey as a vital resource in terms of people's food security is one of the world's luckiest countries. It bears the responsibility to protect and use this important wealth rationally for the welfare of future generations. Because Euro-Siberian, Mediterranean and known as the Iran-Turan three biogeographic region and they have the transition zone and because of bridge between the two continents, climatic and geographical features as a result of changes in short intervals Turkey gained a small continent in terms of biodiversity. Turkey, forest, mountain, steppe, wetland, coastal and marine ecosystems, is a country with different forms and combinations of these ecosystems.

Turkey, a country that is very rich in terms of species and diversity of ecosystems and genetic features. The main reasons for this are that it is located between continents, has

extremely different landforms at very short distances and constitutes the most active region in the world in terms of climatology. In addition to all these factors, migrations and anthropological effects have also greatly increased the biological diversity in Anatolia. The diversity of our country's geographical structure provides high endemism and genetic diversity (Demirsoy, 1996).

The effect of continent formation on fauna

All the continents of the world were formed 250 million years ago by breaking away from a single piece of land called Pangea. This single continent fragmented over time and thus the distribution of living things in the continents occurred. 65 million years ago, when the first mammals began to be seen in this disintegration process, the Anatolian peninsula had related to Europe many times and separated again (Babür and Kurnaz, 2018). In this process, the seas of the Anatolian peninsula also changed its appearance many times, as a result of the rise and fall of the sea many times, the Black Sea was sometimes combined with the Caspian Sea, and sometimes it became a lake without connection to other seas. Animal species that escaped from the cold during the glacial periods Europe has gone through in the last two million years came to Anatolia from the Balkans and the Caucasus. In addition to these migrations from the north, some animal species from the south and east migrated to Anatolia and settled down. Some desert animals from the south could not cross the mountain ranges in the south of Anatolia and only settled in the south (Wildclub, 2012).

Terms of condition fauna biodiversity in Turkey

Animals in Turkey is all members living within the borders of Turkey. It attracts attention with its hosting of many different animal species (Demirtaş et al., 2018). Anatolia contains 75% of the plant species found in Europe, and approximately one third of them are endemic species, that is, they are unique to Anatolia (Çepel, 2003).

The main reason for hosting the extremely diverse animal fauna community of Turkey is located between the continents of the Anatolian peninsula. Some animal species in the North and South of our country remained on the Anatolian peninsula due to the climate changes that occurred in the fourth time, and thus, species richness has emerged. Accordingly, we can consider the natural vertebrate fauna existing in our country in five main groups as fish, as well as mammals, birds, reptiles, and frogs (Akın, 2007).

There are many animal genera in Turkey. It also shows a great variety in terms of animal species and habitat. In terms of fauna, the existence of approximately 60 thousand animal species in Europe is known. In Turkey, it is stated that this figure is around 40 thousand. According to some scientists, there are approximately 60-80 thousand animal species in Turkey. it is still possible to have a unspoilt land in Turkey. These figures show that studies on insects are insufficient. Despite this, the faunistic list of some insect groups has been largely determined. Turkey's presence on the world's two major bird migration path, increases the importance of birds as feeding and breeding areas (Babür and Kurnaz, 2018).

The different conditions in the geographical regions of our country have naturally been the living environment for different animal communities. The fact that the Anatolian peninsula is located on a transition area is another reason for the high number of animal species living in our country. In addition, the climatic changes that occurred in the quaternary provided the animals (fauna) living in the north and south to shelter in our country. Accordingly, there is a wealth of species belonging to various families in terms of fauna in our country. In addition, it can be said that there are approximately 400 fish species in country, which is surrounded by seas on three sides, but there are not many of them on different coasts with commercial value (Akın, 2007).

Turkey's Biodiversity

It is assumed that there are 8.7 million living species in the world. But most of the biodiversity we have at the species level has yet to be identified. Between 1970 and 2006, the number of vertebrate species in the world decreased by approximately one third, this decrease was 59% in the tropics and 41% in freshwater ecosystems (Ministry of Forestry and Water Affairs, 2013).

When the countries in the temperate zone are compared in terms of biological diversity, it is observed that plant and animal biological diversity is quite high in our country. Despite the lack of data, invertebrates constitute the largest number of species identified. The number of invertebrate animal species is approximately 40,000, of which approximately 4,000 species / subspecies are endemic. Vertebrate fauna with many studies conducted in Turkey is determined to a great extent. The total number of vertebrate animal species determined to date is close to approximately 1500. Over 100 species of vertebrates, 70 of which are fish species, are endemic (Çağatay et al., 2013).

Climate different from one another, each of the seven geographic regions that exist in Turkey, shows the characteristics of flora and fauna. The extraordinary ecosystem and habitat diversity have brought along significant species diversity. In Turkey, 120 mammals, 454 birds, 93 reptiles, 18 amphibians, 276 marine fish, freshwater fish, and 60-80000 192 (estimated) of insect species are known to exist. The large number of animal species and identified where homeland is Turkey. All these aspects with Turkey, has shown signs of a continent in terms of biodiversity has a unique place in the world (Ertürk, 2009).

Anatolian fauna also draws attention with its richness of species. Anatolia is the homeland of fallow deer and pheasant, and it is known that traces of the Anatolian leopard, which is thought to have disappeared, are found in Anatolia, which hosts mammals such as brown bear, wild boar, wolf and lynx. Another interesting aspect in terms of biological richness is that it is a shelter for rare species such as bald ibis (*Geronticus eremita*), sea turtles (*Caretta caretta* and *Chelonia mydas*), Mediterranean monk seal (*Monachus monachus*) (Çepel, 2003). Being on bird migration routes, Turkey is a key country for many bird species. Some of these are globally threatened species.

Turkey is an open-air museum with continental characteristics in terms of biodiversity. Turkey this feature, it began with the breakup of Pangea 250 million years ago found in one continent, the geological processes that make up today's world and, in the process, also comes from the ice age occurred four times.

The very different climatic zones that have occurred depending on the different surface shapes formed have created a wide variety of living spaces. In addition, during the warmer periods between the glacial periods and the ages, Anatolia has been a good shelter for living species migrating from the north and south (Uzun, 2004). Turkey is located at the intersection of Mediterranean and Near East region genes. Differences of Turkey’s geographical structure provide high endemism and genetic diversity (Demirayak, 2002).

Turkey, like the variety of plants, is a rich country in terms of animal diversity. In Turkey, once such as a lion, tiger, leopard lived animals. Today, naturally, large mammals such as wolves, bears and lynxes live (Council of Europe, 2006). For example, Turkey in return for the richness of biodiversity in flora and fauna along with other members 'Turkey Bird Fauna', holds an important place in the Western Palearctic Region. There are approximately 500 species of birds in Turkey. West Palearctic bird species reaches its limit distribution in the territory of Turkey. However, Turkey basically is the western most end point for the 23 bird species breeding in the range of the Asian continent. Two of Turkey's most important bird migration routes in the West Palearctic region, Southeast Anatolia- to host on the territory of Western

Thrace and Anatolia Southeast Anatolia-Northeast routes, which increases the importance of bird fauna (Anonymous, 2007).

If we look at the diversity of farm animals; archaeological findings show that sheep and goats were domesticated in regions neighbouring Anatolia. The species and racial diversity of domestic animals seen in Anatolia is an indicator of the welfare of different cultures that have lived in this region for different periods. Today, Turkey's wealth of animal genetic resources is due to the elections with a wide range of environmental and geographical conditions of animal breeders. For the period 2006-2016; approximately 47% of cattle are high-yielding breeds, 41% hybrid breeds and 12% domestic breeds (Muminjanov and Karagöz, 2019).

If we look at the cattle breeds; A significant portion of the total milk and red meat needs Turkey with 14 million head of cattle meets the presence of cattle breeding. Holstein cattle breed is the most widely bred breed, followed by Brown Swiss and Simmental. It is grown in Anatolian Buffalo in our country. Indigenous breeds such as the Native Black, Zavot, Eastern Anatolian Red, Kilis and Gray Race are under the threat of extinction (Muminjanov and Karagöz, 2019).

Sheep breeds: Native sheep breeds constitute most of the total sheep population of 32 million. With 45 registered sheep breeds, the diversity of sheep genetic resources is higher than other farm animals. Conservation programs have been initiated for some races in danger of extinction. Native sheep genetic resources such as South Karaman, Dağlıç, Herik, Tuj, Curly and Hemsin breeds are endangered. Chios, Çine Çaparı and Norduz are in critical condition. Ödemiş and Ringed races disappeared. Each domestic sheep breed has different and valuable features. For example, the meat quality of the curly breed, the adaptation of the Gökçeada breed to the harsh conditions, the fertility rate and milk yield of the Chios breed are the prominent advantageous features (Muminjanov and Karagöz, 2019).

Insect diversity is very rich in Turkey as well as all over the world. Turkey insect fauna on the estimated numbers are available. The insect species have been identified so far in Turkey it is about 40.000, but the number is estimated between 60.000-80.000. It can be deduced from these numbers that studies on insects are insufficient. Despite this, the fauna list related to some insect groups has been made to a great extent. For example; dragonflies in Turkey (Odonata) 114, locusts (Orthoptera) 600, beetle on (Coleoptera) 10.000, half-winged on (Heteroptera) 1400, iso-winged on (Homoptera) 1500 and butterflies (Lepidoptera) 6500 (600 day and others at night) is represented by species (Kanca et al., 2019).

The Sea of Marmara is a unique ecosystem as the transition medium between the Mediterranean and the Black Sea. Despite the deterioration in environmental conditions on the northern shores of the Marmara Sea due to anthropogenic impacts, the Marmara Sea still has high biodiversity, but it is reported that the number of aquatic species is decreasing due to overfishing, pollution and habitat destruction. Although the Mediterranean has relatively more species, it is limited in stock size and quantity. The most abundant species here are sparids, perch, European eel, sharks, seahorses, tunny, oysters, some shrimp species, octopus, squid, and the like (Muminjanov and Karagöz, 2019).

If we look at the biological diversity in inland waters; lakes, reservoirs, rivers, and inland water resources, consisting of wetlands constitute approximately 1.5% of Turkey's surface. Many inland waters fish populations are divided into small isolated subspecies or forms, some of which are endemic, due to mountain heights and various physical barriers in rivers (Muminjanov and Karagöz, 2019).

Living space some freshwater fish species of Turkey: Pearl mullet (*Alburnus tarichi*), Timar pearl (*A. timarensis*), Seaweed fish (*Aphanius anatoliae*), khramulya (*Capoeta capoeta kosswigi*), Stone eater fish (*Cobitis simplicispina*), Chub (*Petroleuciscus kurui*), Beyşehir fat fish (*Pseudo-phoxinus battalgili*), and Abant alas (*Salmo trutta abanticus*)). According to an estimate from 2014, there are 371 species belonging to 27 families and 92 genera. In this list,

Cyprindae (Cyprinidae) is the most dominant family with 193 species (52%), followed by Nemacheilidae (11%), Gobiidae (7%), Cobitidae (5%) and Salmonidae (5%) (Muminjanov and Karagöz, 2019).

Threats to biodiversity

In rural areas, economic pressure and legislative gaps caused by rapid population growth, problems in parcelling agricultural lands cause a decrease in farmers' income. This leads small farmers to activities that destroy biodiversity, such as deforestation to gain land, overgrazing and destruction of rangelands and over-gathering of plants. In steppe areas, Traditional and unsustainable farming methods, destruction of pastures to gain fertile land are the biggest threats to biodiversity. Land speculation in coastal areas, particularly in the Aegean and Mediterranean regions, results in a second housing boom. The ineffectiveness of the institutional structure to prevent environmental degradation and the deficiencies in the legislation cause the loss of natural habitat, which is the biggest threat to biological diversity. Overfishing, gathering of wild animals and birds, inadequate control of hunting processes, and lack of monitoring are the biggest threats to the survival of many species. On the other hand, the insufficiency of the control mechanisms that regulate the fishing periods and periods in both inland waters and seas threaten the biodiversity in marine and freshwater environments. The incentives given to the tourism sector since the 1980s have created an explosion in the construction of mass tourism investments, causing the irreversible destruction of all coastal habitats (such as sea turtles breeding areas, Mediterranean monk seal habitats), dunes, lagoons, coastal forests and fertile agricultural areas (Demirayak, 2002).

Some living species in the world, most of which are not yet known, are under the threat of extinction. In the last quarter of the 20th century, parallel to the developments in the world, environmental pollution, industrial and urban settlements, rapid population growth, and misuse and excessive pressure on natural resources negatively affect this rich fauna in our country (Doğan, 2005). One of the factors that threaten Turkey's existing biodiversity, especially of endemic species is collected and taken abroad in various ways.

The entry of invasive species into the country's seas continues to be a constant and growing threat to marine biodiversity. The coasts of Turkey in the year 2010 400 invasive aquatic species have been reported. Of these, with 105 species, the most dominant group is mollusks, followed by polychets (75 species), crustaceans (64 species) and fish (58 species) (Karagöz and Muminjanov, 2019).

An important part of this invasive species has now been identified as residing in Turkey and has revealed the increasing size of the problem. Aquatic invasive species include periwinkle (Rapana venosa), scleractin coral (Oculina patagonica), scalloped medus (Mnemiopsis leidyi), Caribbean shipworm (Teredothyra dominicensis), parasitic copepod (Taeniacanthus lagenaeus), Japanese shrimp (Marsicuphali) (Percnon gibbesi), crocodile fish (Champsodon capensis) and poisonous balloon fish (*Lagocephalus sceleratus*). The increase and spread of non-native species with climate change continue to be a major threat to marine ecosystems and native species in the biogeographic regions of the Mediterranean and Black Sea (Karagöz and Muminjanov, 2019).

Extinct animals in Turkey

The most interesting among mammals extinct in Turkey Asian lion (*Panthera leo persica*), Asian elephant (*Elephas maximus*), Caucasian ox (*Bison bonasus caucasicus),* Caspian tiger (*Panthera tigris virgata*), Anatolian leopard (*Panthera pardus tulliana*) are types. The Asian elephant and aurochs lived in Anatolia until the beginning of the 1st century BC (Macit, 2019).

Turkey's Hakkâri, Uludere latest tiger, the most recent Anatolian leopard was shot in the 1970s. However, there are data showing that the Anatolian leopard was seen in the Eastern

Black Sea in the early 2000s (Macit, 2019). The lion, known to have lived in the western, central, southern and southeastern regions of Anatolia, was last seen in the second half of the 19th century. 8 bird species have disappeared in Turkey in the last 50 years. Among these, forest rooster (*Tetrao urogallus*) and Snake neck (*Anhinga melanogaster*) birds have been officially declared extinct. Apart from these, there are species that are not known with certainty whether they are extinct or not due to insufficient research; The best example of this is beaver (*Castor* sp.) (Macit, 2019).

In our country; As the most striking example, the Snake Neck bird, which was determined to have incubated in Lake Amik and its surroundings and disappeared with the drying of the lake, has become extinct. Animals belonging to the mammals' class, which includes the most advanced species of the animal kingdom, occupy an important place in nature. Because of their attachment to their living environment, they are greatly affected by the negativities in the environment they live in (Erman, 2009).

Extinction of living species

The biodiversity we see around us is the result of a change that has been going on for 3.2 billion years. The disappearance of the species has always been part of this process. It is accepted that 99% of the living species that have ever been born and lived have been extinct for any reason (Leveque and Mounolou, 2004). However, the extinction forms of species today are completely different. Although the number of species extinct naturally in a year is 1-2, the consumption amount due to human impact is estimated to be 1000 per year (Çepel, 2003).

Many studies have been conducted on the status of biodiversity in our country, current and potential risks, and measures to be taken. Agricultural studies as the most threatening factor biological diversity in Turkey (the expulsion of pastures, overgrazing, burning of stubble, excess fertilizer and pesticide use, take the place of farmers' varieties of high yielding varieties), urbanization, industrialization, roads and dams, from nature They have listed factors such as excessive harvesting and harvesting, excessive forest cutting and forest fires, second housing acquisition, rapid developments in the tourism sector and a lack of trained people (Karagöz et al., 2010; 2016).

The loss of species diversity, which is one of the most important points where biological diversity is discussed in popular and political fields, is classified as endangered species and species that may be endangered. Endangered species are defined as species that are in a spreading area of extinction in all or a significant part of their range. The species that are in a state of extinction in all or a significant part of their distribution areas in the future are species that may be endangered (Campell and Reece, 2008).

With global warming, 56 thousand endemic plant species and 3700 endemic vertebrate species in the world are in danger of extinction. According to the latest estimates, it is stated that 25% of all mammal species and 12% of all bird species in the world are in danger of extinction due to the events related to climate change. According to a scenario claiming that 20% of the moist soils in the coastal regions may be lost in 2080 due to the rise of sea level, this rise in sea level may cause significant losses in marine and coastal ecosystems globally. This uplift will cause land erosion and will result in the extinction of many coastal creatures (Ergin, 1999).

With increasing CO_2 ratios, the pH level in the seas will decrease and the acidity of the sea water will increase. This event will adversely affect the shellfish (Crustacea). The exoskeleton of crustaceans contains $CaCO_3$ and degrades in an acidic environment. It is predicted that the food chain on the sea floor may change due to the extinction of some small crustaceans (Özdemir and Altındağ, 2009).

OECD (2008), Turkey is threatened mammals, more than 20% of our country according to the Environmental Performance Evaluation Report. Some mammals such as red deer (*Cervus*

elaphus), wild sheep (*Ovis gmelinii anatolica*), gazelles (*Gazella* sp.) and otters (*Lutra lutra*) (Figure 1a) are in decline and are presumed to be in danger of extinction. Although the Anatolian leopard (*Panthera pardus saxicolor*) (Figure 1b) is thought to have disappeared, it is known that traces are still visible.

a)  b) 

Figure 1. a) Otter (*Lutra lutra*), b) Anatolian leopard (*Panthera pardus saxicolor*)

The Mediterranean monk seal, tadpole sea turtle and green sea turtle have been endangered for years. The numbers of dolphins and whales are decreas. On the other hand, many species of birds such as flamingos (*Phoenicopterus roseus*) (Figure 2a) and upright tail (*Oxyura jamaicensis*) (Figure 2b), which face extinction in Europe are breed in Turkey (Ertürk, 2009).

a)  b) 

Figure 2. a) Flamingo (*Phoenicopterus roseus*), b) Upright tail (*Oxyura jamaicensis*)

The population of the Mediterranean Monk Seal in the Aegean, Mediterranean and Black Sea is not stable, and this species is about to disappear in the Black Sea and Marmara (Öztürk and Öztürk, 2002; Demirayak, 2002). However, all these sea creatures; It is threatened by deliberate killing due to unconsciousness, the destruction of the coast due to tourism and excessive urbanization, and the reduction of fish stocks due to excessive fishing.

Many studies carried out on vertebrate animals in Turkey and continue to be done. Therefore, there are healthy data on the endemism status of vertebrate animals, their danger classes and the species taken under protection. Accordingly, 141 reptile and amphibian species in Turkey is 16 endemic species, 10 of them are under threat. There are no endemic species of bird in Turkey. However, 5 species and 32 subspecies of mammals, 16 species and / or subspecies of reptiles, and 70 species / subspecies of freshwater fish are endemic (Çağatay et al., 2013).

Extinction and decline of species have always been a natural part of the evolutionary process. However, the extinction ways of species today are disturbingly different than before (Çıplak, 2008). Biodiversity is the commonwealth of the whole world. Biodiversity must be

preserved to meet the needs of today and transmit this diversity to future generations (Biodiversity, 2018).

Biodiversity conservation and sustainability

In parallel with the studies carried out for the protection and development of biological diversity in the world in recent years, studies for the protection of biological diversity in forest ecosystems have been started in our country. Leaving old trees in the forest in places in order to ensure the reproduction of wild birds in the forest; It is recommended to leave lying on the ground, old and dry trees in the forest, provided that they do not constitute a source of fungal and insect diseases. Especially in areas where the risk of insects and fungi is low, it is very important to preserve the overturned or rooted trees in the forest as it is, and to leave high bottom logs during tree cuttings in terms of protecting habitat areas.

Biodiversity is very important economically, ecologically, and culturally for human life. Therefore, many steps have been taken to protect biodiversity. Turkey shows that the emphasis on endemic species and endangered species in biodiversity conservation. Turkey's biodiversity in situ and ex situ effectively be protected by a mixture of programs, strengthening the policy framework, the creation of effective laws and regulations are among the topics.

An alternative indicator that can be used in the conservation of biological diversity in our country is species or habitats. There are many different ecosystems in our country. Different indicator or key types can be determined for these ecosystems. Sea turtles (*Caretta caretta*, *Chelonia mydas*) and Mediterranean monk seals (*Monachus monachus*) are used as flag species conservation biologists in Turkey. Again, the otter (*Lutra lutra*) is used as an indicator of a healthy ecosystem. Today, the number of black vultures in Turkey is estimated to be between 50-100 pairs. Forest ecosystems of old larch can be used as an indicator of the existence and health (Kaya, 2003). The brown bear, which needs large and undisturbed forest areas in order to protect the natural old woodlands and alpine areas in the Eastern Black Sea Region and the Caucasus from the negative impact of humans as much as possible can be a good indicator species (Başkent et al., 2004).

Biodiversity conservation efforts have led to the establishment of protected areas in many parts of the world. Protected areas are very important in terms of protecting and maintaining biological diversity (Putz et al. 2001; Masozera and Alavalapati, 2004).

It is suggested that the protected areas reserved for the purpose of protecting Biodiversity such as National Parks, Nature Protected Areas, Natural Parks, Gene Protection Forests, Special Environmental Protection Areas should be at least 5-6% of the country size. Of the countries surface area allocated for this purpose in Turkey it is around 1%. The amount of such areas is 4.2% in India, 3.8% in Germany (Negi and Stimm, 1997), 3.6% in Finland, 3.7% in Sweden, 11, 12% in America, and 10.9% in the Central African Republic (Raivio et al., 2001; Özçelik, 2006).

Sustainable development can be defined as increasing and improving the quality of life in harmony and balance with the ecosystems that human beings are a part of and provide the basic support for their survival. Article 2 of the Convention on Biological Diversity defines the sustainable use of biological diversity as "the use of biological diversity elements in a way and at a rate that does not cause a decrease in biological diversity in the long term, and thus preserves the potential of biological diversity to meet the needs and aspirations of present and future generations". Sustainable use of biological diversity involves, on the one hand, ensuring the equality between regions while meeting the needs of today's generations, on the other hand, ensuring the rights of future generations (Demirayak, 2002).

CONCLUSIONS

The importance of national parks and other protected areas in preservation of biological diversity should be explained to the public. Alternative sources of income must be created, especially for the livelihood of local people living on the edge of protected areas. It should be ensured that protective activities are carried out not against the people but with the participation of the public (Masozera and Alavalapati, 2004).

In order to make conservation programs more effective, studies of collecting biodiversity inventory and collected data in a database in protected areas should be accelerated and completed in a short time. Again, in another important measure that can be taken in this regard; It is the necessity of coordination between the persons and organizations responsible for the management of protected areas and the relevant organizations (Özçelik, 2006).

As in other countries of the world today is comes up accelerated loss of natural ecosystems of Turkey in the process on a global scale. Especially important in terms of flora and fauna, wetlands and yet protect the habitat of many endemic species that attain the status of protection of Turkey's fauna is of great importance in terms of wealth is not lost. The insufficiency and ineffectiveness of legal regulations, issues related to plans and programs, insufficiency of institutional and individual capacities are also included in the UBSEP (Anonymous, 2007).

In the Biological Diversity Convention, "habitat diversity" is included in the definition of biological diversity (Anonymous 2016a). In addition, the main subject of the Convention to Combat Desertification is land degradation (Anonymous 2016b), and a relationship has been established between climate change and land degradation in the Framework Convention on Climate Change (Anonymous 2016c).

There are many legislations protecting natural life. Some of these directly target biological diversity, while other provisions contribute indirectly. Taking biodiversity into consideration more and especially emphasizing the concept of public benefit is the most important reason for the protection of biological diversity, and this situation is reflected in the theoretical and judicial field. The Convention on Biological Diversity signed in Rio in 1992, the Helsinki agreement in 1993, the Santiago Declaration in 1995 and the Montreal Convention were a turning point in which effective steps were taken towards the operation and protection of biological resources in accordance with the sustainability principles and regional processes around the world began (Özçelik, 2006).

The 20th century has destroyed biodiversity and natural resources at a rate never seen in human history because of unsustainable development. The damage to biological diversity is irreparable by only regulating land use and determining some protected areas. In this context, it is necessary to take concrete steps to make sustainable development a policy of countries. Sustainable use of biological diversity is necessary as a political implementation tool, and structural changes need to be carried out to integrate all sectoral policies with original policies in a way to observe the aim of protecting biological diversity. Every living creature has the right to live in a healthy and balanced natural environment.

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DOES CERIUM AFFECT CADMIUM ACCUMULATION IN ROCKET PLANT AND SOIL?

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ABSTRACT

Cerium (Ce) is widely used in nanotechnology, agriculture, and food industry, and it has been associated with toxic outcomes on different organisms as it ends in different environmental matrixes after use. In this research, rocket (*Eruca vesicaria* subsp. *Sativa* (Miller)) plant was grown by applying Ce element. The five doubling concentrations of Ce varying from 25 to 400 mg kg^{-1} were introduced in to the pots in parallel to the control pots in three replicates. The relation of the level of pollution by spiking Ce in pots together with the cadmium in the soil and the plant was investigated. Experiments were carried out in triplicates according to Randomized Complete Blocks Design. Then, rocket plants were grown in these pots and Ce and Cd analyzes were carried out with the required methods in soil contents and plant.

Ce and Cd were found to be toxic at the plant and soil levels and statistically significant at 5% level. The amount of cadmium, in both the soil and the plant, has increased in Ce pots applied in increasing doses and control pots. In proportion to the applied Ce a positive effect on the cadmium uptake in the soil was observed that caused to increased cadmium concentration in the plant. As a result of Duncan analysis, the amount of cadmium in the soil was statistically different between the control pots and the Ce pots applied. However, the cadmium contents among the doses applied to Ce were included in the same group. Ce application was effective but it was determined that the applied different amounts did not significantly affect Cd accumulation.

Keywords: *Toxicity, Ce, Cd, Heavy metal toxicity*

INTRODUCTION

The rare elements (Rare Earth Elements-REE), which found in very few regions and small amounts on the earth's surface, are used and consumed rapidly in parallel to technological developments. Among these, Cerium (Ce), which used in semiconductors, which has become necessary in the electronics industry, could be counted as the most common elements. Technology Critical Elements (TCE) have gained very important commercial meaning and have become an indispensable today with the developing technology and new technological fields. These include many semiconductors in the electronics industry and solar cells. However, their concentrations are increasing in the nature with their increased usage. In the risk assessment studies, which started to gain intensity, has been shown that TCEs can cause dangerous biological effects on a living being (Duester et al., 2014; Westerhoff et al., 2011).
Even though cerium is one of the rare earth elements, it generates up about 0.0046 per cent of the earth by weight. It is the most common rare earth element among lanthanides (Liu and Cohen, 2014).

Cadmium, which is one of the main parameters that generated the ecosystem, can be contaminated to the soil from many different sources. Especially the contaminations which originating from industrial areas take an important place. The toxicity of cadmium, which has a toxic effect on plants, occurs especially when the amount of soluble cadmium exceeds 0.2 mg kg^{-1} in the soil (Alloway, 1995).

Cadmium toxicity detected in the soil directly infects plants grown on soils. The cadmium in the plant is transmitted to humans and other living things by the food chain. Many organs are affected, primarily the digestive system in humans (Hoffman et al. 2008).

In a study performed in Australia, the Cd content is found 3 times higher in the soils where 30-45 years of superphosphate fertilizer application (1000-4500 kg/ha) is applied compared to the soils without superphosphate fertilizer (Altınbaş et al. 2008). This result shows that a significant amount of Cd is contaminated from phosphorus fertilizers to the soil.

Ruttkay - Nedecky et al. (2017) summarized a wide variety of the plant species used in plant toxicity investigations of NPs in the literature. However, among them rocket plant has not been reported yet. In this study, the relationship of rare elements of Ce and heavy metal Cd pollutants which transmitted to the soil in various ways were evaluating which are important parameters of the ecosystem for agriculture.

MATERIAL AND METHODS

Experimental Design

Randomized Complete Blocks Design was considered to plant the experiments that were carried out in triplicates. Five doses of Ce were applied to pots considering the doubling concentration increase strating from 25 to 400 mg kg^{-1}. A control experiment was also prepared in three replications in parallel.

Rocket (*Eruca vesicaria* subsp. *sativa* (Mill)) was used as a plant in the experiment. Before planting, N, P and K requirements for plants were applied to the soil (urea as a nitrogen source, triple superphosphate fertilizer as a phosphorus source and potassium sulphate fertilizer as a source of potassium). Later, freshly prepared cerium solution was poured to the pots of rocket plants together with irrigation water.

The experiments were carried out under controlled conditions. Irrigation water application in rocket plants was applied by considering the beneficial water values.

Plant analysis

In this study, Rocket (*Eruca vesicaria* subsp. *sativa* (Mill)) used as plant material. Planting of plant seeds was made directly in pots. The image of the plants is shown in Figure 1

The plants were harvested after the vegetative growth period of 45 days. Later the fresh weights of the plants in each pot were determined. After that, plants were washed with pure water and were dried at 68°C for 48 hours until their weights were fixed (Kacar and İnal, 2008). Finally, the dried plant samples were submitted to ICP-OES for analysis of Ce and Cd as it was done for soil samples.

Figure 1. Image of rocket (*Eruca vesicaria* subsp) plants grown in the laboratory

Soil analysis

The soil samples used in the experiment were brought to the laboratory before the experiment and were air-dried. After that they were sieved through a 2 mm sieve and were prepared for ICP-OES analysis.

The pH and electrical conductivity (EC) of the soil samples weres determined in a 1/2,5 soil/water mixture according to literature (Jackson, 1967; Özbek, 1995; Lindsay and Norvell, 1978; Sağlam, 2012). the $CaCO_3$ content was measured by Scheibler calcimeter.

The texture of the soil samples was determined according to the Bouyoucos Hydrometer method (Bouyoucos, 1955) and organic matter was measured by modified Walkey-Black method (Kacar, 1995).

Ce and Cd contents were determined by ICP-OES device by applying DTPA method proposed by Lindsay and Norvell (1978).

Statistics

The data obtained from plants and soils were transferred to the computer and statistical analysis were made using PASW® Statistics 18 for Windows package program. To determine the difference between the groups, variance analysis (ANOVA) was performed on the data of the averages and Duncan multiple comparison tests were applied to the data of the averages that were found important.

RESULTS

Properties of the soil used in the experiments

Chemical and physical parameters of the soil sample used in the experiments are shown in Table 1. According to Table 1, in the neutral reaction of the soil, it is seen that there is a salt-free, low-lime and low amount of organic matter content, a sufficient amount of available phosphorus, a high amount of exchangeable potassium content, as well as a sufficient amount of exchangeable Ca and Mg content.

Table 1. ICP-OES results of the soil sample

Parameters	**Values**
pH, 1: 2,5 soil/water	7,09
Salinity, %	0,08
Lime ($CaCO_3$), %	2,64
Organic matter, %	1,57
Exchangeable Ca, %	0,61
Available P, mg/kg	40,31
Exchangeable K, mg/kg	242,76
Exchangeable Mg, mg/kg	477,21

Ce-Cd relation in plants and soil after increasing doses Ce application

The effect of increasing doses of Ce on Ce and Cd content of the rocket plant is given in Table 2 below. When Table 2 is examined, the amount of Ce that can be removed from the rocket plant and the amount of Ce left in the pots after harvesting increased with increasing Ce doses. This increase was found to be statistically significant at 5% level. However, when the plant was examined, the accumulation in Ce applications was observed at initial doses of Ce to be 100, 200, 400 mg kg^{-1}.

The highest accumulation was seen in pots containing 200 mg kg^{-1}. After this value, the plant was adversely affected, and the accumulation values affected in such a way that they were in different groups. This situation can be explained that the toxic level in the soil increased and continued to be taken by the plant. The limit value of Ce in plants was reported to be 2-50 mg kg^{-1} by Jones et al. (1991).

When the Cd values in Table 2 are examined, it is seen that there is a decrease in potting soil applied to Ce compared to control vessels. Statistically, this situation was found to be significant at the level of 5 %. According to the Duncan test, all applications except control were found to be in the same group. This indicates that the applied Ce doses cause the Cd values in the soil to remain constant.

The increase in Cd values in the facility shows that Cd content causes more accumulation than Ce applied containers compared to control vessels. When these findings are evaluated in terms of soil pollution, we can say that there is a contrasting relationship between Ce and Cd.

When pollutants were examined for accumulation in the plant, it is observed that the rocket (*Eruca vesicaria* subsp. *sativa* (Mill)) plant is a hyperaccumulator.

Table 2. The relationship of Ce applications with increasing doses of Cd in the soil and plant samples

Ce concentration	Ce		Cd	
	Plant	**Soil**	**Plant**	**Soil**
Control	1,13±0,37[d]	1±0,00[b]	0,61±0,05[b]	7,87±0,28[a]
25 mg kg^{-1}	21,06±16,2[d]	66,36±28,5[b]	0,57±0,07[b]	6,25±024[**b**]
50 mg kg^{-1}	35,03±6,30[d]	37,4±94,0[b]	0,65±0,06[ab]	6,59±014[b]
100 mg kg^{-1}	579,76±62,26[a]	36,5± 165,1[b]	0,69±0,04[ab]	6,36±0,18[b]
200 mg kg^{-1}	435,13±18,06[b]	131,13±124[b]	0,75±0,03[ab]	6,63±0,05[b]
400 mg kg^{-1}	339,33±26,61[c]	400±105,4[a]	0,83±0,08[a]	6,22±0,05[b]

*: average of three replications, **: each parameter evaluated individually, ($p \leq 0.05$).

DISCUSSION

It was reported that Cerium at low concentration (10 mg/L) slightly increased oxidation resistance, while high concentration (20-80 mg/L) negatively affected the development and photosynthesis (Jiang et al. 2017).

In one study, the physiological mechanisms of different materials that causing plant accumulation were investigated in soybeans. Combinations of three levels of cadmium (0 and 0.25 and 1 mg.kg^{-1} in dry soil) and two concentrations of CeO_2 (0 and 500 mg.kg^{-1} of dry clay) were investigated. Measurements of plant biomass and physiological parameters showed that CeO_2 molecules lead to higher variable light and maximum light ratio. Cd significantly increased with Ce accumulation in plant roots, especially roots and older leaves (Rossi et al., 2017).

Nano bionic approach of a plant that localizes negatively charged, spherical cerium oxide nanoparticles (nanoceria) inside chloroplasts enhances the sweep and in vivo Arabidopsis thaliana plants' photosynthesis under extreme light (2000 μ mol m^{-2} s^{-1}, 1.5 h), heat (35 °C, 2.5 h) and dark cooling (4 °C, 5 days). Nonendocytic means transfer Nanoceria to chloroplasts are unaffected by electrochemical plasma membrane potential gradient. The low rated Ce^{3+}/Ce^{4+} (35,0 %) PNC decreases hydrogen peroxide, superoxide anion and including hydroxide radical leaf ROS levels by 19 %. There found no known plant enzyme scavenger for the lesser ROS (Wu et al., 2017).

On the other hand, important trace elements in natural environment are present in concentrations of less than 0.1%. In biochemistry studies, trace element concentrations in plant and animal tissues are below 0.01%. In terms of nutrition, the term microelement is used for elements with a concentration lower than 0.002%. Metals that have environmental pollution and toxicity effects and are known as heavy metals due to their density higher than 5.0 g / cm^3 are also the elements needed for organisms at low concentrations (Karatepe, 2006).

It has been determined that some plants are highly effective against heavy metal intake from the soil and that such plants are tolerant of heavy metal toxicity. Among these plants, which are also known as hyperaccumulator plant families such as *Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae* and *Scrophulariaceae* can be counted. The fact that heavy metals in the soil come to the form that can be taken by plant roots is one of the factors affecting the success of phytoremediation, new technology in the removal of heavy metals (Adiloğlu 2016; Adiloğlu, 2017; Akay and Adiloğlu, 2020).

There are different methods in phytoremediation, a name given to the technology of removing organic and inorganic pollutants using plants, depending on the plants used in plant breeding and the pollutants to be removed. In the selection of these methods, factors should be considered such as the intake and removal mechanisms of the pollutants by plants, the physical and chemical properties of the pollutant environment, the suitability of the method to be applied to the pollutant, the concentration of the pollutant, the depth of the pollutant in the soil and the climatic conditions (EPA, 2000; Adiloğlu 2016; Adiloğlu, 2017; Akay and Adiloğlu, 2020).

CONCLUSION

The desire of people to achieve a better standard of living has brought some problems to the agenda. Environmental pollution is one of these problems. The pollution of the environment we live in negatively affects the especially lives of human and all living things. In this study, for plant toxicity researches, soil samples were contaminated by applying Cerium (Ce) to the pots at a toxic level. Later, rocket plants were grown in these pots and Ce and Cd changes in the contents of the plants and post-harvest soil were examined.

According to the of this study results, when the cerium doses applied were compared with the control, cerium accumulation showed the highest value in the application of 100 mg kg^{-1} Ce, then it was determined that there was a decrease in Ce accumulation in the plant.

It has been observed that Ce plant applied to rocket in increasing amounts does not have any negative effects on cadmium accumulation that was confirmed by statistical significance level of 5%.

When compared with the control dose, with the application of 100 mg kg^{-1} Ce, the accumulation values of the plant were in different groups, and it was determined that the cerium had toxic effects.

Also, when the soil pollution values were examined, it was determined that Ce concentrations negatively affect the extractable amount of the Cd heavy metal of soil. It was observed that there was a competition between Ce and Cd in the soil.

Phytoremediation, which is an economical and easily applicable method, can be used to remove cadmium heavy metal and Ce pollutants from rare elements, which are contaminated to agricultural lands in many ways (traffic, industry, etc.), especially fertilizer and medicine, and rocket (*Eruca vesicaria* subsp. *sativa* (Mill)) plant can be used as a hyperaccumulator among these pollutants.

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STUDY OF MANGANESE CONTENT IN THE EXCHANGEABLE AND CARBONATE PHASES IN THE SEDIMENTS OF THE BADOVCI AND BATLLAVA LAKES (KOSOVO)

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ABSTRACT

The study was carried out to investigate the manganese content in the sediments of the lakes. The water analyzes of the Badovci Lake, showed a higher concentration compared to the limits of the Directive EC 98/83, while the waters of the Batllava Lake were below these limits. The analysis of the lakes sediments by the fractionation method showed high values of the manganese content in the carbonate and exchangeable phases. These values are worrying because the manganese bound in these phases can very easily pass into equilibrium in the solid/liquid phase, and become bioavailable in the aquatic ecosystem. Also, according to the risk criteria (Risk Assessment Code), manganese content in the sediments of two lakes was classified as high risk, because in both lakes the sediments showed manganese content higher than 30%.

Keywords: *Manganese, exchangeable, sediment, Kosovo*

INTRODUCTION

The sediments generally serve as accumulating reservoirs of toxic metals that can be transmitted to the aquatic system by the influence of various factors. The accumulation of metals in sediments and the degree of their binding from water to sediments depends on the granulometric composition of the sediments Kulbat and Sokołowska (2019). Depending on the type of metals, depends also and the intensity of its absorption by suspended particles (Wang et al. 2016). The manganese oxidation states Mn (IV) and Mn (II), show different properties in the natural aquatic system, such as solubility, adsorption, and complexation and by these ways can be released from the sediment into the water in the reductive form and can be reoxidized within the oxic part of the water (Schaller et al. 1997). Analyzing the total metal content in the lake bottom sediments is insufficient to assess their risk. Analyzing the metal speciations is very important because the bioavailability of the metal depends on the form of adaptation for a given metal in the ecosystem (Ke et al. 2017; Wojtkowska et al.2016; Pejman et al. 2015; Baran and Tarnawski 2015; Fernandes and Nayak 2014). The ionic charge, ionic radius, ion complexing power, oxidation state, hydration state of metals, and many other factors influence on the transport of them in the sediments (Wojtkowska, M. (2012). The exchangeable sorption of metals in the sediment is the most common type of their binding and is a reversible process from which the speed of this process depends on pH, organic matter, colloids, temperature, and concentration of the anions (Dube et al. 2001). Considering the physicochemical conditions of the sediments and waters, assessing toxic metal concentration due to their impact on their mobility and bioavailability, analyzing metal fractions must be taken seriously. The high values of manganese content in the analyzed sediments and high concentration in the analyzed water especially in the Badovci lake, have aroused interest in studying its behavior in different extraction solutions.

MATERIALS AND METHODS

Study area

The Badovci Lake is located near the capital city Prishtina, while Batllava Lake is in the Podujevë district. The distance from the capital city to Badovci Lake is about 17 km. The Badovci Lake lies in the northeast part of the Pristina city and also in the same geographical position extends Batllave Lake. Badovci Lake Coordinates: 42.6234 ° N and 21.2412 ° E, respectively Batlava Lake: 42 ° 49′16 ″ N and 21 ° 18′28 ″ E (Gashi et al. (2016, Sahiti et al. 2018).

Sampling and analysis

A total of 38 water and sediment samples were taken. Ten samples of sediment and ten samples of water were taken at Badovci Lake, and nine samples of sediment and water were taken from the Batllava Lake with the coordinates described in Table 2. For sampling water, the sampler type (KLL-S) by SEBA, Hydrometrie GmbH & Co.KG) were used while for sediment samples the sampler type (Windaus-Labortechnik.GmbH & Co.KG.d-38678 Clausthal-Zellerfeld) were used. The sediment samples were taken at the bottom of the lakes beds at a depth of 15 cm and water samples were taken one meter above of it. The water samples were placed in acidified and rinsed plastic bottles with re-distilled water, and acidified with nitric acid, while some physicochemical parameters Temp, pH, DO, TSS were measured in situ using portable instruments such as WTW 340i conductor, Hach 5465011 Portable PH / Dissolved Oxygen Meter. About 200 g of the sediment sample is placed in plastic wrap and transported to the laboratory together with water samples in the refrigerator. The water samples were evaporated to 20 ml then nitric acid was added to achieve a pH of about 1 (Malsiu et al. 2020) and filtered with 0.45-micron Watman filter paper, and leveled to 100 ml with re-distilled water according (USEPA 3005A) guidelines. The sediment samples were dried for 30 days in the open air and after in oven at 45 °C to constant weight, then was grinded using crushers and sieved with 0.045 mm sieve type (DIN 4188). The approximately 1g sediment sample was taken and added acids mixture according (USEPA 3050B) guidelines.

Table 1. The procedure based on (Tessier et al. 1979) for metals extraction

Fraction	Extractants	Time (h)	Temp. ºC	Designation
F1	8 ml 1.0 M $MgCl_2$, pH=7	1	20	exchangeable
F2	20 ml 1.0 M CH_3COONa/CH_3COOH, pH=5	5	20	Bound to carbonates
F3	20 ml 0.04 M $NH_2OH.HCl$/25 % HAc solution.	5	96±3 with occasional agitation	Reducible (bound to iron and manganese oxides)
F4	5.0 ml 0.02 M HNO_3, 5.0 ml H_2O_2 30% 6.0 ml H_2O_2 CH_3COONH_4 /20% (v/v) HNO_3	2 3 0.5	85 20	Oxidizable (bound to organic matter)
F5	5ml HNO_3+ 10ml HF +10ml $HClO_4$	until completely dissolved	warm	Residual (bound to the soil matrix)

The measurements were realized at the Institute of Chemistry - Methodius University, Skopje - North Macedonia by ICP-AES method, instrument type (Varian 715-ES- ICP Optical Emission Spectrometer). Multi-Element Standard 11355 - ICP stock solution was diluted to 1.0, 5.0, and 10.0 µg/l then was used to prepare the calibration curve.
To eliminate any specific errors, a blank sample was included and samples were analyzed in triplicate. The extraction of metals at various stages using suitable extractants were based on the method of Tessier et al. 1979, described in Table 1.

Statistical analysis

The statistical calculations such as average, maximum, minimum, and standard deviation, the programs minitab 16 and windows excell 2010 were used.

RESULTS

The average values of the water physicochemical parameters of the two lakes are compatible with international guidelines. While parameters were analyzed according to the depth of the lakes, observed changes such as dissolved oxygen, where lower values occur at higher depths (Table 2). Conductivity and total suspended solids were higher at depth, and there were no major differences between the two lakes. The pH of the analyzed waters does not represent any major change at different depths, other physicochemical parameters are approximately similar in both lakes.

Table 2. The coordinates and physicochemical parameters in the Badovci and Batllava lakes

Badovci Lake								
	GPS Position		Prameters					
Sample sites	Latitude	Longitude	Temp °C	pH	DO mg/L	EC µS/cm	TSS mgL	Depth m
S1	42°37'27.7"N	21°13'15.3"E	9.7	8.75	6.5	350	225	27
S2	42°37'11.2"N	21°13'12.9"E	9.8	8.35	6.55	342	171	30
S3	42°37'14.9"N	21°13'33.2"E	10.1	8.81	6.87	348	172	25
S4	42°37'07.4"N	21°13'53.2"E	10.3	8.2	7.02	342	185	22
S5	42°37'10.7"N	21°14'13.9"E	10.4	8	6.99	346	176	20
S6	42°37'24.9"N	21°14'27.5"E	10.4	7.95	7.1	352	165	17
S7	42°37'35.0"N	21°14'41.8"E	10.2	8.1	8.05	376	170	15
S8	42°37'42.3"N	21°15'03.7"E	10.9	7.8	8	365	172	10
S9	42°37'39.4"N	21°15'35.8"E	10.9	8	8.2	388	175	5
S10	42°37'46.0"N	21°16'08.5"E	10.9	8.2	8.2	388	178	3
Average			10.36	8.216	7.348	359.7	178.9	17.4
±SD			0.43	0.33	0.68	18.25	17.05	9.17
Batllava Lake								
	GPS Position		Parameters					
Sample sites	Latitude	Longitude	Temp °C	pH	OT mg/L	EC µS/cm	TSS mgL	Depth m
S1	42°49'36.52"N	21°16'57.67"E	9.8	7.66	2.45	263	133	27
S2	42°49'39.40"N	21°17'23.61"E	10.2	8.08	5.15	276	138	22

S3	42°49'32.91"N	21°17'48.66"E	10.7	7.95	5.25	277	136	20
S4	42°49'21.84"N	21°18'9.81"E	11.3	8.2	6.31	279	138	18
S5	42°49'15.79"N	21°18'34.81"E	11.1	8.05	6.29	281	140	17.5
S6	42°49'2.76"N	21°18'54.79"E	11.4	8.13	6.52	281	141	15
S7	42°48'59.14"N	21°19'20.55"E	11.4	8.17	6.26	284	141	12
S8	42°48'54.24"N	21°19'46.30"E	10.8	8.17	6.87	284	143	7
S9	42°49'01.5"N	21°20'29.1"E	9.5	8.16	7.05	258	149	3
Average			10.68	8.06	5.79	275.8	139.8	15.72
±SD			0.70	0.17	1.40	9.22	4.53	7.46

Table 3. Manganese concentrations in water and sediments of the Badovci and Batllava lakes, and other references

	Badovci Lake		Batllava Lake	
	Mn (water)	Mn (sediment)	Mn (water)	Mn (sediment)
Samples	mg/L	mg/kg	mg/L	mg/kg
S1	0.076	268	0.146	629
S2	0.053	243	0.001	1845
S3	0.063	420	0.135	1134
S4	0.051	557	-0.001	867
S5	0.111	513	0.001	575
S6	0.285	682	-0.003	207
S7	0.150	820	-0.001	241
S8	0.042	1008	-0.001	265
S9	0.025	1502	0.045	304
S10	0.09	584	/	/
Max	0.285	1502	0.146	1845
Min	0.09	243	0.001	207
Average	**0.096**	**660**	**0.036**	**674**
[a]EEC	0.050	/	0.050	/
[b] Dojran Lake	/	18000	/	18000
[c] Symsar Lake	/	850	/	850
[d] Gulf of Gemlik	/	634	/	634
[e] Kongsfjorden	/	440	/	440
[f] Keratsini Harb	/	95-1101	/	95-1101

[a] EU Directive 1998/83, Drinking Water Standards; https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31998L0083; [b]Rogan et al. (2015); [c]Kuriata-Potasznik, A et al. (2016); [d]Ünlü et al. (2008); [e]Lu et al. (2016); [f]Galanopoulou et al. (2009)

The average concentration of manganese 0.096 mg/L in the water of the Badovci Lake shows higher concentration compared to EC 98/83, while in the Batllava Lake was 0.036 mg/L, below EC 98/83 limits (Table 3). The average manganese content in the studied sediments of the Badovci and Batllava lakes 660 mg/kg and 674 mg/kg, compared to the reference sediment values (Table 3.), do not show significant deviations, but the changes in the oxidizing states as a result of many factors, such as oxic and anoxic conditions, influence in the increase of its concentration in the water. The area where Badovci and Batllava lakes lie is known as the

Vardar zone, which is rich in various minerals (Hyseni et. al. 2010). The metals present in the sediments are bonded at different phases and with different strengths, and based on this, have different potentials of remobilization in the aquatic environments. The values of the binding strength of metals for sediments indicate reactivity and presence in the aquatic environments. Therefore, it is important to assess their risk based on risk assessment criteria (Table 4.)

Table 4. Risk Assessment of metals by criteria in exchangeable and carbonate fractions, Jain et al. (2007)

Risk Assessment Code (RAC)	Criteria
No risk	< 1 %
Low risk	1-10 %
Medium risk	11-30 %
High risk	31-50 %
Very high risk	> 50 %

Table 5. The manganese content (in mg/kg and %) from five fraction phases at sampling points, and cumulative percentage of carbonate and exchangeable in the Badovci Lake

	S1		S2		S3		S4		S5		S6		S7		S8		S9		S10	S10
Manganese	mg/k	S1 %	mg/k	S2 %	mg/k	S3%	mg/k	S4 %	mg/k	S5 %	mg/k	S6 %	mg/k	S7 %	mg/k	S8 %	mg/k	S9 %	mg/k	%
Residual	114	21.7	101	18.5	76	11.6	108	13.3	111	9.0	143	7.7	101	7.2	113	8.4	236	13.2	149	20.8
Organic	55.1	10.5	56.1	10.3	74.1	11.4	101	12.4	183	14.9	254	13.6	164	11.7	132	9.8	153	8.6	82.2	11.5
Fe-Mn ox	163	31.1	169	30.9	242	37.1	324	39.9	500	40.7	911	48.8	646	46.0	564	42.0	628	35.2	249	34.7
Carbonate	175	33.4	198	36.2	236	36.2	243	29.9	269	21.9	337	18.1	274	19.5	252	18.8	352	19.7	175	24.4
Exchangeable	17.5	3.3	22.6	4.1	24.7	3.8	35.9	4.4	166	13.5	220	11.8	220	15.7	283	21.1	414	23.2	62.7	8.7
Carb +Exch		36.7		40.4		39.9		34.4		35.4		29.9		35.2		39.8		43.0		33.1

Table 6. The manganese content (in mg/kg and %) from five fraction phases at sampling points, and cumulative percentage of carbonate and exchangeable in the Batllava Lake

	S1		S2		S3		S4		S5		S6		S7		S8		S9	
Manganese	mg/k	S1 %	mg/k	S2 %	mg/k	S3 %	mg/k	S4 %	mg/k	S5 %	mg/k	S6 %	mg/k	S7 %	mg/k	S8 %	mg/k	S9 %
Residual	43.5	3.8	48.3	1.6	59.9	4.7	47.4	3.9	53.2	6.0	45.4	7.7	44.2	6.5	41.6	9.2	108	18
Organic	69.6	6.0	240	7.9	74	5.8	57.8	4.8	82.7	9.4	68.3	11.6	40.7	6.0	44.8	9.9	62.8	10
Fe-Mn ox	214	18.5	639	21.1	242	18.9	245	20.2	156	17.7	131	22.3	107	15.6	68	15.1	206	34
Carbonate	265	22.9	1021	33.7	242	18.9	251	20.7	186	21.1	123	20.9	171	25.0	71.2	15.8	201	34
Exchangeable	566	48.9	1085	35.8	663	51.8	611	50.4	405	45.9	221	37.5	321	46.9	226	50.0	22.1	4
Carb +Exch		71.8		69.4		70.7		71.1		66.9		58.4		71.9		65.8		37

The carbonate and exchangeable phases considered to be mostly weak bonds in the sediment matrix, which become equilibrating with aqueous phases and can be very bioavailability. On the basis of the results (Tables 5 and 6), it is noticed that the manganese in the carbonate and exchangeable phases are found in a higher percentage.

DISCUSSION

The high concentration of manganese in the waters of the Badovci Lake may come as a result of the mineralogical content of its natural compounds in the extension area.

Also, the high content of manganese in the analyzed sediments may have influenced the increase of its concentration in water, especially in the Badovci lake. Given that manganese is bound to the sediment matrix in the carbonate and exchangeable phase, then the possibility of equilibrium between the solid/liquid phase (sediment/water) is very high. Both carbonate and exchangeable phases are weakly bonded to the sediment matrix, but it is noticed that in the sediment samples of the Badovci Lake, the highest percentage of manganese occurs in the carbonate phase, while in the sediment samples of the Batllava Lake the highest percentage occurs in the exchangeable phases. According to the Risk Assessment Code criteria, the percentage of manganese in the carbonate and exchangeable phases is presented in high values, which are categorized from medium to very high risk. In the sediment samples of the Badovci Lake, the percentage of manganese varies from 29.9 to 43.0, from where based on sampling sites were assessed from medium to high risk. A different situation was in the sediment samples of the Batllava Lake, where the percentage of the manganese varies from 37 to 71.9 and assessed from high to very high risk. In both lakes, the results of our study indicate a bad state of manganese content in their sediments. These high percentages of manganese in the carbonate and exchangeable phases must be monitored continuously.

CONCLUSIONS

According to the obtained results, the analysis of the water and sediments of the Badovci and Batllava lakes were being noticed that:

The water of the Badovci Lake contains a higher concentration of manganese compared to the directive EC98/83, while the water of the Batllava Lake contains the concentration below the permitted levels according to the aforementioned directive. The manganese content in the analyzed sediments of both lakes is close similar to the contents of the reference lakes, but the bonding with the analyzed sediment matrix is worrying.

During the fractional analysis of manganese, it was noticed that the highest percentage of manganese was bounded to carbonate and exchangeable forms, and in these forms, the bond of manganese is very weak and the possibility of its equilibration between the solid/liquid phase is very evident. Using evaluation criteria such as Risk Assessment Code (RAC) were observed from high risk to very high risk. Based on these data, periodical analysis of the sediment of both lakes is recommended.

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THE LEAD MOBILITY AND TOXICITY ASSESSMENT IN THE SEDIMENTS OF THE BATLLAVA AND BADOVCI LAKES (KOSOVO) THROUGH POLLUTION INDICATORS

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ABSTRACT

The purpose of this paper was to evaluate the mobility and toxicity of lead in the sediments of the Batllava and Badovci lakes. Pollution indicators were used to confirm the level of sediment pollution such as contamination factor, pollution load index, geoaccumulation index and enrichment factor. The study shows that sediments from these lakes have lead contents that exceed values by international guidelines limits. According to lead content classification, lake sediments are classified as light to medium toxic. Lead concentration was also analyzed in the waters of two lakes, and concentration measured was at very low values compared to EC Directive 98/83, respectively below the detection level < 0.01 mg/L. The average value of Pb in the Badovci Lake sediment was 166 mg/kg, while in the Batllava Lake 30.6 mg/kg. Comparing with international guidelines and by some lakes in the region, the sediment of the Badovci Lake exceeds the international guidelines values and the referent lakes of the region. The pollution indicators in the sediment of the Badovci Lake were CF 10.42 classified as very high, PLI 10.42 extreme, Igeo 2.09 medium to high, and EF 1.88 minimum. Whereas in the Batllava were CF 1.92 medium, PLI 1.92 medium, Igeo 0.38 low to moderate, and EF 1.72 minimal.These lakes should be monitored more frequently due to the possibility of the changing lead content by changing environmental conditions. The Batllava Lake sediment in terms of lead content, according to the pollution indicators used it was in better condition compared to the Badovc Lake sediment.

Keywords*: toksicity, mobility, lead, Kosovo*

INTRODUCTION

The risk of contamination of the sediments and soil with heavy metals has fueled the interest of scientists in recent decades. The recirculation of toxic metals in sediments and soils can best be analyzed by sequential extraction (Idriss and Ahmad 2012). The quality of freshwater and food chain are significantly endangered by the high content of lead (Wang, Y. (2019). The development of industry and agriculture affected the pollution of lakes by lead and other heavy metals and has increased the level of content significantly (Lintern et al. 2016; Han et al. 2018). Iron and manganese oxides, organic matter, dissolved oxygen, conductivity and pH value can affect lead mobility significantly (Olaniran et al. 2013). The basic or acidic environment plays important role in increasing or decreasing lead content in sediments, e.g. at alkaline pH, the lead may be bound in the form of carbonates or phosphates which are insoluble while at a pH lower than 5.2 the lead may be soluble and bioavailable (Kushwaha et al. 2018). In cases where dissolved oxygen values are higher, the lead may adsorb or co-precipitate with oxides of iron and manganese and vice versa at lower dissolved oxygen values (Munger et al. 2016). Dissolved organic matter greatly affects the mobilization of lead in the sediments. The focus of this study has been to evaluate the sediment content of lead by analyzing its total content in the Badovci and Batllava lakes and calculate its mobility using pollution indicators.

MATERIALS AND METHODS

Study area

The Badovc Lake lies in the northeast part of the Prishtina, the capital city of Kosovo with a distance of about 17.4 km with coordinates of 42.6234 ° N and 21.2412 ° E Gashi et al. (2016). Also, the Batllava Lake, lies northeast of the Prishtina at a distance of about 37 km with coordinates of 42 ° 49′16 ″ N) and (21 ° 18′28 ″ E Gashi et al (2017). Badovci and Batllava lakes supply more than half of Pristina city with potable water, as well as the city of Podujeva. These two lakes lie in the area known as the Vardar zone consisting of a basic and ultrabasic massif rock (Hyseni et. al. 2010).

Sampling and analysis

In the Badovc Lake, 10 sediment samples were taken at different sites along it. At the same sediment sampling sites also were taken the water samples of 1.5 m above the lake sediment bottom. The samples in Btallava Lake were sampled with the same methodology. About 200 g of sediment sample was taken with the sampler type (Windaus-Labortechnik.GmbH & Co.KG.d-38678 Clausthal-Zellerfeld) and the samples were placed in the plastic wrap and 1 L of water using the type sampler (KLL-S) sampler by SEBA, Hydrometrie GmbH & Co.KG) and placed in pre-acidified PET bottles and rinsed with distilled water, then acidified by adding 1ml of concentrated HNO_3. For the analysis of some physicochemical parameters, portable instruments such as WTW 340i conductor, Hach 5465011 Portable PH / Dissolved Oxygen Meter were used. Samples were transported by the refrigerator to the laboratory of the Department of Chemistry-University of Prishtina. The sediment samples collected were dried at room temperature for 30 days, then in the oven at 45 ºC until constant weight. The water samples were concentrated to 20 ml by evaporation then concentrated HNO_3 was added, filtered with 0.45-micron Watman filter paper, and leveled to 100 ml according to the (USEPA 3005A) method and then measured by ICP-AES method, instrument type (Varian 715-ES-ICP Optical Emission Spectrometer) at the Institute of Chemistry - Methodius University, Skopje - North Macedonia. To measure total metals in sediments, the sediment was grinded, then sieved with 0.045 mm sieve type (DIN 4188), approximately 1 g sample was taken and acid mixtures were added according to the procedure described in the method (USEPA method 3050B).

Statistical analysis

The statistical calculations such as average, maximum, minimum, and standard deviation, the programs minitab 16 and windows excell 2010 were used.

RESULTS

Tables 1 and 2 show the physicochemical parameters of the waters of two lakes, the location, coordinates, and the average of the presented parameters. As can be seen from these tables, the waters of both lakes were in good condition referred to physicochemical parameters. The average parameters in the Badovci and Batllava lakes are as follows: pH about 8.2-8.0, temp 10.3-10.6 °C, DO 7.3-5.7 mg/L, EC 359.7-275.8 μS/cm, and TSS 178.9-139.8mg/L. Changes appear in the sediment of both lakes in lead content. According to Table 3, it is observed that lead concentration in the waters of both lakes is below the detection level while the average lead content in the Badovci Lake sediment is 166.8 mg/kg and in Batllava Lake 30.6 mg/kg. Compared with the values of Dutch target (New Dutsch List, 2000), lead content in the sediment of the Badovc Lake exceeds about twice but the sediment of the Btallava Lake is about two and a half times lower. Also, referring results, shown in Table 3, it is observed that

the lead content in the Badovci Lake sediment is 166.8 mg/kg, even higher than the Dorjan and Koumoundourou lakes 43 mg/kg and 46.13 mg/kg. Lead values in the Batllave Lake sediment are lower compared to these two lakes.

Table 1. The coordinates and physicochemical parameters in the Badovc Lake

Badovci Lake								
Sample sites	GPS Position		Prameters					
	Latitude	Longitude	Temp °C	pH	OT mg/L	EC µS/cm	TSS mgL	Depth m
S1	42°37'27.7"N	21°13'15.3"E	9.7	8.75	6.5	350	225	27
S2	42°37'11.2"N	21°13'12.9"E	9.8	8.35	6.55	342	171	30
S3	42°37'14.9"N	21°13'33.2"E	10.1	8.81	6.87	348	172	25
S4	42°37'07.4"N	21°13'53.2"E	10.3	8.2	7.02	342	185	22
S5	42°37'10.7"N	21°14'13.9"E	10.4	8	6.99	346	176	20
S6	42°37'24.9"N	21°14'27.5"E	10.4	7.95	7.1	352	165	17
S7	42°37'35.0"N	21°14'41.8"E	10.2	8.1	8.05	376	170	15
S8	42°37'42.3"N	21°15'03.7"E	10.9	7.8	8	365	172	10
S9	42°37'39.4"N	21°15'35.8"E	10.9	8	8.2	388	175	5
S10	42°37'46.0"N	21°16'08.5"E	10.9	8.2	8.2	388	178	3
Averag			10.36	8.216	7.348	359.7	178.9	17.4
±SD			0.47	0.33	0.68	18.2	17.0	9.17

Table 2. The coordinates and physicochemical parameters in the Batllava Lake

Batllava Lake								
Sample sites	GPS Position		Parameters					
	Latitude	Longitude	Temp °C	pH	OT mg/L	EC µS/cm	TSS mgL	Depth m
S1	42°49'36.52"N	21°16'57.67"E	9.8	7.66	2.45	263	133	27
S2	42°49'39.40"N	21°17'23.61"E	10.2	8.08	5.15	276	138	22
S3	42°49'32.91"N	21°17'48.66"E	10.7	7.95	5.25	277	136	20
S4	42°49'21.84"N	21°18'9.81"E	11.3	8.2	6.31	279	138	18
S5	42°49'15.79"N	21°18'34.81"E	11.1	8.05	6.29	281	140	17.5
S6	42°49'2.76"N	21°18'54.79"E	11.4	8.13	6.52	281	141	15
S7	42°48'59.14"N	21°19'20.55"E	11.4	8.17	6.26	284	141	12
S8	42°48'54.24"N	21°19'46.30"E	10.8	8.17	6.87	284	143	7
S9	42°49'01.5"N	21°20'29.1"E	9.5	8.16	7.05	258	149	3
Averag			10.68	8.063	5.794	275.8	139.8	15.72
±SD			0.70	0.17	1.40	9.22	4.53	7.46

SD=standard deviation

Table 3. Total lead content in waters and lakes sediments, average values and comparison to international data and some region lakes

	Badovci Lake		Batllava Lake	
Samples	Pb (water)	Pb(sediment)	Pb (water)	Pb(sediment)
	mg/L	mg/kg	mg/L	mg/kg
S1	<0.1	183		
S2	<0.1	170	<0.1	40.1
S3	<0.1	208	<0.1	42.9
S4	<0.1	265	<0.1	58
S5	<0.1	205	<0.1	49.8
S6	<0.1	143	<0.1	63.1
S7	<0.1	132	<0.1	74.3
S8	<0.1	147	<0.1	56.8
S9	<0.1	158	<0.1	40.7
S10	<0.1	57.3	<0.1	30.6
Max	/	265	/	/
Min	/	57.3	/	74.3
Average	/	166.8	/	30.6
[a]EEC	0.01	/	0.01	/
[b] Dutch target	/	85	/	85
[c]FTRV	0.002	/	0.002	/
[d]Ohrid Lake	0.003	/	0.003	/
[e]Prespa Lake	0.002	/	0.002	/
[f]FSTRV	/	31	/	31
[g]Dojran Lake	/	43	/	43
[h]K Lake	/	46.13	/	46.13

[a] EU Directive 1998/83, Drinking Water Standards; https://eur-lex.europa.eu/legal content/EN/TXT/?uri=celex%3A31998L0083; [a] Dutch Target and Intervention Values.Version, februari 4th, 2000. www.esdat.net.; [b]FTRV (Freshwater Toxicity Reference Values), proposed by (USEPA. 1999); [d,e] Shehu et al. 2017; [f]FSTRV (Freshwater Sediment Toxicity Reference Values), proposed by (USEPA.1999); [g]Rogan et al. 2015; [h]Hahladakis et al. 2012

DISCUSSION

Chemical fraction of lead in sediments

Obtaining information about the ways and strength of the toxic metals accompanied by sediment is proposed the procedure by sequential extraction Tessier at al. (1979). The percentage of lead content, extracted from the sequential extraction is summarized in Fig. 2. Analyzing the lead content in the Badovci and Batllava lakes sediments can be seen the different associated fractional in descending order. Badovci lake sedimente: Fe-Mn oxide s> organic > residual > carbonate > exchangeable and Batllava lake sediment: organic > residual > Fe-Mn oxides > carbonate = xchangeable.

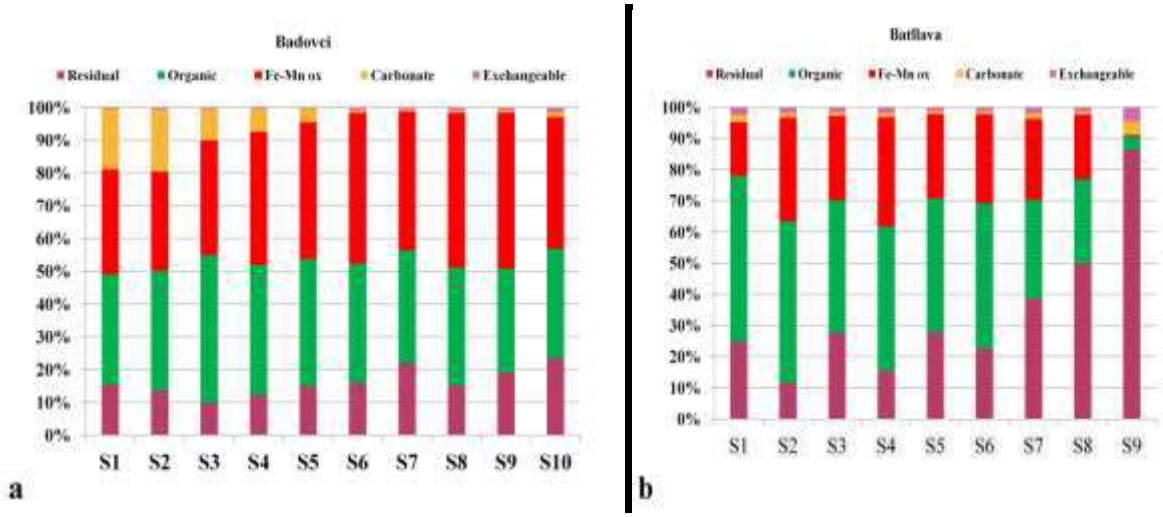

Figure 2. Relative distribution of lead in the sediment of (a) Badovci and (b) Batllava lakes

Pollution indicators

Getting more accurate information on the toxicity and mobility of lead in sediments of both lakes, it is necessary to use several indicators of contamination, like contamination factor, pollution index, geoaccumulation index, and enrichment factor.

Contamination factor

Contamination caused by chemical substances in an environment can be represented by the contamination factor. According to Hakanson (1980), pollution assessment is done by reference to the concentration of metals to their pre-industrial values.

$$C_f^i = \frac{C^i}{C_n^i},$$

This equation is described: C^i is the mean value of metal content in sediment and C_n^i is the reference value for metals in sediments such as Cd-0.2, Cr-71, Pb-16, Zn-127, Fe-900, and Cu-32 mg / kg Martin and Witfield (1983). The contamination factor has been classified in Table 4 according to Hakanson (1980).

Table 4. Classification of Pollution Indicators by Authors (***Hakanson, 1980; Tomlinson et al. 1980, Müller 1969 and Sakan et.al 2009)***

Pollution indicators			
CF	PLI	Igeo	EF
Hakanson, 1980	*Tomlinson et al. 1980*	*Müller 1969*	*Sakan et.al 2009*
Cif < 1 low	PLI < 1 unpolluted	Igeo ≤ 0 no pollut	EF <1 no enrichment
1 ≤ Cif < 3 moderate	1 < PLI < 2 moderate	0 ≤ Igeo ≤ 1 no to moderat	EF < 1 minimal
≤Cif <6 considerable	2 < PLI <3 heavy	1 ≤ geo ≤ 2 moderate	EF < 1 moderate
Cif ≥ 6 very high	3 < PLI extremely	2 ≤ geo ≤ 3 mod to havely	EF < 1 mod to severe
		3 ≤ geo ≤ 4 heavely	EF < 1 severe
		4 ≤ geo ≤ 5 heavely t extremly	EF < 1 very severe
		5 ≤ geo ≤ 6 extremly	EF < 1 extremely seve

Pollution Load Index

The sediment load pollution referringTomlinson et al. (1980) gives a simple relative average of a toxic metal pollution rating scale and is expressed as:

$$\text{PLI} = (CF1\ x\ CF2\ x\ CF3\ x\ \dots CFn)^{1/n},$$

According to this equation, n presents the number of metals, while CF presents the contamination factor. Pollution Load Index according to Tomlinson et al. (1980) is shown in Table 5.

Geoaccumulation index

This pollution indicator, first proposed by Müller (1969), provides a reference for identifying the degree of contamination by metals and can express as:

$$\text{Igeo} = \log 2\left(\frac{Cn}{1.5Bn}\right) \quad ,$$

According to this equation, Cn represents the measured metal concentration n, Bn the background values of n element according to Martin and Witfield (1983) and 1.5 present the correction factor by the possibility of changing background values from lithological influences.

Enrichment factor

For expression of the enrichment factor, Sakan et.al (2009), used the equation according to Ergin et al. (1991).

$$EF = \frac{\left(\frac{\text{C}}{\text{Fe}}\right)\text{sample}}{\left(\frac{\text{C}}{\text{Fe}}\right)\text{backgound}},$$

The Fe element was taken as reference, 35900 mg/kg Martin and Witfield (1983) for geochemical normalization to express the enrichment factor, where (C/Fe)sample express the ratio of the element concentration tested to Fe concentration in the sediment, and (C/Fe) background express the ratio of the Fe concentration as reference.

Table 5. The sediments classification of Badovci and Batllava lakes for lead content through pollution indicators

	Pollution indicators			
Lakes	*CF*	*PLI*	*Igeo*	*EF*
Badovci	10.42	10.42	2.09	1.88
Batllava	1.92	1.92	0.38	1.72

As can be seen from Table 5, the sediment of Badovc Lake is much more lead-loaded than the Batllava Lake sediment. The contamination factor, pollution load index, sediment load in the Badovci Lake with lead is classified as very high and extreme, the geoaccumulation index average to high, and the enrichment factor minimum. The Batllava Lake sediment according to pollution indicators is presented as CF - average, PLI - heavy, Igeo - no pollution to average and EF - minimum. The higher sediment load of the Badovci Lake may be due to the geological structure of the land where the lake lies. This area is known as the Vardar zone with high mineralization Durmishi et al. (2010).

CONCLUSION

Based on the obtained results in this study using some pollution indicators, it can be concluded that: The waters of the Badovci and Batllava lakes have very low lead concentrations under < 0.1 mg/L. The sediments of the lakes differ from each other. According to the total values of Pb content in the analyzed sediments, Pb exceeds the international permitted values as Dutch List, 2000 and (USEPA 1999, FSTRV). Also, comparing the Pb content values in the sediments of two lakes with the region lakes, the Badovci Lake sediment shows much higher values, while the Batllava Lake sediment appears at similar levels. The higher percentage of lead fractions has been in the organic fractions and in the Fe-Mn oxide fractions in the sediments of both lakes. The high values especially in the organic fraction present concern for sediment quality. According to the values of pollution indicators such as CF, PLI, Igeo, and EF, observed large load pollution in the sediment of the Badovc Lake, where CF value 10.42 indicates very high pollution and PLI value 10.42 is classified as extreme. The Igeo values 2.09 are classified as medium to high and EF values 1.88 are classified as minimal. These indicators in the sediment of the Batlava Lake are classified as minimal to average and generally do not show significant pollution. As the indicators mentioned above represent the lead load due to the geological structure of the land where the Badovc Lake lies, the monitoring of its waters and sediment should be periodical.

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AGRICULTURE ENGAGEMENT: EFFECTIVE LEADERSHIP CAN IMPACT THE PRACTICE OF URBAN GARDENING

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ABSTRACT

Leaders in urban centers could serve as key individuals in promoting urban gardening practices by students and adults. With the support of leaders, urban gardening could be encouraged and supported in various settings as community valued projects. The benefits of urban gardening could help the sustainability of people within their own communities, even on a small scale, to gain access to fresher food supplies, improving health issues, expanding knowledge, spending less time and travel to the grocery store and value-added in becoming more self-sufficient in growing some of their own foods in a natural manner. Students and intergenerational individuals, as adults, could participate in urban gardening projects collaboratively. This collaborative experience could further give older adults an additional sense of purpose, worth, and belonging. Urban gardening could help to create and maintain a more engaged community where people would do more healthy eating, socializing in building a cleaner and safer environment. With the appropriate permit, urban gardeners could sell some of their produce to the public. However, this study did not intend to play a key role in eliminating food scarcity in urban districts, but to illustrate the potential of how individuals within communities and students in schools could learn and appreciate the ability to grow some small portions of plants for food. The major purpose of this study was to highlight the importance of community leaders in promoting the growth of gardens by students and adults as a valuable experience in their own community, resurgence of gardening during COVID-19, benefits of urban gardening across several disciplines in the school's curriculum, how community gardening can serve as a catalyst in bringing people together in the community for a common good and how gardening may help students and adults think about and practice eating in a healthier manner.

Keywords: Community Leaders, Gardening, Urban Communities

INTRODUCTION

Leadership can come from such organizations and others like the Green Team, Municipal Agricultural Advisory Committee, Open Space and Environmental Commissions, Public Works Department, Parks and Recreation Commissioners, School Leaders and School-based Parent Organizations, Community Health Educators, Planning Board and Zoning Board of Adjustment members, and Community Nonprofit Organizations which can all play a key role in promoting gardening experiences for students and for adults (Cultivating Community Gardens, 2011). Government leaders could play a key role by providing resources to help create healthy, livable neighborhoods through gardening projects. Such projects could lead to broader expansion of gardening in urban centers. This expansion could help cities to become more self-reliant in having more plant-based foods being grown on unused vacant lots in urban centers (Grewal & Grewal, 2012).

School gardens have been a living part of United States schools since 1890, and the first school garden was established in Roxbury, Massachusetts (Patman, 2015). Since the turn of the 20th-century school gardens have greatly expanded to include inner-city schools in some of the

largest metropolitan areas of the country where a number of individuals have shown an interest in gardening. Since the early 1990s, school gardens have continued to increase in popularity and have been incorporated into the curriculum at a number of schools in the United States (Hillison, 1998).

For school gardens, it is important to have buy-in from the Board of Education, school leaders, staff, teachers, buildings, and grounds staff, as well as students and parents, who can all become partners in the whole gardening initiative (Hayden-Smith, 2011). Using a timeframe in starting a new community or school garden can take 6-9 months for planning and implementation. Project costs and resource needs for community or school garden costs may include a small salary for a part-time garden manager or a responsible individual may volunteer to oversee many aspects of the garden. Owners of garden tools may be kind enough to donate needed materials for the garden. If there are useful donated materials, the total costs for setting up and maintaining a garden could as little as $500 (Community Gardening Resources, 2008). However, these costs can be significantly reduced through donations of materials, volunteer labor, donations of equipment, and partnerships with community organizations to help manage the gardens. Why is it important? Community and school gardens can provide a wide range of benefits including improving the quality of life for people in the garden community by: 1). acting as catalysts for neighborhood and community development; 2). stimulating social interaction; 3). teaching the idea self-reliance; 4). expanding access to nutritious foods; 5).creating opportunities for recreation, exercise, therapy, and education; 6). providing opportunities for intergenerational and cross-cultural connections; 7). helping students understand where their food comes from; 8). how their food choices impact their bodies, environment, and their communities at large plus emphasizing how to avoid wasting food. Community individuals involved in planting, growing and maintaining the garden are invaluable, because when schools are not in session, the community partners and supporter can step in to ensure that the growing and maintaining of gardens in the community are still being taking care of by dedicated members of the community (Surls, 2009).

LITERATURE REVIEW

Historical Perspective At a Glance

Community or school gardens can provide a wide range of benefits to both the gardeners and the broader community. For students, school gardens can serve as living classrooms that teach lessons as simple as “where our food comes from” to complex lessons on ecology, resource management, nutrition, and healthy lifestyles. Community gardens provide access to land, offer technical support, and build community connections that can move beyond the garden fence. The local government will need to provide meaningful support and resources for gardening initiatives. Who should lead and be involved with this action? To carry out this action, it is important that a local representative is committed to supporting the implementation of local food production and gardens in the school environment and in the community (Graham, Lussier, McLaughlin & Zidenberg-Cherr, 2005.

Expansion of Urban Gardening During COVID-19

Looking at current gardening as an agricultural practice, a number of families are using small and large lots of land and are finding a strong interest in growing their food products since the beginning of COVID-19. Community gardens offer many benefits too, which are for gardening knowledge, social bonding, and most importantly the increased production and consumption of nutritious, fresh, and locally grown fruits and vegetables for the community residents (Alaimo, Packnett, Miles & Kruger, 2008).

Because of the COVID-19 pandemic, on the other hand, some people may be unsure if it is safe to visit a community garden or even if the garden remains open. Garden access may be contingent, when the gardener has to carry out those regulations or tasks such as check temperature, require mask covering and washing hands. These new tasks and responsibilities may be uncomfortable for gardeners being in charge of the garden site. It is essential that garden leadership teams clearly and quickly inform all audiences of any policy changes to garden access because of COVID-19. Even when gardening is in a public setting, the Centers for Disease Control recommends wearing gloves and cloth face coverings when being involved with any type of gardening. According to Hayden-Smith (2011), there are two major types of gardens such as allotment gardens, known as community gardens and these gardens offer individual plots on which community members grow and harvest produce for themselves and their families. Demonstration gardens are also known as educational gardens, because these gardens host instructions on gardening practices, healthy living, and plant science, with products that are donated to food pantries or cooking classes and sharing gardens. This type of garden is also known as food pantry gardens which operate on the principles that those who use them help maintain them and only take food that they know they will use, because it is important not to waste food (CDC, 2020).

Managing a Garden During COVID-19

To make decisions on garden availability and access, for example, Purdue Extension advises that community garden leadership should consult with local government leaders to determine if their space is considered essential (Purdue Agriculture, 2020). Therefore, these are best practices for community garden management during the COVID-19 Pandemic. The space for gardening must be viewed from a managerial point of view as follows: 1). Communicate to all potential audiences that they should not visit the garden if they feel ill if they have tested positive for COVID-19 within the last two weeks or have had known contact with someone who has tested positive for COVID-19 within the last two weeks 2) Do not allow anyone with signs of illness on the site; volunteers may need to control the entrance and be checked themselves. 3). discontinue having community events/tours, as well as carry-ins, potlucks, or self-serve food. 40. Leave garden gates open during hours of operation to avoid frequent contact closely with others. 4). Keep all tables, chairs clean, and remove all trash from the area. 5) Limit, by policy, only 8 to 10 individuals. 6) Introduce a staggering time for entry to the garden in order to limit the crowds. 7). Place major attention to all areas being clean such as tools, latches, doorknobs, padlocks, gates, contact surfaces, outhouses, and keep available hand sanitizer for everyone (CDC, 2020).

A Glance at the Benefits of Urban Gardening

The garden, located on municipal property, also gives a new purpose to previously unused land and provides a home for native plants and wildlife. In addition to these valuable benefits, the garden offers a wide variety of workshops and free speakers for the public, which could be received enthusiastically. By including such topics as pruning, constructing rain barrels, composting, and weed identification could serve to inform and educate current and new gardeners. Again, this experience could be educational for all. Urban gardening has many benefits, by serving as effective tools available for growing environmental awareness during students' experiences as they learn new information. Lohr & Pearson-Mims (2005) suggested that while all interaction with plants during childhood is associated with positive values of trees into adulthood. By participating in active gardening as a student, could help one to see and become influenced in knowing the value of plants and the growth and benefits of trees in the

environment. Another study by Blair (2010) also determined that active gardening in childhood was the most important predictor of whether trees had personal value in the total health of the environment. Also, urban gardening builds important life-long social skills and teaches responsibility, communication, teamwork, ownership, and leadership. Gardening also fosters a sense of community, bringing parents, teachers, students, and community members together. In light of the huge success of the garden, the community is looking forward to improving the garden further. Additionally, workshops and running water could make gardening an attractive hobby for more individuals within the community (Lehrer & Dunne, 2011).

Urban Gardening at School

Urban gardening does not have to stop in individual neighborhoods or in backyards. Schools nationwide have shown a steady interest in building urban gardens as well. Schools in urban settings can be an ideal place to start gardening projects for teachers and students. Urban gardens in school settings across the country have contributed significantly to improving students' lives and academics. A school garden can be integrated into the classroom in many ways. Typically gardens have been used to enhance science lessons. Lessons pertaining to plants are the easiest to teach with a garden but should not limit one's self to only these types of lessons. With enough creativity and an engaged teacher, the garden and student activities can be applied to just about any lesson or discipline in the classroom. By teaching from the garden, that could inspire and create a number of professional opportunities and careers that students may not know when they6 exist from the gardening experiences. For example, Science and Math have been the subjects most frequently associated with the infusing of gardening (Linsley & Caplow, 2008). Yet, so many other sciences can be applied including soil compositions, composting, etymology, and the interaction of plants and insects. Allowing students to plan and help design the garden can help them learn to use their skills and knowledge in measuring, engineering, and budgeting as well as gaining entrepreneurial skills as a profitability and basic business foundational knowledge (Duncan, Collins, Fuhrman, Knauft & Berle, 2016).

However, looking at urban gardening from a historical perspective could be applied in history and language arts lessons. Teaching different cultural realities, and all the different foods that are grown all over the world could open up students to the world around them regarding the essences of gardening in urban and other settings. Many poets and writers have been writing about gardens for centuries, and students may relate to this information, if they can be involved in living the experience of gardening in real time. A gardening focused curriculum is a perfect place for hands-on acute learning, and the more it is used, the more students will learn real-world experiences. The origin of foods and how foods could be grown from and at locations such as traditional land, hothouse, and rooftop would be a great learning experience for students. Beyond growing gardens, students could learn about the various benefits of the rooftop garden such as producing oxygen, converting CO_2 emissions, reducing the heat of buildings and decreasing energy costs. By creating a habitat for wildlife, reducing ambient temperature, reducing stormwater runoff and discharge and capturing and harvesting rainwater that could be recycled for gardening plants (Lundberg, 2009).

Additionally, teaching about gardening in the school's curriculum with relevancy could educate students about how food is grown, produced, and consumed. With such knowledge, students will know more about the growing principles of plants and perhaps students may practice eating healthier foods as they are developing socially, emotionally, and academically. School Gardens offer many benefits to teachers and to their students that go way beyond the classroom. The urban garden could also be a great resource by offering small supplies of food

items to low-income families within the community (Swartz, Ranum, Phillps, Cavanaugh & Bennett, 2003). School Gardens provide the opportunity for students, teachers, and members of the community to interact for a common cause. This interaction may allow for an improvement in interpersonal social skills and can teach students how to work cooperatively with each other and their elders in the community to care for garden plants. Without proper care and maintenance, gardens can die or often become "overgrown." By giving students the responsibility to water and care for the plants they grow may instill in them a sense of pride, responsibility, and accountability. Patience is another virtue that students may learn through garden participation, as it takes time for the plants to develop into flowers then fruit. Students today could use some digital tools to monitor the growth of plants so this could be a broader plan to interest students more in gardening as a favorite project (Duncan, Collins, Fuhrman, Knauft & Berle, 2016).

As the garden grows and becomes fruitful and beautiful, students and teachers can take pride in their efforts as a team. This pride helps to bolster self-esteem, and allows students to take pride in the beautification of their school, participating in research in their own community, and generating ideas and having a vision how to sustain our planet. A school garden allows students to work in a non-threatening outdoor environment where they can interact, and learn about nature. Studies find that students who are allowed to learn in an outdoor environment such as a garden have helped to improve their attitudes. School gardens are a wonderful and exciting way to enhance school subjects and make them more interesting, and meaningful to students. Gardens create an environment that encourages creative thoughts, acute learning and interpersonal skills. The garden is a living entity that could serve as an excellent resource to teach subjects while allowing students to learn in an environment that is a typical to the less engaging classrooms to which students are accustomed to in most school environments (Alaimo, Packnett, Miles & Kruger, 2008). Teachers throughout the nation are discovering how useful and educational a garden can be, and are using gardens to integrate every aspect of the learning curriculum thru S.T.E.M. programs. When factoring in the mathematics in the measuring, in the addition and the subtraction the garden can also be utilized also in teaching Social studies, as the students learn where and how foods are produced in various parts of the world (Hou, Johnson & Lawson, 2009).

LIMITATION

This study was limited to actions of leaders within a community being willing to support curriculum and community urban gardening. This study gave information related to the benefits of urban gardening. This research study was limited to the perspective of urban districts.

METHODOLOGY

This was a qualitative study that examined how leaders from the community could support urban gardening that benefits student learning and people engagement within neighborhoods in urban districts. Data for this study were qualitative phenomenological, because the two researchers looked at some readable documents, articles, reports and resources pertaining to how leaders can be the catalyst to encourage students, parents, and others within the community to take an interest in growing plants. The two researchers in this study looked for the most reliable sources concerning the value of urban gardening.

FINDINGS

The findings from this qualitative study based on reports and the review of various documents suggested that urban gardening can 1). support the demand for local food in the

community environment, in a small way, where traditional farms cannot thrive; 2). create opportunities for small businesses and food entrepreneurs as a business; 3). identify the perfect solution for vacant and underutilized lots scattered throughout cities; 4). utilize rooftops on buildings; 5). increase access to affordable, healthy and fresh produce; 6). provide an opportunity for communities to learn about nutrition and how to grow different food plants; 7). reduce the impact of agriculture on the local environment; 8). improve the time spent in transporting food items from faraway places; 9). continue to adopt environmentally-friendly growing practices by avoid using fewer pesticides and chemical fertilizers; 10). help students to learn about the sources and origin of their foods and to encourage individuals to practice growing and eating healthier foods.

RECOMMENDATIONS

For a future study, researchers could use a quantitative survey in collecting data on the expansion and benefits of urban gardening in communities during COVID-19; second, to infuse a gardening curriculum across broader disciplines in schools that addresses ways to eat healthy when families have limited resources; third, to ensure healthier eating habits for all individuals within the community; fourth, to solicit the support of political leaders to enact policy that promotes urban gardening on a broader scale in cities and towns where there is unused land.

CONCLUSION

Leadership from the community and school districts can help to promote urban gardening. Starting a school-based community garden is a unique process that requires the collaboration of the school and neighboring community members from the early stages of the gardening project. Community gardeners are central to the sustainability of a garden on school grounds or in the community. Students who participate in gardening clearly learn how to study plant life and access fresh fruits and vegetables. However, the skills students can learn from gardening goes beyond themselves and their peers, because students can become participants in environmental stewardship when introduced to agricultural practices. Through gardening, students have a chance to become responsible caretakers (Lohr & Pearson-Mims, 2005). Students have an opportunity to engage in agricultural practices on a small scale with community adults, learning about the responsibilities and impact of land cultivation. However, in order to expand urban gardening practices on a broader scale within the community, community political leaders must also approve and make available a wider use of land and space in urban settings to grow plants for human consummation. Community political leaders and educational leaders could collaboratively lead by encouraging parents, students and neighbors to grow urban gardens for all seasons, if possible based of the climate or weather in the area. Political leaders also have within their own authority and power to enact written policy and offer incentives to individuals within the community to use vacant lots of land and rooftop buildings to grow gardens as a human asset to the community.

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DETERMINATION OF SOME MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *SOLANUM OCHRANTHUM*

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ABSTRACT

Wild species is used as sources biotic and abiotic stress tolerance and morphological traits to increase the performance of modern tomato cultivars. In this study, morphological and molecular parameters of *Solanum ochranthum* wild tomato genotype was determined. Seedling of plants were grown in greenhouse with a randomized complete block design was applied with 2 replications, each consisting of 20 plants. According to UPOV, morphological traits were evaluated. Also using the MAS method, Tomato Yellow Leaf Curl Virus (TYLCV), Tomato Spotted Wilt (TSWV), Tomato crown and root rot (*Fusarium oxysporum* f.sp. *radicis lycopersici* = FORL) and Fusarium Wilt (*Fusarium oxysporum* f.sp. *lycopersici* = FOL) were determined for *Ty-3*, *Sw-5*, *Frl* and *I-2* genes, respectively. To development new varieties tomato breeding programs have been systematic and the breeders need to use different genotypes in gene pool. Wild tomato species have a broad genetic diversity and also, these materials can be used as a genetic source for their valuable traits.

Keywords: *Breeding, Molecular marker, Morphological traits, Wild tomato*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to *Solanaceae* family and has 2n=24 chromosomes (Peralta et al., 2006). Tomato is originated from Peru, Equator, Galapagos Islands and mountainous sections of Chili (Chetelat et al., 2009). According to the Food and Agriculture Organization (FAO), world annual tomato production is currently around 182 million tons from about 4.8 million hectares. China, EU, India, US and Turkey are the leading tomato producers of the world (FAO, 2017). Tomato breeding studies were started at the beginning of the 20th century in both public institutes and private companies (Bai and Lindhout, 2007) and many tomato varieties have been developed with breeding studies. Parallel to intense production activities, many diseases result in significant economic losses every year so disease resistant and stress tolerance is the most important factor of plant breeding (Sharma et al., 2019). Wild species is very important for breeding programs and tomato plants were changed in many traits of the wild species ancestors (Tanksley and McCouch, 1997; Doebley et al., 2006). The origin of wild tomato species is to western South America, Ecuador, Peru to northern Chile, and in the Galápagos Islands (Darvin et al., 2003). Wild species is used as sources biotic and abiotic stress tolerance and morphological traits to increase the performance of modern tomato cultivars (Jenkins, 1948; Rick, 1976; Peralta and Spooner, 2005). Wild tomato species have resistance genes of a number of pathogens, including bacteria, viruses, fungi, nematodes and insect pests. Thus a lot of resistant tomato cultivars has been improved this species (Foolad, 2007). The aim of this study, to determine some morphological traits and resistance disease conditions of *S. ochranthum* to use hybrid breeding programs.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse in Antalya Turkey. Seeds of *S. ochranthum* (LA2166) genotype were sown in contained peat and perlite mixture (3:1). Seedling of plants

were grown in greenhouse with a randomized complete block design was applied with 2 replications, each consisting of 20 plants. According to Jones et al., 1991 cultural applications, irrigation, fertilizer and pesticide application were applied. Plants from each replicate were evaluated for morphological traits. According to UPOV, seventeen morphological traits were evaluated in Table 1.

Table 1. Morphological traits of tomato plants

Plant attitude	weak, medium, strong
Seedling: anthocyanin coloration of hypocotyl	absent, present
Inflorescence type	uniparous, forked, multiparous or irregular
Plant growth habit	determinate, indeterminate
Stem hairs	weak, medium, strong
Flower sepal color	yellow, orange
Fruit green shoulder (before maturity)	absent, present
Leaf: intensity of green color	light, medium, dark green
Leaf: attitude	erect, horizontal, drooping
Leaf blistering	weak, medium, strong
Anthocyanin coloration of leaf	absent, present
Fruit color intensity at maturity	dark, light
Fruit weight (g)	average of 10 fruits
Predominant fruit shape (After the fruit turns color)	flattened, oblate, circular, oblong, elliptic, or obovate
Fruit color (at maturity)	cream, yellow, orange, pink, red, brown, green
Fruit shape at blossom end	indented, indented to flat, flat, flat to pointed, pointed
Fruit length (mm)	average of 10 fruits

DNA isolation, molecular marker and pcr amplifications

DNA isolation was performed in according to CTAB (hexadecyltrimethyl ammonium bromide) isolation method developed by Doyle and Doyle 1987. Extraction buffer which was consist of 1.4 M of NaCl, 20 mM of EDTA, 100 mM of Tris– HCL (pH 8), 2 % CTAB, and 0.2 % of beta-mercapto ethanol was added in 0.6 mL of 0.2 g of fresh tomato tissue. The suspension was mixed with vortex and incubated at 60 °C for an hour. Next, chloroform–isoamyl alcohol (24:1) extraction was added to the solution which was mixed with vortex for 10s and centrifuged at 10000 rpm for 3 min. The supernatant was transferred to a fresh tube and cold isopropanol (−20°C) was added inside the micro tubes. The pellet formed after centrifugation at 13,100gn for 10 min was washed twice with 0.75 mL of 76 % ethanol and 10 mM of ammonium acetate, and then re-suspended in sterile distilled water. The solution was incubated at 37 °C for 1 h, after stored at −20 °C until use. Resistance genes *I-2* for race 2 respectively of *Fusarium oxysporum* f. sp. *lycopersici* (Fusarium wilt), *Ty-3* for tomato yellow leaf curl virus (TYLCV), *Frl* for fusarium crown rot disease caused by *Fusarium oxysporum* f. sp. *radicis lycopersici*, *Sw-5* for tomato spotted wild virus (TSWV) were screened to use molecular marker (Table 2). All PCR products were separated on a 1.5% agarose gel (Sigma, St. Louis, MO), visualized with ethidium bromide under UV light

Table 2. Resistant gene, primer sequence and references of primer

Gene	Primer Sequence	References
I2	F:ATTTGAAAGCGTGGTATTGC R:CTTAAACTCACCTTAAATC	Staniaszek et al.,2007
Ty-3	F:GGTAGTGGAAATGATGCTGCTC R:GCTCTGCCTATTGGTCCCATATATAACC	Ji etal.,2007
Frl	F:CATCTGTTTTTAGTCTATTC R:TTGGCCATTGAATGAAGAAC	Mutlu et al.,2015
Sw-5	F:AATTAGGTTCTTGAAGCCCATCT R:TTCCGCATCAGCCAATAGTGT	Dianese et al.,2010

RESULTS AND DISCUSSION

Morphological traits are very important for tomato breeding programs. Because these traits have been used to determine genetic performance of breeding materials (Bhattarai et al., 2016). In our study, seventeen morphological traits were evaluated according to UPOV (Fig 1)

Fig 1. *S. ochranthum* A, leaf, B, Plant habitus, C, inflorescence, D,E, fruits.

Plant attitude, seedling anthocyanin coloration of hypocotyl, inflorescence type, plant growth habit, stem hairs, flower sepal color, fruit green shoulder (before maturity), leaf intensity of green color, leaf attitude, Leaf blistering, anthocyanin coloration of leaf, fruit color intensity at maturity, fruit weight, predominant fruit shape (after the fruit turns color), fruit color (at maturity), fruit shape at blossom end and fruit length were observed and showed in Table 3.

Wild tomatoes have differences in morphological traits (Peralta and Spooner, 2005). S. ochranthum occur exclusively at mid-elevations in rainforest regions from Colombia to southern Peru (Albrecht and Chetelat, 2009). In our study, S. ochranthum were grown in greenhouse and located in Antalya, Turkey and to determine some morphological traits. The seeds of this species are large and winged (Rick 1979; Rick 1988; Child 1990). In our study we observed the seeds in Fig 2.

Table 3. Morphological and observed traits of *S. ochranthum*

Morphological traits	Observed traits	Morphological traits	Observed traits
Plant attitude	medium	Leaf: attitude	drooping
Seedling: anthocyanin coloration of hypocotyl	absent	Leaf blistering	weak
Inflorescence type	multiparous or irregular	Anthocyanin coloration of leaf	absent
Plant growth habit	indeterminate	Fruit color intensity at postpurity	light
Stem hairs	weak	Fruit weight (g)	12 g
Flower sepal color	yellow	Predominant fruit shape (After the fruit turns	circular
Fruit green shoulder (before maturity)	absent	Fruit: color (at maturity)	green
Leaf: intensity of green color	medium	Fruit shape at blossom end	flat
		Fruit length (mm)	4,5 cm

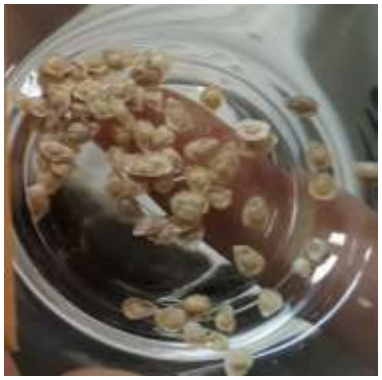

Fig 2. The seeds of *S. ochranthum*

Solanum ochranthum can potentially be used in tomato improvement as a source of resistance to insects, bacteria, fungi, viruses (Rick, 1986; Rick et al., 1990), and P. infestans (Kobayashi et al., 1994). Also Moretti et al., (1990) determined that S. ochranthum was not attacked by leafminer (Liriomyza trifolii Burgess).

In our study we determined some disease resistance of *S. ochranthum* with MAS (Table 4). *S. ochranthum* genotype had Ty-3 and Frl genes with heterozygous and homozygous resistance respectively but Sw-5 gene was found homozygous susceptible.

Table 4. Accession number, species and resistance gene (RR: Homozygous resistance, rr: Homozygous susceptible, Rr; Heterozygous resistance, - Ambiguous results)

Accession	Species	*I2*	*Ty-*	*Frl*	*SW-5*
LA2166	*S. ochranthum*	-	Rr	RR	rr

CONCLUSIONS

Wild genotypes is the source of resistance genes in tomato. And disease resistance in most commercial cultivars has been derived from the related wild species. Also morphological

traits is the most important factor to reduce time and effort this wild genotypes. To determine disease resistance condition and morphological traits can be used in breeding studies. Thus genetic variation will be increased in obtaining new tomato varieties.

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COMPARATIVE STUDY OF ELEVEN APRICOT CULTIVARS IN THE CONDITIONS OF COASTAL REGION OF ALBANIA.

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ABSTRACT

Title: Comparative study of eleven apricot cultivars in the conditions of coastal region of Albania.

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Abstract

The study of eleven apricot cultivars was carried out in a collection planted in the Experimental Base of ATTC Vlore during the period 2017-2020, to evaluate, compare and list among apricot varieties, according to a common protocol for vegetative, pomological, horticultural and technological characteristics, in order to give assistance to farmers who grow apricots, that often fail to plant new cultivars suitable for the conditions of their farms. Antonio Errani, Bulida, San Castrese and Pellecchiella cultivars were planted in 2010, while Spring Blush, Luna, Bora, Magic Cot, Prima, Tsunami, Rubista cultivars were planted in 2014, having the same rootstock (Myrobalan 29C). The results of the study showed that these varieties have different habitats and capacity of growth. The cultivars Bora, Prima, San Castrese, and Spring Blush resulted in strong growth, while the cultivar Rubista resulted to have less potential of growth. Early flowering was recorded in Magic Cot and San Castrese, while the late flowering recorded the Rubista cultivar. Spring Blush, Magic Cot, Prima, Tsunami, Luna cultivars need pollinating plants, while others are self-pollinating. Fruit ripening period, compared to the standard (San Castrese), were recorded; for Tsunami (-28), Spring Blush (-27), Luna, Magic Cot (-25), Prima (-23), Rubista (-19), Antonio Errani (-18), Bora (-15), Bulida (-12) and Pellecchiella (+15) which was the cultivar with latest ripening period. The comparison of fruit size pointed out as cultivars with largest fruit, Antonio Errani, Bora, Bulida and Magic Cot, while with smallest fruit Spring Blush, San Castrese and Rubista. The highest acidic content is recorded in San Castrese cultivar. The total yield has shown variability throughout the years and has confirmed the impact that environmental conditions have on apricot productivity. Bora and Pellecchiella cultivars resulted to have more cracked fruit compared to other varieties, during rainy years. Economic analysis showed that cultivars with earlier ripening are more effective than late ripening varieties. Obviously, an expanded study is required for spreading these varieties in whole regions of the country.

Keywords: apricots, cultivars, self- pollination, maturity, regionalization

Introduction

The climatic soil conditions of Albania and the diversity that characterizes it as a Mediterranean country, offer many opportunities for the cultivation of apricots, even in steep terrain, not very favorable for many other crops [9]. This has led that apricot is being cultivated throughout the

country but without succeeding to dominate as the main culture. In 2018 the number of apricots in Albania reached 260,360 plants, 337 ha planted, with a production of 5131kv and an average yield of 23 kg / plant. The number of new plantings reaches 6673 roots, but in reduced quantities in blocks (3ha) (INSTAT). It is more spread in Korçe, Fier, Berat, Vlore and Peshkopi regions [8, 16].

Apricot is also known as a crop that has poor adaptability to environmental conditions 1, 17, 19]. In this vast area of cultivation with different zone and microzones, the total yield of apricots is not always favoured, there are many risks, mainly during the post-dormant period (winter dormancy) [1, 9]. Spring frosts that hit the plant during flowering when the buds are more sensitive, often accompanied by excess moisture, or conversely relatively high temperatures that inhibit microsporogenesis, can have serious economic consequences for apricot orchard [13, 14, 17]. Climatic factors of the environment can greatly affect the dormancy of flower buds, affecting the entity with the appearance of flower malformations [22, 24]. Also the limiting factors that prevent the extent of apricot cultivation are the climatic conditions related to accumulation of the frosts, a factor that has a significant impact on productivity [1, 19]. Selecting the most suitable variety for the terrain and climate in an ecological zone remains the main factor in improving successfully the yield, stability and profitability of apricot production [2, 4, 12, 20, 21]. First planted cultivars in Albania have local and foreign origin, which have been adapted to the conditions of the areas where they are positioned [9]. The increase of areas with this crop could not be tempted by the presence of new competing cultivars in the markets, of high quality, originating from Greece, Italy, France, Spain and the USA. The introduction of these new cultivars is a promising opportunity in the private and national agricultural economy, for the quantitative, qualitative and commercial improvement of apricots. Meanwhile, the experience of many countries and researchers have shown that apricot cultivars are rarely cosmopolitan [3, 11]. Even in Albania, most of the old cultivars have not been adapted outside their environment of origin [9], thus causing lack of manifestation of the best qualities for which they have been selected.

Rich germplasm with a variety of organoleptic and commercial qualities, size, color, taste and flavor of the fruit, with resistance to manipulation and refrigeration and which offer the opportunity to extend the harvest season until September, requires a period of time and testing before being analyzed for their inclusion in new planting schemes [23]. In apricots, the growing conditions enhance the influences of different natures and various biological, physiological and phenological intensiteties interrelated with the nature of growth and fruiting [1, 6, 10].

The behaviours of cultivars manifested in the flows of vegetative-productive growth, even if not completely identical with their features, are important not only for the selection of the cultivar, but also for the system of keeping the corolla and the method and time of pruning [5, 7].

To assist apricot growers, who often fail to plant new cultivars suitable for their farm conditions, has been realized a study of 11 new apricot cultivars, most of which were not previously tested, in a collection planted in The Experimental Base of ATTC Vlore, during the period 2017-2020, to evaluate, compare and list among them according to a common protocol for vegetative, pomological, horticultural and technological characteristics.

Materials and methods

The study of eleven apricot cultivars was carried out in a collection planted in the Experimental Base of ATTC Vlore, during the period 2017-2020. The cultivars Antonio Errani, Bulida, San Castrese and Pellecchiella, were planted in 2010, are also the cultivars that have been

introduced earlier in Albania market, meanwhile the cultivars Spring Blush, Luna, Bora, Magic Cot, Prima, Tsunami, Rubista were planted in 2014, representing new cultivars, without being tested before. They all have the same rootstock (Myrobalan 29C), planting distance (5x5 m) and are corolled according to the open vase system. For each cultivar were studied the following indicators:

1. Indicators of plant vegetative development, referred to the method used by Viti, R. and Guerriero, R. (2006) [25]. The annual increase in trunk diameter and corolla dimensions was measured to calculate the volumetric index from the ratio of height to corolla diameter. In the biennial branches emerging from the representative branches (selected according to the geographical coordinates since the first year), the elements of the branch insertion angle in relation to the vertical axis, the power of annual growth, the types and spread model of the sprigs are measured. All this measurments are carried out in order to determine the strength and habitat of the growth and fruiting of each cultivar.
2. The flowering period, considering the beginning when 10% of the flowers on the tree have blossomed, the full flowering when 70% of the flowers on the tree have blossomed, and the end when 70% of the petals have fallen.
3. The ripening period by observing the beginning and the end, comparing with the San Castrese cultivar which is considered as a reference cultivar.
4. Biometric indicators of fruit (dimensions Dx d, weight, color, form), other fruit indicators such; sugar content and total acidity, measured randomly in 30 fruits at maturity stage of each variety at ATTC Vlore bio-chemical laboratory.
5. Production for each tree calculating the production / ha for each cultivar according to years.
6. In this analysis were taken four plants for each cultivar, labeling since the winter pruning according to a randomized scheme, where each tree has been treated as a replication, while the representative branches were labeled and selected in N-S-E-W positions, preserving them for four years of experiment. The analysis of statistical indicators was carried out with the Comparisons for all pairs method using Tukey-Kramer HSD, for the error level 0.05.

Results and discussion

Referred to Table number 1, examined varieties are characterized from significative differences of vegetative growth indicators and growth habit. The differences are distinct within the same group regarding to indicators of growth habit, manifesting different growth habit for these varieties. Evidences on growth habit of each variety, ratio between different types of shoots and their position on trees are crucial in order to define correct pruning technique [7, 9]. In apricots, growth habit and fruiting performance are strongly interrelated. [6, 25].

Table 1. Main indicators of vegetative growth and growth habit for all the compared cultivars. Every growth habit is classified according to the reference classes of each parameter, referred to Viti, R. and Guerriero, R. (2006).

Cultivar	Growth habit	Tree height (m.)	Tree canopy diameter (m)	Volumetric Index (h/l)	Branch insertion angle (°)
Antonio Errani	Regular	4.2 bc	4.1 abc	1.04 c	40.6 d
Bora	Upright	4.1 bcd	3.1 e	1.35 b	48.8 b
Bulida	Regular	4.6 a	4.1 ab	1.11 c	43.3 cd
Luna	Spur	3.8 de	3.3 de	1.15 c	50.1 b
Magic Cot	Upright	4.3 ab	3.2 de	1.35 b	48.6 b
Pellecchiella	Spur	3.9 cd	3.6 cd	1.10 c	49.3 b
Prima	Upright	4.2 bc	2.7 e	1.53 a	39.0 d
Rubista	Regular	3.1 f	2.8 e	1.11 c	42.5 cd
San Castrese	Open	3.4 ef	4.4 a	0.79 d	63.8 a
Spring Blush	Open	3.8 cde	4.5 a	0.84 d	67.2 a
Tsunami	Spur	4.1 bcd	3.8 bc	1.08 c	46.3 bc

The flowering stages of apricot cultivars are shown in Table 2. Magic Cot and San Castrese were the earliest cultivar to bloom, and Rubista was the latest. Full flowering period of the cultivars ranged between March 1 and March 20.

Table 2. Main characteristics of recently introduced apricot cultivars (average values of four years 2017-2020)

Cultivar	Flowering period	Autofertility*	Maturity period, compared to S. Castrese
Antonio Errani	2-10 March	SC	-18
Bora	4-16 March	SC	-15
Bulida	5-17 March	SC	-12
Luna	7-20 March	SI	-25
Magic Cot	27 February-8 March	SI	-25
Pellecchiella	8-17 March	SC	+15
Prima	5-15 March	SI	-23
Rubista	10-25 March	SC	-19
San Castrese	1-17 March	SC	
Spring Blush	2-12 March	SI	-27
Tsunami	3-15 March	SI	-28

* Autofertility : SC – auto-compatible, SI - auto-incompatible.

As turned out in the Table number 2, Spring Blush, Magic Cot, Prima, Tsunami, Luna cultivars need pollinating plants, while others are self-pollinating.

Fruit ripening period, compared to the standard (San Castrese), resulted to be (in number of days) for Tsunami (-28), Spring Blush (-27), Luna, Magic Cot (-25), Prima (-23), Rubista (-19), Antonio Errani (-18), Bora (-15), Bulida (-12) and Pellecchiella (+15) which was the variety with latest ripening period. Comparative assessment of ripening period, shows that most

of the varieties recorded early fruit ripening. Maturity time of these cultivars, creates a production conveyor in the market from the beginning of May until the third decade of June.

As it's shown in the chart number 3, there are some significative differences between traits of analyzed cultivars. The comparison of fruit size pointed out as varieties with largest fruit cultivars Antonio Errani, Bora, Bulida and Magic Cot, meanwhile with smallest fruit Spring Blush, San Castrese and Rubista. The highest malic acid content was measured for San Castrese variety.

Table 3. Some fruit characteristics of apricot cultivars (mean values of four years).

Cultivar	Fruit					
	Diameter (mm)	Weight (g)	Pulp /seed ratio	Soluble solids (°Brix)	Acidity (%)	SSC/TA*
Antonio Errani	51.8 a	73.9 a	22.8 bc	16.8 a	1.12 d	1.49 a
Bora	53.1 a	78.1 a	19.5 cd	16.5 ab	1.28 d	0.97 cd
Bulida	51.6 a	71.5 a	19.7 cd	12.5 de	1.7 b	0.97 cd
Luna	50.6 ab	58.9 bc	20.9 cd	10.2 e	1.75 abc	0.57 e
Magic Cot	51.9 a	72.3 a	27.1 ab	12.1 de	1.41 bcd	0.86 de
Pellecchiella	45.8 cd	53.3 c	18.7 cd	13.5 cd	1.27 d	1.06 bcd
Prima	47.8 bc	71.1 ab	21.3 cd	13.8 abcd	1.13 d	1.21 abcd
Rubista	41.4 ef	40.5 d	16.7 d	14.1 bcd	1.06 d	1.33 ab
San Castrese	43.4 de	46.5 cd	19.1 cd	11.8 de	2.1 a	0.56 e
Spring Blush	39.6 f	39.8 d	21.3 cd	11.6 de	1.32 cd	0.87 de
Tsunami	45.1 cde	51.4 cd	29.4 a	15.9 abc	1.05 d	1.22abc

*SSC/TA - Ratio of soluble solids (Brix) to titratable acidity.

SSC/TA ratio is a good indicator of fruit quality. As higher the ratio, the sweeter the fruit taste is perceived. Varieties such Antonio Errani and Rubista resulted with the sweetest taste.

Table number 4 shows average and cumulative values related to total production of analyzed apricot plants. Due to differences in planting period, varieties like Antonio Errani, Bulida, Pellecchiella and San Castrese are planted earlier and showed high total yield indicators. In terms of comparative assessment of yield/trunk section area, the most productive variety result Rubista, followed by Magic Cot and Bora.
Comparative survey of these apricot varieties throughout four years, clearly indicated significative differences in total productivity during different years (data not shown). The lability observed is related to fluctuating temperatures effects during and in the end of flowering period, especially occurred in 2018 and 2020 [15]. The last year resulted the year with the most damages in productivity because of drastic low temperatures in the end of the flowering.

Other climatic occurrences have affected apricot's productivity. Rainfalls associated with fluctuating temperatures have affected the fruit cracking. Varieties Bora and Pellecchiella are recorded to have more cracked fruits compared to other varieties. Climate changes are frequently causing extreme climate events, as apricot is easily influenced, it would be beneficial that the most preferred commercial varieties to be in observation for long terms.

Economic analysis showed that cultivars with earlier ripening are more effective than later ripening varieties.

Table 4: Crosscut surface of stem 30 cm above ground and yield parameters of the apricot cultivars (average of four years)

Cultivar	Crosscut surface of stem 30 cm above ground	Yield (kg/tree)	Yield (t/ha)	Cumulative yield (kg/tree)	Cumulative yield (t/ha)	Yield/trunk section area (kg/cm2)
Antonio Errani	258.7 a	16.8 a	7.4 a	67.5 a	29.6 a	0.06 e
Bora	92.4 c	12.7 b	5.6 b	50.9 b	22.4 b	0.14 bc
Bulida	219.7 ab	17.1 a	7.4 a	68.1 d	29.9 a	0.08 cde
Luna	87.5 c	9.5 cde	4.2 cde	38.2 cde	16.8 cde	0.12 bcde
Magic Cot	85.1 c	12.5 bc	5.5 bc	50.1 sc	22.1 bc	0.16 b
Pellecchiella	209.2 b	16.4 a	7.2 a	65.7 a	28.9 a	0.08 cde
Prima	89.8 c	11.1 bcd	4.8 bcd	44.5 bcd	19.5 bed	0.13 bcd
Rubista	46.6 d	8.9 de	3.9 de	35.9 de	17.7 de	0.22 a
San Castrese	225.6 ab	18.7 a	8.2 a	75.1 a	33.1 a	0.08 de
Spring Blush	84.7 c	7.6 e	3.3 e	30.1 e	13.5 e	0.09 cde
Tsunami	79.3 cd	8.5 de	3.7 de	34.2 de	15.01 de	0.11 bcde

CONCLUSIONS

Comparative study of eleven apricot varieties in coastal regions of Albania, proved potential of these varieties for cultivation in this region. Despite significative differences amongst them, regarding most of the indicators estimated, depend on investors to select the proper variety, based on their targets and market inclinations [23]. Evidences of this study provide enough data to determine the cultivation technology for these varieties. An expanded study is essential for spreading these varieties in whole regions of the country.

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EVALUATION OF MULTIELEMENT EXTRACTANTS FOR PREDICTION OF AVAILABLE PLANT NUTRITIENTS IN SLIGHTLY ALKALINE SOILS IN KAHRAMANMARAS-TURKEY

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ABSTRACT

Multi-element soil tests such as ammonium bicarbonate-DTPA (AB-DTPA) and Mehlich 3 have been employed by soil testing laboratories in some countries, since they reduce labor, time, and chemical losses and allow simultaneous measurement of many elements by using the Inductively Coupled Plasma (ICP). However, information on their efficiencies for assessing the availability of nutrients in Turkey's soils is still inadequate. The objective of this study was to evaluate AB-DTPA and Mehlich 3 in comparison to routine soil tests (Olsen for P; NH4OAc for K, Ca, Mg, and Na; DTPA for Fe, Mn, Cu, and Zn; Hot water for B) for simultaneous measurements of 10 elements (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and boron (B)) in slightly alkaline Kahramanmaras soils. Pearson correlation analysis were applied to investigate the relationship between AB-DTPA or Mehlich 3 and the respective soil test. The results can be summarized as follows: 1) the best correlations were found for K and Cu with both extractants and routine soils tests; 2) The AB-DTPA method showed significant correlations with routine test for all the elements studied but Mehlich 3 did not have any correlations with routine tests for Na and B; 3) the highest correlation coefficients were achieved in Zn (r= 0.998**) with AB-DTPA and in K (r= 0.876**) with Mehlich 3 compared with routine soil tests; 4) while a low negative relationship at the level of 1% was found with AB-DTPA for Ca, a moderately positive relationship at the level of 1% was determined with Mehlich 3. The findings of the study strongly encourages new research on multielement methods and plant nutrient uptake studies in the region.

Keywords: *Multielement extractants, AB-DTPA, Mehlich 3, available plant nutrition, slightly alkaline soils.*

INTRODUCTION

Diagnosis of plant nutrients in the soil by chemical analysis is essential in determining the amount of fertilizer to be applied (Yanai et al., 2000). The suitability and usability of the soil analysis method in terms of the territory of the region reveals the quality of that analysis process. Therefore, a diagnostic method is required both to save labor and time and to quickly identify nutrients in the soil, because many soil samples are taken at once in a season (Uwasawa, 1994). The United States has a history trying to implement and develop soil analyzes since the early 1940s (Jones, 1998). Due to improvements in soil testing techniques, Quebec and Canada's Prince Edward Islands (Liu and Bates, 1990), the Netherlands (Houba, 1998), Malawi (in East Africa) (Chilimba et al., 1999), Spain (Monterroso et al. ., 1999), Turkey (Yıldız et al., 1999), Czech Republic (Zbiral and Nemec, 2000), Thailand (Aramrak, 2007), India (Joshi et al., 2014) and many other countries are trying to adapt these methods to

their own soils. These developed techniques allowed the plant nutrient to be determined by reading it with a single extraction solution by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Yıldız et al. (1999), engaged in research related to the first multi-extraction methods in Turkey. In this study, Mehlich 3 extraction method was tried in 20 Erzurum plain soils and 14 acid soils, but no relation was found between Mehlich 3 method and extraction of available nutrients to the plant. Later, Gürbüz and Günay (2013) measured the amount of nutrients P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B in the acid, neutral and alkaline soils with conventional and multiple extraction methods and determined the correlations between them. According to this study, it was reported that Mehlich3 and acid ammonium acetate-EDTA (AAAc-EDTA) methods in acid soils and Mehlich3 and AB-DTPA methods in neutral and alkaline soils gave high correlations. However, it still remains uncertain since there is not enough work done on this subject in our country. The purpose of this study; using multiple elemental analysis methods such as AB-DTPA and Mehlich 3, to determine the amount of available plant nutrients in the soil and to obtain the relationship between the methods and the routine soil tests.

MATERIAL AND METHODS

Soils

The soil samples were collected from Afsin and Elbistan districts of Kahramanmaras province (Figure 1). A total of 239 surface (0-20 cm) soil samples were taken from Afsin (99 samples) districts and from Elbistan districts (140 samples). Soil samples were collected from agricultural lands by determining their coordinates with GPS device. The soil samples brought to the laboratory were laid in clean polyethylene tubs, and stone, all kinds of garbage and plant particles were removed and dried in the open air. The dried soils were beaten with wooden mallets, passed through a 2 mm steel sieve and made suitable for analysis.

Laboratory analysis

Some properties of the soil samples were determined like soil texture determined according to the hydrometer method (Bouyoucos, 1951), pH in saturated soil (saturated mud) was measured by pH meter with glass electrode (Thomas, 1996), electrical conductivity (EC) measured from saturated mud with electrical conductivity device (Richards, 1954), total lime it was determined volumetrically in Scheibler calcimeter (Klute, 1986), organic matter (OM) it was determined by the modified Walkley-Black method (Nelson ve Sommers, 1996), available P determined by spectrophotometer device according to Olsen method (Olsen et al., 1954), available K, Ca and Mg and extractable Na determined by measuring with ICP-OES according to ammonium acetate method (Helmke ve Sparks,1996), available Fe, Mn, Cu and Zn filtered soil solutions obtained from soils extracted with DTPA solution were determined by measuring with ICP-OES (Lindsay and Norvell, 1978) and available B determined according to the hot water method (Klute, 1986), AB-DTPA (Soltanpour and Schwab, 1977) and Mehlich 3 (Mehlich, 1984).

Ammonium bicarbonate-DTPA method

On the basis of this method developed by Soltanpour and Schwap (1977), 20 ml of AB-DTPA solution (0.005 M DTPA + 1 M NH_4HCO_3, pH: 7.6) was added to 10 g of the soil sample ready for analysis, shaken in shaker for 15 minutes and then filtered. Amounts of P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn and B passing from soil samples to the solution were determined by the ICP-OES device.

Mehlich 3 method

According to the method determined by Mehlich (1984), 15 ml of Mehlich 3 solution (0.2 N glasial acetic acid (CH_3COOH), 0.25 N ammonium nitrate (NH_4NO_3), 0.015 N ammonium fluoride (NH_4F), 0.13 N nitric acid (HNO_3) and 0.001 M EDTA (Ethylene diamine tetra acetic acid), pH: 2) was added to 2 g of the soil sample ready for analysis, shaken in shaker for 5 minutes and then filtered. Amounts of P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn and B passing from soil samples to the solution were determined by the ICP-OES device.

Statistical evaluation

Relationships between routine analysis results and multiple elemental analysis results such as AB-DTPA and Mehlich 3 were determined according to Pearson correlation analysis in SPSS (2018) statistical software program.

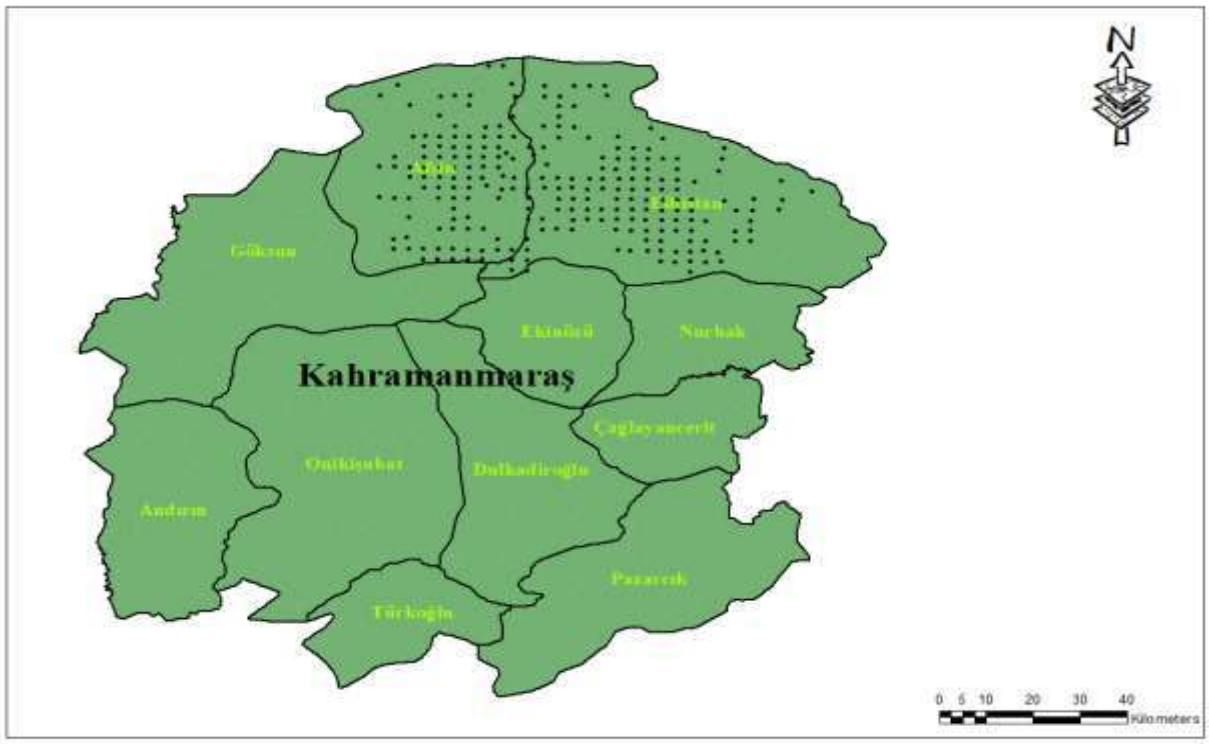

Figure 1. Map showing the points where soil samples were collected

The significance level of the correlation coefficients between routine and multiple extraction methods were evaluated according to the data of Atan (Gürbüz and Günay, 2014) in Table 1.

Table 1. Correlation coefficient significance level (Gürbüz and Günay, 2014)

Correlation coefficient	**Significance level**
0 - 0.25	Very low relationship
0.26 - 0.49	Low relationship
0.50 - 0.69	Moderate relationship
0.70 - 0.89	Highly relationship
0.9 – 1.0	Very highly relationship

RESULTS

Soil Properties

Determined soil parameters are given in Table 2. According to this; the amount of sand was between 10.4% and 81.2% and the average was 40.4%, the amount of silt was between 1.0% and 54.1%, the average was 16.9%, the amount of clay was 15.2% and 81.2%, and the average was 42.7%. The average texture class of the soils was found as clay. Soil pH changed between 6.47-8.18, and average was 7.65 (slightly alkaline), soil EC ranged from 0.38 dS m^{-1}-2.66 dS m^{-1}, and mean was 0.95 dS m^{-1} and slightly saline, total lime of soil changed between 0.88-39.56% and the average was 20.53% too chalky and, the organic matter of the soils ranged from 0.42% to 4.81%, and the average was determined as 2.14% and medium class.

Table 2. Minimum, maximum and average values of the determined soil properties

Determined soil parameters	**Sand (%)**	**Silt (%)**	**Clay (%)**	**Texture**	**pH**	**EC (dS m^{-1})**	**$CaCO_3$ (%)**	**OM (%)**
Range	10.4-81.2	1.0-54.1	15.2-81.2	C	6.47-8.18	0.38-2.66	0.88-39.56	0.42-4.81
Mean	40.4	16.9	42.7		7.65	0.95	20.53	2.14

The minimum, maximum and average values of the soils determined as results of these analyzes are given in Table 3. According to the mean values for routine, AB-DTPA and Mehlich 3 methods, macronutrients were found as 14.92, 8.91 and 52.73 mg kg^{-1} for P, 381, 255 and 386 mg kg^{-1} for K, 8089, 247 and 15767 mg kg^{-1} for Ca, 373, 130 and 343 mg kg^{-1} for Mg, and 50.97, 9.91 and 345 mg kg^{-1} for extractable Na respectively. Microelements were determined as 4.48, 7.05 and 40.64 mg kg^{-1} for Fe, 10.19, 8.83 and 110 mg kg^{-1} for Mn, 1.46, 2.87 and 3.53 mg kg^{-1} for Cu, 1.06, 1.37 and 2.51 mg kg^{-1} for Zn, and 0.71, 0.03 and 6.46 mg kg^{-1} for B respectively.

Table 3. Minimum, maximum and average values (mg kg^{-1}) of some parameters of soils obtained by routine and multiple elemental analysis methods

Tests	**Range-Mean**	**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Mn**	**Cu**	**Zn**	**B**
Routine tests	**Range**	2.25-55.50	69.49-1070	1138-13977	91.94-2524	4.46-198	0.80-20.66	2.35-69.43	0.30-6.21	0.09-68.79	0.01-3.07
	Mean	14.92	381	8089	373	50.97	4.48	10.19	1.46	1.06	0.71
AB-DTPA	**Range**	0.58-711	34.60-823	123-352	9.67-607	1.41-107	1.16-46.81	0.88-73.11	0.38-12.26	0.10-76.35	0,00-0,25
	Mean	8.91	255	246.80	130	9.91	7.05	8.83	2.87	1.37	0.03
Mehlich 3	**Range**	2.21-596	48.42-1289	1492-30031	90.03-2608	214-697	5.48-261	26.30-426	0.63-16.46	0.30-26.40	0.24-52.74
	Mean	52.73	386	15767	343	345	40.64	110	3.53	2.51	6.46

After the properties of each soil were determined by the routine, AB-DTPA and Mehlich 3 extraction methods, the relationships between these methods were also examined (Table 4). According to this; all the relationships among the methods are at 1% level and very important. Among all the correlations, it is seen that there is only in Ca element a negative relation. The negative correlation was found between ammonium acetate and AB-DTPA methods. However, this negative relationship is significant at the level of 1% again. The highest correlations were determined between the routine and AB-DTPA methods for Zn (r= 0.998**) and K (r= 0.878**). With the Mehlich 3 method, the highest correlation was obtained in the K element (r= 0.876**). The highest relationship between AB-DTPA and Mehlich 3 multielemental analysis methods was determined in K (r= 0.804**). No significant relationship was found between Routine and Mehlich 3 for sodium and boron (NS). Similarly, no significant relationship was detected for calcium and boron (NS) also between AB-DTPA and Mehlich 3.

Table 4. Relationships between extraction methods

Methods and correlations	**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Mn**	**Cu**	**Zn**	**B**
Routine x AB-DTPA	0.283 **	0.878 **	-0.256 **	0.675 **	0.509 **	0.869 **	0.850 **	0.783 **	0.998 **	0.468 **
Routine x Mehlich3	0.426 **	0.876 **	0.596 **	0.637 **	NS	0.622 **	0.454 **	0.740 **	0.310 **	NS
AB-DTPA x Mehlich3	0.636 **	0.804 **	NS	0.276 **	0.381 **	0.559 **	0.207 **	0.747 **	0.329 **	NS

****:** Significant at p< 0.01; **NS:** Not significant

DISCUSSION

Mean values

It is seen that the same nutrient elements are extracted in different amounts by all three extraction methods. It is thought that this is due to the fact that each extraction method has different pH values and different shaking times. Looking at the averages, it is understood that the order of the methods that extract the most nutrients is Mehlich 3 > Routine > AB-DTPA in macro elements. However, in the ammonium acetate method, it is seen that the mean of Mg is higher than the Mehlich 3 method. This can be explained by the fact that the shaking time of 60 minutes in the routine analysis method caused more of the magnesium to pass to the soil solution than the clay surfaces according to the Mehlich 3 method. The order of methods that extract the most nutrients in microelements is Mehlich 3> AB-DTPA> Routine, for iron, copper and zinc. It is understood from Table 3 that this ranking altered to Mehlich 3> Routine> AB-DTPA for manganese and boron. This is because power of AB-DTPA to extract Mn and B is less than the DTPA solution of which pH is 7.3 and the shaking time is 2 hours.

Correlations

It is seen that all correlations obtained between extraction methods are significant at the level of 1% ($p< 0.01$).

P correlations

In this study, for the obtained P values, between the Olsen method (routine method) and the AB-DTPA method was found a significant at the level of 1% ($p< 0.01$) but a low relationship ($r= 0.283^{**}$). While there was determined a significant at the level of 1%, but low relationship between Olsen and Mehlich 3 extraction methods, there was found a moderate relationship between AB-DTPA and Mehlich 3 extraction methods ($r= 0.426^{**}$ and $r= 0.636^{**}$, respectively). Gürbüz and Günay (2014) stated that both the AB-DTPA-P method ($r= 0.426^{**}$) and Mehlich 3-P method ($r= 0.413^{**}$) showed significant at the level of 1% but low relationships with the Olsen-P method on 123 neutral and alkaline soils collected from the agricultural lands. Elrashidi et al. (2003) reported that in alkaline soils both AB-DTPA and Mehlich 3 showed high correlations with Olsen method, but Mehlich 3-P gave a stronger relationship with Olsen method ($r= 0.926^{**}$) than AB-DTPA-P ($r= 0.689^{**}$). The amount of P obtained by AB-DTPA and Mehlich 3 multi-nutrient extraction methods were strongly correlated ($r= 0.636^{**}$).

K, Ca, Mg and Na correlations

Correlations between ammonium acetate (AA) (routine method) x AB-DTPA and ammonium acetate (AA) x Mehlich 3 for potassium ($r= 0.878^{**}$ and $r= 0.876^{**}$, respectively) and magnesium ($r= 0.675^{**}$ and $r=0.637^{**}$, respectively) were found very close to each other. Both AB-DTPA-K and Mehlich 3-K methods gave a high relationship for potassium, while both methods showed a moderate relationship for magnesium. Some researchers also reported that they obtained high correlations for K and Mg between both AA x AB-DTPA and AA x Mehlich 3 methods in alkaline soils (Elrashidi et al., 2003; Gürbüz and Günay, 2014). Relationships between AB-DTPA and Mehlich 3 methods were also found to be high in K and low in Mg. It showed that these two methods are more reliable for K than Mg. For calcium, the correlation between AA-Ca and AB-DTPA-Ca methods was significant at the level of ($p< 0.01$), but a negative and low relationship was found ($r= -0.276^{**}$). In other words, while the amount of Ca in AA increased, the amount of Ca in AB-DTPA decreased. Or the opposite is the case. A similar relationship ($r= -0.273^{**}$) was also reported by Elrashidi et al. (2003), Gürbüz and Günay (2014). Precipitation of lime explains the failure of the AB-DTPA multiple extraction method in alkaline soils, where the amount of soluble Ca is too high (Soltanpour, 1991). A moderately significant relationship at the level of 1% level was obtained between AA-Ca and Mehlich 3-Ca ($r= 0.596^{**}$), but no significant relationship was found between AB-DTPA-Ca and Mehlich 3-Ca (NS). For extractable Na, a moderately significant relationship at 1% level between AA-Na and AB-DTPA-Na ($r= 0.509^{**}$), and a weak relationship at 1% level between AB-DTPA-Na and Mehlich 3-Na ($r= 0.381^{**}$) were determined. On the other hand, no significant relationship was found between AA-Na and Mehlich 3-Na (NS). Elrashidi et al. (2003) reported that they obtained significant correlations at the level of 1% between routine analysis and AB-DTPA ($r= 0.946^{**}$) and Mehlich 3 ($r= 0.996^{**}$) methods in alkaline soils. Madurapperuma and Kumaragamage (2008) stated in their study that they found a significant relationship at the level of 0.1% ($p <0.001$) between AA and AB-DTPA methods ($r= 0.78^{***}$) for Na.

Fe, Mn, Cu, Zn and B correlations

For Fe, Mn, Cu and Zn elements, significant relationships were determined at the level of 1% among all methods. For B, only between hot water-B (routine-B) and AB-DTPA-B extraction methods were obtained a significant relationship. Gürbüz and Günay (2014) found a similar result for the B nutrient element between routine and AB-DTPA (r= 0.300**) in a study they conducted in alkaline soils. No significant relationship was found between routine-B x Mehlich-3-B and AB-DTPA-B x Mehlich 3-B methods (NS). While between DTPA (routine) and AB-DTPA was determined a significant and high relationships at 1% level for Fe, Mn and Cu (r= 0.869**, r= 0.850** and r= 0.783**, respectively), a very high correlation was found for Zn (r= 0.998**). While all of the relationships between DTPA and AB-DTPA were high for the Fe, Mn, Cu and Zn elements, the correlation of only Cu element was high in the other two (r= 0.740** and r= 0.747**). Some researchers had also reported that the relationships between routine and AB-DTPA for Fe, Mn, Cu and Zn in alkaline soils were high (Elrashidi et al., 2003; Gürbüz and Günay, 2014).

CONCLUSIONS

In the light of the data, it was concluded that AB-DTPA extraction method could be used for macroelements (P, K and Mg), and extractable Na and microelements (Fe, Mn, Cu, Zn and B) in alkaline soils. However, Mehlich 3 multielement extraction method could also be recommended for simultaneous measurement of all these elements except for Na and B. Yet, upon careful examination, it can be considered that AB-DTPA is a better method for these soils than Mehlich 3. Further research is necessary in order to test the extractants in several soil types in Turkey and to determine other nutrients particularly when ICP is used for measurement of the quantity.

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COMPARISON THE BIOREMEDIATION PERFORMANCE OF SOME MICROCOCCUS STRAINS ON IMIDACLOPRID INSECTICIDE VIA TOTAL ORGANIC CARBON AND TURBIDITY

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ABSTRACT

The efficiency of some micrococcus strains on imidacloprid ($C_9H_{10}ClN_5O_2$) bioremediation with total organic carbon (TOC) and population dynamics of these bacterial strains revealed out during biodegradation phase under agitated culture conditions. Micrococcus sp. DR44, Micrococcus sp. HEXBA04, Micrococcus sp. Pv8, Micrococcus sp. BP3_1A, Micrococcus sp. NCTC2665 are identified with 16sRNA in Polymerase Chain Reaction (PCR) and used in this study. To prepare the bioremediation media, 1 ml of imidacloprid and 1 ml of enriched bateria (each of micrococcus strains) were added to 98 ml 0.8 % isotonic saline water. The imidacloprid was prepared in the concentration of 1600 ppm (advised concentration for farmers). Turbidity measurements were taken from imidacloprid media at 650 nm (Photolab 6600 UV-VIS Spectrophotometer) while TOC assays were performed with High Temperature Combustion Method. As a result, the highest and lowest TOC removal efficiency was determined by Micrococcus sp. BP3_1A as 94% and Micrococcus sp. HEXBA04 as 53%, respectively at the end of the 144th hour. According to the results TOC removal efficiency showed some differences depend on the bacterial species. It was also observed that Micrococcus sp. BP3_1A can be a suitable bacteria for bioremediation of receiving environments contaminated by imidacloprid.

Keywords: Imidacloprid, Bioremediation, Total Organic Carbon, Turbidity

INTRODUCTION

The environmental contamination caused by the presence and accumulation of pesticide residues in soil, in addition to surface and ground waters should be assessed (Yonten et al., 2011). Water pollution by pesticides is considered as a pervasive problem, since these compounds affect the living organisms adversely (Kolpin et al., 1996). Some organisms have pesticide remediation ability which is primarily based on their biodegradation activity levels, although bioremediation was initially achieved with bacteria or fungi (Fernandez et al., 2012).

Biodegradation is necessary for remediation of pesticide residues in soil and in underground waters.The microorganisms that are required for such process are present in the soil (Vaccari et al., 2006). Study on the remediation of the active substance Ethior in anaerobic conditions by mesophilic bacteria isolated from soil polluted by pesticides suggests that the bacteria Pseudomonas and Azospirillum achieve effective remediation (Foster et al., 2004).

There are no studies into the degradation of imidcloprid using micrococcus strains, the majority of which identified low-efficiency degradation, and identified that the degradation of metabolites is more persistent and toxic than that of the parent compound (Siddique et al., 2003). Many microorganisms which are able to degrade imidacloprid, including bacteria and have been isolated from different sources (Singh and Singh 2011). In this study, bacteria which

have been isolated from an agricultural area with no previous exposure to imidacloprid were used to measure microbial degradation.

The aim of this study is to evaluating the efficiency of some micrococcus strains to degrade the suggested concentration of insecticide imidacloprid ($C_9H_{10}ClN_5O_2$) under agitated (160 rpm) submerged culture conditions (1600ppm) via remediation of total organic carbon (TOC) and reveal out the degradation time via the turbidity measurements to give a suggestions to scientists who try to eliminate the opposite effects of these kind of pesticides from receiving environments.

MATERIAL AND METHODS

Isolation and Identification of Bacteria

The bacteria were isolated from collected soil samples in agricultural area about 0-15 cm of depth in tomato farming area in Kirklareli province of Turkey and this agricultrural area is unexposed to imidacloprid before (Figure 1). Characteristic properties of agricultural soil is given in Table 1. About 10 grams of these collected soil samples are diluted in isotonic water containing 0.8% sodium chloride and 0.1 ml of diluted samples were poured to plate count agar plates in steriline conditions. These plates were taken to bacteriological indicator at 25^0 about 5-6 days. After the growing phase of the bacteria completed, these bacteria were sent for identification process.

Table 1. Properties of the studied agricultural field (Adapted from Erguven et al., 2016)

Parameters	**Unit**	**Methods**	**Resuts**	**Evaluation**
pH		Richards (1954)	7.6	Mild alkali
Lime		Tuzuner (1990)	16.6	Unsalted
Salt		Richards (1954)	0.05	Cayed loamy
Saturation		Tuzuner (1990)	64	Moderate
Organic material	%	Walkley and Black (1934)	2.1	Moderate
Total Nitrogen		Bremner (1965)	0.1	Moderate
Cation exchange capacity		Tuzuner (1990)	31.5	Sufficient
Silt		Bouyoucos (1962)	5	Low
Sand			32	Sufficient
Humidity			21	Sufficient

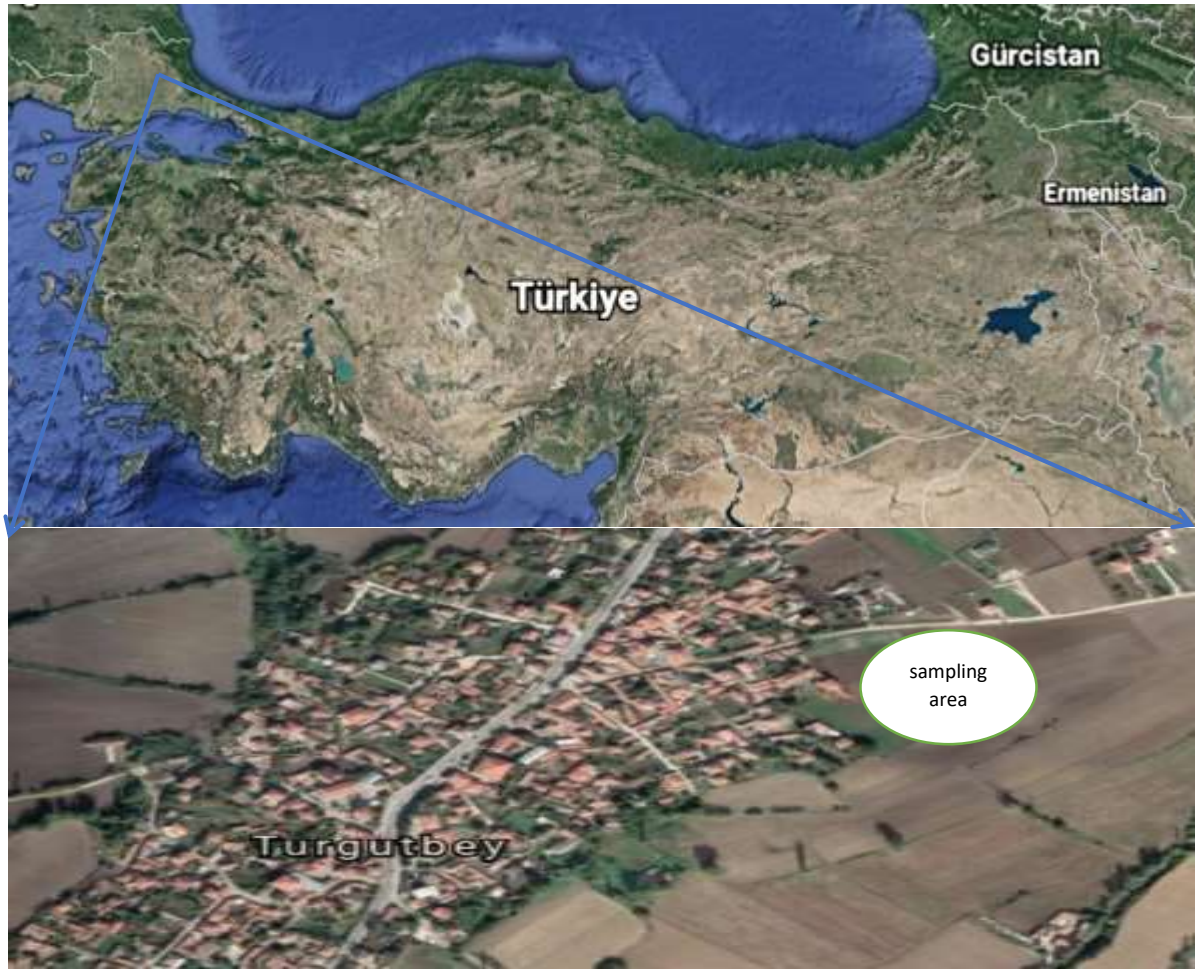

Fig.1. Soil sampling area

Molecular characterization

The Deoxyribonucleic Acid (DNA) obtained were stored at −20^{0}C. The Polymerase Chain Reaction (PCR) method was applied and thermal cyclers were used to reproduce 16S rRNA genes of DNA of them. The mixtures, which were followed by identification of methanogenic species and analysis of Denaturing Gradient Gel Electrophoresis (DGGE) sequences. Isolating the Genomic DNA from gram positive and Gram Negative Bacteria was studied. To turn them into a single colony, streak plate plantation method was applied to homogenize the colonies marked in petri dishes. Phire Hot Start II DNA Polymerase was utilized to obtain bacterial 16S ribosomal general primers and (1000– 3000 bp) of PCR bands of various lengths were taken. Identification of bacteria isolated was carried out in line with 16S RNA Universal Primers were as; 16S rRNA for AGA GTT TGA TCC TGG CTC AG, while 16S rRNA Rev ACG GCT ACC TTG TTA CGA CTT.

Microbial biodegradation studies

To gain knowledge about the degradation ratio of the imidacloprid from the micrococcus strains, firstly, enriched bacterial cultures were grown with shaker controlled at 25°C through the enrichment techniques in broth media. In this enriched media, total organic carbon (TOC) measurement was carried out in 24-hour intervals. These monitoring stopped when the enriched media's TOC value is equal to the 1600 ppm of imidacloprid value. The TOC assays were done according the high temperature burning method dentified in the Standard Method 5310A with

TEKMAR - DOHRMANN - Apollo 9000 device (APHA 2009) and after the end of the fifth day, 1 ml of enriched cultures were taken and added into liquid media with 1600 ppm of imidacloprid solution, and bioremediation studies were started at 160 rpm. The bioremediation studies were carried out in 24 hours intervals. Every 24 hours, TOC assays were calculated via the turbidity measurements

RESULTS

Monitoring microbial activity and TOC reduction of Imidacloprid in liquid media through turbidity

The results of the turbidity study conducted with Micrococcus sp. DR44, Micrococcus sp. HEXBA04, Micrococcus sp. Pv8, Micrococcus sp. BP3_1A, Micrococcus sp. NCTC2665 in imidacloprid media related with TOC reduction are given in Figures 2-6, respectively.

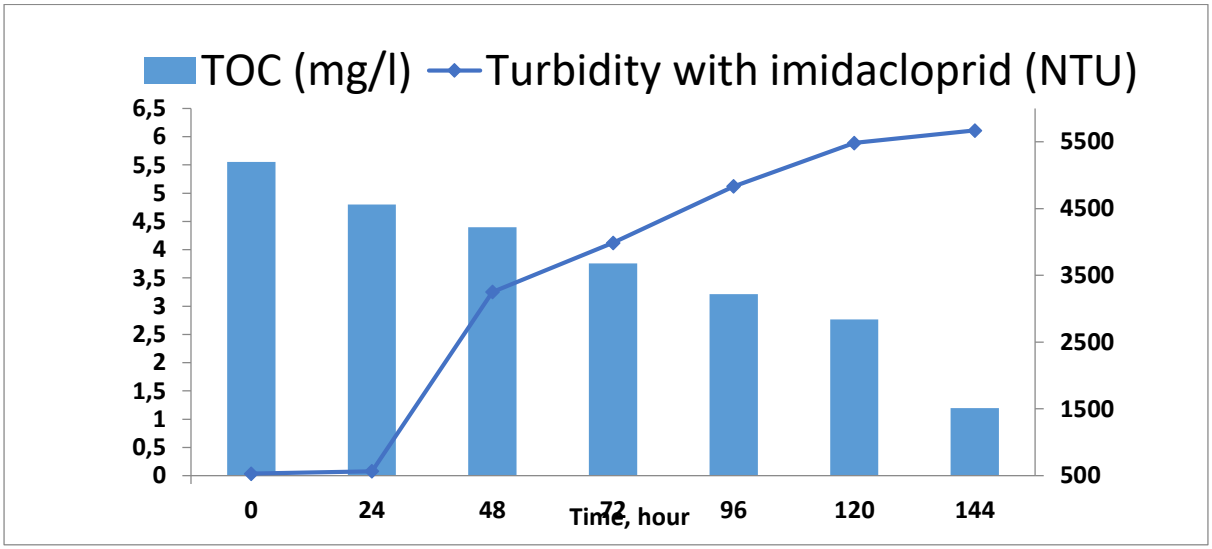

Figure 2. Reduction of TOC of imidacloprid via Micrococcus Sp. Dr44 related with turbidity

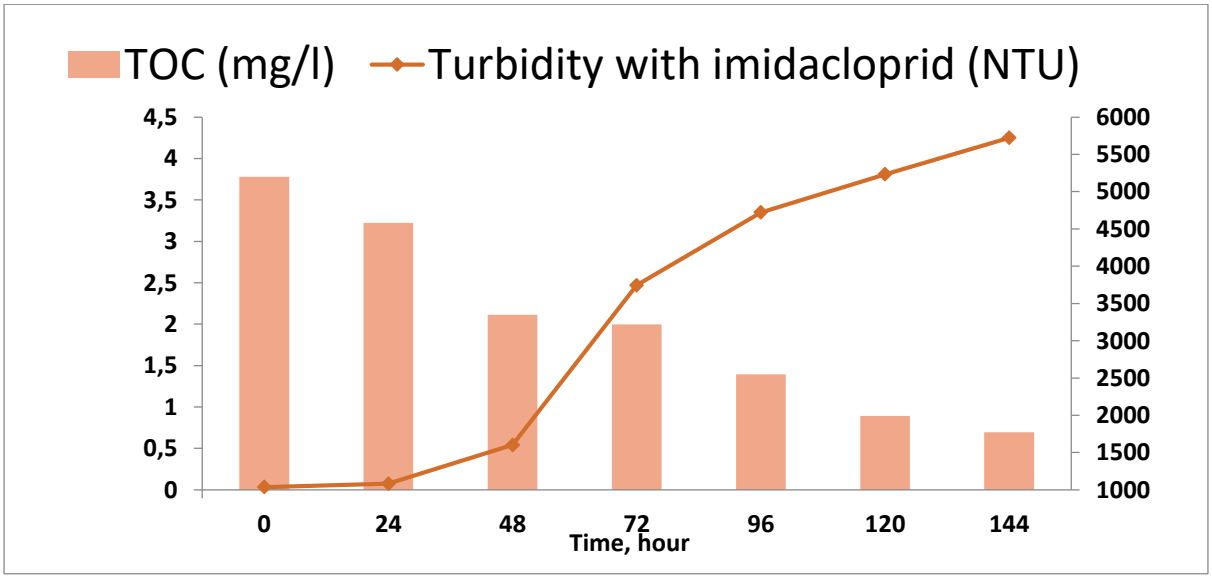

Figure 3. Reduction of TOC of imidacloprid via Micrococcu sp. HEXBA04 related with turbidity

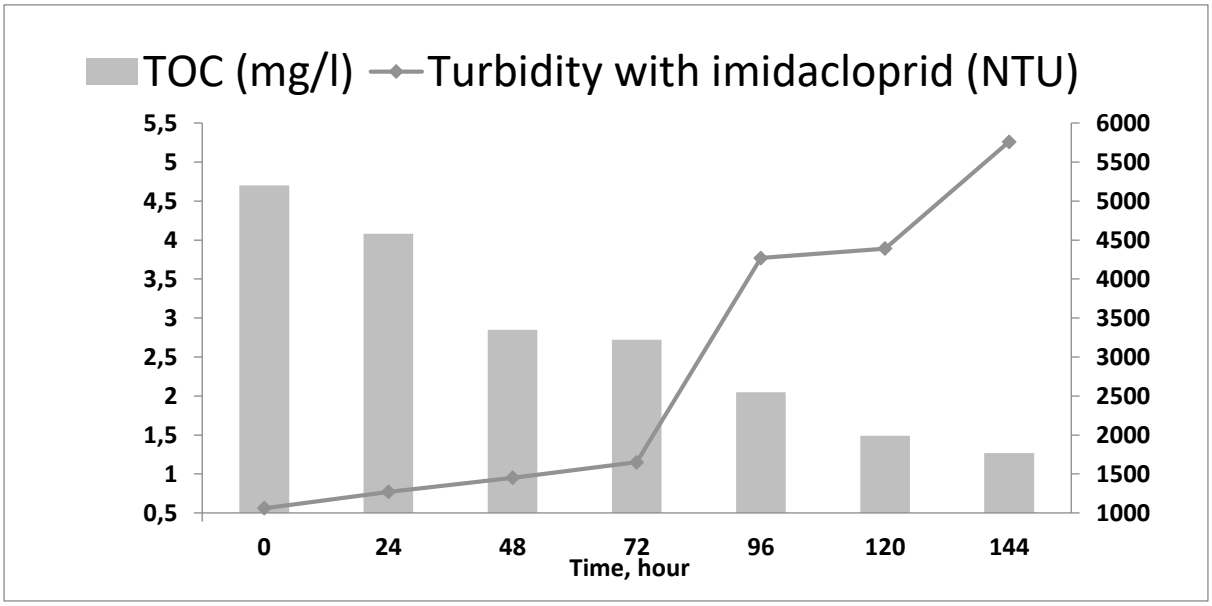

Figure 4. Reduction of Toc of Imidacloprid via Microroccus Sp. Pv8 Related with Turbidity

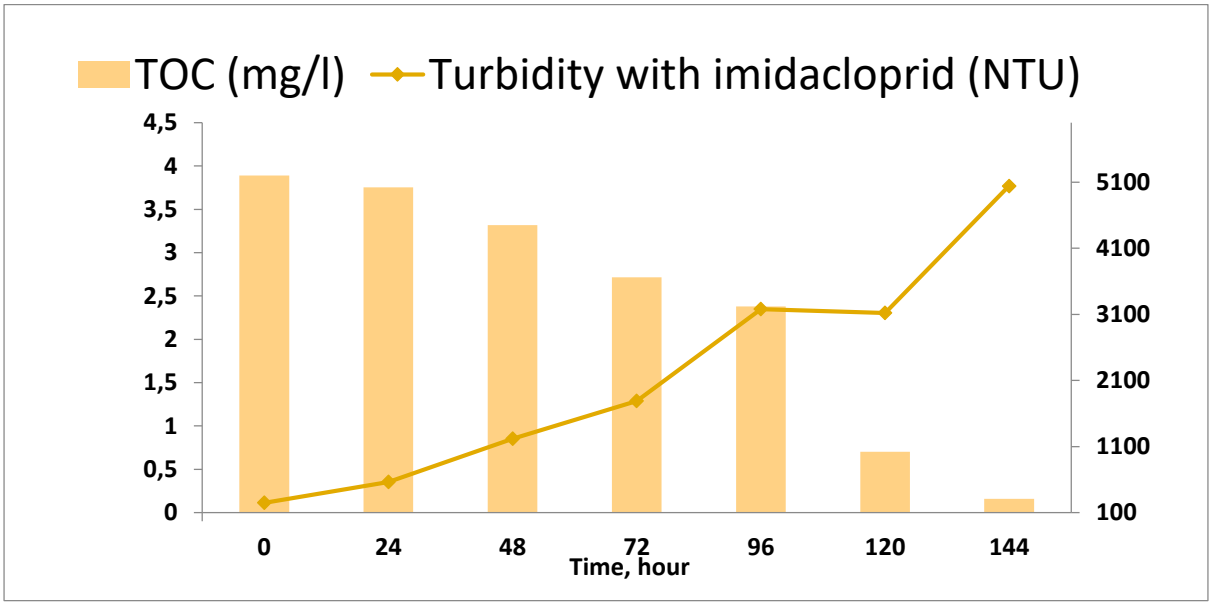

Figure 5. Reduction of Toc of Imidacloprid via Micrococcus sp. BP3_1a Related with Turbidity

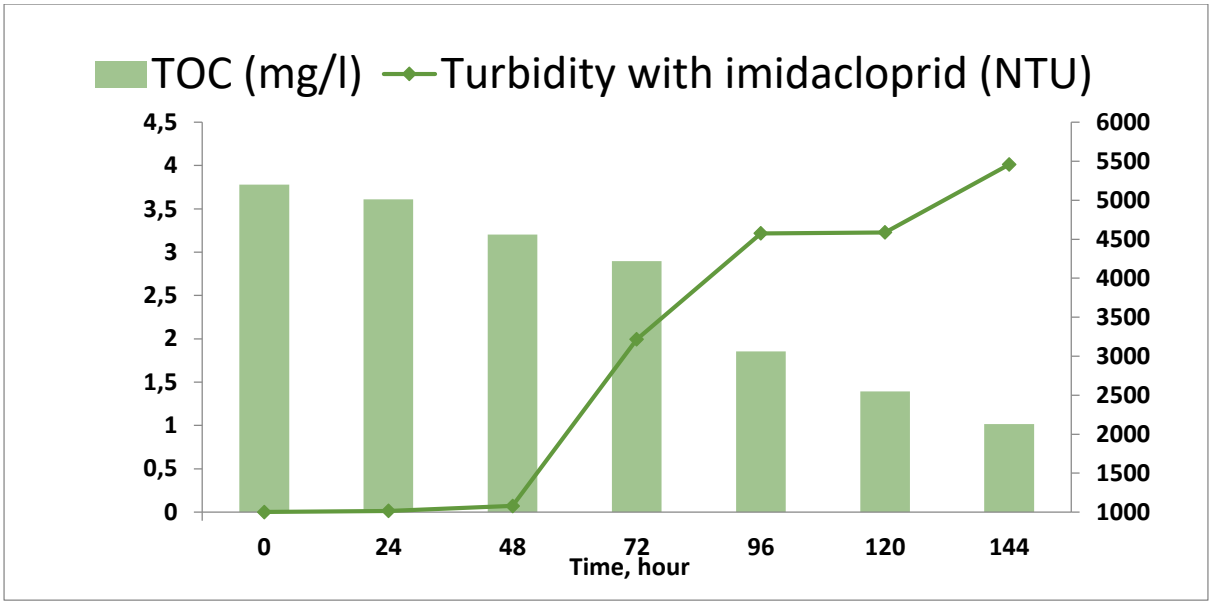

Figure 6. Reduction of Toc of Imidacloprid via Mıcrococcus sp NCTC2665 Related with Turbidity

In the media with imidacloprid, TOC reduction rates have showed different results depend on differences in species of bacterial strains. The TOC reduction efficiencies of Micrococcus sp. DR44, Micrococcus sp. HEXBA04, Micrococcus sp. Pv8, Micrococcus sp. BP3_1A, Micrococcus sp. NCTC2665 species were 71%, 53%, 66%, 94% and 59% respectively in 144th hours.

At the end of the 6th day, there were negligible changes. According to these results, the highest TOC removal level was achieved by Micrococcus sp. BP3_1A as 94%. At the end of this time period, approximately 5200 mg/l TOC of imidacloprid was reduced to 310 mg/l. The lowest TOC removal efficiency was seen by Micrococcus sp. HEXBA04 as 53% This means 5200 mg/l to 2440 mg/l

DISCUSSION

Biological degradation is the frequently used method for the remediation of pesticides in receiving environments. The experiments results have shown that levels of biodegradation depend on the removal of imidacloprid residuals. Biodegradation/bioremediation is a theoretically alternative process that does not result in toxic final chemicals (Massiha et al., 2011). Different removal efficiency in the submerged culture media have showed different results depend on differences in bacterial species (Erguven, 2018). Insecticide amounts remaining in agricultural fields are due to how they are retained by the soil components. This situation can be explained by the properties of the receiving environment, such as soil water content and the application period of pesticides (Arias-Esteves et al., 2008).

Pesticides differ in chemical composition and react differently when incorporated into the agricultural soils or rivers of other aquatic media due to differences in their chemical properties and interactions with soil components and environmental factors. Microbial bioremediation has been shown to be an important pathway leading to the dissipation of imidacloprid residuals in receiving bodies. Bioremediation was high in the experiments; however, Vieno and Toivikko (2014) studied some micropollutants, including Aclonifen, in a conventional biological and tertiary wastewater treatment plant and found that some environmentally harmful molecules are still present at important values after treatment.

Erguven and Demirci (2019) studied the bioremediation performance of *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* bacteria and their consortia to reduce the pesticide imidacloprid. At the end of two weeks period days, they found full reduction rates for imidacloprid active material for each bacterium and their mixtures, while COD reduction rates were 97% and 96% for all types of bacteria. They also investigated TOC and BOD_5 removal rates. Nearly 97% reduction was seen for both types and their consortia in the same time period. Erguven (2018) observed that removal rates of acetochlor in agitated culture media obtained by *M. owariensis, M. cylindrospor*ae and *V.chlamydosporium* were 70%, 55% and 50% as TOC reduction. Yanga et al. (2014) studied a bacterial strain with the ability to utilize chlorimuron-ethyl as the carbon source in phosphate-basal minimal medium cultures. They found this pesticide was provided as the sole carbon source, the increase of growth rate of microbial strain accompanied with the degradation of chlorimuronethyl, and more than 95% of herbicide at an initial concentration of 50 mg l^{-1} was degraded at the end of a 4th day. Erguven et al. (2016) prepared five different units with the soil samples taken from the Thrace Region and added 1900 μg l^{-1} aclonifen to each of them. According to the results, the highest bioremediation ratio was observed in the soil sample to which 10 ml of mixed culture of microorganisms was added and cclonifen, COD, BOD_5, and TOC remediation was observed as 93%, 98%, 99%, and again 99%, respectively at the end of the 5th week.

CONCLUSIONS

Bioremediation can be an effective method to handle environment contamination with imidacloprid or this kind of insecticide. There are many sources of microorganisms with the ability to biodegrade persistent organic pollutants. Since pesticides are mainly applied to agricultural crops, soil is the medium that mostly holds these chemicals, besides wastewater, natural waters, sediments, and areas surrounding the manufacture of the pesticides, and they are also present in live organisms. The access more knowledge about degradation of persistent organic pollutants, it is better to isolate and identify some agricultural soil bacteria or fungi and try to adapt them to the biodegradation process rapidly. Acording to the results of this study, some micrococcus strains showed a good reduction performance for imidacloprid insecticide.

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DEVELOPMENT AND CHARACTERIZATION OF ENTERIC-COATED PECTIN BEADS CONTAINING PREGABALIN

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ABSTRACT

The oral route is the most popular and most convenient way in the administration of drugs. There are many polymeric biomaterials such as naturally sourced proteins and polysaccharides used in oral drug delivery systems. The use of pectin, one of them, is highly interesting as a carrier for many active substances in order to achieve controlled release in pharmaceutical applications. Pregabalin (PGB), a member of the gamma-aminobutyric acid class, is considered one of the first drugs to come to mind for the treatment of neuropathic pain (NA). PGB has been proven to be effective for both central and peripheral NA and provide rapid pain relief. However, increasing the amount of dose in daily intake has been associated with the formation of stomach ulcers. For this purpose, in our study, enteric-coating of pectin beads containing PGB was carried out with two different techniques, and thus it was aimed to prevent stomach ailments, especially stomach ulcers that may develop due to PGB dosing. In this context, PGB's quantification method was developed and validated, bead formulations were developed and enteric coatings were performed. Subsequently, in-vitro characterization studies were carried out. As a result, the coating thickness, high encapsulation efficiency (77%) of the E6 coded formulation we developed was found to be quite satisfactory. It may also be an alternative carrier system in the future for patients who experience stomach complaints due to PGB use.
Keywords: *Pregabalin, Pectin, Beads, Enteric-Coating, Neuropathic Pain, Stomach Ulcers.*

INTRODUCTION

NA is defined as “pain initiated or caused by a primary lesion or dysfunction in the nervous system”. The characteristic abnormality in NA is a lesion or dysfunction of the sensory pathway with hyperexcitability of the nervous region. It causes depression, fatigue, anxiety, sleep disorders, and a decrease in general physical functions accompanied by prolonged pain (Dongre and Swami, 2013).

PGB, a member of the gamma-aminobutyric acid class, is considered one of the first drugs that come to mind for the treatment of NA. It has been proven that PGB is effective for both central and peripheral NA and provides rapid pain relief. Pain reduction in the first week after treatment can be achieved with a dose of 150 to 600 mg/day (Dongre and Swami, 2013). However, it was found that there was a significant increase in stomach ulcers with increasing the daily dose (Kaygisiz et al., 2020).

The oral route is the most popular and most convenient way in the administration of drugs. Therefore, new carrier systems are being developed for the oral delivery of drugs. There are many polymeric biomaterials such as naturally sourced proteins and polysaccharides used in oral drug delivery systems. These natural biomaterials have advantages such as safe, stable, non-toxic, renewable, ease of processing, and abundant in nature (Veronovski et al., 2014).

Pectin, one of these polymers, is used as a carrier for many active substances in order to provide controlled release in pharmaceutical applications (Sriamornsak, 2003). Pectin is a water-

soluble ionic polysaccharide found naturally in the cell walls of many plants and contains α-D-galacturonic acid in straight chains (Sriamornsak, 1998; Alvarez-Lorenzo et al., 2013; Sriamornsak and Nunthanid, 1998). It is resistant to protease and amylase enzymes active in the upper parts of the gastrointestinal tract and is digested in the lower parts of the gastrointestinal tract. Therefore, it can be used orally to the colon as a drug carrier (Veronovski et al., 2014).

There are many techniques for drug delivery systems prepared with pectin. In particular, the ionotropic gelation technique is often used. The reliability of this technique is quite high in terms of toxicity (Sriamornsak, 2003). In the ionotropic gelation technique, polysaccharides (alginate, gellan, and pectin) are dissolved in water or in a weakly acidic medium. Then they are dropped into the solution containing the opposite charged ion. Solvent toxicity and other undesirable effects are prevented by recyclable cross-linking as a result of electrostatic interaction (Racovita et al., 2009).

Kollicoat® MAE 100P; Methacrylic acid: ethyl acrylate (1: 1) copolymer is a reliable and high-performance enteric-coating agent registered in American Pharmacopoeia (USP) and European Pharmacopoeia (Ph. Eur.). It has been partially neutralized to redisperse quickly and is easily dispersible in an aqueous environment. While pH does not dissolve at 1.2, it dissolves above pH 5.5 and initiates the active substance release (Martell et al., 2009).

In this study, the enteric-coating of pectin beads containing PGB with two different techniques was carried out, in this way, it was aimed to prevent stomach disorders, especially stomach ulcers, which may develop due to the dosing of PGB. It will also be possible to prolong drug release as the pectin is resistant to enzymatic activities in the upper parts of the gastrointestinal tract and begins to dissolve in the lower parts of the gastrointestinal tract. In this way, especially in patients suffering from neuropathic pain, stomach complaints will be reduced and patient compliance will be increased with less dosing.

Material and Methods

Development of PGB quantitation method

Modifications have been made and re-developed on the assay method developed by Mohan et al. The standard solution of PGB was prepared in ultrapure water at a concentration of 200 µg/mL and a calibration curve and equation were obtained by making the necessary dilutions (Mohan et al., 2014). For HPLC was used the temperature adjust to 25 °C RP-C18 column (Sliachram® Slicyle, 150 x 4.6 mm) and Phosphate Buffer (pH adjusted to 6.5 with potassium hydroxide) :MeCN: ACN (75:10:15) as mobile phase. PGB analysis was performed at 197 nm using a 0.7 mL/min flow rate, 20 µL injection volume, and PDA detector.

Validation of the quantification method

Analytical method validation is to test the validity and reliability of the method to be used in order to achieve the targeted results under specified conditions. For this purpose, criteria such as linearity, accuracy, precision, sensitivity (LOQ and LOD), selectivity (specificity) were examined in the validation of the analytical method (Sahbir, 2003; Sreekanth et al., 2017; Mohan et al., 2014, Srivastava and Kumar, 2017).

Development of pectin bead formulations containing and non-containing PGB

Ionotropic gelation method was used in the preparation of beads containing and non-containing PGB (Veronovski et al., 2014; Sriamornsak, 1998; Sriamornsak and Nunthanid, 1999; Lohani, et al., 2016). For this purpose, different concentrations of pectin were homogeneously dispersed in pure water at 750 rpm on a multi-point magnetic stirrer for 30 minutes. PGB was

added to this dispersion in determined amounts (such as 5, 10 mg) and completely dissolved. The prepared containing PGB dispersions were dropped into $CaCl_2$ solution (such as 0.1M, 0.2M, and 0.3M) by an insulin injector from a fixed height. The gel-like beads were formed and mixed for a while on their own to complete the complexation. After the beads were formed, filtration was applied and the beads were collected (min. n=3). The collected beads were washed several times with ultrapure water and then kept in the -20 °C overnight. They were then lyophilized for one day and stored in a desiccator at room temperature for further studies. The preparation of non-containing PGB beads (blank beads) was also carried out without the addition of PGB as described above.

Enteric-coating of beads containing and non-containing PGB

Two different methods were tried and the coating studies of the beads were made. The first is to throw fresh (non-lyophilized) beads into the coating solution, and the second is to drop dry lyophilized beads into the coating solution. In order to accomplish the first, freshly prepared and filtered pectin beads containing and non-containing PGB were added to Kollicoat® MAE 100P solution prepared in two different doses of ethanol (5% and 10%, w/v) and mixed for 10 minutes at 750 rpm. Then, filtration was applied again and the beads were kept in the -20 °C overnight then lyophilized for 24 hours (min. n=3). Lyophilized beads kept in the desiccator at room temperature for further studies. In the second method, lyophilized pectin beads containing and non-containing PGB were added to Kollicoat® MAE 100P solution prepared in two different doses of ethanol (5% and 10%, w/v) and mixed for 10 minutes at 750 rpm. Afterward, the filtration was applied again and the beads were dried in the oven for a while and kept in the desiccator at room temperature for further studies (min. n=3) (Ali et al., 2014; Sun et al., 2014). In order to determine the color of the coatings, 0.01% w/v FD&C Red No:3 was added to the coating solution.

Determination of coating efficiency

The determination of the efficiency of the enteric coatings of the prepared beads was carried out using simulated gastric fluid (pH 1,2 HCl buffer) (USP30-NF25). A certain amount of beads from the formulations were weighed and placed in vials containing 10 mL of pH 1,2 HCl buffer and study carried out for 2 hours in a horizontal shaker water bath set at 37 °C/50 rpm. At the end of the period, the supernatants of all samples were taken and filtered through a 0,45 µm membrane filter. PGB quantity was determined by the HPLC method, which was validated (min. n=3) (Anwer et al., 2017; Ahmed et al., 2014).

Determination of drug loading capacity and encapsulation efficiency

In order to determine the amount of PGB in the content of pectin beads containing enteric-coated PGB, a certain amount of beads were weighed and placed in vials containing 7 mL of the organic mixture (5 mL mobile phase + 2 mL ethanol). At 750 rpm, the beads were mixed until the beads were completely disintegrated in the magnetic stirrer, and subsequently, all the supernatants were filtered through a 0.45 µm membrane filter and PGB quantities determined using the validated HPLC method, which (min. n=3). Dilutions were made with the mobile phase. The following equations were used in the calculation of drug loading capacity and encapsulation efficiency of enteric-coated beads containing PGB (Govender et al., 1999; Su et al., 2009)

$$\textbf{Drug Loading}\ \% = \frac{\textbf{Theoretical PG Amount} - \textbf{Practical PG Amount}}{\textbf{Amount of Obtained Floating Drug Delivery System}}\ \boldsymbol{x}\ \mathbf{100}$$

$$\textbf{Encapsulation Efficiency}\ \% = \frac{\textbf{Theoretical PG Amount} - \textbf{Practical PG Amount}}{\textbf{Theoretical PG Amount}}\ \boldsymbol{x}\ \mathbf{100}$$

Morphological and organoleptic control of bead formulations

All enteric-coated and uncoated bead formulations containing and non-containing PGB were visually evaluated in terms of their features such as shape, size, size distribution, and color. For this purpose, a certain number of samples were taken from freshly prepared and lyophilized beads dimensions were measured with a caliper (min. n=30). Results are given as mean and standard deviation. In addition, digital photographs of freshly prepared and lyophilized beads were taken.

Surface and structural morphology

The morphology and surface properties of lyophilized (containing and non-containing PGB) beads, which were enteric-coated by both methods, were examined with the "Zeiss Sigma 300" scanning electron microscope. Since the samples are non-conductive that approximately 100 Å thick gold was coated before measurement.

Fourier transform infrared spectroscopy (FT-IR)

It is a method used to explain the composition of the chemical structure and bond arrangements of substances (Sunil et al., 2012). In order to examine the interactions of pure substances, polymer, and prepared bead formulations with each other, measurements were made with "Bruker VERTEX 70v" brand FT-IR (ATR) spectrophotometer in the range of 4000-400 cm-1 wavenumber.

Results

Development of PGB quantitation method

The quantitation method was drawn from 11 points (1, 5, 10, 25, 50, 75, 100, 125, 150, 175, 200 µg / mL) to increase the sensitivity, and R2 was found as 0.9999. The calibration curve and equation are given in Figure 1.

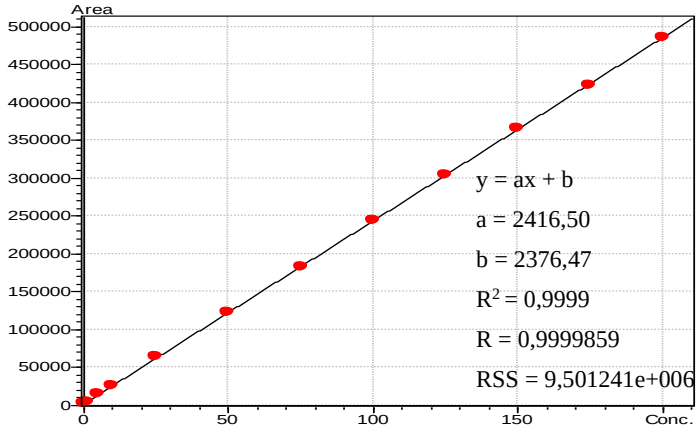


Figure 1. Calibration curve and equation

Validation of the quantification method

The method developed showed linearity between 0.3 µg/mL pregabalin dose and 200 µg/mL dose. It has been determined that all formulation components without PGB do not peak at the same place as PGB and the method is selective for PGB. Experimentally LOD was determined as 0.3 µg/mL and LOQ was determined as 1 µg/mL. As a result of the intra-day and inter-day (repeatability and reproducibility) studies, it has been determined that % relative standard deviation (RSD%) and % relative error (RE%) are below 2%.

Development of pectin bead formulations containing and non-containing PGB

Different combinations were tried by changing various formulation parameters. Formulation components and rates are given in Table 1 below. Formulations were selected based on quantification, coating efficiency, and yield.

Table 1. Formulation components and proportions

	PGB (mg)	**Pectin (mg)**	**$CaCl_2$**	**Kollicoat®**	**Drug Phase:Aqueous Phase: Coating Phase Ratio**
E1	5	20	0.1 M	%5	1:5:10
E2	5	20	0.2 M	%5	1:5:10
E3	5	20	0.3 M	%5	1:5:10
E4	5	20	0.1 M	%10	1:5:10
E5	5	20	0.2 M	%10	1:5:10
E6	5	20	0.3 M	%10	1:5:10
Boş-E1	-	20	0.1 M	%5	1:5:10
Boş-E2	-	20	0.2 M	%5	1:5:10
Boş-E3	-	20	0.3 M	%5	1:5:10
Boş-E4	-	20	0.1 M	%10	1:5:10
Boş-E5	-	20	0.2 M	%10	1:5:10
Boş-E6	-	20	0.3 M	%10	1:5:10

Enteric-coating of beads containing and non-containing PGB

All formulations were coated with ethanol solution containing 5% and 10% Kollicoat®. Digital images of formulations before and after coating are given in Figure 2..

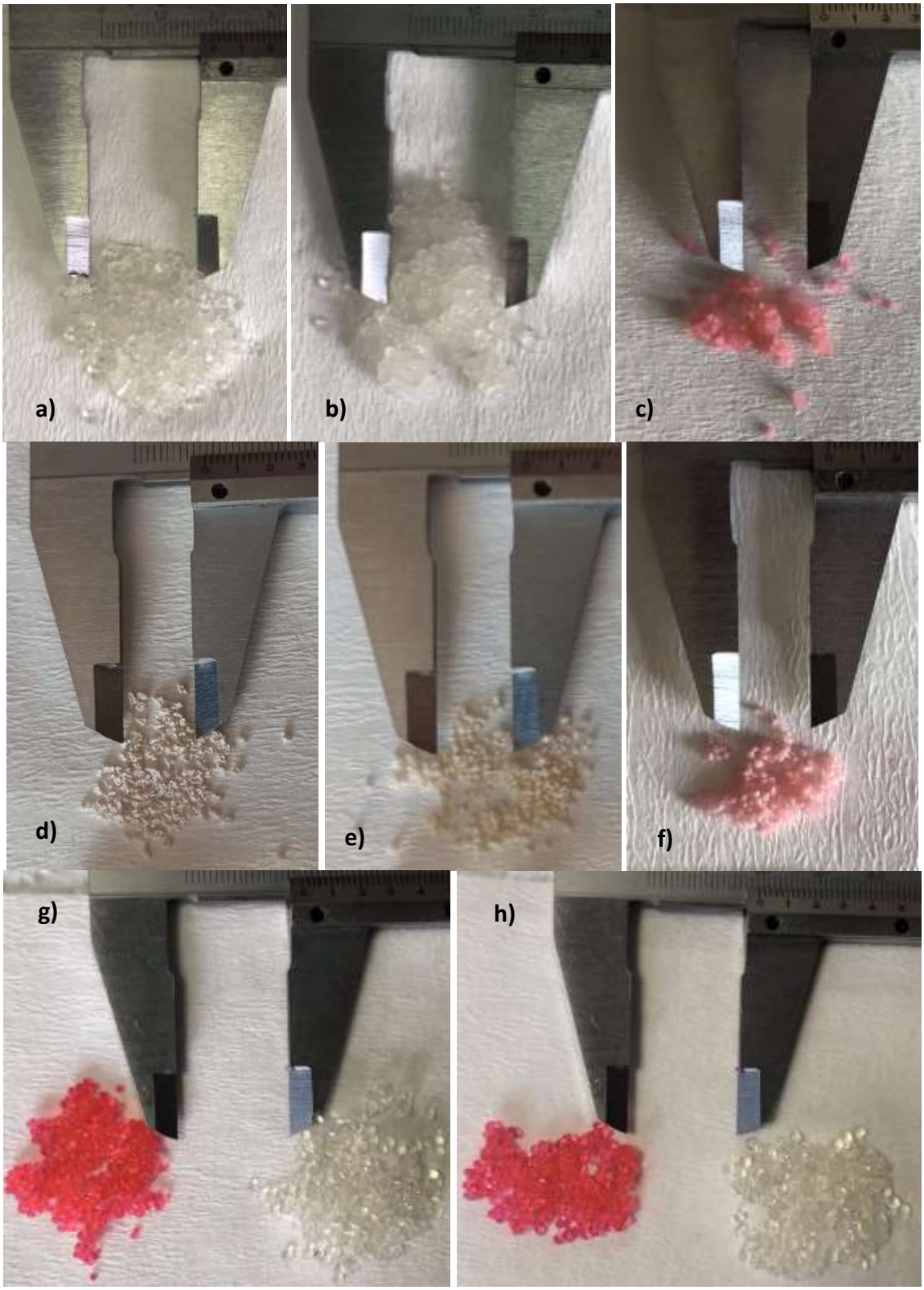


Figure 2. a) freshly prepared blank pectin beads b) freshly prepared pectin beads containing PGB c) after lyophilization enteric-coated blank pectin beads, d) lyophilized blank pectin beads e) lyophilized pectin beads containing PGB f) after lyophilization enteric-coated pectin beads containing PGB, g) enteric coating of freshly prepared blank pectin beads, h) enteric coating of freshly prepared pectin beads containing PGB.

Determination of coating efficiency

The amounts of PGB released at the end of 2 hours in pH 1.2 HCl simulated gastric fluid are given in % in Table 2 below. According to these results, the formulations showing the least PGB release have been E3 and E6.

Table 2. Coating efficiency results of formulations

Formulations	PGB (%)
E1	0,23678
E2	0,19054
E3	0,18918
E4	0,20788
E5	0,23526
E6	0,15312

Determination of drug loading capacity and encapsulation efficiency

The drug loading capacity and encapsulation efficacy data of the selected formulations are given in Table 3 as mean and standard deviation.

Table 3. Results of encapsulation efficiency and drug loading capacity of formulations

Formulations	% EE	% DL
E3	51.18±9.82	6.71±0.38
E6	77.19±11.88	7.14±0.11
Lyo-E3	69.38±18.67	4.11±0.28
Lyo-E6	78.15±10.81	4.04±0.11

Morphological and organoleptic control of bead formulations

All formulations were subjected to organoleptic control as a result of formulation developing studies. At this stage, both their general appearances were checked and their dimensions are given in Table 4 as mean and standard deviation. Digital photographs of E3-E6, which are the best results from coating efficiency and quantification, and after lyophilization enteric-coated pectin beads containing PGB (Lyo-E3, Lyo-E6) are given in Figure 3.

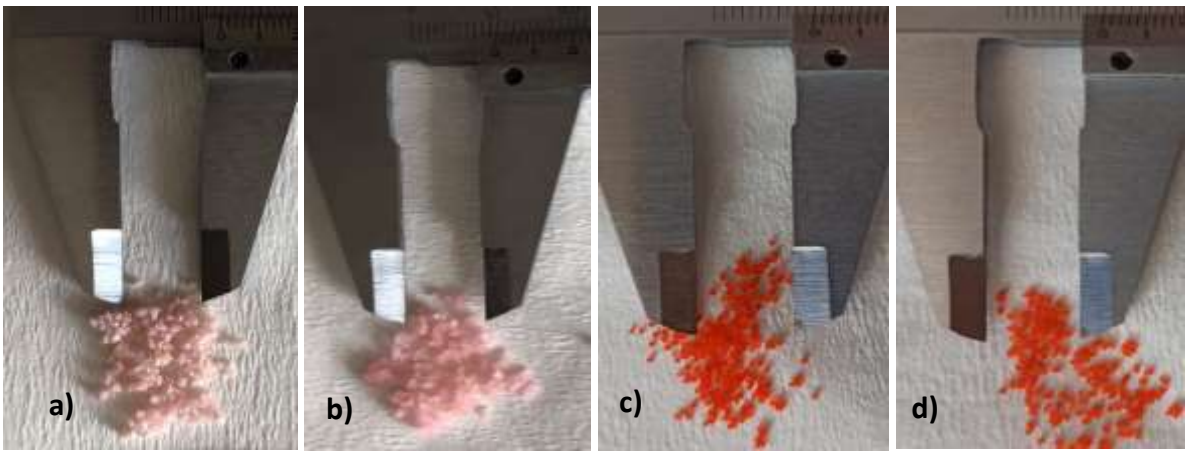


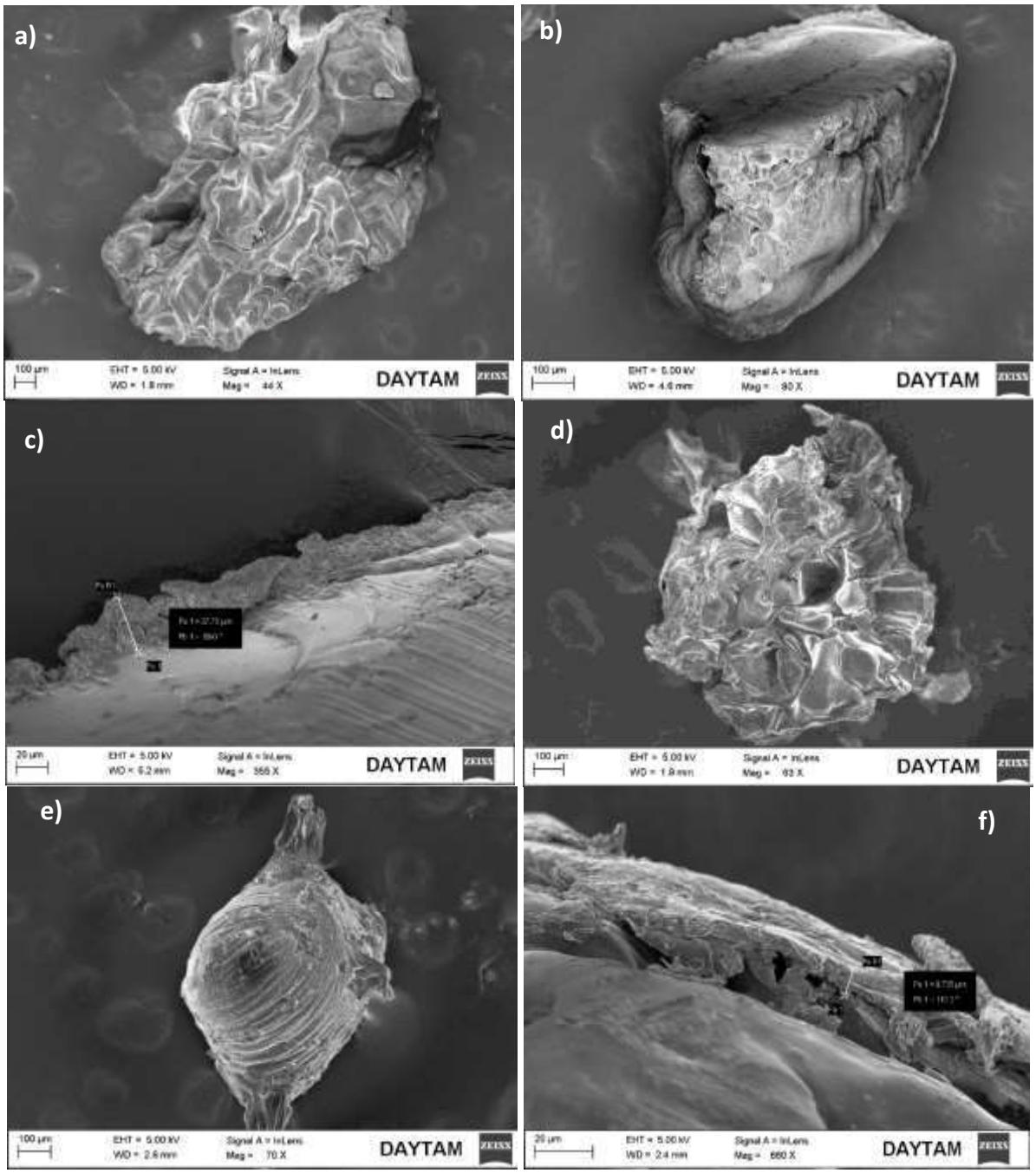
Figure 3. a) Lyo-E3 b) Lyo-E6 c) E3, d) E6

Table 4. Sizes of developed formulations (mean ± SD)

	Blank-E3	Blank-E6	E3	E6	Blank-Lyo-E3	Blank-Lyo-E6	Lyo-E3	Lyo-E6
Size (mm)	0.63± 0.11	0.86± 0.13	0.80± 0.08	1.58± 0.19	1.25± 0.12	1.50± 0.21	1.36± 0.15	1.60± 0.14

Surface and structural morphology

The SEM images of freshly prepared and enteric-coated E3 and E6 and their blank beads (without PGB) are given below. Also, the SEM images of Lyo-E3 and Lyo E6 and their blank beads (without PGB) are given in Figure 4 below. In this way, the thicknesses of the enteric coating and the difference of the coating between formulations were observed.



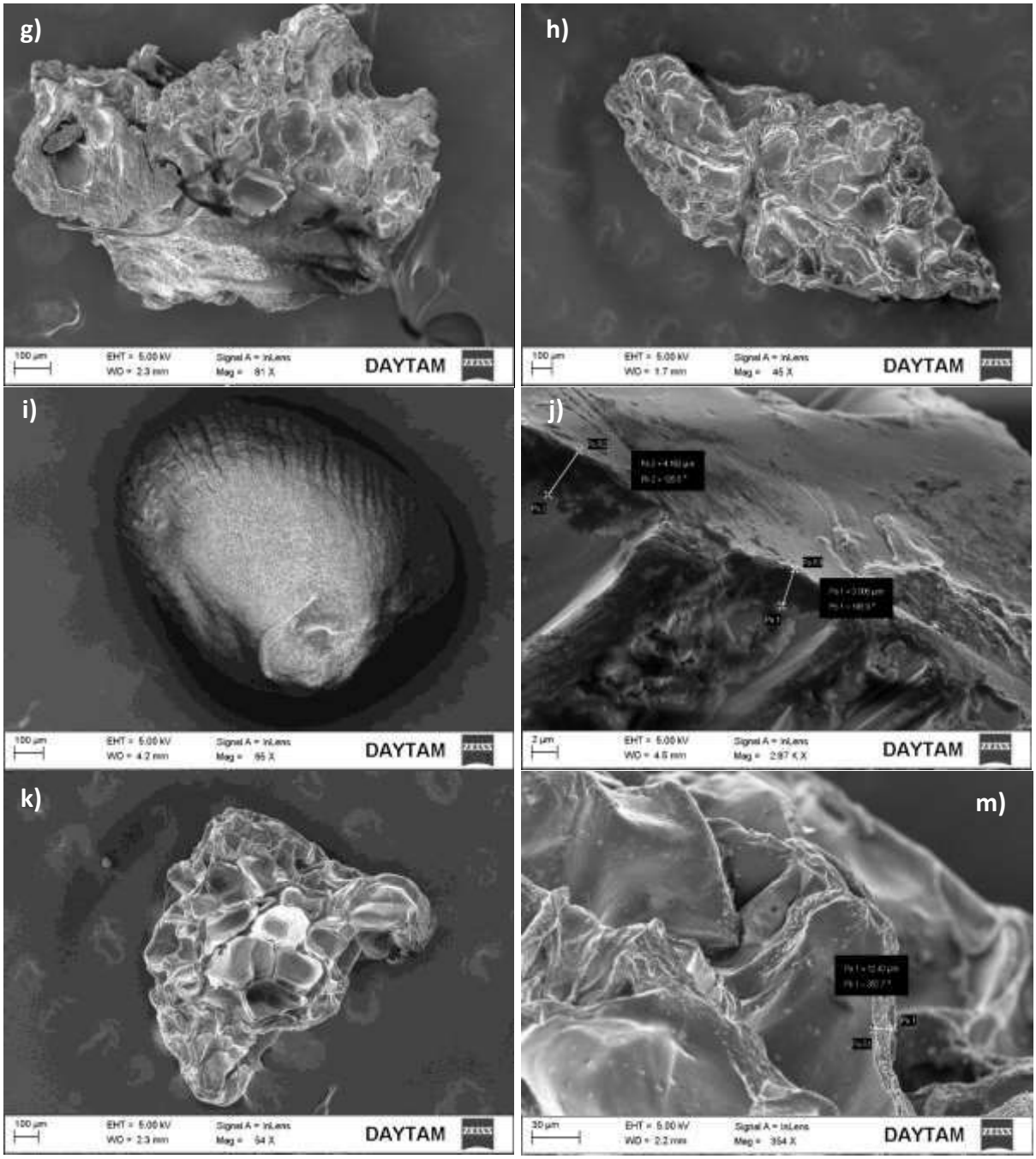


Figure 4. a) Blank-E3, b) E3, c) E3 coating thickness, d) Lyo-E3, e) Blank-E6, f) Lyo-E3 coating thickness, g) Blank-Lyo-E3, h) Blank-Lyo-E6, i) E6, j) E6 coating thickness, k) Lyo-E6, m) Lyo-E6 coating thickness

Fourier transform infrared spectroscopy (FT-IR)

The spectra of the formulations overlapped with pure PGB and no interaction was observed (Figure 5).

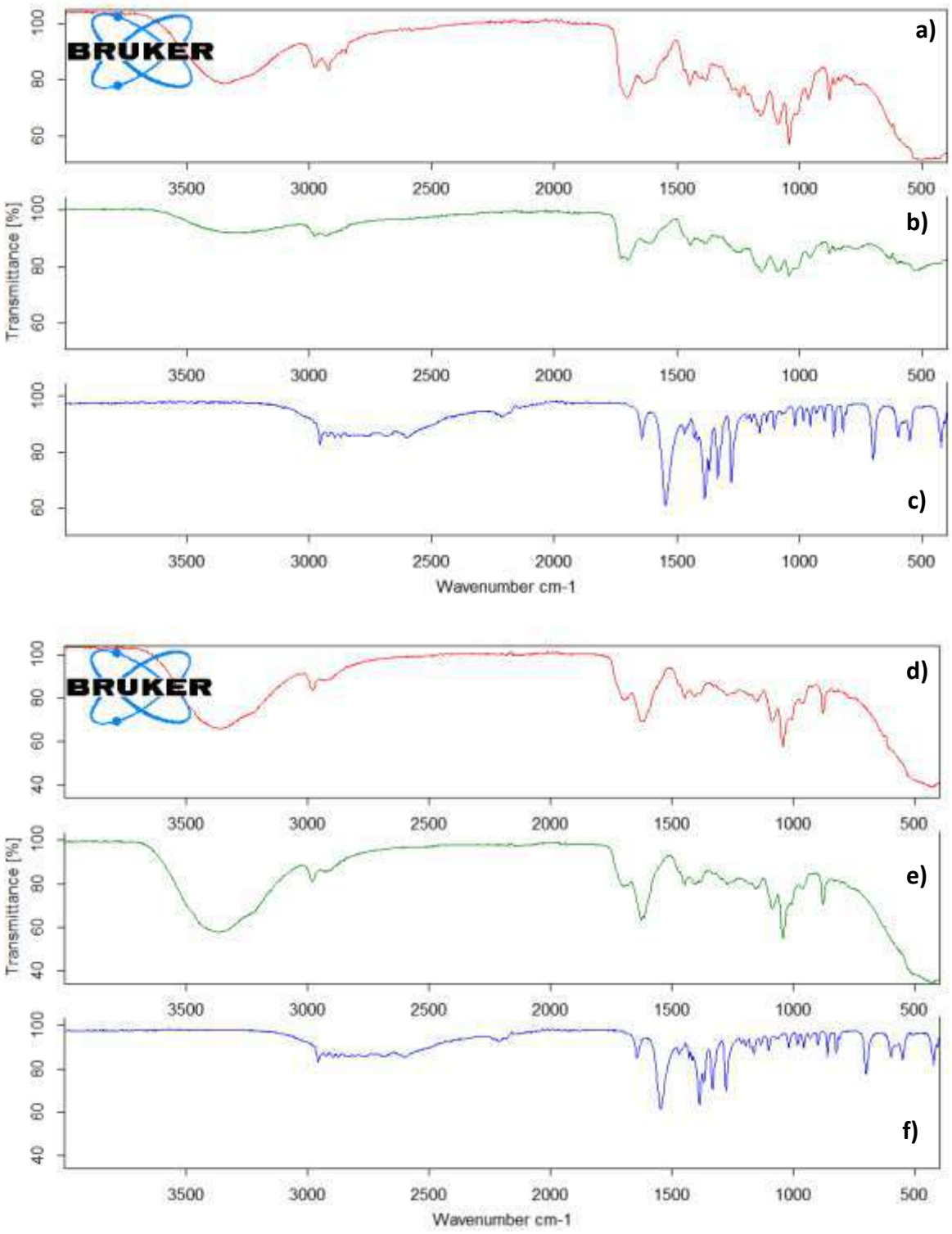


Figure 5. Spectrums of E3 (a), E6 (b), PGB (c), Lyo-E3 (d), Lyo-E6 (e) and PGB (f)

Discussion

The PGB quantification method was developed and subsequently validated to obtain sensitive, reliable, and reproducible results (Sahbir, 2003). Pectin beads containing and non-containing PGB were prepared by the ionotropic gelation method with two different techniques and subsequently, the beads were enteric-coated using Kollicoat® MAE 100P, an enteric coating agent. Various formulations have been developed by considering many process parameters within the scope of formulation development studies. Eventually, E3 and E6 beads were chosen that release at least PGB for 2 hours in pH 1.2 HCl simulated gastric fluid (Ali et al., 2014; Anwer et al., 2017; Haiyan et al., 2014).

In order to compare enteric coating thicknesses, pectin beads containing lyophilized PGB compared to E3 and E6 formulations were added to the enteric coating solution and their coatings were performed (Lyo-E3, Lyo-E6). In this way, the contribution of two different techniques to the coating was also examined. The coating thickness and the difference between formulations in terms of the enteric-coating were observed with SEM. In SEM, all developed formulations were examined and it was determined that the coating thickness of freshly prepared and enteric-coated beads was 4 times thicker than that coated beads after lyophilization (Figure 4, c and f).

The lowest encapsulation efficiency of the formulations was determined as 51% and the highest 78%, and formulations were successfully prepared to contain a high percentage of PGB. It was found that the sizes of the beads in the formulations developed ranged from 630 μm to 1600 μm, that beads containing PGB were larger compared to blank beads, and that the beads coated after lyophilization compared to freshly prepared and coated were larger. It has also been observed that the size distributions are narrow and homogeneous in themselves.

When all spectra were examined, it was determined that the peaks of the PGB were observed similarly in the formulations and that there were no changes. Especially in enteric coatings made after lyophilization, it has been determined that PGB peaks can be observed more clearly (Lyo-E3, Lyo-E6) due to the less coating thickness. PGB peaks were suppressed in proportion to the coating thickness in the freshly prepared enteric-coated beads (E3, E6). From here, it can be concluded that the freshly prepared and then enteric-coated beads suppress the PGB peaks by producing more protective and comprehensive enteric coating. It has been observed that the band voltage peaks of the PGB are trapped in the formulations and there are decreases in the peak intensity. This situation has been observed in similar drug delivery system studies (Anwer et al., 2017).

Conclusions

Pectin beads containing PGB were enteric-coated with two different techniques and characterization studies were carried out. In particular, the coating thickness, high encapsulation efficiency (77%) of the E6 coded formulation was found to be quite satisfactory. It may also be an alternative carrier system in the future for patients who experience stomach complaints due to PGB use with negligible PGB release in the stomach content. At the same time, it has been made possible to prolong drug release as pectin is resistant to enzymatic activities in the upper parts of the gastrointestinal system and begins to dissolve in the lower parts of the gastrointestinal system. With this study, it is aimed to prevent stomach complaints, which may develop due to the dosing of PGB. Patients suffering from neuropathic pain will have been both reduced stomach complaints and increased patient compliance with less dosing.

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CLIMATE CHANGE IN TURKEY

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ABSTRACT

The present study aimed to investigate the climate change inducted by global warming in Turkey. The reasons for climate change experienced due to global warming could be categorized in two groups: natural and artificial reasons. Natural causes of global climate change include magnetic solar storms, deviations in the axis of the Earth, and the increase of the kurtosis of the Earth orbit around the Sun, the El Nino effect, and artificial causes (anthropogenic activities) include fossil fuel consumption, greenhouse gases, and the reduction of the ozone layer. The increase the density of atmospheric greenhouse gases such as Carbon Dioxide (CO_2), Methane (CH_4), chlorofluorocarbon gases (CFC-11, HCF-C22, CF4, etc.) and Nitrogen (N_2O) is the main source of global warming. The carbon dioxide emissions in Turkey are concentrated in two industries: the energy sector (86.3%) and industrial processes (13.4%). Methane emissions are mainly due to agriculture (62.30%) and energy industry activities (21.30), waste emissions (16.4%), while N_2O emission sources include agricultural activities (71%), waste emissions (15.1%), energy sector (10.7%) and industrial processes (3.3%). When compared to 1990, CO_2 emission per capita has increased about 1.5-fold today, which is a serious warning to take precautions. Consequently decrease in *Marmaris salamander, Otis tarda*, *Karaman grasshopper*, *Turkish red dragonfly* populations and *Eğirdir longsnout scraper, European eel, Acıgöl sailton pupfish*, and *marine turtle* reproduction decreased to extinction levels evidenced that global warming and climate change are observed in Turkey.

Keywords: Global warming, climate change, species diversity, Turkey.

INTRODUCTION

Turkey is located between eastern and western cultures in allocation where continents of Asia and Europe. Turkey is there for Asian and Europan country. Turkey is one of the countries in that are risk in terms of potential effects due to the global climate change. In recent years in Turkey the number of hot days have been increasing. In 2019 an average temperature of 14.7 C^0 is obtained in Turkeyan this value is 1.2 C^0 higher than 1980-2010 temperature (Şeker, M., et al., 2020)

Although climate change has been an ongoing phenomenon throughout history, it was never as rapid as it is today. In recent years, several atmospheric events occurred in many regions of the world unique in violence, affects, duration and location. These changes also threaten the life on earth and socioeconomic development. Various parameters trigger climate change (Saraçoğlu, N., 2018).

Although the causes of climate change are generally anthropogenic, there are also natural causes: The solar effect, the Earth's precise movement, and the El Nino Effect. The sun generates cosmic radiation. Excessive cosmic radiation increases cloud cover, alters the soler radiation, and leads to an increase global temperature. Serbian scientist Milankovic explained the Precision Movement of the Earth. It was demonstrated that the Earth's orbit around the sun slightly flatten every 95000 years. It was also reported that there is a linear shift in Earth’s axis every 41000 years and a circular deviation every 23000 years. This is one of the reasons for

the linear global warming. The El Nino Effect entails 2-5^{0}C higher than normal sea surface temperatures in the tropical eastern Pacific Ocean between 1990 and 1998. The temperatures increased in record levels during 1997 and 1998. It was suggetsed that the main effect of global warming in 1998 was El Nino (Saraçoğlu, N., 2018).

After the industrial revolution in the 18th century, mechanization and technological advanced led to the consumption of fossil fuels in energy generation, currently called traditional energy sources. When fossil fuels are burned, they release CO_X, SO_X, NO_X, CH_4, H_2O, and O_3 gases, called the greenhouse gases. These gases released by the burning of fossil fuels increase the atmospheric air temperature and lead to global warming and climate change. The most important environmental problem induced by the use of fossil fuels in energy generation is the CO_2 emissions. Furthermore, other harmful emissions that lead to acid rain and destruction of the ozone layer, include sulfur oxides (SO_X), and nitrogen oxides (NO_X).

2.1 The Industries with the Highest Impact of Greenhouse Gas Emissions in Turkey:

It was accepted that Carbon dioxide (CO_2), Methane (CH_4), Nitrous Oxide (N2O), Hydro-fluoride carbons (HFCs), Perfluoro carbons (PFCs), Sulfur-hexea fluoride (SF6) are the main factors that lead to the greenhouse effect. In Turkey, greenhouse gases are released during various processed that include:

Forest Fires: Although forests are usually carbon consumers, forest fires release carbon dioxide, methane, carbon monoxide, nitrogen dioxide and other nitrogen oxides to the atmosphere.

Power Plants: They lead to air pollution due to NO, CO and high CO_2 emissions based on the raw material.

CFCs: The common use of CFCs (Chlorofluorocarbons) include refrigerants, car air conditioners, insulation material, perfumes and deodorants, and they react with the ozone in the ozone layer and lead to the breakdown of ozone and destruction of the ozone layer.

Paddy Fields: Nitrous oxide (N_2O) gas is released by nitrification and denitrification in paddy fields. In addition, methane (CH_4) gas emissions are high due to anaerobic breakdown. Paddy cultivation is among the major agricultural activities in the world and Turkey.

Fertilization: N_2O is released when chemicals are added to fertilizers to increase crop yield.

Cattle Husbandry: Methane (CH_4) is released by manure stored in pastures.

Landfills: It was determined that regular old landfills without removal facilities have high levels of methane emissions.

Factory Chimneys: PAH, SO_2, CO, NO_x and vapor are released by the factories based on the raw material and manufacturing process employed in the factories when the chimneys are not controlled and routinely cleaned, the filters are not routinely changed, or the compounds are not burned or half-burned.

Vehicle Exhausts: It is known to contain carbon monoxide (CO), hydrocarbons (HC) and nitrous oxide (NOx), which are dangerous for human health and the environment, in addition to harmless substances such as vapor, carbon dioxide and nitrogen dioxide.

The most effective greenhouse gases include CO_2, CH_4 and N_2O, respectively. It is known that the greenhouse effect of methane is 25 times more than nitrogen oxide and 298 times more than carbon dioxide; however, CO_2 emissions is considered to be the main cause of global warming due to the increase in CO_2 emissions every day (Lallanilla M., 2019).

The most effective greenhouse gases include CO_2, CH_4 and N_2O, respectively. The industrial distribution of the greenhouse gases in Turkey are presented in Figures 1, 2, and 3.

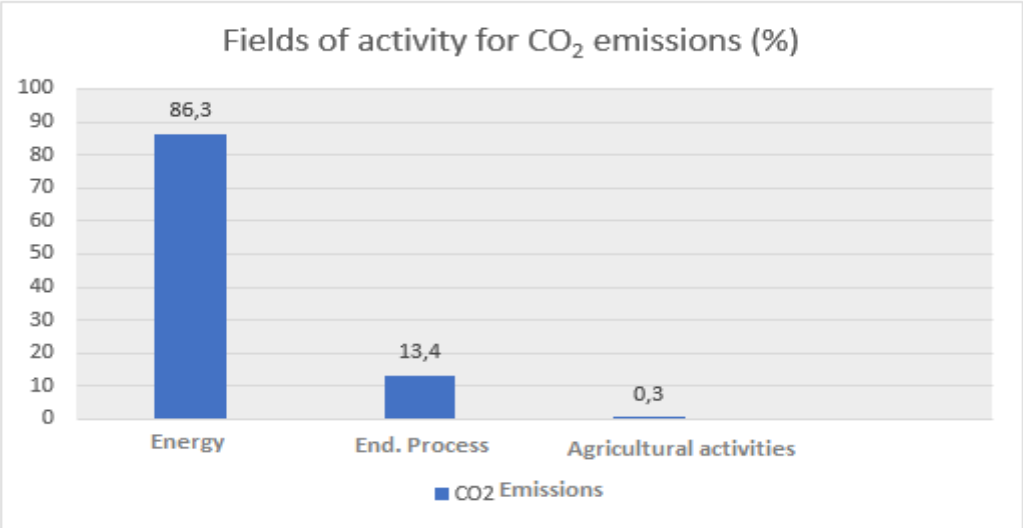

Figure 1: CO_2 Emissions Based on Industry

The analysis of CO_2 emissions based on industry revealed that the power plants ranked the first (86.3%). Based on carbon dioxide emissions, industrial processes ranked second (13%) and agricultural activities ranked the last (0.3%). The highest CO_2 emissions are produced by the power plants. Since the power plants, which are directly affect the exponential increase of the greenhouse gases in the atmosphere, employ fossil fuels as raw material, the CO_2 emissions are at very high levels. CO_2 emissions in industrial processes, on the other hand, represent the gases released due to the substances processed in factories and released from the factory chimneys (http://www.tuik.gov.tr/, 12.02.2020).

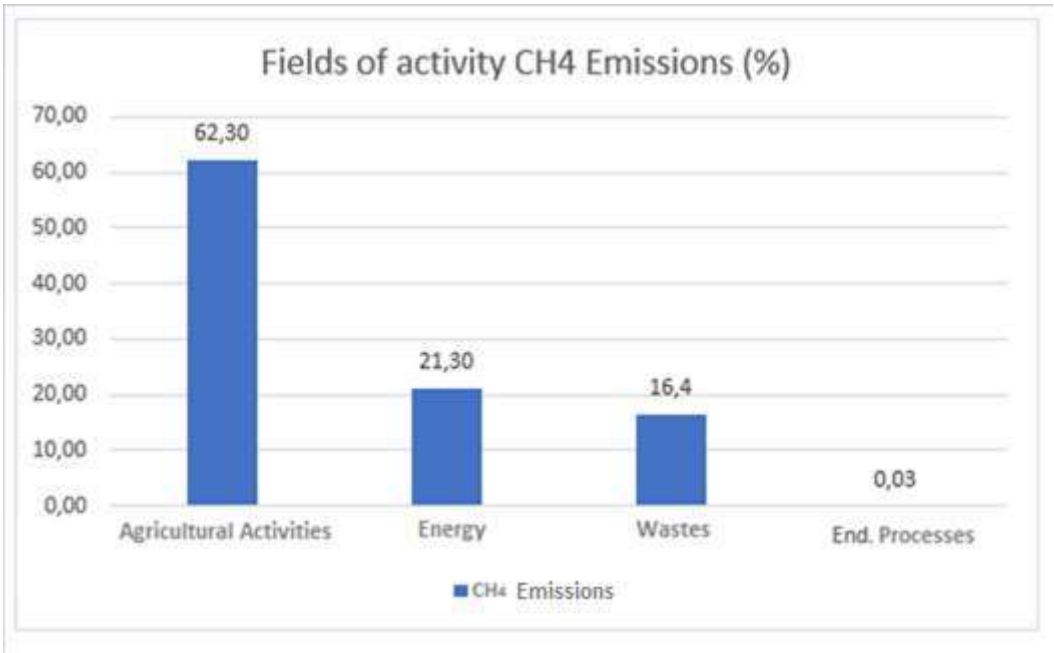

Figure 2: CH_4 Emissions Based on Industry

The CH_4 emission data based on industry revealed that agricultural activities ranked the first (62.30%). It was followed by energy activities (21,30%), waste-induced emissions (16,4%), and industrial processes (0.03%). The highest methane emissions was observed in agricultural activities due to animal husbandry activities and paddy cultivation. The main factor behind methane emission, the greenhouse effect of which is known to be 25 times more than carbon dioxide, is the decay of organic matter in an oxygen-free or low-oxygen environment (http://www.tuik.gov.tr/, 12.02.2020).

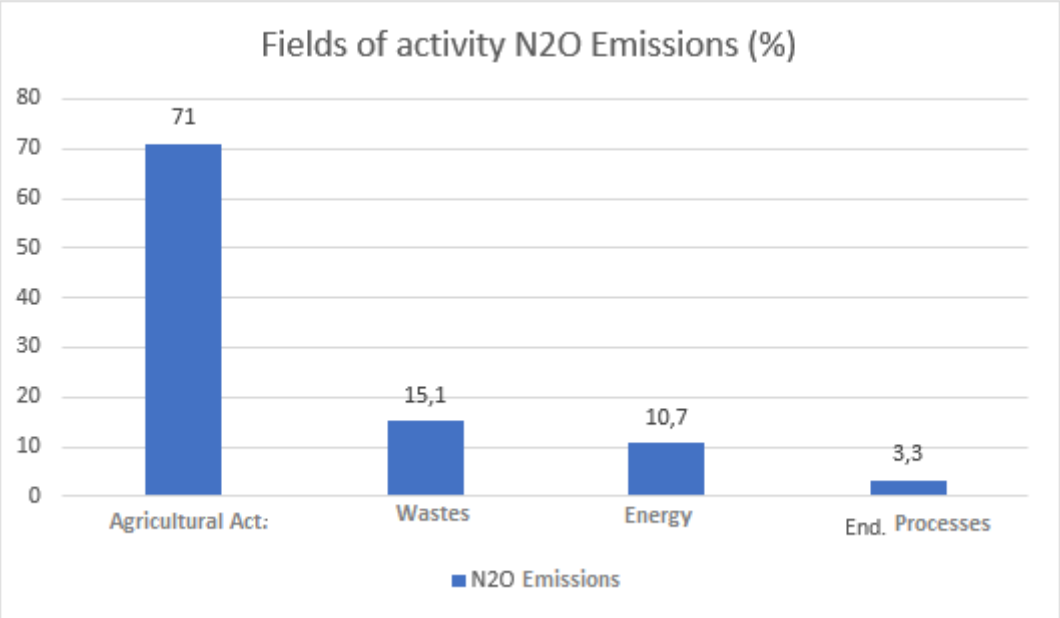

Figure 3: N_2O Emissions Based on Industry

The analysis of N_2O emissions based on industry revealed that the agricultural activities led to the highest emission rate (71%). Nitrous oxide (N_2O) emissions are observed in agricultural activities due to fertilization. Various chemicals are included in fertilizers to improve crop yield. Thus, increasing N_2O emissions. The agricultural activities were followed by waste (15.1%), energy industry (10.7%), and industrial processes (3.3%) (http://www.tuik.gov.tr/, 12.02.2020).

Measures to reduce greenhouse gas emissions: Individual measures to prevent climate change include the preference of public transportation, non-packaged products, washing with water at lower temperatures, turning the electronic devices off completely, preparing the picnic contents at home, and collection of recyclable waste separately.

Turkey has abundant renewable energy reserves when compared to other countries. Turkey has advantages due to the number of sunny days, wind potential, hydroelectric energy potential, and geological and geographical location on an active tectonic belt, leading to geothermal potential. Preference of more sustainable energy sources instead of fossil fuels would contribute to the reduction of greenhouse gas emissions in the country.

Potential Impact of Climate Change on Turkey

Climate change led to reduced snowfall, reduction of evening temperatures, variable climatic events, droughts, floods caused by sudden precipitation, early onset and late termination of the summer season when compared to previous years, etc. in Turkey. As a result, corn, paddy,

wheat, oat, barley, rye, pea, sugar beet, chickpea, lentil, pistachio, sunflower, cotton, olive, grape, soy, sesame, fig, and nut yields have reduced (Güventürk, A., 2013).

Due to increasing soil and temperatures in continental climate regions, *Marmaris salamander, Otis tarda, Karaman grasshopper, Turkish red dragonfly* populations decreased and *Eğirdir longsnout scraper, European eel, Acıgöl sailton pupfish*, and marine turtle reproduction decreased to extinction levels due to the increase in sea and freshwater temperatures.

CONCLUSION

Industrialization, population growth, fossil fuel use, deforestation, depletion of the ozone layer, destruction of the environment, uncontrolled and unplanned urbanization are among the important causes of climate change in Turkey.

Instead of the old public transportation vehicles, new generation vehicles powered by renewable energy should be used, green areas should be improved, residences with thermal insulation should be preferred and the waste should be recycled. Greenhouse gas emissions have increased due to fossil fuel consumption. Renewable energy sources such as geothermal, wind, solar energy and hydroelectricity that exist in Turkey should be preferred instead of traditional energy generation methods that utilize fossil fuels.

Persistence on current conditions could accelerate events such as droughts, desertification and erosion, agricultural production potential could decrease, new issues could be added to the present water resource problems in Turkey, limiting drinking and tap water supplies, coastal areas may submerge, destroying the existing flora and fauna in these areas and increasing the prevalence of viral and bacterial epidemics.

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NATURAL SOLUTION FROM MEXICAN MARIGOLD (T. ERECTA L.) AGAINST THE VAGINITIS PROBLEMS OF WOMEN

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ABSTRACT

One of the most common health problems encountered by women is vaginal discharge that occurs due to various reasons. Vaginitis that manifests itself with these vaginal discharges is the number one disease seen in women. In this study, it was aimed to investigate the effects of extracts, obtained from *T. erecta*, on some microorganisms that cause discharges occurring out of menstrual cycles in women, and to find natural, practical and useful solutions.

In the study, first of all, a survey was conducted to obtain data about how much women have knowledge about this subject and the sample of the Mexican marigold (*T. erecta*) to be used as a material were collected and dried. Three different extracts were obtained from the flowers of the plant, its leaf+stem and its flower+leaf+stem parts. The antimicrobial activities of these extracts were determined on *Enterococcus faecalis, Escherichia coli, Lactobacillus acidophilus* bacteria and *Candida albicans*, which cause vaginitis and urinary tract infections.

As a result of the study, it was determined that those other than the leaf+stem extract were effective on the bacteria and that since the extract obtained from flowers does not have much effect on the *L. acidophilus*, which is important in vaginal flora, this extract must be used in the products to be produced. Using the obtained extract, natural, practical, and easily accessible gel and pads, which are alternative to the medicines that are used when faced with these problems and that are generally synthetic and affect other organs in the body, have been produced. These products can be also helpful in the treatment of urinary tract infections. It is thought that the produced products will be developed and brought into use by experts in the field.

Keywords: Vaginal discharge, vaginitis, T. erecta, gel, pad, urinary tract infections

INTRODUCTION

One of the most common problems that women complain about during their lives is vaginal discharges. The vaginitis that generally manifests itself with these discharges is the number one disease seen in women. Vaginitis is a disease that occurs based on the inflammation of the vaginal area and the external genital organ and that manifests itself with a bad smelling discharge, itching and pain complain. Fungus, bacteria and protozoa infections, irritant substances, used medicines, tumors and hormonal changes cause this disease. In addition to the fact that vaginitis can be transmitted from public toilets, the selection of improper underwear, vagina cleaning soaps, not to keeping the vagina clean, used pad types and also sexual abuse may cause vaginitis (Balcı & Çapar, 2005; Kaygusuz et al., 2014). Depending on the cause of the disease, a wide variety of vaginitis is seen. These are the most common of them;

Bacterial vaginosis: Bacterial infection is the most common cause of vaginitis and manifests itself with a discharge similar to the fish smell. However, there is a vaginal flora in the vagina (pH= 3.8-4.5). In the vagina, all of the microorganisms that do not harm people are called "vaginal flora". The microorganisms that live in the vagina are not harmful to women since they live in certain numbers and in a balance. Bacterial vaginosis occurs as a result of the loss

of Lactobacillus producing hydrogen peroxide due to the change of normal vaginal flora and when the bacteria, such as anaerobic gram negative bacteria (*Prevotella, Porphyromonas* and *Bacteroides*), *Gardnerella vaginalis, Neisseria gonorrhoeae, enterococcus, Streptococcus* etc., dominate the flora by increasing. *G. vaginalis* is the most common. *N. gonorrhoeae* is seen in childhood and postmenopausal periods, ant it is usually asymptomatic.

Fungal vaginitis: *Candida albicans* causes this disorder that manifests itself with severe itching and whitish vaginal discharge. 75% of adult women experience this disorder at least once in their lives.

Trichomonas vaginalis Vaginitis: This vaginitis, which has a high rate of infection, is a sexually transmitted disease and is seen in males and females. This disease is caused by a parasite, which is a flagellated protozoa, called *Trichomonas vaginalis*.

Desquamative Inflammatory Vaginitis: This disease causes epithelial cell loss, plenty of discharge, burning in the vagina, bleeding in the form of occasional spotting. It emerge as a result of the increasing of the streptococcus, and there is no effective treatment of it.

Many different vaginitis caused by estrogen deficiency, improper use of tampons, and some substances such as creams, soap etc. are observed (Kaygusuz et al., 2014). What is important is the treatment of these diseases. If these diseases are left untreated, they can lead to significant health risks such as pelvic inflammatory disease (PID), infertility, chronic pelvic pain, ectopic pregnancy, and uterus, cervical, or ovarian ducts cancers (Breshears et al., 2015). These diseases are tried to be treated with vaginal tablets, creams, antibiotics, and antiparasitic drugs; however, these drugs are not effective in some of these diseases (Balcı & Çapar, 2005; Kaygusuz et al., 2014). One of the most important problems in terms of health is that most of these drugs are produced synthetically. These synthetic substances damage especially the liver and kidneys in our body (During the literature review, the drug names were identified and these drugs were also investigated). For this reason, the use of natural substances as an alternative to synthetics, in short, the development of traditional medical methods is important in terms of health care.

Traditional medicine is the oldest method that uses various parts of plants to treat diseases and infections. The plants used in these methods are called 'medicinal plants or medicinal herbs' and they have active substances (bioactive components = secondary metabolites) used in the treatment of many diseases (Sumathi et al., 2010). These substances can be listed as peptides, unsaturated long-chain aldehydes, alkaloid compounds, some essential oils, phenol, ethanol, chloroform, methanol, and butanol soluble compounds etc. (Seyyednejad et al., 2009). Since these bioactive compounds are widely used as an alternative source in modern medicine (Vanwyk & Wink, 2009), the number of the studies conducted with medicinal plants are increasing day by day.

Tagetes erecta, one of these plants, is located in the Asteraceae (Compositae) family and its origin is North-South America (Motamedi et al., 2015). *T. erecta* known as 'African marigold, American marigold, Aztec marigold and big marigold" is cultivated worldwide as ornamental plant. Besides being ornamental plants, the plant has a long history in traditional and folk medicine thanks to its secondary metabolites and it is used in many countries (Verma & Verma, 2012). The plant, used as food in Thailand and tea, is rich in flavonoids and phenolic compounds (Gallic acid, scopoletin, ferulic acid, and quercetin) and it is anti-carcinogenic (for stomach, bladder, bowel, etc.) (Kaisoon et al., 2012). It has been informed that this plant has antifungal and insect killer effect, there are 26 different bioactive components in the leaf of it, and 86% of these components are comprised of (Z)-β-ocimene, dihydrototone, (Z)-tagetone, lemonene, (E)-ocimenone and (Z)-ocimenone (Singh et al., 2003). The plant extract is obtained

today from this plant, which has some features such as antioxidant, antimicrobial, antibacterial, wound healing and accelerating blood clotting, being effective in good sight, moisturizing the skin, being effective in normal rhythmic work of the heart and protecting, and it is sold commercially. In addition, due to its phytochemical content, the plant is recommended for drug production (Regaswamy & Koilpillai, 2014). The flowers of the plant are used as a coloring agent in food items (Barzana et al., 2002), animal feed and textile products (Jothi, 2008). It was found that the flowers contained a large amount of lutein, and due to their nutritious properties, the values of the flowers has increased (Hadden et al., 1999). In addition, *T. erecta* is effectively used in biological fight against nematodes (Natarajan et al., 2006; Tan, 2011) and spiders (Erdoğan, 2017) that live in plant roots and harm plants. During the literature review, very few studies have been found in our country regarding *T. erecta* and these are usually related to plants.

In this study it was aimed 1) to investigate the effects of T. erecta extract on some microorganisms that cause vaginal discharge out of the menstrual cycle in women 2) to investigate the impact of it on some of the microorganisms that cause urinary tract infections 3) to be able to produce gel by taking advantage of these extracts, and 4) to be able to use it in pad production in order to provide practical and widespread usage.

METHOD

2.1. Plant, Bacteria and Materials

In this study, *Tagetes erecta* L. (Mexican marigold) was used the material. Fresh leaves and flowers belonging to the plant were collected from various areas (i.e., university and school gardens, parks, etc.) and samples taken from yellow and orange-flowered plants are packaged separately (Figure 1).

Figure 1: Collection of the plant

In the study in which various media (Nurient Agar, MRS Broth, Mueller Hinton Broth, Mueller Hinton Agar, Sabouraud Dekstroz Broth, Sabouraud Dekstroz Agar) were used, the experiments were performed using *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Lactobacillus acidophilus* (ATCC 11975) and *Candida albicans* (ATCC 90028) yeast strain.

2.2. Survey Study

In order to obtain an overview about the topic of the study, a survey questionnaire including some specific questions (i.e., Do you have such complaints? Do you go to the doctor when you have any complaints? Which medicines do you use? or Is there any natural methods that you apply? etc.) was prepared. The questionnaire was applied to 3 groups: under 18 years old, 18-40 years old and over 40 years old.

2.3. Drying of Collected Samples

After rinsing under tap water, the collected samples were sterilized by washing with 5% alcohol (isopropyl alcohol) solution. The sterilized leaves and flowers were dried at room temperature for 10-15 days (Figure 2) (Verma & Verma, 2012).

Figure 2: Cleaning and Drying of the Materials

The dried samples were turned into dust by grinding separately in blender and coffee machines (Figure 3). The obtained materials were stored in airtight containers.

Figure 3: Grinding of the samples

2.4. Preparation of *T. Erecta* L. Extracts

In the study, the extracts were obtained using two different methods. First, one gram from each of the dried materials (yellow flowers, orange flowers, stalks and stems of the yellow plant, stalks and stems of the orange plant) was weighed and placed in centrifuge tubes. 10 ml ethanol-distilled water (8: 2) solution was added to the tubes and centrifuge (3000 rpm) was applied for 15 minutes (Figure 4). After centrifuge, the supernatant was harvested and the process was repeated 3 times. Three tubes were prepared for each sample. The tubes were left open at room temperature (5-6 days) and evaporation of solvents was ensured (Motamedi et al., 2015). The tubes that had not vaporized completely were kept in the drying-oven (41°C).

Figure 4: Plant extract preparation stages (1st Method)

In the other method used for obtaining the extract, 3 groups were formed from the material. There were 5 g yellow flowers + 5 g orange flowers in the first group, 5 g stalk and stem mixture belonging to the plants in the second group, and 2.5 g material from each sample in the third group. The weighed 10-gram samples were put in glass beakers, 100 ml 80% ethyl alcohol (8: ethyl alcohol, 2: distilled water) was added on them and then they were placed in mechanical mixers (Figure 5). Twenty-four hours later, the mixtures in the beakers were filtered and an evaporator was used to remove ethyl alcohol.

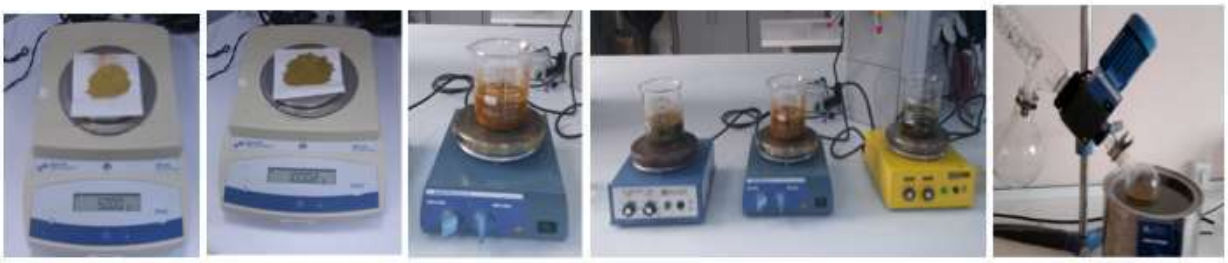

Figure 5: Plant extract preparation stages (2nd Method)

2.5. Preparation of Media

The media that would be used to determine the effects of extracts on microorganisms were prepared as follows;

Preparation of Nurient Agar (NA): After dissolving the 20 g powder medium with distilled water, it was completed to 1000 ml volume and sterilized in the autoclave at 121°C and 1-atmosphere pressure for 15 minutes.

Preparation of MRS Broth: After the 52.2 g dust medium was dissolved with 1000 ml distilled water, it was distributed to tubes with cotton and sterilized in the autoclave at 121°C and 1-atmosphere pressure for 15 minutes.

Preparation of Mueller Hinton Broth (MHB): After 21 g powder media was completed with distilled water to 1000 ml, it was distributed to the tubes with cotton and sterilized in the autoclave at 121°C and 1-atmosphere pressure for 15 minutes.

Preparation of Mueller Hinton Agar (MHA): The 34 g powder medium was completed with distilled water to 1000 ml and sterilized in the autoclave at 121°C and 1-atmosphere pressure for 15 minutes.

Preparation of Sabouraud Dekstroz Broth (SDB): After 30 g powder medium was completed with distilled water to 1000 ml, it was distributed to tubes with cotton and sterilized in the autoclave at 121°C and 1-atmosphere pressure for 15 minutes.

Preparation of Sabouraud Dekstroz Agar (SDA): After dissolving the 65 g medium with distilled water, it was completed to 1000 ml and sterilized in the autoclave at 121°C and 1-atmosphere pressure for 15 minutes.

The agar medium was sterilized with autoclave, cooled to 45-50 °C and poured into petri containers.

2.6. Determination of the Antimicrobial Activity of the Extracts

For antimicrobial activity, the extracts obtained from flowers, stems and all parts of the Mexican marigold were used. Antimicrobial activity of the extracts that can dissolve in water was studied by modifying disk diffusion method. In order for the determination of the antimicrobial activities, *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Lactobacillus acidophilus* (ATCC 11975) bacteria and *Candida albicans* (ATCC 90028) yeast strain were used. Bacterial stock cultures were planted to NA (*E. faecalis* and *E.coli*) and MRS Agar (*L. acidophilus*) to revive them and yeast culture were planted to Sabourud Dextroz Agar (SDA) to revive it, and they were left to incubation at 37 °C for 16-18 hours. After the incubation, it was taken from the colonies developing on the media and transferred to the tubes including MH, MRS broth, and SDB according to 0.5 MC Farlanda; that is, the turbidity of suspensions including approximately 10^8 CFU/ml for bacteria and 1-5x106 cells for yeast was adjusted using the densitometer. The prepared microorganism suspensions were planted in the medium with the help of swab. The extracts in the concentrations of 25 µl, 50 µL and 100 µl were placed into the wells opened in the solid media (Figure 6). The bacteria that were cultivated were incubated at 37°C for 16-18 hours. Ciprofloxacin (CIP, 5 mcg/disc) was used as a positive control. The study was conducted as three repetitions. Diameters of the inhibition zone occurring against the microorganisms were measured and their reproduction was evaluated.

Figure 6: Preparation of sample petri dishes to investigate the effects of extracts on microorganisms

In this study, Gardenella vaginalis was also studied, but the reproduction of it could not be observed although it had been obtained from a well-known institution and although a large number of planting was performed using various media. In fact, it was obtained from the patients by cooperating with the hospitals; however, a problem was experienced in production. Trials related to production are ongoing.

2.7. The Production of Protective Gel against Some Microorganisms that Cause Vaginal Discharge from the Extract obtained from the Flower of *T. Erecta*:

In order to make the extract obtained from the flowers of Mexican marigold useful, the Faculty of Pharmacy has been consulted. It was suggested that the extracts could be converted to various products (e.g., pills, gel, suppositories, etc.). Since it is more practical in terms of usage and in order for it to be able to affect the problematic area immediately, our idea of gel production has been supported.

The laboratories of the faculty were used in gel production. In gel preparation, in addition to the plant extract, distilled water, humectant, gelling agents, PH regulators, and preservatives were used. First, the gelling agents and distilled water were placed in the same glass container and inflated by keeping for 24 hours without mixing. Then, by adding the extract and the other substances, it was blended in a homogenizer at 500 rpm for 10 min until it was homogenized and the gel was produced (Figure 7) The names of the ingredients of the structure of the gel were given generally, but the type and quantity of them were not written since the product was original.

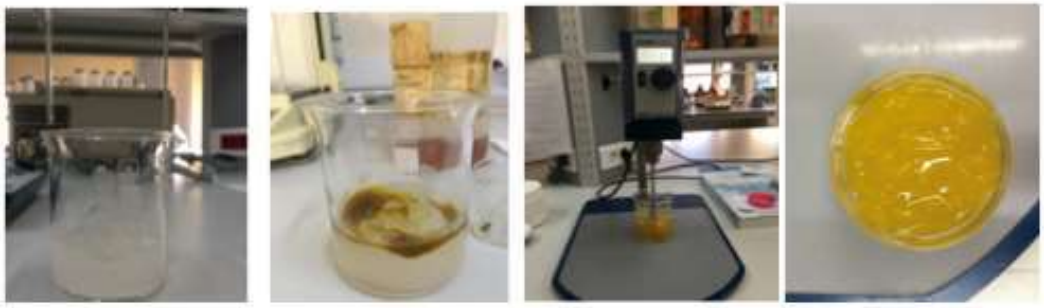

Figure 7: Gel production

2.8. Use of Extract Obtained from the Flower of *T. Erecta* in Pad Production

In order for the extract obtained from the plant to be more practical and useful in struggling with the vaginal discharge problem and for all women to be able to reach easily, it was thought that it would be beneficial to include it into the structure of the pad during the production process or any stage of this process. For this reason, the R&D department of an expert firm has been consulted. It was stated that in general, if a new substance was to be added to the pads, trials were done through applying this substance by syringe or spray method, if it was found appropriate, it was tried in big machines which were used for production and the production was stopped at that time.

Processes were started with the provided information and some trials were made to determine in which solvent the obtained extract was dissolved better (acetone, water, ethyl acetate). It was determined that the extract was dissolved in water and ethyl acetate, but ethyl acetate was more effective. One gram extract was dissolved in 100 ml ethyl acetate and the obtained liquid was applied to the pads with syringe and spray methods (Figure 8).

Figure 8: The use of the extract obtained from flowers in the pads

Since the production needs to be stopped in order to make trial in the machines used in the main production, the negotiations are going on (Figure 9).

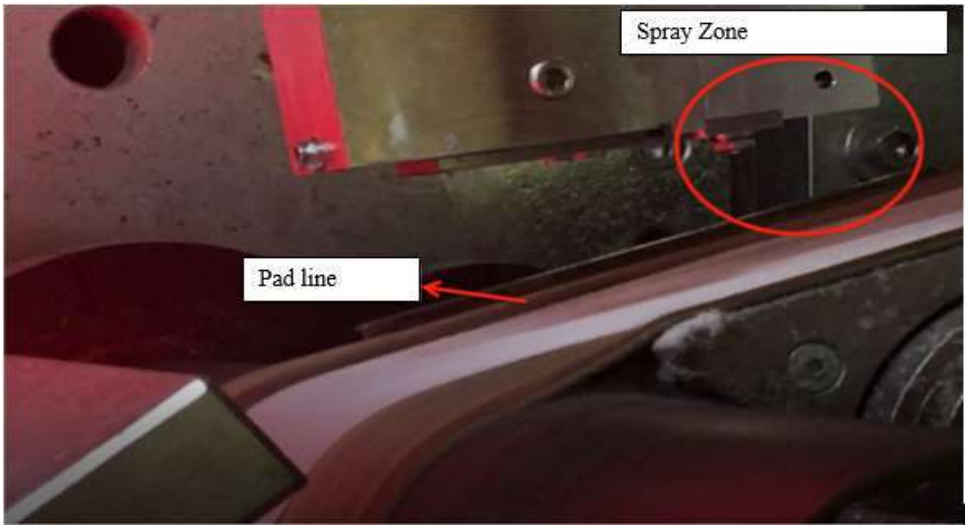

Figure 9: The machine used in pad manufacturing

3. Findings

As a result of the survey, which was the first stage of the study, it was determined that single women under 18 years old usually had vaginal discharge problems twice a month, especially before menstruation, these discharges were thick and smelly, but in this case they did not consult a doctor or someone with knowledge in this field and did not use any medication. It was also determined that the majority of married and unmarried women between the ages of 18 to 40 had vaginal discharge problem, while some of them experience it one time in a month, some of them experience it continuously. Majority of this women did not have information about these discharges and they were not using a medicine to avoid this problem. Also, although thick and smelly discharge was mostly seen, a specialist were not consulted. The majority of them did not have a routine check-up and that they did not have any information about diseases such as uterine cancer. Finally, It was also determined that women over 40 years old did not usually experience this problem. For those who experience it, it was seen once a month before the menstruation. They experienced it more often when using a normal pad than when using a daily pad. They did not do anything for these discharges and did not have any information. Thick and smelly discharge was not seen very much, and the number of those who had check-up routinely and the number of those who did not have were equal.

Antimicrobial activities of the extracts obtained by two different methods were investigated. The desired results were not obtained from the extracts obtained in the first method. However, it was determined that the extracts obtained by the second method were very effective. By evaluating zone diameters of these extracts, it was determined that they showed antimicrobial activity. It was determined that the extract including all parts of the plant showed the best activity against all bacteria. On the other side, it was identified that the extract of flower showed activity especially against *E. faecalis* and *E. coli*. However, any antimicrobial activity of the stem extract against microorganisms was not determined. In addition, any effect of all extracts against *C. albicans* was not determined and antifungal activity was not identified (Figure 10, Table 1)

Table 1: The inhibition zones (mm) created by extracts against microorganisms.

Microorganism	Extract	25 µl	50 µl	100 µl	CIP
E. coli	Flower	11	16	18	-
	Stem	0	0	0	22
	All	22	26	31	-
E. faecalis	Flower	15	17	20	-
	Stem	0	0	0	20
	All	20	22	24	-
L. acidophilus	Flower	0	0	16	-
	Stem	0	0	0	30
	All	14	16	27	-
C. albicans	Flower	0	0	0	-
	Stem	0	0	0	-
	All	0	0	0	-

The obtained data were shared with the Faculty of Pharmacy and the companies producing pads, gel was produced to be able to use the extracts obtained from the plant effectively (Figure 11), and necessary studies were carried out to be able to test it in pads (Figure 8).

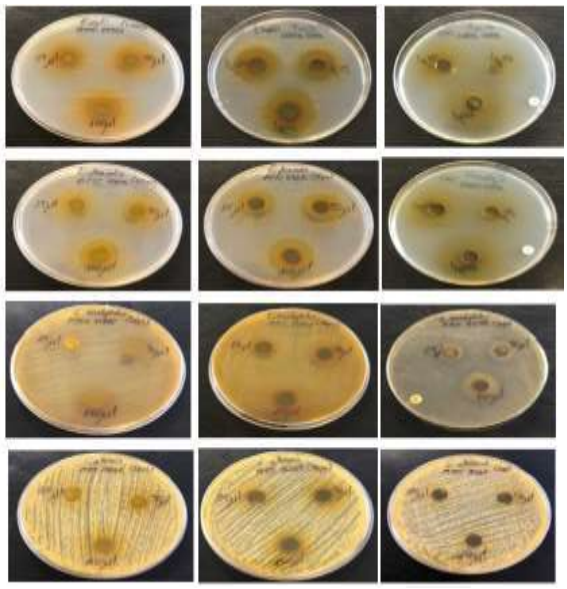

Figure 10: The antimicrobial activities of the extracts on *E. coli, E. feacalis, L. acidophilus* bacteria, and *C. albicans*.

Figure 11: Gel produced from flower extract

RESULTS AND DISCUSSION

Thanks to its antioxidant, antibacterial and antifungal properties (Kaisoon et al., 2012; Tripathi et al., 2012, Verma & Verma, 2012; Motamedi et al., 2015; Dasgupta et al., 2016), the Mexican marigold (T. erecta), which is cultivated for the ornamental purposes in our country and decorate almost every park, is effective in many areas such as wound healing, accelerating blood clotting (Dasgupta et al., 2016), and protecting the skin. For these reasons, the extract obtained from the plant is sold commercially and it is recommended for drug production due to its phytochemical content (Regaswamy & Koilpillai, 2014). Many studies related to the plant have been conducted abroad, but no studies have been done to resolve vaginal discharge or urinary tract problems as in our project. In our country, there is no studies other than a few studies (Erdogan, 2017) done with this plant. This situation reveals the originality of our study and increases its importance.

The results of this study are presented and discussed below:

When the applied survey questionnaires were examined, it was determined that vaginitis (reasons may be different) was seen in the majority of women. The most important data obtained in this survey is that when faced with this problem, it is usually not consulted to an expert and medications are not used. The majority of women do not know that this problem is important and that it may cause cancers such as the uterine cancer and so on if it is frequently

repeated. With the help of our study, it may be ensured that women to be informed about this issue, to have regular health examinations and to take the necessary measures on time.

There are many different compounds such as phenylpropanoids, carotenoids, flavonoids, thiophenes in the structure Mexican marigold used as the material in this study (Marrotti et al., 2004). The amount of these compounds (including plant parts) produced by plants may vary depending on many factors such as harvest season, methods and techniques used, plant development phase and geographical area (Burts, 2004). Therefore, the extracts were obtained with two different methods and the studies were carried out together. Whereas the intended results were not obtained from the extracts obtained by the first method, the extracts obtained by the second method showed the antimicrobial activity.

Antimicrobial activity of 3 different extracts obtained from *T erecta* were tested on *E. faecalis, E. coli, L. acidophilus* bacteria and C. albicans yeast strain. It was determined that the extract containing all parts of the plant except the root was effective on all microorganisms and the extract consisting of stems and leaves was not effective. It was found that flower extract showed activity against *E. faecalis* and *E. coli*, and if used in high doses, it was effective on *L. acidophilus* (Figure 9, Table 1). This result is very important in terms of our study because *L. acidophilus* plays an important role in the balance of vagina flora. If the L. acidophilus decreases in number, pH changes, other bacteria proliferate, and vaginal discharges occurs (Kaygusuz et al., 2014). Extracts that do not affect this microorganism much more and whose antimicrobial activities on the other bacteria are high are more suitable. Therefore, extracts obtained from flowers were used in gel and pad production.

In previous studies, it has been reported that *T. erecta* is effective on *E. coli* (Behidj-Benyounes et al., 2014; Chakraborthy, 2009; Das & Mishra, 2011; Motamedi et al., 2015; Padalia et al., 2015; Rhama & Madhavan, 2011; Salehi et al., 2018; Verma & Verma , 2012). Our study also supports this result. Although E coli is present in the normal flora of the body, when it increases in number, it causes urinary tract infections, inflammation in the intestines and wounds where it accumulates. Because they live in the gaps of the reproductive organ, they cause the deterioration of the vaginal flora, the formation of vaginal discharge and urinary tract infections. Because of their resistance to antibiotics, it is needed to consider the new solution recommendations.

In the literature review, no study was found to investigate the effect of extract obtained from the plant on *L. acidophilus*. Therefore, it can be said that this study was the first study to investigate the effect of *T. erecta* extract on the bacterium. This bacterium is important for vaginal flora. Because of this, the flower extract, which does not harm this bacterium and has effect on others, were used for the production of gel and pad.

It was determined that the extracts obtained in the study were not effective on *C. albicans*. However, many studies have reported that *T. erecta* extract has an effect on *C. albicans* (Behidj-Benyounes et al., 2014; Chakraborthy, 2009; Padalia et al., 2015; Salehi et al., 2018). This may be due to the harvest season of the plant, development period, the variation of the active substances according to the geographical area, the used methods, and the being differences in the used doses (Burts, 2004). In addition, these studies have been usually carried out in countries where the plant grows naturally. On the other hand, the culture forms of the plant were used in this study since it does not grow naturally in our country. Since C. albicans is the reason of fungal vaginitis, which is an important problem for women, obtaining extracts from the materials of the plant by using different methods and conducting experiments on C. albicans are going on. If our suggestions for the solutions to these problems (gel and pad) are desired to be developed by experts, the natural plant can be used.

If the diseases caused by these microorganisms are left untreated, they may cause the development of especially the uterine, cervical and ovarian cancers (Breshears et al., 2015). These diseases are attempted to be treated by vaginal tablets, creams, antibiotics, and antiparasitic medicines, but some of these medicines are not effective (Balcı & Çapar, 2005; Kaygusuz et al., 2014). On the other hand, one of the biggest problems of today is the fact that the microorganisms gain resistance to antibiotics. In addition, the majority of the medicines used are synthetics and adversely affect many organs such as the stomach, kidneys, and liver. For these reasons, solutions that are natural and affect only the infected region was tried to find in this study and two different solutions were proposed.

One of our solutions is to produce gel using flower extract of the plant. In this regard, we discussed with the experts of the Faculty of Pharmacy and shared the data, after the exchange of views, our idea of producing gel was also supported by them and the gel was produced. In the studies carried out in the laboratories belonging to the faculty, various substances that have the ability to increase consistency were used. Because of the absence of a study done with the extract obtained from this plant in our country and the absence of a product such as gel produced from this plant, the names of the ingredients in the structure were not written in the report. The produced gel can be easily applied to the desired area and only affects that area.

During the pad production phase of the study, it was discussed with the R&D department of an expert firm in the field. After the necessary information was received from the authorities, the extracts were tried to be absorbed into the pads by spraying and syringe methods. Experiments have been observed to be successful. As stated in the method section, it was negotiated with the company to conduct a test at the production tape; however, since a large number of pads were produced in the production tape and it was needed to stop production for trial, it was decided to wait the time when the firm will conduct trial. As a result, the officials working in this field stated that by using spraying method, the obtained extract could be used in the production of toilet papers, daily pads and other pads, they also emphasized that if it was used especially in the production of Spunbond Nonwonen (Nonwonen Tela), which is used in the coating of the upper surface of the pads, it would be more useful. It is known that various antibacterial substances and odors are added to the structure while these surface materials are produced.

It is known that the vaginitis, which is tried to explain in detail in the study and which is one of the problems of women even if it is not brought to the agenda, has increased in recent years with sexually transmitted diseases. The reasons of it may be very different, but generally this increase occurs due to some factors, such as socio-economic conditions of societies, increasing migration from village to cities and other countries, changing living conditions, increasing domestic and foreign tourism with the developing transportation opportunities, starting of sexual activities at early ages, ensuring sexual freedom by family planning measures, the increasing of hidden prostitution especially in cities, and leaving dirty the common use areas (e.g., toilet) (Karaman et al., 2006). It is not possible to find a solution in a short time for the majority of them. However, thanks to this study, it was determined how important it is for women to be informed about this issue. As a result of this study, it has been seen that when faced with these problems, natural alternatives may come to the fore instead of synthetic medicines harming some organs in the body, natural, practical and cheap treatment methods or measures can be taken thanks to the gel and pads produced. In addition, it has been also understood that these gel and pads can be used not only for vaginitis, but also for other diseases, such as urinary tract infections, thanks to the investigated microorganisms. The data obtained can be evaluated and developed by experts in the field, the treatment of the mentioned diseases can be done or spread of them can be prevented.

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ALOEHEC BAND-AID AND DIAPERS

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ABSTRACT

Some of the most important problems in injuries are wound care and hygiene. Bleeding and discharge in the wounds cause some problems, microorganisms gain resistance against the chemical antibiotics used. Today, various polymeric films are produced and studies are carried out for this purpose. In this study; It has been investigated whether the films produced using aloe vera gel and HEC (Hydroxyethyl cellulose) natural polymer can be used as band-aid and in diaper production.

Gel was obtained using mature leaves of the plants being grown. In the study, four different films were produced, not containing aloe vera gel, in the amounts of 1%, 5% and 10% gel. Dry weights of the films were measured by taking samples of 1 cm^2 from the films. The samples were weighed again after waiting for 10 minutes in an aqueous environment and their water absorption capacities were calculated. It has been determined that the water absorbing capacity of the film containing 10% gel is 70% and this film was used in the tests made with diaper and band aid.

It has been determined that the films produced by using the plant with antimicrobial and tissue regenerating components can be used in wound treatment due to the high liquid absorption capacities, especially wound tracking can be performed in open wounds, and diapers can be produced for children with sensitive skin by using aloe vera instead of chemical water absorbents and antibiotics. In addition, it has been observed that answers to current problems such as antibiotic resistance and recycling can be found naturally.

Key Words: Aloe vera, HEC, Band-Aid, Diapers

INTRODUCTION

Aloe vera, which is a member of the *Liliacea* family and also called *Aloe barbadensis Miller*, has over 275 species in the world (Rahman et al., 2017). Four species of these are used for commercial purposes. Aloe vera is a plant that has been used in the medical field since 1552 BC (Udupa et al., 1994). It has been found that Aloe vera has beneficial effects on skin wounds, burns, diabetes, obesity, and cholesterol balance (Avila et al., 1997). For the tissue regeneration during the repair of wounds, collagen in the interstitial fluid is important. Aloe vera stimulates the growth of fibroblasts, accelerates their cleavage, and increases collagen synthesis (hence the amount of hyaluronic acid) (Thompson,1991; Sripriya et al., 2008). In addition, in plants' wound healing process, different mechanisms, such as keeping the wound moist, increasing epithelial cell migration, and faster maturation, also play a role (Reynolds and Dweck, 1999; Gupta and Malhotra, 2012). For the use of Aloe vera gel in tissue engineering, films have been produced by using polymers such as chitosan (Silva et al., 2013; Rahman et al., 2016), alginate (Pereira et al., 2013), poly (methacrylic acid) (Anjum et al., 2016), oxidized pectin-gelatin (Tummalapalli et al, 2016), sodium alginate, and polyvinyl alcohol (PVA) (Pereira et al., 2014) and they have been tested in many areas. Films containing natural polymer are used in a wide range of fields such as pharmaceutical production, cosmetics, and tissue engineering and their wound-healing effects and features such as biocompatibility and biodegradability are

investigated (Pereira et al., 2013). Hydrogels are 3B hydrophilic polymer networks and they can swell and maintain their structure without dissolving in aqueous solutions (Pasqui et al., 2012). It should be noted that numerous studies related to the plant have been done in many different areas of the world. Studies such as polyamide nanocapsule production (Esmaeili and Ebrahimzadeh, 2015), investigation of its usability as a natural plasticizer (Pandey et al., 2016), creating tissue scaffold by using with nano fibers, and producing gel and film by using biomolecules such as polycaprolactone (PCL), chitosan, poly (ethylene glycol) (PEG) and polyvinyl alcohol (PVA) (Uslu et al., 2010; Rahman et al., 2017) have been conducted. Thanks to the characteristics of the plant, obtaining gel and powder from the plant and processing of them have become a major industry (Rahman et al., 2017).

In this study, the usability of films produced using Aloe vera gel and HEC (Hydroxyethyl cellulose) natural polymer in the production of adhesive bandages/pledget and diapers was investigated.

MATERIAL AND METHOD

Obtaining Gel from the Aloe vera Plant

The mature leaves of the plants bought and grown as seedlings were selected and cut; then, the green parts of the leaves were peeled with a knife and the gel part was taken. Large parts were made smaller by cutting and the green-colored vein parts were weeded out. The samples collected in the plastic container were turned into gel by thoroughly broken down with the help of blender and mixer (Figure 1).

Figure 1: Obtaining Aloe vera gel.

Use of Aloe Vera Gel for Film Production

In the study, HEC was tried as a biopolymer for film production. The following stages were followed to obtain four different films that did not contain Aloe vera gel and contained 1%, 5% and 10 % Aloe vera gel:

98 ml water, 2 ml glycerol, and 1g HEC were put into the glass beaker and they were mixed in the magnetic mixer (500 - 600 rpm, 2 unit temperature) until all the particles of the polymer dissolved in water and the gel consistency was obtained (1.45 - 2 hours).

After putting 97 ml water, 2 ml glycerol, and 1g HEC into the beaker and mixing for 1 hour in the magnetic mixer (500-600 rpm, 2 unit temperature), 1 ml aloe vera gel was added and it was continued to mix for 30 minutes more. The gels, which came to the appropriate consistency, were poured into medium-sized glass double Petri dishes. The same processes were also repeated to create films containing 5% and 10% Aloe vera gel by setting the water amount. Petri dishes were placed in a vacuum sterile cabinet to remove excess water (Figure 2).

Figure 2: Production of the film containing Aloe vera gel

After the samples were dried (1.5-2 days later), they were taken from Petri dishes and dried a little more, and stored in the refrigerator for some analyses (Figure 3).

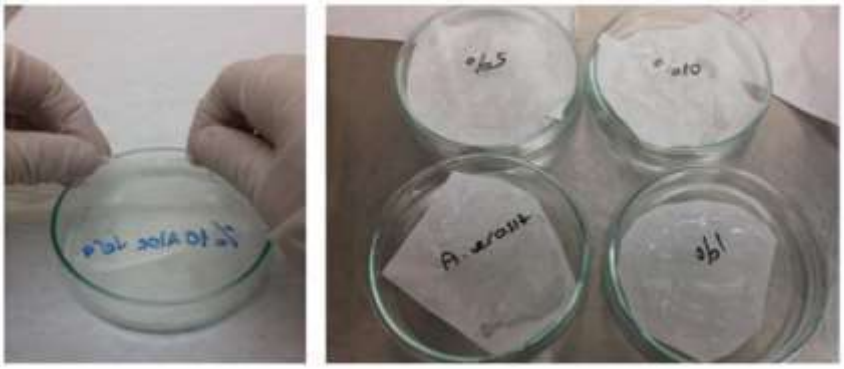

Figure 3: Taking of the sample films from the Petri dish

Determination of the Water Absorption Capacities of the Films

The last two phases of the study were conducted to evaluate each product obtained during the study in one area. For this purpose, the water absorption capacity of the first prepared sample films was examined. Pieces of 1 cm^2 were cut from the sample films (containing no Aloe vera gel, and containing 5% and 10% gel) and their weight was measured (Figure 4).

Figure 4: A) Dry weight of the film without Aloe vera gel, B) Dry weight of the film containing 5% gel, C) Dry weight of the film containing 10% gel.

After adding 10 ml of distilled water on the samples, they were kept for 10 minutes and weight measurements were performed again (Figure 5). The samples were analyzed in three copies and their average was taken.

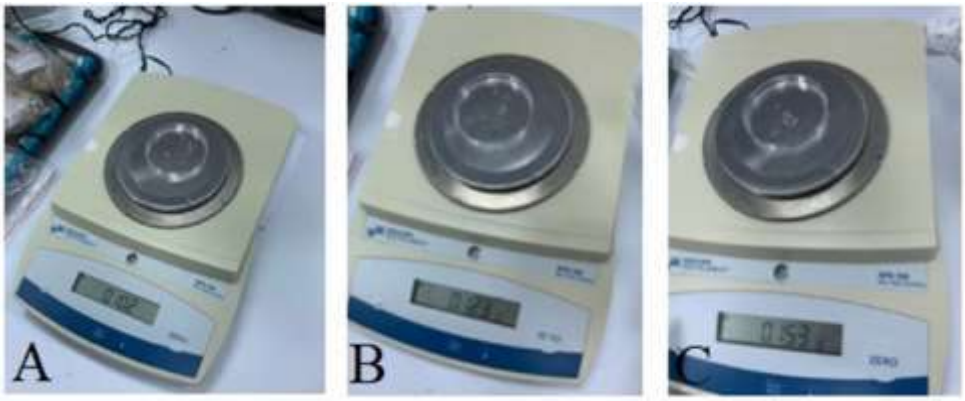

Figure 5: A) the wet weight of the film without Aloe vera gel, B) the wet weight of the film containing 5% gel, C) the wet weight of the film containing 10% gel.

Investigation of the Usability of Films as Pledget and in the Production of Diapers

The inner surface of one of the diapers was opened, the polymer ensuring the water-holding property was removed and the film containing 10% gel was placed inside of it. 100 ml of water was poured over the diapers and they were kept for 10 minutes; then, observations were recorded (Figure 6).

Figure 6: Use of the film containing Aloe vera gel in diaper production

With Aloe vera gel, gels and films have been produced mostly for use in the healthcare field, but no trial has been conducted with the HEC polymer. With the produced films containing HEC, can we follow the improvements without opening the wound since the films are transparent? Can the excess fluid in the wound be absorbed by the film? In order to answer these and similar questions, observations were made on the artificially created wound (Figure 7).

Figure 7: Use of the film containing Aloe vera gel as an adhesive bandages or bandage

RESULTS

The test results related to the water holding capacity of the produced films are given in Table 1.

Table 1. Water holding capacity results of the films

Sample name	Dry weight	Wet weight	Water holding capacity (%)
0% Aloe vera	0.087 g	0.102 g	%17
5% Aloe vera	0.088 g	0.123 g	%40
10% Aloe vera	0.089 g	0.153 g	%72

In the experiments conducted with the diaper, it was determined that whereas water holding in the film-placed diaper was equal throughout the film, there was swelling in the polymer-dense area of the normal diaper compared to other parts. On the other side, related to the wound bandage applied to the model, it was observed that the film adhered to the model and the improvements in the wound could be clearly seen.

DISCUSSION AND CONCLUSIONS

One of the most common dangers encountered in daily life is injuries. The most important problems in injuries are wound care and hygiene. Bleeding and discharge occurring in wounds cause some problems and microorganisms gain resistance to the chemical antibiotics used. Today, a wide variety of creams, solutions, adhesive bandages, and dressing materials are used for wound care. Among these products, polymeric dressings are effective for wound treatment (Sripriya et al., 2004). This is because polymeric films have advantages over other films in the repair of wounds due to their properties such as providing easiness in the distribution of liquid or semi-solid drugs to tissue, ability to adhere to damaged tissue, making absorption, irritating the tissue at different rates, and not creating toksic effect (Hoffman, 2002; Bartolo et al., 2012; Pasqui et al., 2012). In addition, they also prevent the body to lose water, protect the wound from external contamination and show antimicrobial effect to inhibit infection (Jurjus, 2007; Drago et al., 2010; Goldberg and Diegelmann, 2010). However, polymeric dressings are not sufficient in infected wounds; materials with antibacterial and antiseptic properties are needed. Therefore, new materials are produced by adding synthetic drugs to some natural polymer dressings. However, when synthetic drugs are used continuously, antibiotic-resistant microorganisms develop and problems are experienced in treatment (Sripriya et al., 2004). Therefore, the use of plants with natural antimicrobial properties when producing polymeric films becomes important.

In families with children, apart from injury, the irritation made by diapers on the child's skin is also a major problem. It is known that the water-holding material in diapers is the Super Absorbent Polymer (SAP – slush powder). This substance was banned sometime in the past; however, it was allowed to be used again after the 1990s. In this study, HEC, a natural polymer, and aloe vera gel, which has water-holding property, were used; they may be alternatives to SAP by some additional studies to be performed. Also, the aloe gel contains about 55% polysaccharide (especially Glucomannan and Acemnnan ensuring the tissue regeneration), 17% sugar, 16% mineral, 7% protein, 4% lipid, and 1% phenolic compounds (Ahlawat and Khatkar, 2011). Due to its numerous bioactive components in its structure, it is effective in

tissue regeneration and growth (wound healing) with its antifungal, antiseptic, antiviral, antibacterial, anti-inflammatory, antioxidant, immune modulator, and antidiabetic effects (Rahman et al., 2017). Thanks to these properties, it can be an alternative to various chemical or synthetic substances used for hygiene in diaper production.

This study showed that the films produced using HEC and Aloe vera gel which had antimicrobial, tissue-regenerating components could be used in wound treatment and that wound follow-up can be done easily, especially in open wounds. Due to its high capacity for liquid absorption, it was determined that for children with sensitive skin, it could be used in the production of diapers instead of chemical water holders and antibiotics. In addition, it was seen that natural and more cost-efficient solutions could be found for the issues of antibiotic resistance and recycling, which are among today's problems.

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INVESTIGATION OF THE EFFECTS OF WALL PAINT AND FILMS PRODUCED USING ALOE VERA GEL IN RADIATION PROTECTION

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ABSTRACT

Emission or transmission of energy in the form of electromagnetic waves or particles is called radiation. Besides natural radiation, people are also intertwined with electromagnetic waves due to the development of technology. In this study; It is aimed to investigate the effect of Aloe vera in the protection of ionizing and non-ionizing radiation by producing wall paints and films containing aloe vera gel.

The plants, in the form of seedlings, were multiplied and the gel was obtained when the leaves got matured. Gel was used to produce wall paint and film. The protective effect of the wall paint against ionizing radiation was analyzed by using neutron counting with Polymaster device and gamma counting with HpGe detector. The shielding effect of non-ionizing radiation features was determined by Using the 3 film, produced with different properties, shielding effect of non-ionizing radiation was determined by RF analysis.

As a result of the study, it has been determined that the protective feature of the wall paint against ionizing radiation was not the desired size, but the shielding feature of the films against non-ionizing radiation was at the same level with the standards. By using the data obtained, glass films, tulle, curtains and other protective products can be produced by using natural materials instead of chemicals and materials that are difficult to recycle, answers to natural problems can be found naturally.

Key Words: Aloe vera, Wall Paint, Film, Ionizing and Non-Ionizing Radiation

INTRODUCTION

The emission of energy in space in the form of waves or particles is called “radiation”. Radiation is classified as ionizing and non-ionizing radiation (Bor, 2015). The ionizing radiation is the radiation that forms ion pairs by removing electrons from the orbits of the atom it encounters. The ionizing radiation is divided into two;

Particle-type radiation: They are very fast-moving particles with specific mass and energy. Alpha (α) and beta (β+, β-) radiations can be given as examples.

Wave-type radiation: It is a type of radiation that has a certain energy but is massless. This group contains X and gamma (γ) rays.

Non-ionizing radiation is radiation that does not form ions in the substance it interacts with. Radio waves, microwaves, red and ultraviolet light, and visible light are examples of this type of radiation (Ince, 2002; Togay, 2002).

All living beings are exposed to ionizing radiation from natural sources and this is an indispensable feature of the natural life. There are two main sources of the natural radiation exposure. These are high-energy cosmic ray particles entering the atmosphere and radioactive nuclei found in the earth's crust. In addition, there is also artificial radiation exposure that is often caused by medical applications. The annual radiation doses, which humans are exposed

to, are 0.39 mSv from high-energy cosmic ray particles, 0.46 mSv from earth and 0.23 mSv from in-body irradiation. On the other hand, the most common cause of radiation in the world is Radon gas with its annual effective dose of 1.3 mSv (Çimen et al., 2017). Since understanding the presence of radiation is not possible with the sense organs, its detection and measurements are made with radiation-sensitive devices (e.g., radiation detector) (Dönmez, 2017). When these radiations exceed certain doses, they cause various ailments instantly or after a period of time. For example, it has been identified that there are radiation-induced increases in breast, thyroid, colon, stomach, ovary, esophagus, bladder, liver, and lymph cancers. Moreover, it is known that radiation causes hereditary disorders, nervous and immune system disorders, cataract, hyperparathyroidism, microcephaly, and growth-development and mental retardation (Sugarman et al., 2009). Although radiation sources have negative effects on living beings, they are used for diagnostic and therapeutic purposes in medicine and for beneficial purposes in industry, nuclear reactors, and various research activities. For radiation protection, those, working in this area, often use folding screens, gonadal protectors, lead glasses, lead aprons, gloves, glasses, and neck protectors (Çimen et al., 2017). Studies are also carried out for protection from natural radiation, and dyes, glasses, screen protectors, and films are produced using various metals and chemical materials. The most important problem today is the use of too many chemicals, their recycling, and the pollution and disturbances that they cause. For struggling with this problem, by using plants such as aloe vera, natural solutions should be sought and tried.

Aloe vera, which is a member of the *liliacea* family and also called *Aloe barbadensis Miller*, has over 275 species in the world (Rahman et al., 2017). Four species of these are used for commercial purposes. It is attracted to attention that numerous studies related to the plant have been done in many different areas in the world. Studies such as polyamide nanocapsule production (Esmaeili and Ebrahimzadeh, 2015), investigation of its usability as a natural plasticizer (Pandey et al., 2016), creating tissue scaffold by using nanofibers, and producing gel and film by using biomolecules such as polycaprolactone (PCL), chitosan, and polyvinyl alcohol (PVA) (Rahman et al., 2017) have been carried out. Furthermore, the effect of aloe vera extract on the radiation-induced oxidative stress (Nada et al., 2013) and the effect of it on wounds occurred on skin due to radiation in the radiotherapy process have been investigated (Olsen et al. 2001; Haddad et al., 2013; Ahmadloo et al.,2017; Rao et al., 2017). In our country, the antimicrobial effect of the plant has been studied (Çete et al., 2005) and by adding it into the structure of fishmeal, the effect of it on the growth of fish has been investigated (Yılmaz et al., 2019). By using poly (vinyl alcohol) (PVA), poly (ethylene glycol) (PEG) and PVA + PEG together with Aloe vera, Uslu et al. (2010) produced a nanofiber dressing material.

In this study, it was aimed to investigate the effect of Aloe vera on protection from ionizing and non-ionizing radiation by producing wall paint and film containing Aloe vera gel.

MATERIAL AND METHOD

Obtaining Gel from the Aloe Vera Plant

The mature leaves of the plants bought and grown as seedlings were selected and cut; then, the green parts of the leaves were peeled with a knife and the gel part was taken. The samples collected in the plastic container were turned into gel by thoroughly broken down with the help of blender and Mixer (Figure 1).

Figure 1: Obtaining Aloe vera gel.

Producing Dye Containing Aloe Vera Gel

While the dye was produced, after 275 ml of water was put into the container where the materials would be mixed, 75 g of rejuvenating liquid, 10 g foam blocker, 90 g natrosol preventing dye collapse, 35 g antibacterial protective material preventing dye odor, 50 g antifreeze preventing dye freezing, and 135 g powder filler (omyacarb calcite) were added as auxiliary materials. Then, they were mixed and cooked at the appropriate temperature until it reaches the desired consistency. After the dye cooled, 30 g acrylic glue, 90 g foam blocker, 10 g thinner, and 200 ml water were added, and it was made ready by mixing for 1 hour. In the second phase of the study, after the dye cooled, instead of 200 ml water, 100 ml water+100 ml Aloe vera gel was added and mixed. The resulting dyes were used to paint the clay-made building bricks, which were the main elements of constructions (Figure 2).

Figure 2: Preparation of the painted samples

Investigation of Ionizing Radiation Shielding Properties of the Produced Dye

In the study, which was conducted at a university's Energy Institute, two methods (gamma and neutron analysis) were used. In the analyses, bricks painted with the produced dyes were used. After the painted bricks were placed in the device, the analysis began and the necessary measurements were taken. In the analysis, the neutron count was performed with the Polimaster (PM1401K) device (Figure 3).

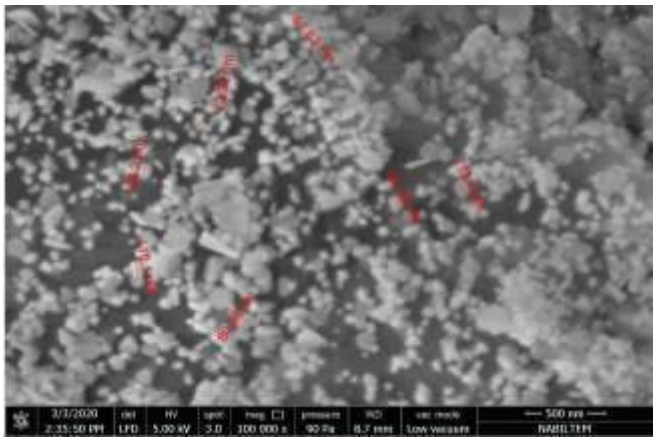

Figure 3: Neutron analysis with the Polimaster device

Another analysis related to the ionizing radiation, on the other hand, was conducted by using the HpGe detector and performing gamma counting (Figure 4).

Figure 4: Gamma counting with the HpGe detector

Film Production for Non-Ionizing Radiation Analysis

At this stage, 3 different films that did not contain Aloe vera gel, contained 10% gel and contained 10% gel + nano-silver were produced. First, nano-silver particles were obtained. 20 ml aloe vera gel and 2ml AgNO3 were added to the glass beaker and they were mixed in a magnetic mixer; the examination was performed at the spectrophotometer (380-500nm) every 30 minutes. After being washed with pure water 7 times (5000 rpm, 3 min) by using a centrifuge, it was dried, and measurement was carried out by taking photos with SEM (Figure 5).

Figure 5. Silver nanoparticles

To obtain a film that does not contain Aloe vera gel, the glass beaker containing 190 ml distilled water, 10 ml glycerol and 2 g HEC was placed in a magnetic mixer (500-600 rpm, 2 unit temperature) and mixed for 1.5 hours until the gel consistency was obtained. In the production of the film containing 10% gel, 170 ml distilled water, 20 ml gel, 10 ml glycerol, and 2 g HEC were used. In the production of the third film, by setting the quantities, the same method was also followed. The prepared gels were poured into glass containers and placed in a vacuum sterile cabinet for drying (Figure 6).

Figure 6: Production of the sample films for analysis

After two days, it was determined that the films were dried in the desired level. Then, the films were taken out of the containers and packaged (Figure 7).

Figure 7: Sample films; A: Gel-free, B: 10% gel, C: 10% gel + silver nanoparticle

Investigation of Non-Ionizing Radiation Protection/Shielding Properties of the Produced Films

The produced films' protection or shielding effect against the non-ionizing radiation emitted from computers, mobile phones, or internet modems, which we continuously keep in touch, were examined by using the RF-Analyzer (HF 58B-R). To evaluate the result of the films, X (made of Polyester + silver) and Y (made of cotton, polyester, and stainless steel) samples produced by a company working in this field were used for comparison purposes (Figure 8).

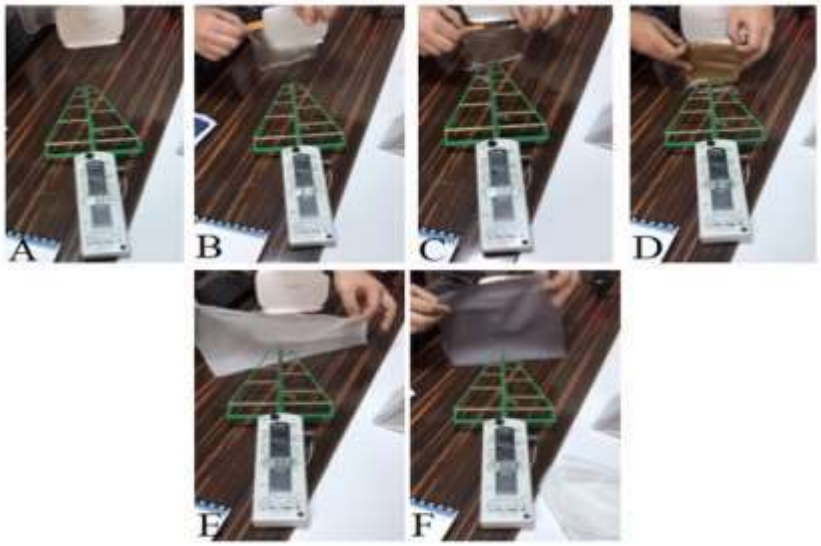

Figure 8. A: Amount of electromagnetic radiation emitted from the modem, B: Film without Aloe vera gel, C: Film containing 10% Aloe vera gel, D: Film containing 10% Aloe vera gel+silver nanoparticle, E: sample X, F: sample Y

RESULTS

The neutron count, in which the protection or shielding effect of the dye samples containing and not containing the produced Aloe vera gel is analyzed, is given in Table 1.

Table 1. Neutron count results

Measurement Number	Dye containing Aloe vera gel (Cps)*	Dye without Aloe vera gel (Cps)*
1	250.6	275.3
2	250.3	278
3	250	275.5
4	250.7	275.2
Mean	250.4	276

*Cps: Number of neutrons per second

The results of the gamma count, which is the second analysis of the produced dyes' protection or shielding effect against the ionizing radiation, are given in Table 2. At this stage, in order to get a healthy result, the HpGe detector was first operated as empty 3 times for 10 minutes and the average was taken. Then, when it is empty, when the brick painted by the dye containing Aloe vera gel was placed in it, and when the brick painted by the dye that did not contain Aloe vera gel was placed in it, the detector was operated for 3 hours for each condition and measurements were done (3 hours is accepted as standard). The measurements were repeated 2 times.

Table 2. Gamma count results

	Detector	Sample with dye that do not contain A.v. gel	Sample with dye containing A.v. gel
1st Measurement	14100	39667	39754
2nd Measurement	13600	37936	38560
Mean	13850	38801.5	39157

In Table 3, the results of the analysis in which 3 different films' non-ionizing radiation protection or radiation shielding effects were examined by using the internet modem found in almost all houses and workplaces are given through comparing them with the standard X and Y samples.

Table 3. Radio frequency analysis results of the produced film and the standard samples

Samples	RF Analyzer (mW/m^2)	Shielded amount (mW/m^2) and its percentage
Modem	3.04	-
Film that do not contain A.v. gel	0.18	2.86 (%94.07)
Film containing 10% A.v. gel	0.02	3.02 (%99.34)
Film containing 10% A.v. Gel + silver nanoparticle	0.16	2.88 (%94.73)
X	0.02	3.02 (%99.34)
Y	0.02	3.02 (%99.34)

* The shielded amount was calculated according to the difference between the modem and the samples.
* mW/m^2: Milliwatt/square meter

DISCUSSION AND CONCLUSIONS

There is a view that is known and practiced in most places in our country: "If plants such as cactus and aloe vera are placed next to the computer, the person will not be affected by the radiation emitted". Is it true or not? Although we do not know a clear answer, these views arouse curiosity in people and lead to many scientific studies. In this study, it was aimed to produce wall paint and film by using Aloe vera gel and to investigate the effect of aloe vera in protection/shielding from ionizing and non-ionizing radiation by doing various analyses with these materials.

In the investigation of the plant's usability in the protection from ionizing radiation, two different analyses were performed. Based on the results of the neutron count analysis conducted with the Polimaster device (Table 1), it was determined that while the neutron count of the brick painted with dye containing Aloe vera gel was 250.4 Cps, the neutron count of the brick painted with gel-free dye was 276 Cps on average. There are natural sources of radiation in the structure of the brick too. However, it is clearly seen that there is no significant difference between the dye with aloe vera gel and the dye without gel in terms of neutron permeability; the neutron absorption property of the gel is quite low. Therefore, aloe vera gel can be used as material in studies requiring neutron permeability. When Table 2 in which gamma count results are presented was examined, it was determined that the gamma value was 13850 for HPG detector where the analysis is conducted, 38803.5 on average for the brick painted with the dye that did not contain the plant gel, and 39157 on average for the brick painted with the dye containing gel. If the analyzed samples had a protective effect against the ionizing radiation, the value of the brick painted with the dye containing the gel should have been less than the sample painted with the dye that did not contain the gel. When the experts in the field were consulted, they stated that the fact that this value was found in the 35000s may be sufficient to explain that it showed a positive effect. In the results of the two analyses conducted, it should not be overlooked that the brick is rich in radiation factors such as thorium and uranium which it obtained from nature. If the dye produced by adding gel is tried only on cement or different

materials used in building construction, different results can be obtained. When studies related to Aloe vera and radiation are examined, it is seen that studies are mostly done on the ailments caused by radiotherapy. While in some sources, it is stated that the plant is effective in wounds caused by ionizing radiation during the radiotherapy process (Olsen et al. 2001; Haddad et al., 2013; Rao et al., 2017), in some other sources, on the other hand, it is explained that it is not effective in prevention and healing of these wounds (Ünlü et al., 2016; Ahmadloo et al., 2017). In their experiments conducted on rats, Nada et al (2013) reported that aloe vera extract created a protective effect against the radiation-induced oxidative stress. Apart from method and techniques, the differences in the results may be due to the fact that the quality of the aloe vera gel varies depending on its species type, growth conditions (e.g., climate, water, fertilization), harvest, extraction method, and ambient conditions (temperature, sterilization) (Rahman et al., 2017).

It is known that the techniques of protecting and shielding people from the electromagnetic radiation (non-ionizing radiation) emitted by the technological products that we use very often in our daily lives are becoming a need everywhere. In this context, curtains, fabrics, dyes, and glass films are produced and studies are keeping on. In this study, film production by using HEC natural polymer was attempted and non-ionizing radiation-related studies were analyzed using these produced films. In the previous studies, films containing aloe vera gel had been produced. However, in those films, substances such as PCL, PVA, PEG, chitosan, alginate, and pectin gelatin were used as polymers (Pereira et al, 2013, 2014; Silva et al., 2013; Anjum et al., 2016; Rahman et al., 2016; Tummalapalli et al, 2016) and films were tested in health or tissue engineering studies. When the results of 3 different gels produced for non-ionizing radiation trials were examined by comparing them with the standard samples (X and Y) (Table 3), the fact that the radiation shielding property of all samples was determined to be around 99% and the fact that with 99.34% shielding rate, the film containing 10% aloe vera gel was at the same value with the standard samples attract attention. It was observed that silver nanoparticles did not increase the gel effect, but rather inhibited somewhat. In terms of standard samples, X was produced from polyester and silver, while Y was produced from cotton, polyester, and stainless steel; both of them were products available commercially. These products are usually produced in Germany, Italy, and Switzerland, and their prices per square meter vary between 75-175 Euros. X is produced in our country and sold for 75 euros per square meter. Taking into account the data obtained, it can be said that metals can be protected from non-ionizing radiation through producing natural products, which are cheaper and do not cause environmental pollution (because they are recyclable), by using plants (without using various chemicals).

In the conducted literature review, studies on non-ionizing radiation were not be encountered much. In their study, Sheikh et al (2013) reported that aloe vera leaves showed a significant increase in current with a slight increase in voltage at first when they exposed to microwave, but then set themselves to their normal value. Studies support each other.

As a result of the study, it was determined that the dye produced by adding aloe vera gel was not effective in protection against ionizing radiation since its ion absorption property is low. The absorption property can be increased by adding some different metals to the gel. It was determined that the shielding property of the film, produced by adding Aloe vera gel, against non-ionizing radiation was equivalent to the standards. It is thought that by developing these data, cheap, natural and recyclable glass film, tulle, and fabric can be produced.

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EFFECTS OF DIETARY L-ALLIIN AND OLEUROPEIN ON GROWTH PERFORMANCE AND BODY COMPOSITION OF *TILAPIA NILOTICUS* (*OREOCHROMIS NILOTICUS*)

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ABSTRACT

The study was conducted to evaluate the growth performance and body composition of *Oreochromis niloticus* (average initial body weight 34.37 ± 0.29g) fed diets containing different levels (0.25, 0.5, 1 mg/kg) of L-Alliin and Oleuropein, for a period of 60 days. The addition of L-Alliin to the feed increased the final weight ($p<0.05$). The level of L-Alliin and Oleuropein in fish diets did not affect body crude protein, ash contents, body moisture and lipid ($p>0.05$).

Keywords: *Tilapia Niloticus*, L-Alliin, Oleuropein, growth performance, body composition

INTRODUCTION

Medicinal plants have been reported as a cheaper and more sustainable alternative to chemotherapy in aquaculture because they show numerous bioactivities such as anti-stress, immunostimulants and antiparasitic (Reverter et al., 2014). Garlic has various biological functions such as antioxidant, antimicrobial and antihypertensive effects (Konjufca et al., 1997; Sivam, 2001). Oleuropein and its metabolite hydroxytyrosol have been reported to be a potent antioxidant, antiangiogenic and anticancer agents (Vissers et al., 2006; Visioli et al., 2002). The effects of L-Alliin and Oleuropein on growth performance in Nile tilapia (*Oreochromis niloticus*) have not been reported in the literature to date. For this reason, we carried out this study on the Nile tilapia under controlled conditions.

Material and Methods

The experiment was conducted at Aydın Adnan Menderes University, Faculty of Agriculture, Aquaculture Research and Application Unit. The analyses were realized at Aydın Adnan Menderes University Agricultural Biotechnology and Food Safety Application and Research Center (TARBIYOMER). Before the feeding experiment, the fish were adapted to the experimental environment for 15 days. In trial, 210 Nile tilapia (*Oreochromis niloticus*) which has average weight of ±SD =34.37±0.29 was used. No additive was supplied to the control feed. Alliin and Oleuropein were added to the experimental feeds at the rate of 0.25 mg/kg, 0.5 mg/kg and 1 mg/kg. In the 60-day trial, the fish were hand-baited 3 times a day at a rate of 2% of their body weight. The experiment was conducted in 21 aquariums in the 200 liter. In the experiment, 30 fish were put into each tank in 3 replications (Yılmaz et al., 2018). Alliin and Oleuropein were obtained from Synergy Laboratory ind.trade.co.ltd. Commercial feed (Gümüşdoğa Trout Feed) was used as an experimental feed (Table 1). Alliin and Oleuropein were added to the feeds by dissolving in water and ethyl alcohol and spraying as reported in the literature (Bilen et al., 2014).

Table 1. Fish diet (pellet size: 1mm)

	%
Crude Protein	53
Crude Lipid	15
Crude Cellulose	0.9
Crude Ash	11

During the experiment, temperature, oxygen, and conductivity measurements of the test aquariums were monitored every other day with the help of WTW Multi 3420 portable multi-parameter device. Moisture, protein and ash AOAC (1998) and lipid analysis were made according to Folch et al. (1957). The analysis of the data obtained in the experiment was done using SPSS 21 statistical software. One-way analysis of variance (ANOVA) was applied to the data and Tukey multiple comparison tests were performed. Differences between the groups were evaluated as $p<0.05$ (Logan, 2010).
This study was approved by the local Ethics Committee for animal experimentation at Aydın Adnan Menderes University (decision number 2018/107) as appropriate for the ethics of animal experimentation.

Results

During the trial, the water temperature was 27.20±0.56°C, oxygen was 8.17±0.06 mg/l, pH was 7.07±0.01. The growth performance and feed conversion rates of L-Alliin and Oleuropein-added (0.25 mg/kg, 0.5 mg/kg, and 1 mg/kg) and additive-free (control) feeds are shown in Table 2. Adding 1 mg/kg L-Alliin to feed positively affected the average weight of the fish at the end of the trial ($p<0.05$).

Table 2. Growth performance in tilapia niloticus that were fed diets containing different levels of L-Alliin and Oleuropein (0.25, 0.5, 1 mg/kg of feed; diets control, A1, A2, A3,O1,O2 and O3, respectively) for 60 day

	Control	A1	A2	A3	O1	O2	O3
IW (g)	34.30±0.32^{a}	34.65±0.48^{a}	34.40±0.48^{a}	34.34±0.15^{a}	34.01±0.3^{a}	34.31±0.21^{a}	34.63±0.15^{a}
FW (g)	69,00±1,78^{b}	69.90±1.78^{ab}	70.04±0.84^{ab}	72.59±0.49^{a}	69.34±1.86^{b}	69.68±0.90^{ab}	69.83±0.72^{ab}
WG (g)	34.63±0.36^{a}	35.24±2.09^{a}	35.63±0.68^{a}	37.68±1.27^{a}	35.32±1.98^{a}	35.37±0.78^{a}	35.20±0.65^{a}
SGR (%/d)	1.16±0.00^{a}	1.16±0.05^{a}	1.18±0.02^{a}	1.23±0.03^{a}	1.18±0.05^{a}	1.18±0.01^{a}	1.16±0.01^{a}
FCR	0.87±0.03^{a}	0.83±0.05^{a}	0.85±0.10^{a}	0.79±0.02^{a}	0.87±0.02^{a}	08.3±0.02^{a}	0.85±0.02^{a}

Values are mean ± SE (n = 3). Within a row, means with differing letters are significantly different ($P < 0.05$).
A: L-Alliin O: Oleuropein

The findings of chemical nutrient composition in the flesh of fish at the end of the trial in groups fed on feeds containing L-Alliin (A1, A2, A3) and Oleuropein (O1, O2, O3) are displayed in Table 3. As shown in Table 3, there was no statistical difference between the groups in the ratio of moisture, protein, ash and lipid in fish meat ($p>0.05$).

Table 3. Whole-body proximate composition (%) of tilapia niloticus fed diets with different levels of L-Alliin and Oleuropein for 60 day

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Control	73.83±2.64[a]	14.21±1.21[a]	4.28±0.26[a]	5.44±1.24[a]
A1	75.06±4.05[a]	14.21±1.72[a]	5.14±0.53[a]	4.92±0.48[a]
A2	73.87±5.15[a]	15.03±1.81[a]	4.70±0.28[a]	4.85±0.60[a]
A3	74.05±1.60[a]	15.37±1.40[a]	4.35±0.99[a]	4.71±4.66[a]
O1	76.13±11.56[a]	13.66±5.56[a]	5.72±1.01[a]	3.18±1.86[a]
O2	74.46±5.49[a]	15.77±2.93[a]	4.01±2.15[a]	4.15±0.94[a]
O3	74.59±6.93[a]	14.21±2.70[a]	4.68±0.97[a]	4.57±2.24[a]

A: L-Alliin O: Oleuropein

Discussion

As a result of this study, it was determined that the addition of L-Alliin in feed increased the fish's end-of-experiment weight gain. Similarly, different studies have reported that garlic added to fish feed has a positive effect on the growth performance of fish (Talpur and Ikhwanuddinb, 2012; Büyükdeveci et al., 2018). In a different study, Nile tilapia (19.7±0.94 g) was fed for 12 weeks with the ether extract of olive leaf (1 g/kg) and ethanol extract of propolis (4 g/kg). The results revealed that feed additives increased the fish's growth performance, body composition, feed conversion rate, immune parameters (Shahin et al., 2019).

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THE EFFECT OF A NISIN PRODUCER STRAIN *L. LACTIS* SUBSP. *LACTIS* LL27 ON THE FORMATION OF *LISTERIA* AND *SALMONELLA* BIOFILM STRUCTURES

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ABSTRACT

In this study, the antibiofilm efficacy of the *Lactococcus lactis subsp. lactis* LL27 strain isolated from raw milk and identified as nisin producer, against *Salmonella* Typhimurium and *Listeria monocytogenes* biofilms were investigated. In the research primarily, autoaggregation capabilities of each strain were determined. The strain with the highest autoaggregation ability was determined as *L. monocytogenes* ATCC7644 (0.677), followed by *S.* Typhimurium 14028 (0.55) and *L. lactis* subsp. *lactis* LL27 (0.184). In the cocultures of *L. monocytogenes* ATCC7644 *and S. Typhimurium* 14028 treated separately with *L. lactis* subsp. *lactis* LL27, coaggregation rates (average 0.46) were found close to the autoaggregation rates of the aforementioned pathogens. However, these rates were found to be statistically significant ($p < 0.05$) higher than the autoaggregation rate of *L. lactis* subsp. *lactis* LL27 strain. Biofilm experiments were started, as this indicates that the nisin producer strain of *L. lactis* subsp. *lactis* LL27 may affect the formation of biofilms by reducing the autoaggregation ability of the pathogens in question. *Salmonella* Typhimurium 14028 biofilm capacity statistically significant increase ($p < 0.05$) in the cocultures of *L. lactis* subsp. *lactis* LL27 + *L. monocytogenes* ATCC7644 and *L. lactis* subsp. *lactis* LL27 + *S.*Typhimurium 14028. The most interesting result in the study was the statistically significant decrease ($p < 0.05$) in biofilm production capacity on the 3rd day in *L. monocytogenes* ATCC7644 and *S.* Typhimurium 14028 cocultures. These data indicate that direct use of starter culture strains in the fight against biofilm structures of pathogens may promote biofilm production, as opposed to the antagonistic effect expected.

Keywords: *L. lactis*, *S.* Typhimurium, *L. monocytogenes*, biofilm

INTRODUCTION

Biofilm forms created by pathogenic microorganisms are the main source of permanent infections, especially in the medical and food industry. Biofilms are multicellular-like life forms consisting of one or more species. In these forms, after the cells attached to organic or inorganic surfaces form microcolonies, they usually surround them with an extracellular polymeric matrix and develop and form this matrix, embedded biofilm forms. Biofilm structures do not only emerge as coexistent behaviour but also act as a community whose genetic and biochemical regulation is common and responds to environmental effects together. This led to the definition of biofilm forms as a very different structure from planktonic life forms (1,2).

It is characteristic that the biofilm forms and the cell or cells that make up the biofilm show much higher resistance to environmental stress conditions than their independent (planktonic) forms. Since they are much more resistant to antibiotics and other antimicrobial agents, heavy metal ions, drying, nutrient deficiency, competitive microflora, pH and salt stress, the prevention and eradication of these forms has become one of the main problems of clinical and industrial microbiology. For this reason, the identification of effective combating agents against biofilm forms has become an intense focus of research (3,4). The critical factor in these

studies is that biofilm control agents do not have negative effects on consumer health and the environment. For this reason, many chemical agents and antibiotics have been removed from the class of preferred agents in the fight against biofilms. Today, among the environmental and consumer health-friendly agents, the search for the use of lactic acid bacteria, which are used as starter culture strains in the production of fermented products, in the fight against biofilm structure is due to this requirement (5,6,7).

In this study, the effectiveness of direct use of *Lactococcus lactis* subsp. *lactis* LL27 strain against *Listeria monocytogenes* and *Salmonella* Typhimurium pathogens isolated by our research group, which is isolated from raw milk and defined as a manufacturer of nisin (a peptide-based lantibiotic produced by lactococcus strains) was investigated. Research findings showed that contrary to expectations, *L. lactis* subsp. *lactis* LL27 strain had a synergistic effect, not antagonistic against biofilm forms of the pathogens in question.

Material and Method

Bacterial Strains and Growth Conditions

L. monocytogenes ATCC7644, *S.* Typhimurium 14028 and *L. lactis* subsp. *lactis* LL27 strains were obtained from Ankara University Faculty of Science, Department of Biology Prokaryotic Genetics laboratory culture collection. Stock cultures were stored at -80 ℃ for *L. monocytogenes* ATCC7644 and *S.* Typhimurium 14028 in LB broth media containing 60% glycerol and for *L. lactis* subsp. *lactis* LL27 in M17 broth media containing 60% glycerol (Merck, Germany).

Auto-Aggregation and Co-Aggregation Assays

In the examination of the autoaggregation properties of bacteria, the method proposed by Kos et al. 2003 (8) was taken as a basis, but some changes were made (9). *L. monocytogenes* ATCC7644 and *S.* Typhimurium 14028 strains were developed at 37 ℃ in LB broth medium and *L. lactis* subsp. *lactis* LL27 strain at 30℃ in M17 broth medium for 12 hours. Cells were centrifuged at 5000 x g for 15 minutes. After discarding the upper liquid, the pellet was washed twice with distilled water. Subsequently, these media were diluted in PBS with approximately 10^8 CFU / mL cells. Cell suspensions with a volume of 4.0 mL were mixed for 15 seconds in a mechanical mixer and autoaggregation rates were determined for 5 hours incubation at 25℃. For this, 0.1 mL samples taken every hour were mixed with 3.9 mL PBS and their absorbance at 620 nm was measured. The autoaggregation rate was defined as $(1- A_t/A_0) \times 100$ (where, A_t represents the absorbance at t = 1, 2, 3 and 4, and A_0 represents the absorbance at time t_0). Coaggregation trials were also carried out in the same way as autoaggregation trials. Merely, the experiments were continued after the strains used in coagulation were mixed in equal volumes (total volume of 4.0 mL). Coaggregation rate was determined using the formula as $[1- 2A_{mix} / (A_{probio} + A_{indicat})] \times 100$. (where, A_{probio} and $A_{indicat}$ refer to each of the two strains in the control tubes and A_{mix} refers to the mixture of these two strains) (10).

Biofilm Assay

To create biofilms on polystyrene surfaces, 18-hour active cultures were adjusted to 0.07 at OD570 nm and inoculated into LB medium. After 24 hours incubation at 20 ° C, biofilm formation on these surfaces was quantified in the ELISA reader at the end of the crystal violet binding test. The efficacy of the *L. lactis* subsp. *lactis* LL27 strain on the biofilms of *L. monocytogenes* ATCC7644 and *S.* Typhimurium 14028 strains was determined using the same method after mixing this strain with pathogens in microtitre wells in a ratio of 1/1 (11).

Statistical Analysis

Statistical analysis of the data obtained as a result of the experiments was carried out using SPSS 18.0 software. Data with a P value of <0.05 were considered statistically significant.

Results

Autoaggregation and coaggregation

When the autoaggregation properties of the studied strains were examined, the highest values were obtained in *L. monocytogenes* ATCC7644 strain for all times (0.573-0.667). This was followed by *S.* Typhimurium 14028 (0.36-0.55) and *L. lactis* subsp. *lactis* LL27 (0.153-0.184). In the coaggregation of *L. lactis* subsp. *lactis* LL27 strain with pathogens, the said ratios are; for *L. lactis* subsp. *lactis* LL27 + *L. monocytogenes* ATCC7644, it was (0.366-0.480) and for *L. lactis* subsp. *lactis* LL27 + *S.* Typhimurium 14028, it was around (0.268-0.464) (Table 1 and Figure 1). Compared to pathogen autoaggregation, decreases in pathogen coaggregation with *L. lactis* subsp. *lactis* LL27 were found statistically significant (p <0.05)

Table 1. Autoaggregation and coaggregation rates of strains used in the experiment

Time	14028	ATCC7644	LL27	LL27/ATCC7644	LL27/ 14028
0. h	0,55	0,677	0,184	0,48	0,464
1. h	0,436	0,623	0,177	0,453	0,303
2. h	0,431	0,637	0,168	0,427	0,295
3. h	0,36	0,619	0,153	0,442	0,342
4. h	0,467	0,573	0,181	0,366	0,268

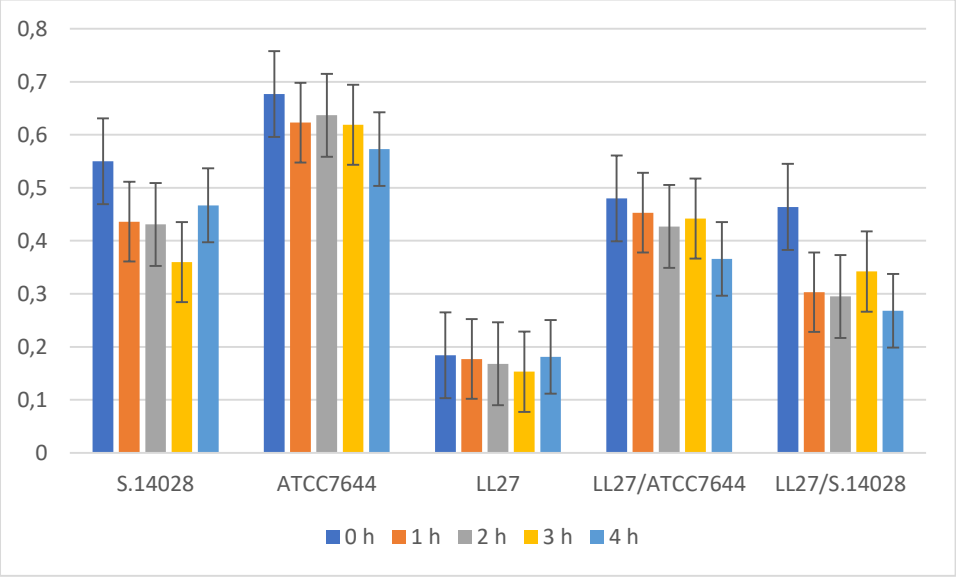

Figure 1. Autoaggregation and coaggregation graph

The effect of *L. lactis* subsp. *lactis* LL27 strain on the formation of pathogen biofilms

In the first stage, the biofilm formation ability of pathogens was monitored for 3 days to determine the effect of *L. lactis* subsp. *lactis* LL27 strain on the biofilm-forming ability of pathogens. As a result of these trials, *S.* Typhimurium 14028 reached the highest biofilm production level (2.214) in 24 hours, while *L. monocytogenes* ATCC7644 produced the highest biofilm production (1.447) in 72 hours. In *S.* Typhimurium 14028 co-cultured with *L. lactis* subsp. *lactis* LL27, the amount of biofilm increased statistically ($p < 0.05$) in all incubation times. The same was determined for the biofilm structures formed in the cocultures of *L. lactis* subsp. *lactis* LL27 and *L. monocytogenes* ATCC7644. However, in *L. monocytogenes* ATCC7644 and *S.* Typhimurium 14028 cocultures, biofilm amounts were lower than in monocultures at all times (Table 2 and Figure 2).

Table 2. The effect of *L. lactis* subsp. *lactis* LL27 strain on the formation of pathogenic biofilms

Strains / Time	ll	sal	lis	ll+lis	ll+sal	lis+sal
24 h	1,059	2,214	0,428	1,832	3,784	2,602
48 h	0,853	1,285	1,045	1,723	3,513	2,126
72 h	0,62	1,048	1,447	2,35	2,193	0,399

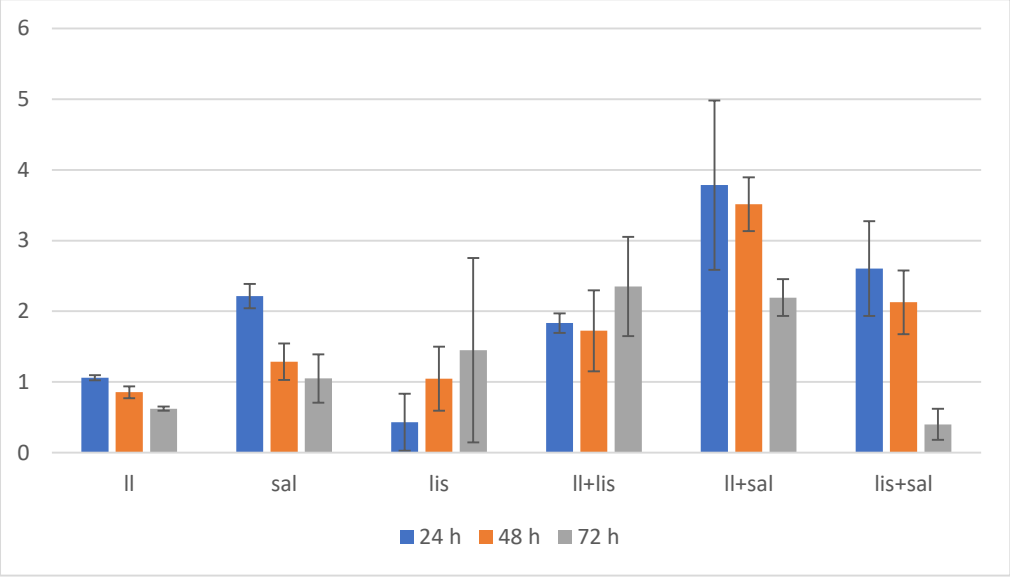

Figure 2. The effect of *L. lactis* subsp. *lactis* LL27 strain on the formation of pathogenic biofilms.

Discussion

In order to prevent the formation of biofilms of pathogens, the use of lactic acid bacteria or the metabolites produced by them is studied extensively because these organisms are reliable in human and animal consumption (GRAS). One of the common tests for determining effective strains in these studies is the ability of lactic acid bacteria to form aggregates with pathogens.

In these tests, lactic acid bacteria, whose coaggregation is determined to be high with pathogens, are predicted to have an antagonistic effect for biofilm formation (12,13,14,15). However, in our study, *L. lactis* subsp. *lactis* LL27 strain, which shows a very good co-aggregation ability with pathogens and is a producer of nisin, has had a synergetic effect on the biofilm formation of both pathogens used in the study. This is probably due to the fact that *L. lactis* subsp. *lactis* LL27 strain helps pathogens with its high coaggregative ability in adhering to the biofilm. Besides, the possibility that the proteins produced by this strain can contribute to the reversible attachment of pathogens to the surface should not be excluded.

Conclusion

In the fight against pathogen biofilms, the use of live microorganism cultures should be investigated by considering the fact that it can increase biofilm efficiency by promoting mixed biofilm formation and by examining the physiological effects at all biofilm formation stages. Because when these details are overlooked, it is possible that the opposite effects may occur.

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MICROBIAL AND PHYSICOCHEMICAL PROPERTIES OF KOMBUCHA FERMENTATION AND ALTERNATE SUBSTRATE SOURCES: A REVIEW

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ABSTRACT

Kombucha tea is an alcohol-free, low-acid beverage that is increasingly popular among traditional fermented foods, fermented by the symbiotic culture of yeast and acetic acid bacteria. Kombucha culture is known as tea fungus and it produces microbial cellulose. Acetic acid, glucuronic and gluconic acids are main metabolites that are produced other than microbial cellulose as a result of the fermentation. Because kombucha is a fermented beverage, it has many functional features which contain various phenolic and antioxidant substances, as well as having probiotic and antimicrobial properties. Black tea is generally used as a substrate in the production of traditional kombucha tea. However, in the literature, use of different substrates are available, such as green tea, coconut, oolong tea, peppermint, lemon herb, milk, mulberry leaf, soy and cherry juice. It is stated that biochemical and therapeutic properties of the produced beverage change, according to the type and amount of substrate used in fermentation, carbon source and rate, fermentation time, culture composition. In this review, it is aimed to investigate the kombucha tea fermentation process, the substrates used, the dominant microflora and the metabolites produced.

INTRODUCTION

Kombucha tea is an alcohol-free, low-acid, increasingly popular traditional fermented beverage fermented with a symbiotic culture of yeast and acetic acid bacteria (Villarreal-soto et al., 2018; May et al., 2019). Kombucha fermentation is a type of fermentation in which bacteria and osmophilic yeasts work together and is carried out by the tea fungus called SCOBY. In addition, black tea is generally used in the production of this beverage, which first appeared in Asia. Kombucha, which was consumed in China, Korea and Japan in 221 BC, spread to western countries after it was first brought from Korea to Japan by Dr. Kombu in order to treat the Emperor (Dufresne and Farnworth, 2000; Amarasinghe et al., 2018; De Pilippis et al., 2018; Laureys et al., 2020). Kombucha tea is obtained by incubating at an average of 28 ° C for at least 10 days under aerobic conditions (Velicanski et al., 2013). Two phases occur as a result of fermentation. The first of these is the liquid phase and is the sour-tasting, drinkable part containing the fermentation metabolites. The second is the film layer that develops on the surface. Bacterial cellulose membrane, which is formed as a by-product as a result of Scoby fermentation, is a reversible, organic raw material suitable for human consumption and mostly finds use in the textile and food packing industry (Ashrafi et al., 2018; Vohra et., 2018; Aduri et al., 2019; Sederavičiūtė et al., 2019; Tan et al., 2019; Zhou et al., 2019). As a result of Kombu fermentation, the main metabolites other than microbial cellulose are acetic acid, gluconic and glucuronic acid. Since Kombu is a fermented product, it has many functional properties such as containing various phenolic and antioxidant substances, as well as having probiotic properties and antimicrobial properties. In addition, it can detoxify the blood, lower cholesterol level, lower blood pressure, improve liver function, normalize intestinal activity, balance intestinal flora, reduce obesity and balance appetite, protect against diabetes, increase body resistance against cancer, strengthen the immune system, relieve bronchitis and asthma. It is stated that it has a positive effect on human health (Jayabalan et al., 2011; Jayabalan et al.,

2014; Laureys et al., 2020). Although many such health effects have not been proven by scientific studies, clinical studies on several functional and therapeutic properties of kombucha tea are available in the literature (Yang et al., 2009; Sai Ram et al., 2000; Banerjee et al., 2010; Jayabalan et al. , 2011; Aloulou et al., 2012; Bhattacharya et al., 2013; Chakravorty et al., 2016). In the production of kombucha, generally 1 L tap water and 50-100 g sucrose are boiled. Usually black tea is added at a rate of 0.15-0.6%, infused for 10 minutes and filtered. Then 0.1-0.2 L of previously fermented tea is added and incubated at 28-30 ° C. Fermentation is terminated by following the pH change after at least 7 days of fermentation. It is stated that the critical parameter determining the end of fermentation is titratable acidity rather than pH. Because the buffer substances in kombu tea affect the pH value. It is also expressed that the end of the kombu fermentation may also depend on the thickness of the tea fungus formed (Reiss, 1994; Velicanski et al., 2013; Jayabalan et al., 2014; Değirmencioğlu et al., 2019).

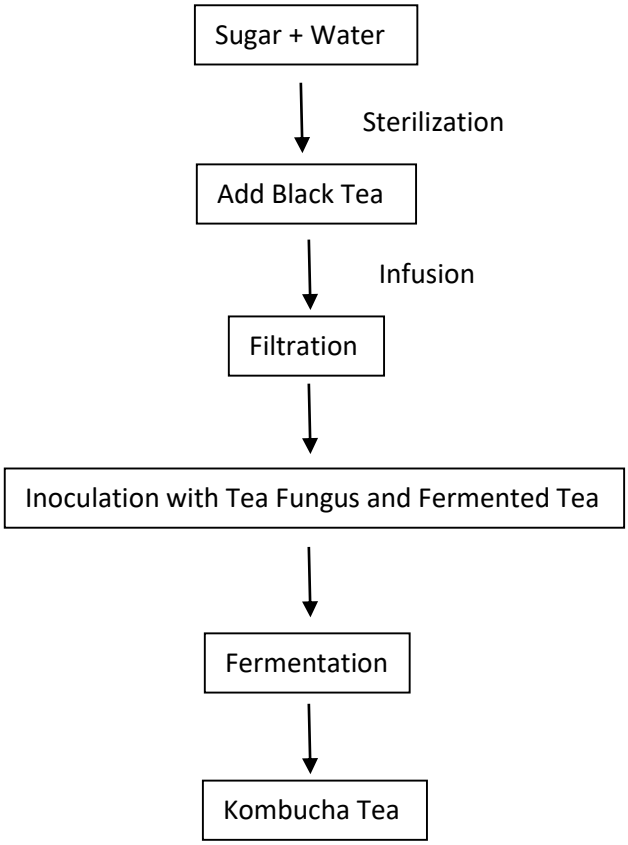

Figure 1. Schematic representation of kombucha production

It is indicated that the type and amount of substrate, carbon source and ratio, fermentation time, culture composition used in fermentation, change in the biochemical and therapeutic properties of the beverage obtained (Jayabalan et al., 2011).

The aim of this review is to investigate the production of kombucha tea, microorganisms responsible for fermentation and the metabolites formed, alternative substrate sources for fermentation.

Microbiota of Kombucha Tea

SCOBY (Simbiotic Culture of Bacteria and Yeast), which is responsible for Kombucha fermentation, usually consists of acetic acid bacteria and osmophilic yeast (Asai et al., 1964). Apart from these microorganisms, it also contains some lactic acid bacteria and the exact microbial composition of kombucha has not been determined. Fermentation temperature and time, inoculation rate, tea and sugar ratio cause diversity in microbiota and metabolites. *Acetobacter, Gluconacetobacter, Gluconobacter and Komagataeibacter* genes constitute the general bacterial profile of tea fungus. The most important species is *Acetobacter xylinum* which forms the cellulosic layer during Kombucha fermentation. The most common yeast genus in this culture is reported as *Saccharomyces, Schizosaccharomyces, Zygosaccharomyces, Brettanomyces / Dekkera, Candida, Torulospora, Pichia* (Markov et al., 2001; Teoh et al., 2004; Velicanski et al., 2013; Jayabalan et al., 2014; Marsh et al., 2014; Chakravorty et al., 2016; Coton et al., 2017). The strains of these yeasts vary widely and one of these strains is *Dekkera bruxellensis* isolated by Matei and others (2018).

In Kombucha, it is occured of two main fermentation, including alcohol and acetic acid. In alcohol fermentation, sugar is converted into ethanol by yeasts. This ethanol formed is oxidized by acetic acid bacteria and forms acetic acid, which is the characteristic metabolite of kombucha.

Table 1. Characteristics the genera of acetic acid bacteria present in kombucha

Characteristic	***Acetobacter***	***Gluconobacter***	***Gluconacetobacter***	***Komagataeibacter***
Flagellation	Peritrichous	Polar	Peritrichous	None
Oxidation of acetate	Positive	Positive	Positive	Positive
Oxidation of lactate	Positive	Negative	Positive	Positive
Growth on 30%glucose(w/v)	Negative	Negative	Negative	Not Determined
Growth on 1% glucose(w/v)	Positive	Positive	Positive	Positive
Utilization of methanol	Negative	Negative	Negative	Not Determined
Gram staining	Negative	Negative	Negative	Negative
Production of acetic acid from ethanol	Positive	Positive	Positive	Positive
Catalase	Positive	Positive	Positive	Positive
Morphology	Ellipsoidal	Ellipsoidal	Rod	Rod

Tea Components of Kombucha

Various metabolites are released as a result of the presence of many types of yeast and bacteria in the Kombucha culture. These metabolites include organic acids (acetic, gluconic, glucuronic, citric, lactic, malic, tartaric, oxalic acid), vitamins (B1, B2, B6, B12, C), amino acids, some enzymes, ethanol, carbon dioxide, minerals (Cu, Fe, Mn, Ni, Zn), phenolic substances (Teoh

et al., 2004; Yavari et al., 2010; Velićanski et al., 2013; Vitas et al., 2013; Jayabalan et al., 2014; Nguyen et al., 2015; De Filippis et al., 2018; Gaggìa et al., 2019). But, Kombucha includes acetic acid, glucuronic acid, gluconic acid as the predominant compounds. In the study of Ivanisove et al. (2019), as a result of fermentation of kombucha prepared with 30 g / L white sugar at 22 C for 7 days, 1.55 g / L acetic acid and 0.05 g / L citric acid were determined. Neffe-Skocin´ska et al., (2017) who determined the amount of 1.26 g/L acetic acid and 0.067 g/L citric acid in kombucha beverage fermented 7th day at 25 C. Acetic acid is one of the important and characteristic organic acids found in kombucha and is an antimicrobial compound that protects this beverage from pathogenic microorganisms. Another important organic compound found in kombucha is glucoronic and gluconic acid. Glucoronic acid is an important phenolic component that has detoxification properties, has a liver protective effect, and is a precursor for vitamin C biosynthesis (Nguyen et al., 2015; Cardosa et al., 2020). Kombucha prepared with 100 g / L sucrose and fermented for 10 days at 30 ° C was determined to contain 0.04 g / L glucoronic acid (Neffe-Skocin´ska et al., 2017).

Different Substrate Sources of Kombucha

In traditional kombucha fermentation, black or green tea is used with sucrose as a substrate. In the last two decades, different substrates have been used instead of tea. In the literature, there are studies in which Kombucha fermentation was carried out using substarts such as yarrow (Vitas et al., 2018), snake fruit (Zubaidah et al., 2019), cherry juice (Yavari et al., 2010), date syrup (Khosravi et al., 2019), goji fruit (Abuduaibifu & Tamer, 2019), coconut water (Phong et al., 2017), green and rooibos tea (Gaggia et al., 2019), mustard leaf (Rahmani et al., 2019), milk (Vitas et al., 2013), soy PAS (Tu et al., 2019), mulberry tea and green tea (Talawat et al., 2006), mint and lemongrass tea (Velićanski et al., 2013). In the literature, yarrow (Vitas et al., 2018), snake fruit (Zubaidah et al., 2019).

Different kombucha production methods may have been carried out to improve the functional properties of the product obtained or to soften the standard acidic taste of kombucha (Malbasa et al., 2014; Essawet et al., 2015; Zubaidah et al., 2019; Coskun and Kayisoglu, 2020).

It is stated that in kombucha enriched with olive leaves and honey, there is an increase in the amount of phenolic substance and the bioavailability of phenolics compared to standard kombucha (Değirmencioğlu et al., 2020). Similarly, Ulusoy and Tamer (2019) produced kombucha with green tea, cherry laurel, blackthorn, black carrot juice concentrate and red raspberry. The total phenolic content and antioxidant activity increased compared to the control combination.

CONCLUSIONS

Today, although Kombucha tea is known all over the world, it is not consumed much because of its vinegarish taste and short shelf life. Bacteria and yeasts in kombucha bring probiotic properties to this drink, as well as functional properties with various metabolites formed by microorganisms. Since the bacteria and yeasts contained in tea fungus such as kefir grains cannot be determined exactly, they cause some difficulties in the production of standard kombucha.

In recent years, different substrate uses in the production of kombucha tea have attracted attention. Apart from black and green tea, various plants, fruits and milk are among the alternative substrates used. These drinks, produced in different flavors and aromas, can increase the consumption of kombucha.

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EVALUATION OF THE STREAMS IN THE CAMILI BIOSPHERE RESERVE AREA (ARTVIN, BORÇKA) ACCORDING TO PHYSICOCHEMICAL VARIABLES AND SOME HABITAT CHARACTERISTICS

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ABSTRACT

Camili District, the first biosphere reserve area of Turkey, is a part of the Caucasus Biodiversity Hotspot. Besides, many conservation areas such as Borçka-Karagöl Nature Park, Camili-Gorgit Nature Conservation Area and Camili-Efeler Nature Conservation Area are also within the boundaries of the region. The present study aimed (1) to determine the sections with reference habitat conditions by locating the anthropogenic effects on the streams forming the Camili Basin, (2) to determine the water quality classes of the sampling sites according to the physicochemical variables, and (3) to classify the sampling sites according to the EU Water Framework Directive, System A and B classification. Within the scope of the study, 23 sampling sites were determined from the region. In each site, water temperature, pH, electrical conductivity, total dissolved solids, the concentration of dissolved oxygen were measured, and then water quality classes of the sites were determined. Besides, by locating whether there was any human-based destruction on and around the sites, reference habitats were determined according to the natural habitat conditions of the sampling sites. In addition, some information such as stream order, current velocity, altitude, stream region, distance from the source, and land slope were also provided. As a result of the evaluation, 17 sites that were not under the influence of agriculture-animal husbandry, permanent settlement and physical destruction were identified as the sites with reference habitat characteristics. Seven of 23 sites were on 1st stream order, 12 of them on 2nd stream order, three of them on 3rd order, and one of them on 4th stream order. According to physicochemical variables, the water qualities of 12 sites were determined as Class I, four sites were Class II, six sites were Class III, and one site was Class IV. According to the results of the assessment of the stream orders, six of the 17 reference sites were located on 1st stream order, 10 of them on 2nd stream order, and one them on 3rd stream order. Any reference site was not found on 4th stream order. As stream order increases, the physicochemical and hydromorphological features of streams change, as well. As a result of these changes, natural deterioration in water and habitat qualities can be observed. However, the distortions observed in the sampling sites that did not meet the reference conditions in the present study were not natural but human-caused. Therefore, the reason for not having any reference site on 4th stream order was due to anthropogenic effects. Streams in an isolated and protected area, such as the study area, are expected to have high water quality. However, as a result of water quality assessments, it was observed that some sites had III. and IV. Class water quality. The variable that caused this situation was pH. The pH value decreases due to episodic acidification, which is generally encountered during melting periods of snow and ice masses. Due to the fact that the periodically occurring and the temporary phenomenon was observed during the sampling period, it resulted in low water quality values. If the situation observed in pH values was ignored, the water quality of all sites corresponded to the I. and II. Class. Continuing conservation work is essential to maintaining the quality of river habitats in the region.

Key Words: *Biodiversity hotspot, Habitat quality, Protected area, Turkey, Water quality.*

INTRODUCTION

The term "Biosphere Reserves" emerged for the first time from the programme on Man and the Biosphere (MaB) of UNESCO (UNESCO, 1970; Batisse, 1982). Biosphere Reserves are the places where terrestrial, marine and coastal ecosystems have international importance and are involved in the MaB programme of UNESCO. Besides, these reserves are a fundamental approach to solving the conflicts between the conservation of biological diversity, economic development and the continuity of cultural values in a sustainable way. (UNESCO, 2020a).

According to the recent records, there are 701 biosphere reserves in 124 countries all over the World (UNESCO, 2020b). Camili District is the first and the only biosphere reserve area of Turkey. In 2005, Camili Biosphere Reserve (Turkey) was designated for inclusion in the World Network of Biosphere Reserves by the decision of the International Co-ordinating Council of the Programme Man and the Biosphere. Since this date, the importance of Camili Basin has gradually increased and the efforts to protect the region have also accelerated.

Besides, Camili Biosphere Reserve area is located in Eastern Part of Black Sea Region of Turkey and within the borders of Caucasus Biodiversity Hotspot (CEPF, 2003; Kazancı, 2009), which is one of the 34 Hotspots of the World (Mittermeier *et al.*, 2005). Many conservation areas such as Borçka-Karagöl Nature Park, Camili-Gorgit Nature Conservation Area and Camili-Efeler Nature Conservation Area are also in Camili.

Due to the biological importance of the Camili Basin and its status as a protected area, it is of great importance to determine the water quality of the rivers in this area and to carry out monitoring studies. The present study aimed (1) to determine the sections with reference habitat conditions by locating the anthropogenic effects on the streams forming the Camili Basin, (2) to determine the water quality classes of the sampling sites according to the physicochemical variables, and (3) to classify the sampling sites according to the EU Water Framework Directive, System A and B classification.

MATERIAL AND METHODS

Study area and sampling sites

The study area is located in the farthest point of the Northeastern part of Turkey and very close to the Georgia border (Figure 1). The streams that forming the Camili Basin covered approximately 1244km^2 area. From these streams, 23 sampling points were selected for the present study.

Physicochemical variables and water quality classes

Six physicochemical variables were measured in the field; water temperature (°C), concentration and saturation of dissolved oxygen (DO_c and DO_s, respectively), electrical conductivity (EC), pH and total dissolved solids (TDS). The physicochemical variables were measured by using YSI 556 multiprobe systems. One time sampling was performed on each site. The water quality classes of the sites were determined by using these variables according to the Surface Water Quality Management Regulation (Ministry of Agriculture and Forestry, 2004, 2015).

Figure 1. The location of the study area in Turkey and the streams* forming the Camili Basin.

(*Only the investigated streams and sidearms were demonstrated).

Habitat characteristics and reference sites

Some information such as stream order, current velocity, altitude, stream region, distance from the source, and land slope were also provided to define the habitat characteristics of the sampling sites. To measure the current velocity, Hydrobios RHCM current meter was used. During the fieldwork, any anthropogenic effects observed on (or around) the sampling sites, such as agriculture-animal husbandry, permanent settlement, physical destructions and dam/reservoir/water regulator, were noted to determine the reference habitat conditions.

System A and System B classification of WFD

In Annex-II of the European Union Water Framework Directive (EU WFD) (Council of European Communities, 2000), it is aimed to determine the initial characterization of surface water bodies. For this purpose, two classification systems were created, System A and System B. The sampling sites in the present study were classified according to these systems to observe whether there were any differences in the sites.

RESULTS

Physicochemical variables and water quality classes

The water temperature (T) values of the sampling sites were between 3.68ºC – 19.15ºC. All T values were corresponded to I. Class water quality (WQ) in the area. Dissolved oxygen saturation (DO_s) values were between 83% and 140%. Only five sites (ART-2, ART-4, ART-5, ART-7 and ART-14) had II. Class WQ according to the DO_s variable, while all the other sites had I. Class WQ. Dissolved oxygen concentrations (DO_c) were between 8.31mg/l – 18.0mg/l. Although II. Class DO_s values were observed in five sites, the DO_c values were corresponded to I. Class WQ in all sites. pH values of the sampling sites were between 5.52 – 8.2. Very low pH values were observed during the study; in seven sites (ART-3, ART-4, ART-9, ART-18, ART-19, ART-20 and ART-21), pH values were below 6.5, corresponded to III.

and IV. Class WQ. Electrical conductivity (EC) values were between 67µS/cm - 253µS/cm. All EC values were corresponded to I. Class WQ. Total dissolved solids (TDS) values were between 2mg/l and 910mg/l. Only in two sites (ART-2 and ART-5), excessive TDS values were observed. However, these values were corresponded to II. Class WQ in these two sites. TDS values of all the other sites were corresponded to I. Class WQ.

Both WQ classes of all variables in each site and the final WQ classes of each site were shown in Table 1. According to the final evaluation, 12 sites had I. Class WQ, four sites had II. Class WQ, six sites had III. Class WQ and one site had IV. Class WQ.

Table 1. WQ Classes of each variable and the final WQ classes of the sampling sites.

Sites	T (°C)	DO_s (%)	DO_c (mg/l)	pH	EC (µS/cm)	TDS (mg/l)	Final WQ Classes
ART-1	I	I	I	I	I	I	I
ART-2	I	II	I	I	I	II	II
ART-3	I	I	I	III	I	I	III
ART-4	I	II	I	IV	I	I	IV
ART-5	I	II	I	I	I	II	II
ART-6	I	I	I	I	I	I	I
ART-7	I	II	I	I	I	I	II
ART-8	I	I	I	I	I	I	I
ART-9	I	I	I	III	I	I	III
ART-10	I	I	I	I	I	I	I
ART-11	I	I	I	I	I	I	I
ART-12	I	I	I	I	I	I	I
ART-13	I	I	I	I	I	I	I
ART-14	I	II	I	I	I	I	II
ART-15	I	I	I	I	I	I	I
ART-16	I	I	I	I	I	I	I
ART-17	I	I	I	I	I	I	I
ART-18	I	I	I	III	I	I	III
ART-19	I	I	I	III	I	I	III
ART-20	I	I	I	III	I	I	III
ART-21	I	I	I	III	I	I	III
ART-22	I	I	I	I	I	I	I
ART-23	I	I	I	I	I	I	I

Habitat characteristics and reference sites

Among the sampling sites, the stream order in the sampling points in the streams were from 1st to 4th stream order. Seven sampling points were on 1st stream order, twelve sampling points were on 2nd stream order, three sampling points were on 3rd stream order, and one sampling point was on 4th stream order. In addition, which stream region the sampling points are on were determined. Accordingly, one point was on eucrenon, four points were on hypocrenon, fifteen points were on epirhithron and three points were on metarhithron zone of the streams. The current velocities at the sampling time were between 0.55m/s – 1.70m/s. Some features such as altitude, distance from the source and land slope were also recorded during the field study (Table 2).

By locating whether there was any human-based destruction on and around the sites, reference habitats were determined according to the natural habitat conditions of the sampling sites. In this scope, human-based destructions were observed in six sites. These were, agriculture-animal husbandry and permanent settlement areas in ART-6 and ART-16; agriculture-animal husbandry and physical destructions in ART-17; agriculture-animal husbandry in ART-20, physical destructions in ART-19 and ART-23. All remaining sites had reference conditions and their natural habitats were protected (Table 2).

Table 2. Other features of the sampling sites and their reference status.

Sites	Stream Order	Stream Region	Current velocity (m/s)	Altitude (m)	Distance from the source (m)	Slope (%)	Reference status
ART-1	1	Epirhithron	1.01	1541	1450	35.3	Yes
ART-2	2	Epirhithron	1.27	2007	3400	11.3	Yes
ART-3	2	Epirhithron	1.7	1998	7470	19.6	Yes
ART-4	1	Hypocrenon	0.97	2270	1860	17.7	Yes
ART-5	1	Hypocrenon	0.65	2190	1880	24.8	Yes
ART-6	4	Metarhithron	1.05	607	15700	18.7	No
ART-7	1	Hypocrenon	1.12	2359	1190	16.8	Yes
ART-8	2	Epirhithron	1.2	2219	2665	13.6	Yes
ART-9	1	Hypocrenon	0.98	2409	2400	18.8	Yes
ART-10	2	Epirhithron	0.95	1816	5100	24.2	Yes
ART-11	3	Epirhithron	1.3	1576	6000	22.5	Yes
ART-12	2	Epirhithron	0.91	1900	3060	25.1	Yes
ART-13	1	Eucrenon	1.2	2320	20	26.3	Yes
ART-14	2	Epirhithron	0.81	1960	2450	24.5	Yes
ART-15	2	Epirhithron	1.1	1830	4130	23.7	Yes
ART-16	3	Metarhithron	1.54	645	12490	16.2	No
ART-17	1	Epirhithron	1.02	1022	1820	33.8	No
ART-18	2	Epirhithron	0.68	915	2790	34.0	Yes
ART-19	2	Epirhithron	0.55	940	3470	33.0	No
ART-20	3	Metarhithron	1.67	780	9830	19.6	No
ART-21	2	Epirhithron	1.08	1016	2360	36.8	Yes
ART-22	2	Epirhithron	1.38	1016	3000	35.3	Yes
ART-23	2	Epirhithron	0.88	1503	4215	26.9	No

System A and System B classification of WFD

The purpose of the initial classifying the sites according to System A and System B is to identify the stream types and to reveal the differences between sites (or habitats). Thus, when reference sites (or habitats) are determined in the later stages, it will be easier and more accurate to compare the sites located in similar environments with each other.

The characterization features used in System A and System B and the classification results were shown in Table 3 and Table 4.

Table 3. Classification of the sampling sites according to System A classification of EU WFD.

System A

	ART-1	**ART-2**	**ART-3**	**ART-4**	**ART-5**	**ART-6**	**ART-7**	**ART-8**
Ecoregion	Y	Y	Y	Y	Y	Y	Y	Y
Altitude typology	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	Mid-altitude (200m-800m)	High (>800m)	High (>800m)
Size typology based on catchment area	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)
Geology	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous

	ART-9	**ART-10**	**ART-11**	**ART-12**	**ART-13**	**ART-14**	**ART-15**	**ART-16**
Ecoregion	Y	Y	Y	Y	Y	Y	Y	Y
Altitude typology	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	High (>800m)	Mid-altitude (200m-800m)
Size typology based on catchment area	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)
Geology	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous

	ART-17	**ART-18**	**ART-19**	**ART-20**	**ART-21**	**ART-22**	**ART-23**
Ecoregion	Y	Y	Y	Y	Y	Y	Y
Altitude typology	High (>800m)	High (>800m)	High (>800m)	Mid-altitude (200m-800m)	High (>800m)	High (>800m)	High (>800m)
Size typology based on catchment area	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)	Very large (>10.000km 2)
Geology	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous

Table 4. Classification of the sampling sites according to System B classification of EU WFD.

System B

	ART-1	**ART-2**	**ART-3**	**ART-4**	**ART-5**	**ART-6**	**ART-7**	**ART-8**
Altitude	1541m	2007m	1998m	2270m	2190m	607m	2359m	2219m
Latitude	41° 28' 51.21"	41° 29' 39.28"	41° 27' 33.78"	41° 26' 07.51"	41° 26' 28.10"	41° 26' 36.02"	41° 24' 04.35"	41° 23' 27.86"
Longitude	42° 02' 22.07"	42° 06' 07.48"	42° 06' 07.34"	42° 03' 59.08"	42° 04' 35.24"	41° 55' 22.10"	42° 03' 24.47"	42° 03' 53.18"
Geology	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Size	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2
Current velocity	1,01 m/s	1,27 m/s	1,7 m/s	0,97 m/s	0,65 m/s	1,05 m/s	1,12 m/s	1,2 m/s
Mean stream width (rainy time)	3m	4m	7m	5m	2m	10m	4m	3m
Mean stream width (dry time)	1m	2m	5m	3m	1m	7m	2m	2m
Mean substratum composition (%)								
Boulder	10	30	40	20	0	30	10	10
Cobbles	30	40	40	40	50	30	45	40
Gravel	40	20	15	30	40	30	35	40
Sand	20	10	5	10	10	10	10	10
Silt	0	0	0	0	0	0	0	0

	ART-9	**ART-10**	**ART-11**	**ART-12**	**ART-13**	**ART-14**	**ART-15**	**ART-16**
Altitude	2409m	1816m	1576m	1900m	2320m	1960m	1830m	645m
Latitude	41° 24' 04.84"	41° 25' 04.65"	41° 19' 27.80"	41° 19' 58.43"	41° 21' 07.04"	41° 20' 14.42"	41° 19' 40.59"	41° 28' 44.72"
Longitude	42° 02' 17.38"	42° 01' 10.18"	41° 55' 25.52"	41° 56' 12.48"	41° 57' 00.71"	41° 56' 10.58"	41° 56' 21.25"	41° 57' 15.67"
Geology	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Size	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2
Current velocity	0,98 m/s	0,95 m/s	1,3 m/s	0,91 m/s	1,2 m/s	0,81 m/s	1,1 m/s	1,54 m/s
Mean stream width (rainy time)	7m	4m	8m	6m	7m	6m	4m	8m
Mean stream width (dry time)	3m	3m	5m	5m	7m	3m	2m	5m
Mean substratum composition (%)								
Boulder	20	10	20	30	40	30	20	10
Cobbles	40	40	40	30	30	40	30	40
Gravel	30	40	30	30	20	20	40	40
Sand	10	10	10	10	10	10	10	10
Silt	0	0	0	0	0	0	0	0

	ART-17	**ART-18**	**ART-19**	**ART-20**	**ART-21**	**ART-22**	**ART-23**
Altitude	1022m	915m	940m	780m	1016m	1016m	1503m
Latitude	41° 28' 00.02"	41° 28' 23.17"	41° 28' 28.91"	41° 28' 42.48"	41° 30' 16.32"	41° 30' 13.50"	41° 23' 11.32"
Longitude	41° 57' 30.77"	41° 58' 58.20"	42° 00' 07.66"	41° 59' 04.64"	41° 57' 28.20"	41° 57' 33.50"	41° 51' 26.70"
Geology	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous	Siliceous
Size	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2	20.263km^2
Current velocity	1,02 m/s	0,68 m/s	0,55 m/s	1,67 m/s	1,08 m/s	1,38 m/s	0,88 m/s
Mean stream width (rainy time)	3m	6m	3m	18m	6m	4m	6m
Mean stream width (dry time)	1m	3m	2m	10m	3m	2m	3m
Mean substratum composition (%)							
Boulder	5	10	10	10	10	20	5
Cobbles	50	50	30	40	40	35	25
Gravel	35	30	50	40	40	35	60
Sand	10	10	10	10	10	10	10
Silt	0	0	0	0	0	0	0

DISCUSSION

The concept of reference conditions is one of the crucial subjects in EU WFD (Council of European Communities, 2000). It represents natural or closely natural habitats. Also, there is not any human-based degradation and physical destruction in the habitat (Buffagni *et al*., 2001). As a result of the evaluation of the reference condition in the present study, 17 sites that were not under the influence of agriculture-animal husbandry, permanent settlement and physical destruction were identified as the sites with reference habitat characteristics.

According to the results of the assessment of the stream orders, seven of 23 sites were on 1st stream order, 12 of them on 2nd stream order, three of them on 3rd order, and one of them on 4th stream order. When stream order and reference sites were evaluating together, six of the 17 reference sites were located on 1st stream order, 10 of them on 2nd stream order, and one them on 3rd stream order. Any reference site was not found on 4th stream order. As stream order increases, the physicochemical and hydromorphological features of streams change, as well (Harrel and Dorris, 1968). As a result of these changes, natural deterioration in both water and habitat qualities can be observed. However, the distortions observed in the sampling sites that did not meet the reference conditions in the present study were not natural but human-caused. Therefore, the reason for not having any reference site on 4th stream order was due to anthropogenic effects.

According to physicochemical variables, the water qualities of 12 sites were determined as Class I, four sites were Class II, six sites were Class III, and one site was Class IV. Streams in an isolated and protected area, such as the study area, are expected to have high water quality. However, as a result of water quality assessments, it was observed that some sites had III. and IV. Class water quality. The variable that caused this situation was pH. The lowest pH (5.52) was measured in ART-4. However, the other variables in ART-4 were in the range of I. and II. Class WQ. The other low pH values were measured in ART-18, ART-3, ART-9, ART-20, ART-19 and ART-21 (6.01, 6.04, 6.05, 6.28, 6.31 and 6.41, respectively). As in ART-4, all other variables were corresponded to I. Class WQ in these sites, as well. This situation indicated that, if we reckon without pH, all of these sites mentioned above had a good water quality. Furthermore, if we can explain why only pH values were corresponded to III. and IV. Class WQ, we can evaluate these sites as the sites with high water quality. The pH value decreases due to episodic acidification, which is generally encountered during melting periods of snow and ice masses (Jacks *et al*., 1986; Davies *et al*., 1993; Kazancı, 2009). Besides, it is a very common phenomenon in the region (Kazancı, 2009; Türkmen and Kazancı, 2020). Due to the fact that the periodically occurring and the temporary phenomenon was observed during the sampling period, it resulted in low water quality values. If the situation observed in pH values was ignored, the water quality of all sites corresponded to the I. and II. Class.

CONCLUSIONS

Camili is one of the most ecologically and biologically significant areas of Turkey since it is the only biosphere reserve area of Turkey as well as it contains many protected areas. In addition to the importance of determining the fauna and flora elements of the region, it is of great importance to determine the quality of the river basins and the river systems that make up the basin and to monitor them regularly. With the present study, some necessary information that

can be used in this type of protection and monitoring studies in the future was given. Continuing conservation work is essential to maintaining the quality of river habitats in the region.

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DIETARY FIBER A NATURAL BARRIER AGAINST ALARMINGLY INCREASING METABOLIC DISORDERS

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ABSTARCT

Dietary fiber a poly saccharide having ten or more monomeric unit. Endogenous enzymes in small intestine are unable to hydrolyze the dietary fiber. In large intestine it is partially or fully fermented. Alarmingly increasing chronic disorders needs the management strategies. Dietary fiber inclusion in daily intakes is the best option for management and prevention of these disorders. Dietary fiber has significant role in reduction of serum glucose level by increasing insulin sensitivity and delayed gastric emptying. Serum cholesterol level is also controlled by its fecal egestion and by preventing bile reabsorption. Dietary fiber also reduces hypertension.

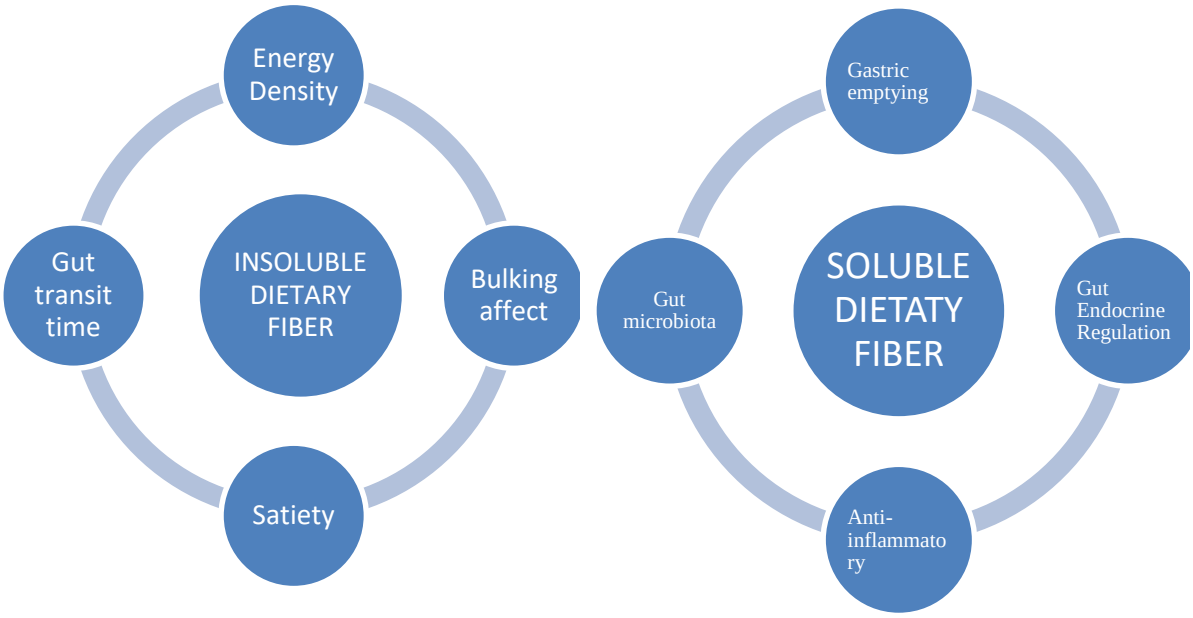
Key words: Dietary fiber, lipid profile, serum glucose level, hypertension

INTRODUCTION

CODEX Alimentarius Commission defined the dietary fiber to reflect the current state of knowledge about dietary fiber, all substances that behave like fiber show physiological benefits, and to promote international harmonization for food labeling and food composition tables (Jones, 2014). Dietary fiber a complex non digestible polymers having impact on e.g. gut microlora, immunity and gut endocrine regulation (El-Salhy, Ystad, Mazzawi, & Gundersen, 2017). Dietary fiber includes cellulose, hemicellulose, pectin, hydrocolloids and lignin. Hydrocolloids and pectin is among water soluble dietary fiber. Oat bran, fruits, vegetables, soybean, legumes and pysillium. Hemicellulose, cellulose and lignin are among the insoluble dietary fiber and whole grain is their good source. Insoluble dietary fiber is those components that are insoluble in water and includes cellulose, hemicellulose and lignin. Whole grains are good sources of insoluble fiber (McKEE & Latner, 2000).Short chain fatty acids are the fermentation product of dietary fiber by the intestinal micro biota, having host physiological impact such as digestion of complex macromolecules, Vitamin synthesis , reduction elimination of pathogenicity and regulation of the immune system.(Holscher, 2017; Koh, De Vadder, Kovatcheva-Datchary, & Bäckhed, 2016). Diet with high fiber contents has positive impact on the physiological functions of the body. Dietary fiber in whole grain considered to be effective in the prevention of type 2 diabetes mellitus.(Kaline, Bornstein, Bergmann, Hauner, & Schwarz, 2007).Dietary fiber contributes to a number metabolic effects e.g. improvement in

insulin sensitivity, modulation of gut hormones secretion and effects on metabolic and inflammatory markers that are associated with the metabolic syndrome.(Weickert & Pfeiffer, 2008).High dietary fiber diet has tendency to reduce the serum lipid profile by minimizing absorption of cholesterol in intestinal area and increasing fecal elimination of cholesterol. LDL-Cholesterol level of the experimental group was decreased by 1 to 13 mg/100ml.(Guo, Shu, & Yang, 2016; Ramos et al., 2011). Diet with dietary fiber 11.5 g/d changed systolic blood pressure by -1.13 mm Hg and diastolic blood pressure by -1.26 mm Hg (Streppel, Arends, van't Veer, Grobbee, & Geleijnse, 2005). Dietary fiber may enhance the body's defenses against oxidative stress, reduce inflammatory markers and reduces level of C reactive protein a major contributory towards cardiovascular diseases.(King, Mainous, Egan, Woolson, & Geesey, 2005).Dietary fiber intake has positive impact on the immune system. Dietary fiber Fibers affects immunity by production of short-chain fatty acids Short-chain fatty acids are produced in the fermentation process of dietary fibers in gastrointestinal track and have many physiological functions including maintenance of epithelial barrier functions, regulation of proliferation and tumor suppression, reduction in oxidative DNA damage and regulation of cytokine production. Dietary fiber also has the anti-inflammatory affect, regulation of immune responses.(Fernstrand, Bury, Garssen, & Verster, 2017).

Figure 1. Soluble and insoluble dietary fiber role



1. Health Benefits of Dietary fiber:

1.1. Effect of Dietary Fiber on Serum Glucose Level:

Dietary fiber intake can reduce fasting glucose level 0.85 mmol. Increasing dietary fiber in the diet of patients with type 2 diabetes is beneficial. Type 2 diabetes can be managed by dietary fiber intake (Post, Mainous, King, & Simpson, 2012). Dietary fiber plays a therapeutic role toward type2 diabetes mellitus prevention. Dietary fiber incorporation in daily intake can

reduce incidence of disease and can save millions lives and economic losses incurred on mediation (Ismaiel, Yang, & Min, 2016). A high intake of dietary fiber, particularly of the soluble type, above the level recommended by the ADA, improves glycemic control, decreases hyperinsulinemia, and lowers plasma lipid concentrations in patients with type 2 diabetes (Chandalia et al., 2000; Tiberius, Carmen, & Evelin, 2017). insoluble dietary fibers as found in whole grain cereal products are considered to be especially effective in the prevention of type 2 diabetes mellitus.(Kaline *et al*., 2007). Dietary fiber was efficient in decreasing glucose levels of diabetic patients. The consumption of greater doses or smaller doses for longer periods of time produced better results(Andrade *et al.*, 2015).Type 2 diabetes is one of the metabolic syndrome having highest economic and social impact, also has detrimental effect on the quality of life. Prevention and management of this metabolic disorders should be focused rather than cure. High dietary fiber content in the diet reduces HbA1c and triglycerides, while improving HDL-c levels. Increasing fiber consumption affect satiety level and delays gastric emptying hence promote blood glucose control(Velázquez-López *et al*., 2016). Dietary fiber when taken as dietary supplement improves intestinal viscosity, nutrient absorption, rate of passage, production of short chain fatty acids and production of gut endocrine hormones.

Due to change in dietary behavior of masses dietary fiber should be incorporated in diet to fetch its physiological merits. This will not only improve the individual life but also will contribute towards decreasing health treatment burden of the developing countries(Lattimer & Haub, 2010). Diabetes increases mortality by almost 2-fold and increases mortality by cardiovascular disease by 2-to 3-fold.The diet rich in fiber reduces glycemic index of the food and is used as nutritional therapy against diabetes mellitus (Post *et al.*, 2012).Dietary fiber has therapeutic effect in reduction of diabetes. Regular intake of dietary fiber in food maintains blood glucose levels, insulin resistance and metabolic profiles. Dietary fiber has not significant impact on the secretory function of the islets of Langerhans(C. Chen et al., 2016). High fiber diets having soluble fiber has impact in weight management, carbohydrate metabolism, lower total and LDL cholesterol. It also plays important role in improvement of insulin sensitivity, modulation of the secretion of certain gut hormones, improvement of various metabolic and inflammatory markers associated with the metabolic syndrome, thus reducing the risk of cardio-metabolic diseases.(Mogoş, Dondoi, & Iacobini, 2017). Among the dietary fiber water soluble dietary fiber has potential to reduce postprandial blood glucose and insulin than insoluble fiber. Dietary fiber with higher viscosity has, the greater the effect. Among the benefits of dietary fiber include delay of glucose absorption, increase in hepatic extraction of insulin, increased insulin sensitivity at the cellular level, and binding of bile acids.(Tabatabai & Li, 2000).

Dietary fiber reduces prevalence of abdominal obesity, hypertension and metabolic syndrome. Dietary fiber intake lower prevalence of albuminuria, low glomerular filtration rate and chronic kidney disease(Fujii *et al.*, 2013).Primarily dietary fiber was considered due to its gastrointestinal affects but presently it is also known as having effect on glycaemia and lipidemia. Dietary fiber is s associated with substantial improvements in glycemic control and reductions in the use of oral medication and insulin doses(Anderson *et al*., 2009). Functional role of dietary fiber is well documented in diabetes management, improving insulin sensitivity, decreasing body weight gain and reducing the risk factors of cardiovascular disease(Li & Uppal, 2010).Soluble dietary fiber from apricot pulp investigated on diabetic rat. Soluble dietary fiber from apricot significantly ($p < 0.01$) changed blood glucose level and body weight after intragastric administration for 28 days (Cui, Gu, Zhang, Ou, & Wang, 2015).

(Kimmel, Michel, Hess, & Ward, 2000) investigated the effect of low fiber, high insoluble fiber diet and high soluble fiber diet diabetic dogs concluded that the blood glucose concentrations and area under the blood glucose curve were significantly lower in dogs fed the high insoluble fiber diet, compared with values in the same dogs fed the high soluble fiber diet or low fiber diet.

Table 1. Mechanism of serum glucose lowering effect of dietary fiber

Mechanism	Reference
Inhibit macronutrient absorption, reduce postprandial glucose response, Improvement of glucose sensitivity, modulation of gut hormones and by affecting metabolic and inflammatory markers	(Weickert & Pfeiffer, 2008)
Prolonged feeling of satiety	(de Leeuw, Jongbloed, & Verstegen, 2004)
Improves glycemic control, decreases hyperinsulinemia, and lowers plasma lipid concentrations	(Chandalia et al., 2000)
Alter small intestine juice viscosity Binding of available glucose Inhibitory effect on α amylase	(Ou, Kwok, Li, & Fu, 2001)
Increase insulin concentration	(Díez et al., 2013)
Body weight Management	(Rohajatien, Estiasih, & Sriwahyuni, 2018; Russell et al., 2016)
Regulating insulin level and body weight management	(Jiang et al., 2012)
Production of short chain fatty acids Regulation of pancreatic insulin release Regulation of glycogen breakdown	(Slavin et al., 2009(Zhao et al., 2018)
Venous and capillary postprandial blood glucose reduction Reduction in postprandial insulin concentration	(Stewart & Zimmer, 2018)
Reduction of micro inflammation Intestinal flora improvement	(Cai, Wang, Wang, & Liu, 2017)
Delayed gastric emptying, reduction of PH of digestion medium	(Alexander, 2012; Asif, 2011; Hyman, 2010; Scazzina, Siebenhandl-Ehn, & Pellegrini, 2013)
Abrogation of obesity	(Han et al., 2015; McRorie Jr, 2015)
Mediating glucose metabolism(Costa, Guimarães, & Sampaio, 2012)	(Costa et al., 2012(Abutair, Naser, & Hamed, 2016)
Obesity Management , insulin resistance, altering endocrine and cytokines production by the adipose tissue	(Galisteo, Duarte, & Zarzuelo, 2008)
Fermentation and altering viscosity of GI	(Dahl & Stewart, 2015)

1.2. Effect On Serum Lipid Profile

Diet with high fiber supplementation decreased the serum cholesterol level. It was observed there was a decrease of 1 to 15 mg/100 ml in the experimental group. The change in the LDL-Cholesterol value was significant at both 5 percent and 1 percent level of supplementation (Khogare, 2012). Soluble fiber and soy protein improved the serum lipid profile of the hypercholesteromic subjects significantly low-density lipoprotein (LDL) cholesterol fraction was significantly reduced (8.5% ± 3.3%, P = .020). A combination of acceptable amounts of soy, vegetable protein, and soluble-fiber foods as part of a conventional low-fat, low-cholesterol therapeutic diet is effective in further reducing serum lipid risk factors for cardiovascular disease(Jenkins *et al*., 2000). Cholesterol rich diets are responsible for Hypercholesterolemia and hypertriglyceridemia. Cocoa fiber showed an important hypolipidemic action, returning triacylglycerol levels in hypercholesterolemia animals to normal values. The hypocholesterolemic effect was also patent, reducing total and low-density lipoprotein cholesterol(Guo et al., 2016; Lecumberri et al., 2007) concluded that the three fibers could improve serum and hepatic lipid profiles in mice fed a high cholesterol diet. Dietary fiber increased the fecal discharge of cholesterol by binding cholesterol through gastrointestinal track. Dietary fiber has lipid-binding ability and the inhibition of lipid peroxidation.

Table 1. Mechanism of serum cholesterol lowering effect of dietary fiber

Mechanism	References
Reduced glycemic response Prevention of bile salt re-absorption Excess fecal bile salt excretion Fiber binds bile salt micelles	(Gunness & Gidley, 2010)
Reduction in endogenous cholesterol synthesis	(Ramos et al., 2011)
Regulation of alpha-hydroxylase activity Reduction in cholesterol absorption	(Babio, Balanza, Basulto, Bulló, & Salas-Salvadó, 2010; Fernandez, 1995)
Production of short chain fatty acids	(Queenan et al., 2007)
Reduction in hepatic cholesterol synthesis Deconjugating bile salts	(Gallaher et al., 2002; Pereira & Gibson, 2002)
Secretion of 7-α-hydroxy-4-cholesten-3-one (Bile acid)increases Depleting cholesterol from liver	(Bazzano, 2008; Moreyra, Wilson, & Koraym, 2005)
Lipid emulsification Lipolysis Postprandial lipemia	(Sánchez-Muniz, 2012)
inhibition of bile acid reabsorption	(Moreyra et al., 2005)
Glucose oxidation Insulin clearance Free fatty acids	(Zunft et al., 2003)
Cholesterol homeostasis	(Z.-Y. Chen, Ma, Liang, Peng, & Zuo, 2011)

Tea dietary fiber has the potential to be used as a functional ingredient to control cardiovascular disease. A higher content of dietary fiber in the diet reduces HbA1c and triglycerides, while improving HDL-c levels. Increasing fiber consumption ensures lowering calorie consumption considered an important preventive reduce body weight and to maintain healthy serum lipid profile.(Velázquez-López *et al*., 2016). Greater dietary fiber intake lower risk of both cardiovascular disease and coronary heart disease. It is recommended to increase fiber intake(Threapleton *et al*., 2013).

Figure 2. Mechanism of cholesterol removal from gastrointestinal track

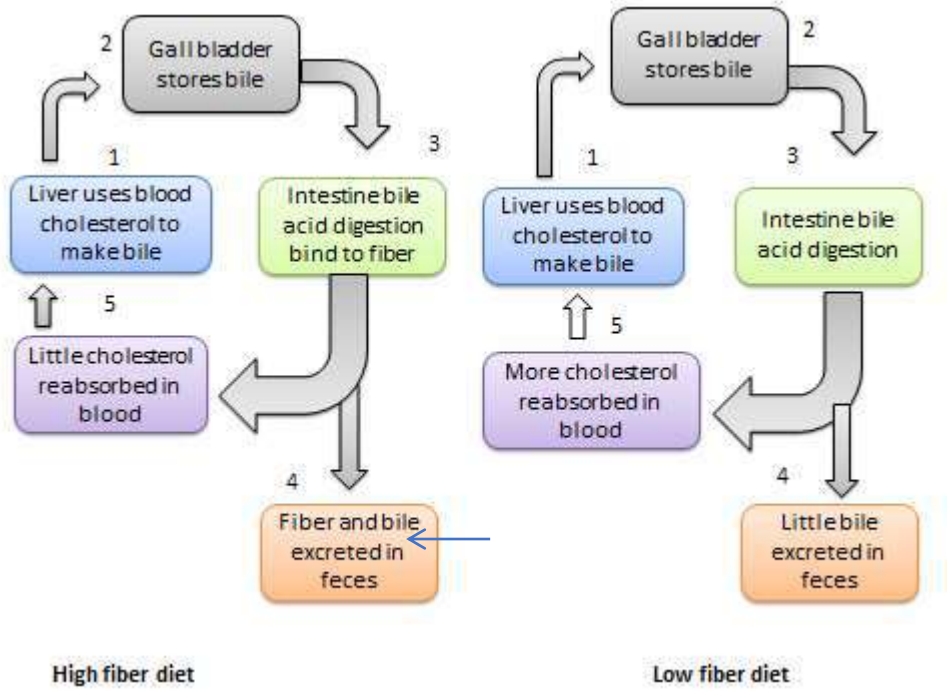


Figure 3.cholesterol reabsorption pathway

1.3 Dietary fiber Role in Hypertension

Dietary fiber is the natural mediator of metabolic disorders(Whelton et al., 2005).Studies revealed that oral intake of dietary fiber plays potential role in the reduction of systolic and diastolic blood pressure(Aleixandre & Miguel, 2016) by 1.13 mm Hg and 1.26 mm Hg respectively(Streppel et al., 2005). Dietary fiber intake reduced systolic blood pressure by 2.9 mmHg and diastolic by 1.5mmHg(Evans et al., 2015). Studies revealed that linear polysaccharide present in oat have significant effect on blood pressure reduction among the hypertensive patients. These mainly contribute towards the arterial blood pressure reduction(Aleixandre & Miguel, 2008; Daou & Zhang, 2012).Study revealed that dietary fiber lowered the systolic blood pressure by −5.95 mmHg and diastolic blood pressure and lowered systolic blood pressure −4.20 mmHg respectively by eight weeks intervention(Whelton et al., 2005).11.5 g /day dietary fiber intake systolic BP by –1.13 mm Hg and diastolic BP by –1.26 respectively. The reduction was more significantly observed among the hypertensive patients

above the 40 years old(Streppel et al., 2005).Dietary fiber has significant effect in reduction of hypertension(Ötles & Ozgoz, 2014).

1.4 Immuno Modulatory Role of Dietary Fiber

Soluble fiber from oat has stimulates macrophages and monocytes thus increasing immunoglobin quantity, NK cells and killer T-cells. Immunomodulation provides natural barrier against parasitic infection and infectious cancerous cells(Daou & Zhang, 2012). Fermentable dietary fiber has immune modulatory effect. Mechanism by which this effect is modulation of gut-associated lymphoid tissues, secondary lymphoid tissues and peripheral circulation(Schley & Field, 2002),Mechanism of immunomodulation includes effects on f lactic acid-producing bacteria , bacterial constituents on immune cells, production of short chain fatty acids and binding of these fatty acid receptor on leucocytes(Kelly-Quagliana, Nelson, & Buddington, 2003; Seifert & Watzl, 2007; Watzl, Girrbach, & Roller, 2005).Studies revealed that dietary fiber has immunomodulatory role in both animals and plants(Delgado, Tamashiro, & Pastore, 2010). Immuno modulatory role of cereal beta glucan is due to its physical structure. Polymer ratio affects its solubility and aggregation. Fiber having low solubility and more aggregation property increase Immuno response(Mikkelsen, Jespersen, Mehlsen, Engelsen, & Frøkiær, 2014).

CONCLUSION

Chronic disorders are not only affecting the individuals but also threat to the developing economies. These increasing disorders can only be wiped out by preventive and management strategies. Dietary fiber is natural barrier against these disorders. Unfortunately, we are not consuming the recommended intake that's why are not fetching its benefits. Massive public awareness can play a vital role in introduction of health effect of dietary fiber at gross root level especially illiterate population of developing countries. This can be only the way to reduce the public health burden round the globe.

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THE EFFECTS OF DEFICIT IRRIGATION ON NET PHOTOSYNTHESIS RATE, STOMATAL CONDUCTANCE, INTRACELLULAR CO_2 CONCENTRATION AND TRANSPIRATION RATE IN SUGAR BEET

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ABSTRACT

In this study, the effects of deficit irrigation applications on net photosynthesis rate (A), stomatal conductance (gs), intercellular CO_2 concentration (C_i) and transpiration rate (E) physiological parameters in sugar beet were examined in Bursa Uludağ University Agricultural Application and Research Center in 2019. Plants were irrigated with drip irrigation method and physiological measurements were made on 12 August, 29 August and 9 September. Irrigations were scheduled based on the replenishment of 100% (S_1), 67% (S_2), 33% (S_3), and 0% (S_4) of soil water depletion from a soil depth of 90 cm using a 7-day irrigation interval. According to the research results; It was determined that all physiological parameters were significantly affected by different water stress conditions ($p< 0.01$) on all of the measurement days. The A, gs, C_i and E values measured in the irrigation treatments have changed in that intervals respectively; 6.03-19.00 µmol CO_2 m^{-2} s^{-1}, 0.0747-0.4742 mol H_2O m^{-2} s^{-1}, 237.83-294.00 µmol CO_2 mol^{-1}, 1.89-5.31 mmol H_2O m^{-2} s^{-1}.

Keywords: *Deficit Irrigation, Sugar Beet, Net Photosynthesis Rate, Drip Irrigation*

INTRODUCTION

In the world, sugar was produced from sugar cane until the end of the 1800s. The existence of sugar in sugar beet was first determined in studies conducted in Germany in the 18th century, and sugar production from sugar beet began at the beginning of the 19th century. The climatic zones located our country are not suitable for sugar cane production. Since our country is located between 36-42° north latitudes in the northern hemisphere and in the subtropical climate zone, all of the sugar produced is obtained from sugar beet due to the suitability of climate and soil conditions (Anonymous, 2018). In 2018, the total sugar beet production area in the world was 4 809 490 hectares. In the same year, the world sugar beet production amounted to 274 886 306 tons (FAOSTAT, 2018). In 2019, 18 085 528 tons of sugar beet were obtained from 310 000 hectares of production area in Turkey (TUIK, 2019). Between the years 1961-2018, according to the average yield for Turkey and the world, it is observed that the two values are close to each other all the time. (FAOSTAT, 2020).

Today, the increasing population due to global warming and world conditions has increased the use of agricultural land and water and reduced the sustainability rate of these resources. In irrigated agriculture areas in the world, especially in arid and semi-arid climates; insufficient precipitation and high evaporation increase soil dryness. Due to the decrease in the quantity and

quality of water resources worldwide, deficit irrigation practices are becoming more important day by day. As a result of the planning of the irrigation system according to limited conditions, there is a significant decrease in water, energy and capital needs, and consequently, the income of the enterprise can be increased (English et al., 1990). For this reason, it has become necessary to consider approaches such as deficit irrigation management that provides water increase in order to be able to irrigate more effectively and irrigate more areas with our existing water resources allocated to agriculture (Ünlü et al., 2008). This approach, which aims to use less water in crop production with acceptable losses in yield parameters in plants with different irrigation levels throughout the growing period, is named with different names; Partial Irrigation, Regulated Deficit Irrigation, ET Deficit Irrigation and Limited Irrigation (English et al., 1990; Kanber et al., 2007). On the other hand, photosynthesis, which is an important parameter of plant physiology, is affected by different irrigation levels. For plants which irrigating by deficit irrigation, the decrease in photosynthesis rate can be attributed to the decrease in leaf width or the water stress. Along with these, it can be attributed to the decrease in the amount of C by physiological closure of the stomata or the decrease in the amount of light per unit area per leaf (Bloch et al., 2006). The level of different irrigations affects photosynthesis parameters independent of stomatal factors. In addition to closing and opening of stomata, inhibition of mesophyll activity (non-stomatal restriction of photosynthesis) occurs only under severe or prolonged drought stress. With photosynthesis values changing with different irrigation levels; changes in net photosynthesis rate, stomatal conductivity, intercellular CO_2 concentration and transpiration values are also observed.

The researches of the effects on physiological parameters of deficit irrigation on sugar beet plants in Turkey and in the world are limited in number (Tognetti et al., 2003; Bloch et al., 2006; Köksal, 2006; Li et al., 2019). Studies on sugar beet irrigation in Turkey are usually about water-yield relationships (Süheri et al., 2007; Poçan, 2008; Tari et al., 2013;). This study aims to determine effects of deficit irrigation applied with drip irrigation on the net photosynthesis rate (A), stomatal conductivity (gs), intercellular CO_2 concentration (Ci) and transpiration (E) in sugar beet in the sub-humid climate.

MATERIAL AND METHODS

The research was carried out on the experimental area of Bursa Uludağ University Faculty of Agriculture Agricultural Application and Research Center. The experimental area is located at an average altitude of 112 m and 40º 13 '33 "north (N) latitude, 28º 51' 34" east (E) longitude. The soils of the experimental area are in the clay structure. For 0-120 cm soil depth, considering 30 cm soil layers, the volumetric mass is 1.35-1.38 g cm^{-3}; the field capacity ranged from 38.17%-43.01%and the wilting point ranged from 23.18%-27.07%. Considering the effective rooting depth of sugar beet (90 cm), the water-holding capacity for 0-90 cm soil depth was calculated as 163.3 mm. Bursa, located in the south-east of the Marmara Region, has a mild climate, with hot and dry summers and cool and rainy winters (Candoğan et al., 2013). The long-term climate data (1960-2019) (Anonymous, 2020a)and the 2019 growing season climate data (Anonymous, 2020b) of the experimental area are given in Table 1.

Table 1. Long terms (1960-2019) monthly averages and experimental area 2019 climate data for growing period.

	Average temperature (ºC)		Relative Humidity (%)		Wind Speed (m/s)		Precipitation (mm)	
Months	2019	1960-2019	2019	1960-2019	2019	1960-2019	2019	1960-2019
May	19.6	17.6	67.3	68.1	2.3	2.0	40.4	46.0
June	23.7	22.0	68.6	62.3	2.9	2.0	51.2	36.7
July	23.6	24.4	64.6	59.6	2.8	2.3	37.9	15.8
August	24.5	24.2	64.3	61.5	3.2	2.3	39.1	18.9
September	21.3	20.3	63.5	66.8	2.9	1.9	11.3	42.7

"KWS Akazia" sugar beet (Beta vulgaris L) variety was used as the plant material. Sugar beet seeds were hand-sown on May 1, 2019, approximately 3 cm deep in the soil, with a row spacing of 45 cm and a plant-plant spacing of 20 cm. After planting, 15-15-15 NPK compound fertilizer at an effective substance dose of 5 kg da^{-1} was perfused by hand on the plots. Irrigation water was applied to the plots by sprinkler irrigation method for the germination and emergence of seeds. The experiment was carried out with three replications according to the randomized blocks design.The area of each trial plot was formed by 5 plant rows in planting; Plot area was designed to be 2.25 m x 6.0 m = 13.5 m^2. In the research, 4 different experimental treatments were created with different irrigation levels. Irrigations were scheduled based on the replenishment of 100% (S_1), 67% (S_2), 33% (S_3), and 0% (S_4) of soil water depletion from a soil depth of 90 cm using a 7-day irrigation interval. Gravimetric method was used to determine the irrigation water to be applied.

According to the water analysis results; Irrigation water to be used in the experimental area is classified as C_2S_1. Irrigation water was applied by drip irrigation. A polyethylene lateral drip line was designed for each row using 16 mm-dia. lateral pipes with 2 L h^{-1} in-line drippers spaced at 20 cm.

Net photosynthesis rate, stomatal conductivity, intercellular CO_2 concentration and transpiration physiological measurements were measured with a portable photosynthesis system (Li-6400, LI-COR Inc., Lincoln, NE, USA) between 13:00 and 14:00. In the measurements, young and mature upper leaves were used without detaching them, and measurements were made on two leaves in two plants in each plot. Portable photosynthesis system uses equations derived by von Caemmerer and Farquhar (1981) as the basis for net photosynthesis rate, transpiration, and stomatal conductivity.

Transpiration

The mass balance of water vapor in an open system is given by 14th equation.

$$sE = u_o w_o - u_i w_i \qquad (14)$$

where s is leaf area (m^{-2}), E is transpiration rate (mol m^{-2} s^{-1}), u_i and u_o are incoming and outgoing flow rates (mol s^{-1}) from the chamber, and w_i and w_o are incoming and outgoing water mole fractions (mol H_2O mol air^{-1}). Since

$$E = \frac{u_i(w_o - w_i)}{s(1 - w_o)} \qquad (15)$$

The relationships between the terms are 14-15 and what the LI-6400 measures are

$$u_i = F/10^6 \qquad (16)$$

$$w_i = W_r/10^3 \qquad (17)$$

$$w_o = W_s/10^3 \qquad (18)$$

$$s = S/10^4 \qquad (19)$$

where F is air flow rate (µmol s^{-1}), W_s and W_r are sample and reference water mole fractions (mmol H_2O (mol air)$^{-1}$), and S is leaf area (cm^{-2}). The equation that the LI-6400 uses for transpiration is:

$$E = \frac{F(W_s - W_r)}{100S(1000 - W_s)} \qquad (20)$$

Total Conductance to Water Vapor

The total (includes stomatal and boundary layer) conductance of the leaf g_{tw} (mol H_2O m^{-2} s^{-1}) is given by

$$g_{tw} = \frac{E\left(1000 - \frac{W_l + W_s}{2}\right)}{W_l - W_s} \qquad (9)$$

where W_l is the molar concentration of water vapor within the leaf (mmol H_2O (mol air)$^{-1}$), which is computed from the leaf temperature T_l (C) and the total atmospheric pressure P (kPa)

$$W_l = \frac{e(T_l)}{P} \times 1000 \qquad (10)$$

The function e(T) in 11th equation is saturation vapor pressure (kPa) at temperature T (C) measured in 12th equation (Buck, 1981).

$$e(T) = 0.61365 e^{\frac{17.502T}{240.97+T}} \qquad (11)$$

Stomatal Conductance to Water Vapor

The stomatal conductance g_{sw} to water vapor (mol H_2O m^{-2} s^{-1}) is obtained from the total conductance by removing the contribution from the boundary layer.

$$g_{sw} = \frac{1}{\frac{1}{g_{tw}} - \frac{k_f}{g_{bw}}} \qquad (12)$$

where k_f is a factor based on the estimate K of the fraction of stomatal conductances of one side of the leaf to the other,

$$k_f = \frac{K^2 + 1}{(K + 1)^2} \qquad (13)$$

and g_{bw} is the boundary layer conductance to water vapor (mol H_2O $m^{-2}s^{-1}$) from one side of the leaf. The boundary layer conductance correction thus depends on whether the leaf has stomata on one or both sides of the leaf.

Net Photosynthesis Rate (A)

The mass balance of CO_2 in an open system is given by

$$sa = u_i c_i - u_o c_o \qquad (1)$$

where a is assimilation rate (mol CO_2 m^{-2} s^{-1}), c_i and c_o are incoming and outgoing mole fractions (mol CO_2 mol air^{-1}) of carbon dioxide. Using (2), we can write

$$a = \frac{u_i(c_i - c_o)}{s} - Ec_o \qquad (2)$$

Portable photosynthesis system makes measurements using the following relationships.

$$c_i = C_r/10^6 \qquad (3)$$

$$c_o = C_s/10^6 \qquad (4)$$

$$a = A/10^6 \qquad (5)$$

where C_r and C_s are sample and reference CO_2 concentrations (μmol CO_2 $(mol\ air)^{-1}$), and A is net assimilation rate of CO_2 by the leaf (μmol CO_2 m^{-2} s^{-1}).

$$A = \frac{F(C_r - C_s)}{100S} - C_s E \qquad (6)$$

Transpiration in the equation (E) serves as a dilution correction. As water vapor is added to the leaf system, transpiration dilutes all other gases containing CO_2.

Intercellular CO_2 Concentration

The intercellular CO_2 concentration C_i (μmol CO_2 mol air^{-1}) is given by

$$C_i = \frac{\left(g_{tc} - \frac{E}{2}\right)C_s - A}{g_{tc} + \frac{E}{2}} \qquad (7)$$

where g_{tc} is the total conductance to CO_2, and is given by

$$g_{tc} = \frac{1}{\frac{1.6}{g_{sw}} + \frac{1.37k_f}{g_{bw}}} \qquad (8)$$

1.6 is the ratio of the diffusivities of CO_2 and water in air, and 1.37 is the same ratio in the boundary layer.

Data for A, gs, C_i, and E were subjected to analysis of variance (ANOVA). The F-test was used to determine the effects of irrigation treatment and block at the 0.05 and 0.01 probability levels, with F-protected least significant difference (LSD) calculated at 0.05, according to Turan (1995).

RESULTS

For four different irrigation treatments applied, results of variance analysis and average values of A, C_i, gs and E values performed on 12.08.2019, 29.09.2019 and 09.09.2019 are given in Table 2. According to the results of variance analysis, all physiological parameters were significantly affected by different water stress conditions for each measutement date ($P < 0.01$).

Table 2. Results of variance analysis and mean values of net photosynthesis rate (A), stomatal conductivity (gs), intercellular CO_2 concentration (C_i) and transpiration (E) under deficit irrigation treatments

	A			gs			Ci			E		
Treatment	12.08.19	29.08.19	09.09.19	12.08.19	29.08.19	09.09.19	12.08.19	29.08.19	09.09.19	12.08.19	29.08.19	09.09.19
S_1	15.63 a[1]	18.18 a	19.00 a	274.67 a	294.00 a	284.83 a	0.37 a	0.47 a	0.44 a	4.48 a	5.31 a	3.43 a
S_2	12.77 b	15.15 b	16.73 b	260.33 b	283.17 a	270.83 a	0.26 b	0.36 b	0.36 b	3.95 b	4.32 b	2.68 b
S_3	10.92 c	12.23 c	13.77 c	232.17 c	237.33 b	235.33 b	0.11 c	0.18 c	0.15 c	2.73 c	3.15 c	1.80 c
S_4	6.03 d	8.27 d	9.72 d	200.33 d	222.83 b	221.83 b	0.07 d	0.09 d	0.08 d	1.48 d	1.89 d	0.97 d
$LSD_{0.05}$	1.56	13.98	0.03	0.5	1.65	15.05	0.05	0.3	1.24	26.08	0.07	0.5
Blocks	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatments	**	**	**	**	**	**	**	**	**	**	**	**

[1]: means with different letters in the same column are significantly different
ns: non significant

**: significant at the 1% of probability level ($P<0.01$)

It was determined that the highest A values were measured in S_1 and were between 15.63 and 19.00, and the lowest values were measured in S_4 and varied between 6.03 and 9.72. The highest gs values were measured in S_1 and were between 274.67 and 284.83 and the lowest values were

measured in S_4 and varied between 200.33 and 222.83. The highest C_i values between 0.37 and 0.47 were measured for S_1, while the lowest C_i values were between 0.07 and 0.09 for S_4. E values were measured in S_1 with the highest values between 4.48 and 5.31, and the lowest in S_4 between 1.48 and 0.97 (Table 2).

The changes of all physiological parameters during the measurement dates are also given in Figure 1. As seen in Figure 1, except A; gs, C_i, E values showed a tendency to decrease in the last measurement (09.09.2019). The reason for this situation can be attributed to the approaching of the sugar beet plant to the harvest period.

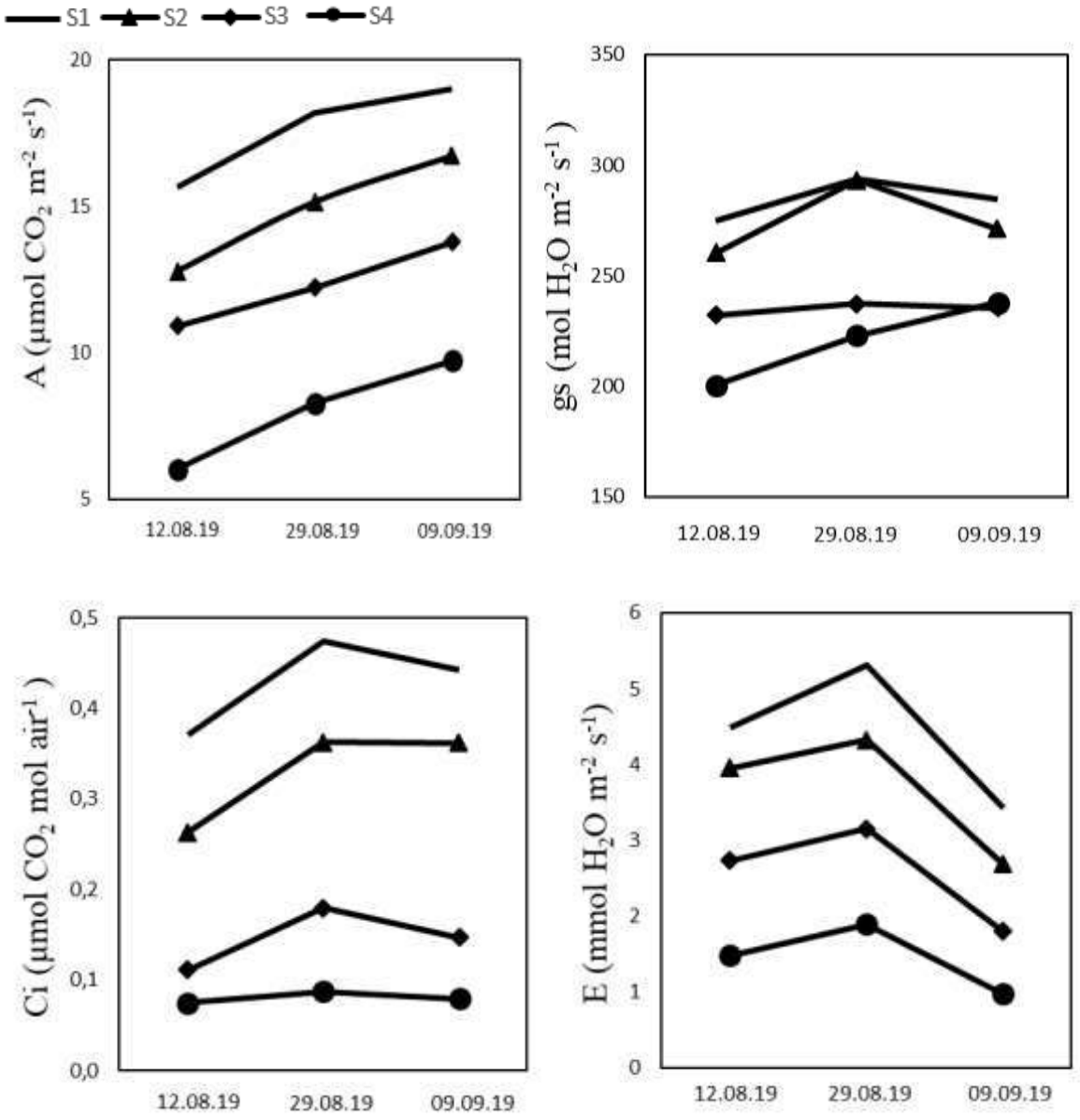

Figure 1. Changes of net photosynthesis rate (A), stomatal conductance (gs), intercellular CO_2 concentration (C_i) and transpiration (E) parameters during the measurement dates

DISCUSSION

When Figure 1 is examined, it is observed that the A, gs, C_i and E values decrease with the decreasing of water application levels. Bloch et al. (2006), in their study on 3 different sugar beet varieties in Germany, observed physiological parameters for different irrigation levels, 100%, 50% and 20%, and determined a decrease in net photosynthesis rate, stomatal conductivity and perspiration values with increasing of water deficit. Photosynthesis values of 3 different genotypes for full irrigation subject varied between 13 and 15 μmol CO_2 m^{-2} s^{-1}, for 50% subject between 7 and 10 μmol CO_2 m^{-2} s^{-1}, and for 20% subject between 3 and 4 μmol CO_2 m^{-2} s^{-1} values. For the same study, transpiration values ranged from 5 to 6 H_2O m^{-2} s^{-1} for full irrigation, 3 to 4 H_2O m^{-2} s^{-1} for 50%, and 1 to 2 H_2O m^{-2} s^{-1} for 20%. At the last, stomatal conductivity values varied between 350-420 H_2O m^{-2} s^{-1} for full irrigation, 160-200 H_2O m^{-2} s^{-1} for 50%, and 20 to 70 H_2O m^{-2} s^{-1} for 20%. Tognetti et al. (2003) in the study conducted in Southern Italy measured the responses of sprinkler and drip irrigation techniques to sugar beet physiological parameters and determined 3 different irrigation levels for the drip irrigation method: 100%, 75% and 25%. In the 2-year results of the experiment, it was observed that as the irrigation level decreased, the net photosynthesis rate (A) and stomatal conductivity (gs) values decreased. For the full irrigation treatment irrigated with drip irrigation, net photosynthesis rate values varied between 25 and 20 μmol CO_2 m^{-2} s^{-1} during the season, between 22 and 17 μmol CO_2 m^{-2} s^{-1} for 75%, and 17 and 14 μmol CO_2 m^{-2} s^{-1} for 50%. Stomatal conductivity values for 100%, 75% and 50% irrigation levels varied respectively, between 450-200, 320-180, 220-140 mol H_2O m^{-2} s^{-1} values. Li et al. (2019) created 3 different irrigation levels in their studies conducted in China, observed the physiological responses of deficit irrigation on sugar beet, and encountered decreases in A, gs, and Ci values as the water stress increased. Experimental subjects are designed at 3 different irrigation levels as 70%, 50% and 30%. At the end of the research average values were found as follows: 25.5, 18.5, and 12.5 μmol CO_2 m^{-2} s^{-1} for the net rate of photosynthesis and for the stomatal conductance; 405, 190 and 110 mol H_2O m^{-2} s^{-1}, lastly for the intercellular CO_2 concentration: 260, 220 and 200 μmol CO_2 mol air^{-1}. Many similarities were found between the results obtained in the study and the results determined in the above studies.

CONCLUSIONS

The effects of deficit irrigation applications on physiological characteristics of sugar beet A, gs, C_i, E in semi-humid climates was found to be significant at the level of $P < 0.01$. As the water stress increased, a decrease was observed in A, gs, C_i and E values. It is thought that these conclusions will be useful in evaluating the results to be obtained in studies to be conducted in similar climatic conditions.

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ASSOCIATION OF CANOPY SPECTRAL REFLECTANCE INDICES AND YIELD COMPONENTS OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Grain yield of wheat is a complex trait made up of the interaction between different yield components and environmental effects. Due to the importance of yield traits, breeders need efficient and precise methods to measure differences among genotypes. Since that spectral proximal sensing is promising for high-throughput non-destructivephenotyping, recent findings suggest that multispectral proximal sensors can be used in place of labour intensive methods to estimate specific plant traits. The objective of this study was to evaluate the impact of different spectral reflectance indices (SRIs) in assessing stem height and spike length in 4 winter wheat genotypes grown in different conditions of seed priming. Seeds of each winter wheat genotypes were primed with different concentrations of zinc oxide nanoparticles (ZnO NPs) and after sown in soil pots. Spectral reflectance from the plants at different growth stages during vegetation was measured using an active multispectral, optical sensor namely Plant-O-Meter. Since that device provides four indicative wavelengths between 465, 535, 630 and 850 nm, several SRIs were calculated. The results revealed that most of SRIs measured at full flowering stage (BBCH 65) of wheat were positively correlated ($P < 0.01$) with stem height of wheat, with r values of up to 0.674. For the trait spike length of wheat the greatest positive association was found at medium milk grown stage (BBCH 75) of wheat under each treatment, with r values of up to 0.798. Overall, statistically significant correlations were more influenced by the phenological stage of estimation and the index used.

Keywords: *Wheat, Spectral vegetation indices, yield traits.*

INTRODUCTION

Wheat is one of the world's most important staple crops and provides 20% of the food calories and similar proportion of daily protein to the world's population (Reynolds and Braun, 2013). Obtaining wheat cultivars with high genetic potential for grain yield, as well as good quality of wheat are the main priority of breeding programs (Petrović et al., 2012). The grain yield of wheat is a complex trait that depends on mainly yield components and environmental factors (Kraljević-Balalić et al., 1995; Zečević et al., 2008). The knowledge of association between grain yield and its components can improve the efficiency of breeding programs by identifying appropriate traits for selecting wheat varieties (Evans and Fisher, 1999; Zečević et al., 2008). Stem height and spike length are important quantitative trait which are in correlations with other yield components. It has been confirmed that nanotechnology poses great potential to increase

plant growth and different grain yield traits. Seed priming with zinc oxide nanoparticles (ZnO NPs) has been shown as prominent to enhance yield components, such as plant height and spike length in wheat (Munir et al., 2018; Rizwan et al., 2019, Ljubičić et al., 2020). On the other side, its sufficient concentratin could raise negative effects and decrease growth. However, due to the importance of yield traits, breeders need efficient and precise methods to measure differences among genotypes. Recent findings suggest that multispectral proximal sensors can be used in place of labour intensive methods to estimate specific plant traits. The spectral signatures reflected from the plant canopy at specific wavelengths provide different types of cumulative information on the substantial and gradual changes that occur in specific plant characteristics or tolerance levels (El-Hendawy et al., 2019). Crop spectral reflectance measurements are being used efficiently for crop growth monitoring as they reflect wide range of biochemical and physiological measures, while spectral reflectance indices (SRIs) are quantitative measurement and present a spectral transformation metric for measuring the presence and state of vegetation (Bannari et al., 1995). Spectral reflectance indices (SRIs) have been developed on the basis of simple mathematical formulae such as ratios or differences in thereflectance at given wavelengths Araus et al. (2001). Its basis is the characteristic photosynthetic response of green vegetation to incident light (Khan et al., 2018). Attempts have been made to evaluate the potential use of SRIs in breeding to differentiate genotypes for yield under differnt conditions (Aparacio et al. 2001; Royo et al. 2003; Babar et al. 2006; Babar et al. 2007). Several studies have deal with different SRIs as indirect selection traits and their potential as an indirect selection tool has been evaluated based on the basis of its genetic correlation and heritability (Babar et al., 2006; Gutierrez et al., 2010; El-Hendawy et al., 2019). The most widespread application of these indices is in the assessment of characteristics related to the total photosynthetic area of the canopy (Aparacio et al., 2000). Among numerous SRIs, the most widely used are the Normalized difference vegetation index (NDVI), Green normalised difference vegetation index (NDVIg), simple ratio (SR) and Transformed difference vegetation index (TDVI). However, the accuracy is influenced by the climate, growth stage of evaluation and the index used (Bolton and Friedl 2013). Since that wheat seed priming with zinc oxide nanoparticles (ZnONPs) is the main constraint in this investigation, the object of this paper was to estimate the performance of different SRIs, during the second half of the crop cycle, in assessing changes in stem height and spike lenght of wheat genotypes primed with different concentration of ZnO NPs**.**

MATERIAL AND METHODS

The present investigation was carried out at the experimental greenhouse facility available in the University of Novi Sad, in Serbia, during the 2018-2019 growing season. The experimental material in this study was comprised from 4 winter wheat genotypes, namely, Pobeda, NS 40S, Ingenio and Futura. Seeds of each wheat genotypes were primed with different solutions containing appropriate concentrations of ZnO NPs (0, 10, 100 and 1000 mg L^{-1}) for 48 h in dark box by continuous aeration. Primed seeds of wheat were after sown in soil pots filled with 5.0 kg of soil, with 60-70% moisture contents during the whole experiment. The trial was set up according to the completely randomized design with three replications of each treatment on chernozem soil. For each winter wheat genotypes within each treatment spectral reflectance was measured at three different growth stages: full flowering (BBCH 65), medium milk (BBCH 75) and fully ripe stage (BBCH 89). For spectral reflectance measurements, an active, multispectral, optical sensor Plant-O-Meter proximal sensor, recently developed in BioSens

Institute, was used. Plant-O-Meter is an active hand-held sensor, which emits light and measures the reflectance at four indicative wavelengths, 465 nm (Blue), 535 nm (Green), 630 nm (Red) and 850 nm (Infrared). The sensor records the reflectance for each band separately providing the ability to calculate more than 30 different indices. Detailed information concerning the work principles and specifications of the Plant-O-Meter multispectral device has been provided in Kitić et al. (2019). Spectral measurements were taken close to noon, between 10:00 am and 2:00 pm on sunny, cloud-free days when the plant canopy and soil surface are dry. Reflectance from the vegetation was measured at 3 growth stages of wheat: full flowering (BBCH 65), medium milk (BBCH 75) nad full rippe stage of wheat (BBCH 89). Based on Blue (465 nm), Green (535 nm), Red (630 nm) and Infrared (850 nm) spectral wavebands the following spectral reflectance indices were calculated: Normalised difference vegetation index (NDVI), Red normalised difference vegetation index (RDVI), Green normalised difference vegetation index (NDVIg), Blue normalised difference vegetation index (NDVIb), Simple ratio (SR), Modified simple ratio (MSR), Infrared Percentage Vegetation Index (IPVI), Structure Insensitive Pigment Index (SIPI), Green Atmospherically Resistant Index (GARI), Soil Adjusted Vegetation Index (SAVI), Green Soil Adjusted Vegetation Index (GSAVI), Green Optimized Soil Adjusted Vegetation Index (GOSAVI), Normalized Pigment Chlorophyll Ratio Index (NPCI), Green Chlorophyll Index (GCI), Green Ratio Vegetation Index (GRVI), Plant Senescence Reflectance Index (PSRI), Non-Linear Index (NLI), Transformed Difference Vegetation Index (TDVI), Visible Atmospherically Resistant Index (VARI), Wide Dynamic Range Vegetation Index (WDRVI), Green Re-normalized Difference Vegetation *Index* (GRDVI), Green-Blue NDVI (GBNDVI), Red-Blue NDVI (RBNDVI), Pan NDVI (PNDVI), Inversion of the simple ratio (ISR), Normalised difference water index (NDWI), Green leaf index (GLI) and Triangular Greenness Index (TGI). Calculation and selection of spectral reflectance indices were according the mathematical formulae described according to the Index *database for remote sensing indices* (https://www.indexdatabase.de/info/idb.php). At the stage of full maturity, ten plants from each replication of each treatment of wheat genotypes were selected for measuring data for stem height and spike length. Average values of three replication trait analysis were used. Pearson correlation coefficient (*r*) was used to study the relationship between vegetation indices and plant height and spike length of wheat. All statistical analyses were carried out using software STATISTICA, version 13 (StatSoft Inc., USA).

RESULTS AND DISCUSSION

Yield components performance.

The presented results revealed wide range between the minimum and maximum values for observed traits of wheat within each treatment. The stem height of each wheat genotype increased with increasing ZnO NPs concentration applied. The greatest increase in stem height was found at 100 mg L^{-1} ZnO NPs for genotypes NS 40S (89.3 cm) and NK Ingenio (86 cm), while genotypes with greatest increase at 10 mg L^{-1} ZnO NPs applied were NS Futura (89 cm) and NS Pobeda (86 cm). Lower values of plant height were observed at control plants, ranged between 73 cm to 80 cm. The lowest values of plant height were found in maximum concentration of ZnO NPs ranged between 61 cm to 69 cm (Table 1).

The present results indicated that different treatments influenced on differences in stem height which was expected since that the stem height is a quantitative and variable trait which

expression highly depends on the environmental factors. With respect to the trait spike length the results indicated that spike length increase with increasing ZnO NPs concentrations in the priming solution, comparing than control conditions. The highest increase in spike length was found in conditions of 10 mg l^{-1} and 100 mg l^{-1}NPs ZnO applied, while the lower values of spike length were found in cultivars in control conditions. The greatest increase in spike length within application of 10 mg l^{-1} ZnO NPs were observed for genotypes Futura (11.3 cm) and Pobeda (9.9 cm). Wheat cultivars with greatest increase at 100 mg l^{-1} ZnONPs applied were NS 40S (9.8 cm) and NK Ingenio (11.1 cm). Lower values of spike length were observed at plants in control conditions which values ranged between 9.3 cm to 10.9 cm. In conditions of maximum concentration of ZnO NPs applied the lowest values of these parameters were found (Table 1). The present results indicated that different treatments influenced the differences in spike length. According to Zečević et al. (2008), spike length is genetically controlled, but it highly depends to environmental factors. In general, results showed that ZnO NPs increased the plant growth and confirmed that ZnO NPs are effective in increasing plant growth and stem height. These results are in accordance with results published by Munir et al. (2018) and Rizwan et al. (2019).

Table 1. The mean values of stem height and spike length of examined 4 winter wheat genotypes

Yield components								
Stem height (cm)					Spike length (cm)			
Environments								
Treatments	K - 0 mg l^{-1}	10 mg l^{-1}	100 mg l^{-1}	1000 mg l^{-1}	K - 0 mg l^{-1}	10 mg l^{-1}	100 mg l^{-1}	1000 mgl^{-1}
Genotypes	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$	$\overline{X}$
Pobeda	79.7	86.0	85.0	67.3	9.3	9.9	9.8	8.7
NS40S	75.0	83.7	89.3	63.7	10.2	11.1	11.2	9.7
Ingenio	74.7	82.7	86.0	65.3	9.9	10.7	11.1	9.3
Futura	75.0	89.0	88.0	64.3	10.9	11.3	11.3	9.9
$\overline{X}$	76.1	85.3	87.1	65.2	10.1	10.8	10.8	9.4

$\overline{X}$ - mean value (cm); *Environment labels represents control (0), 10, 100 and 1000 mg l^{-1} primed concentrations of ZnO NPs applied.

Effect of environment on yield components and spectral reflectance indices (SRIs*).*

The presented results revealed wide range between the minimum and maximum values for spectral reflectance indices (SRIs), as well as for individual spectral bands for green vegetation detection which varied on overall basis. Regarding to the overall mean values, the maximum values most of SRIs were observed during full flowering stage (BBCH 65) and gradually declined through the later growth stages, reaching the minimum values at full ripe stage of wheat (BBCH 89). SRIs, such as a Normalized pigment chlorophyll ratio index (NPCI), Inversion of the simple ratio (ISR) and Normalized difference water index (NDWI), as well as for individual spectral bands, increased from BBCH 65 stage and showed the greatest values at the end of growing season, Table 2.

Table 2. Mean values for the stem height, spike lenght and the spectral reflection indices (SRIs) measured for 4 winter wheat genotypes at 3 growth stages in all environments.

Environments

	K - 0 mg l-1	10 mg l-1	100 mg l-1	1000 mgl-1	K - 0 mg l-1	10 mg l-1	100 mg l-1	1000 mgl-1	K - 0 mg l-1	10 mg l-1	100 mg l-1	1000 mgl-1
						Growth stage						
SVIs		BBCH 65				BBCH 75				BBCH 89		
RED	13.08	10.60	22.91	13.30	29.28	26.81	12.46	32.78	86.90	81.13	71.94	70.52
GREEN	25.02	21.17	41.25	22.05	47.14	48.89	23.41	50.18	114.1	117.0	103.9	84.67
BLUE	15.29	13.09	20.03	10.83	23.86	24.43	13.49	24.53	68.49	74.84	60.40	52.45
INFRARED	102.4	95.57	111.8	82.10	125.1	127.3	106.8	119.7	181.3	180.4	162.0	138.3
NDVI	0.749	0.781	0.901	0.684	0.622	0.641	0.768	0.550	0.367	0.398	0.414	0.339
RDVI	9.182	8.996	9.407	7.934	9.483	9.753	9.389	8.672	9.155	9.567	8.785	7.795
NDVIg	0.583	0.620	0.718	0.560	0.447	0.449	0.615	0.420	0.238	0.259	0.287	0.242
NDVIb	0.740	0.762	0.951	0.767	0.682	0.680	0.772	0.670	0.467	0.450	0.520	0.474
SR	8.949	10.03	6.025	7.077	5.430	5.628	9.552	4.533	2.646	2.665	3.178	2.290
IPVI	0.874	0.890	1.076	0.842	0.811	0.820	0.884	0.775	0.683	0.699	0.707	0.670
GARI	0.619	0.669	0.677	0.484	0.395	0.437	0.628	0.312	0.144	0.210	0.206	0.325
SAVI	1.118	1.165	1.222	1.020	0.929	0.958	1.146	0.822	0.549	0.596	0.619	0.507
GSAVI	0.871	0.926	0.949	0.836	0.669	0.672	0.919	0.626	0.356	0.387	0.429	0.362
GOSAVI	0.583	0.619	0.717	0.559	0.447	0.449	0.614	0.419	0.238	0.258	0.287	0.242
NPCI	-0.047	-0.063	0.333	0.134	0.084	0.047	-0.005	0.171	0.114	0.063	0.126	0.158
GCI	3.444	3.866	2.562	3.105	1.971	2.084	3.995	1.848	0.950	0.873	1.378	0.792
GRVI	0.308	0.325	0.513	0.225	0.254	0.277	0.302	0.184	0.144	0.158	0.146	0.110
NLI	0.996	0.996	1.245	0.993	0.995	0.995	0.996	0.992	0.992	0.994	0.990	0.990
TDVI	1.276	1.308	1.417	1.204	1.130	1.153	1.295	1.032	0.762	0.825	0.835	0.723
WDRVI	0.199	0.268	0.261	0.079	-0.032	-0.003	0.240	-0.133	-0.360	-0.339	-0.305	-0.398
GRDVI	0.430	0.480	0.528	0.369	0.246	0.261	0.467	0.186	-0.029	-0.005	0.029	-0.043
GBNDVI	0.419	0.463	0.557	0.416	0.277	0.277	0.468	0.249	0.019	0.023	0.080	0.018
RBNDVI	0.548	0.590	0.693	0.516	0.410	0.422	0.590	0.348	0.100	0.111	0.159	0.079
PNDVI	0.295	0.347	0.404	0.256	0.118	0.128	0.345	0.065	-0.167	-0.157	-0.108	-0.184
ISR	0.148	0.127	0.471	0.196	0.246	0.231	0.136	0.311	0.491	0.449	0.442	0.517
NDWI	-0.583	-0.620	-0.218	-0.560	-0.447	-0.449	-0.615	-0.420	-0.238	-0.259	-0.287	-0.242
GLI	0.274	0.280	0.539	0.267	0.282	0.287	0.287	0.245	0.189	0.183	0.192	0.169
WDRVI	-0.122	-0.054	-0.057	-0.237	-0.343	-0.318	-0.081	-0.426	-0.607	-0.596	-0.564	-0.639
Plant height	76.08	85.33	87.33	65.17	76.08	85.33	87.08	65.17	76.08	85.33	87.08	65.17
Spike length	10.10	10.75	11.08	9.40	10.11	10.75	10.83	9.40	10.11	10.75	10.83	9.40

*Environment labels represents control (0), 10, 100 and 1000 mg l^{-1} primed concentrations of ZnO NPs applied.

Regarding to the environmental conditions, within stage of BBCH 65, the greatest values was found in conditions of 100 mg l^{-1} than at 10 mg l^{-1}NPs ZnO applied, while the lower values of SRIs were found in cultivars in control conditions. Within BBCH 75, the greatest values was found in conditions of 100 mg l^{-1} and 10 mg l^{-1}NPs ZnO applied, while the lower values of SRIs were found in cultivars in control conditions. Within BBCH 89, the greatest values was found in conditions of 100 mg l^{-1} and 10 mg l^{-1}NPs ZnO applied, while the lower values of SRIs were found in cultivars in control conditions. The lowest values of SRIs were found in maximum concentration of ZnO NPs within BBCH 65, BBCH 75 and BBCH 89 (Table 2). This results indicated that ZnO NPs increased the plant growth and yield components, such as a stem height and spike lenght which was reflected throught spectral reflectance signature.

Relationship between yield components and spectral reflectance indices.

Highly significant ($P < 0.01$) positive correlations between stem height and spike length of wheat were observed at BBCH 65 ($r = 0.689^{**}$) and BBCH 75 stage of wheat ($r = 0.740^{**}$). In present study SRIs expressed better sensitivity than individual spectral bands for green vegetation detection and showed better corelation with observed traits under each treatment. With respect to the association between stem height and SRIs, the result revealed that the positive and highly significant association for most of SRIs were observed at the full flowering (BBCH 65) stage of wheat (Table 3). Positive and highly significant correlations were also observed in BBCH 65 between stem height and GSAVI ($r = 0.674^{**}$), NDVIg ($r = 0.672^{**}$), GOSAVI ($r = 0.672^{**}$), GRDVI ($r = 0.661^{**}$), GCI ($r = 0.661^{**}$) and GCI ($r = 0.661^{**}$). Significant and positive correlations were also observed between stem height and GARI ($r = 0.622^{*}$), PNDVI ($r = 0.605^{*}$), NDVI ($r = 0.600^{*}$), IPVI ($r = 0.600^{*}$), SAVI ($r = 0.600^{*}$), WDRVI ($r = 0.596^{*}$), TDVI ($r = 0.593^{*}$), NPCI ($r = 0.527^{*}$), NLI ($r = 0.524^{*}$), RBNDVI ($r = 0.517^{*}$), SR ($r = 0.515^{*}$), GBNDVI ($r = 0{,}514^{*}$). Positive, but not significant correlations were observed between stem height and RDVI ($r = 0{,}414$), GRVI ($r = 0{,}415$), VARI ($r = 0{,}387$) and GLI ($r = 0{,}121$). Single band which had higher positive correlations were INFRARED ($r = 0{,}353$) and BLUE ($r = 0{,}246$). During the medium milk stage of wheat (BBCH 75), the largest positive and significant relation were observed between stem height and TDVI ($r = 0.522^{*}$), while the weaker and non signiicant relationships was observed in the full ripe stage of wheat. Highly significant negative correlations between stem height of wheat and the SRIs at BBCH 65 were observed for NDWI ($r = -0.672^{**}$). Significant negative corelation was also found for ISR ($r = -0.593^{*}$), SIPI ($r = -0.562^{*}$) while negativne and non significant relation was observed for PSRI ($r = -0.182$) and TGI ($r = -0.014$). At BBCH 75, significant negative correlation were found for ISR and SIPI and non-significant for PSRI ($r = -0.391$), MSR ($r = -0.383$) and TGI ($r = -0.037$), Table 3.

With regard to the trait of the spike length, the greatest positive and highly significant correlations between spike length and SRIs were found at medium milk stage of wheat (BBCH 75). In BBCH 75 the largest corelation were observed TDVI ($r = 0.798^{**}$), NDVI ($r = 0.781^{**}$), IPVI ($r = 0.781^{**}$), SAVI ($r = 0.781^{**}$), WDRVI ($r = 0.745^{**}$), RBNDVI ($r = 0.728^{**}$), SR ($r = 0.671^{**}$), GRVI ($r = 0.663^{**}$). Significant positive association was found for GARI, GDVI, PNDVI, NLI with r values of up to 0.629^{*}. At BBCH 75, between spike length of wheat and the vegetation indices highly significant negative correlations was founfd for ISR ($r = -0.798^{**}$), SIPI ($r = -0.693^{**}$) and negativne and nonsignificant for NDWI ($r = -0.422$), PSRI

(*r* = -0.464) and MSR (*r* = -0.587). At BBCH 65 significant positive correlations were observed between stem height and NDVIg (*r* = 0.732**), GOSAVI (*r* = 0.730**), GSAVI (*r* = 0.725**), PNDVI (*r* = 0.713**), GBNDVI (*r* = 0,712**), GRDVI (*r* = 0.685**), RBNDVI (*r* = 0.662**). Significant positive association was found for NDVI (*r* = 0.600*), IPVI (*r* = 0.600*), WDRVI (*r* = 0.600*), TDVI (*r* = 0.593*), SAVI (*r* = 0.593*), SR (*r* = 0.574*). At BBCH 65, between spike length of wheat and the SRIs significant negative and highly significant correlations were observed for NDWI (*r* = - 0.732**) and MSR (*r* = -0.730**). Significant negative was found for single RED (*r* = -0.609*) and ISR (*r* = -0.595*). Negative and nonsignificant correlation were found for SIPI (*r* = - 0.382), NPCI (*r* = - 0.361), TGI (*r* = - 0.359) and PSRI (*r* = -0.182), Table 3.

Table 3. Pearson's correlations coefficients between stem height (cm), spike length (cm) and evaluated spectral SRIs of 4 winter wheat genotypes measured at 3 growth stages.

	Plant trait					
	Stem height (cm)			Spike length (cm)		
SVIs	BBCH 65	BBCH 75	BBCH 89	BBCH 65	BBCH 75	BBCH 89
RED	-0.122	-0.319	0.028	-0.609 *	-0.498	0.086
GREEN	0.079	-0.141	0.151	-0.366	-0.165	0.110
BLUE	0.246	-0.135	0.153	-0.231	-0.264	0.103
INFRARED	0.353	-0.001	0.200	-0.057	0.061	0.224
NDVI	0.600*	0.503	0.343	0.600*	0.781**	0.344
NDVIg	0.672**	0.229	0.127	0.732**	0.422	0.365
NDVIb	0.060	0.188	0.067	0.412	0.441	0.302
SR	0.515*	0.361	0.402	0.574*	0.671**	0.233
IPVI	0.600*	0.503	0.343	0.600*	0.781**	0.344
GARI	0.622*	0.423	-0.112	0.480	0.609*	-0.328
SAVI	0.600*	0.504	0.344	0.593*	0.781**	0.345
GSAVI	0.674**	0.232	0.127	0.725**	0.424	0.365
GOSAVI	0.672**	0.230	0.127	0.730**	0.422	0.365
NPCI	0.527*	-0.327	-0.426	-0.361	-0.474	0.008
GCI	0.661**	0.260	0.267	0.457	0.446	0.347
GRVI	0.415	0.447	0.327	0.450	0.663**	-0.147
NLI	0.524*	0.410	0.145	0.374	0.515*	0.291
TDVI	0.593*	0.522*	0.325	0.593*	0.798**	0.375
WDRVI	0.596*	0.465	0.374	0.600*	0.745**	0.289
GRDVI	0.662**	0.385	0.233	0.685**	0.629*	0.374
GBNDVI	0.514*	0.230	0.135	0.712**	0.440	0.366
RBNDVI	0.517*	0.439	0.251	0.662**	0.728**	0.375
PNDVI	0.605*	0.362	0.222	0.713**	0.607*	0.385
ISR	-0.593*	-0.521*	-0.324	-0.595*	-0.798**	-0.374
NDWI	-0.672**	-0.229	-0.127	-0.732**	-0.422	-0.365
GLI	0.121	0.444	0.211	0.398	0.663**	-0.090
WDRVI	0.584*	0.440	0.386	0.596*	0.726**	0.265
Stem height	1.000	1.000	1.000	0.689**	0.740**	0.689**
Spike length	0.689**	0.740**	0.689**	1.000	1.000	1.000

*– Significant at P < 0.05 probability level, ** – Highly significant at P < 0.01 probability level.

Between stem height of wheat and SRIs significant positive correlations were observed mostly at full flowering stage. Among the number of diferent SRIs, which are based on a combination of visible and NIR wavelengths, NDVIg (Green Normalised Difference Vegetation Index) on average, performed better than the others and was relatively consistent in all stages for both traits. NDVIg is index which indicates canopy photosynthetic area and exactly it is a modified version of NDVI, which substitutes the green band in place of the red band in the NDVI equation. The GNDVI is sensitive to the chlorophyll concentration in vegetation, when the leaf area index is moderately high. Hence, NDVIg overcomes the problems with saturation, which NDVI exhibits for some vegetation types at later growth stages because it is more sensitive to low chlorophyll concentrations (Gitelson et al., 1996; Mashaba et al., 2016). Consistent and higher correlation between NDVIg and grain yield traits of wheat has been also reported by Babar et al. (2007).

With a few exceptions, between spike length of wheat and SRIs significant positive correlations were observed mostly at BBCH 75 stage of wheat. According to Appparacio et al. (2000) the weaker relationships recorded between the SRIs and spike lentgh during BBCH 65 compared with those for stem height, might have been the result of the difficulties encountered in measuring properly the green area of spike. According to the Shibayama et al. (1986) the relationship between SRIs and canopy parameters has been reported as being disturbed by the presence of the spike. Within BBCH 75 the greatest relation was observed between TDVI which was expected, since that TDVI does not saturate like NDVI and SAVI. Also the greatest positive corelation between spike lenght and NDVI in BBCH 75 was observed, as a consequence of lower amounts of green biomass. It may be explained as during the crop cycle, after flowering, vegetation shows a decrease in reflectance in the near-infrared bands, an increased red reflectance in the chlorophyll active band. According to Appparacio et al. (2000) ontogenetic changes in the spectral signature lead to a decrease in either NDVI and SR. Similarly to the present findings, the highest correlation with wheat grain yield was obtained with the NDVI and at the grain filling start stage was reported by Wang et al. (2014). Negative corelation between NDWI and both traits may be expected since that the NDWI (Normalised Difference Water Index) is a moisture index for determining vegetation water content and mainly indicated as an effective tool for water stress, soil, vegetation moisture conditions and water content in vegetative areas, which was determined by the NIR (Tuvdendorj et al., 2019). According to McFeeters (1996) the NDWI is designed to enhance the reflectance of water by using the green wavelengths, decreasing the low reflectance of NIR by water features and taking into account that vegetation and soil features have a high reflectance of NIR.

In general, except few exeptions, all SRIs had positive correlations with the measured parameters under each treatment. However, these indicate that it is posible to deal with different SRIs as indirect selection traits like the traditional grain yield related traits.

CONCLUSION

Despite the fact that each environment has its own characteristics and influence on plant response the overall results showed that statistically significant correlations were predominantly influenced by the phenological stage of estimation and the index used. This investigation indicated that by combining spectral reflectance data with an appropriate

statistical analysis during diferent grown stages, it is possible to accurately assess the growth and yield components of winter wheat genotypes. The comparison of many indices could also serve for recommending specific indices and can contribute to the optimization of sensors, the selection of measurement dates and specific SRIs.

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FOOD SAFETY PRESENT SCENARIO: A ROAD MAP OF PAKISTAN

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ABSTRACT

Food safety refers to the potentially hazardous agents or contaminants present in food that can cause food borne illness. These extraneous agents are causing morbidity and mortality. Contamination of food by various agents includes physical, chemical and microbiological. Food safety ensures that what we eat, chew or drink is safe for human health. Whole food chain should be monitored right from production to consumption. Public health burden of the developing countries is increasing beyond their resources. In developing countries like Pakistan, food safety issues result in serious health issues. Diarrhea is perhaps most important problems are responsible for more than half of the global burden of foodborne diseases, causing 550 million people to fall ill and 230,000 deaths every year. Children are at particular risk of foodborne diarrheal diseases, with 220 million falling ill and 96000 dying every year. There is persistent rise in the health expenditure of Pakistan. Pakistan is spending 0.5 to 0.8 percent of its GDP. Total health expenditure during 2016-17 remains at 145.97 billion showing an increase of 9 percent over the last year. Pakistan has dire need of revamping the food safety policy and infrastructure.

Keywords: Food safety; food chain; contaminants; foodborne illness; food safety policy

INTRODUCTION

Food safety is multidisciplinary scientific approach emphasizes on reduction and prevention of food borne illness by ensuring safe intake. Food safety is a chain of management starting from production to consumption of food. Food safety measures ranges from simple household preparatory operation to the molecular approach being used to alleviate the arsenic in rice. Food safety measures not only are taken after the harvest of eatable commodities. These should be implemented right from the production premises to the final consumption. Food contamination is caused by the biotic and abiotic factors. Abiotic and biotic factor may be pathogenic and non-pathogenic. Among the abiotic contamination environmental contamination is causing raw food external contamination, transportation in non-sanitized containers, storage inappropriately, processing premises packing material(Nerín, Aznar and Carrizo, 2016).Soil, Irrigation source, animal dung is the major contributory at farm level contamination. Chemical contamination may also occur at farm level non voluntary chemical mixing with eatables. Pesticide bottles are

washed with the irrigation water and produce is also washed by this contaminate water hence makes it unfit for human consumption(Marriott, Schilling and Gravani, 2018).

Large food production volume to feed the increasing population has increased microbiological infestation. Food born and water born contamination causing morbidity and mortality in infants .According to the WHO (World Health Organization) Waterborne diarrheal disease kills about 2.2 million people annually(Marušić, 2011). Food borne illness is threat to the developing countries. Mode of transmission has wide range from physical to microbiological contamination. Among the microbial pathogenicity *Clostridium botulinum, Vibrio cholera, Salmonella typhus, Campylobacter spp., Toxoplasma gondii, Listeria monocytogenes* and Nosocomial rotavirus which causes gastroenetrocolitis in children (Javed, 2016; Nitsch-Osuch, Kuchar, Kosmala, Zycinska and Wardyn, 2013).

Food borne contamination is global threat not only for developing countries where the sanitary and hygienic conditions are not up to the mark thus facilitating its outbreaks. Comprehensive food safety measures are in need to be opted to restrict the economic burden. Food safety measures are imposing 7 billion dollar economic burden on USA.(Hussain and Dawson, 2013). Public health burden of Pakistan is increasing and imposing social as well as economic constraints. Total health care expenditure of Pakistan in 2000-01 was 24.28 billion rupees and in 2017-18 it has increased up to Rs. 384.57 billion for fiscal year 2017-18. Alarmingly increasing health care budget of Pakistan is imposing extra economic burden. UN member states have to include 169 targets and 17 goals to frame their socioeconomic policies. Sustainable goal 3 and 6 are related to the health of people. This will be a milestone in decreasing public health burden of Pakistan.

Social behavior and activities of the people impart a major impact on the food safety and food borne illness. Safe feeding of the 9 billion populations is a challenge of present scenario. Epidemiological studies are also important to evaluate the association between unsafe feeding, morbidity and mortality. Developed countries have decreased the public health burden caused by unsafe intakes by practicing appropriate measures. Food safety is not merely related to the health but also has potential social and economic impact.

1. Multidisciplinary contamination of Food

Globalization and domestication is major contributory of food born contamination and illness. Mode of contamination varies from microorganism to the physical substances which are being mixed up in food stuff intentionally or non-intentionally. Contamination may be external or internal. Where external contaminations caused by the physical factors such soil, water, air, dust, irrigation water, insects, rodents and by different farm operations. (Marriott et al., 2018).

Figure 1. Potential sources of contamination

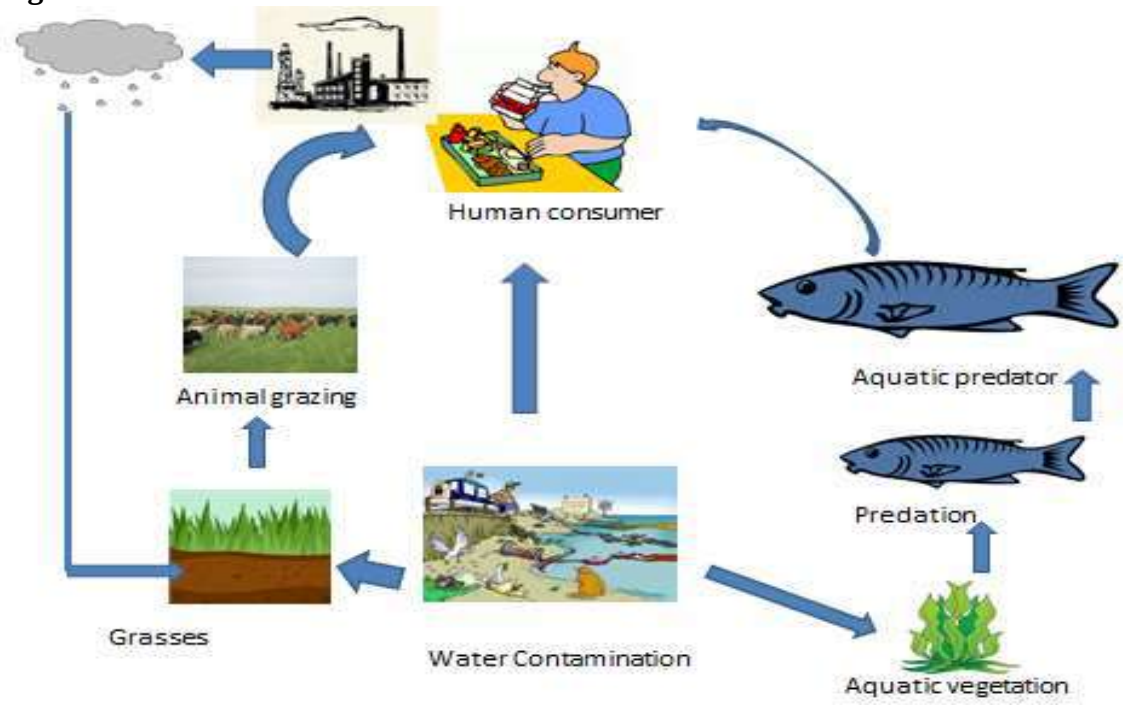


Figure 2. Contaminants flow in food chain

Table.1. Water contaminants and their health hazards

Toxicant	**Disorder/Disease**	**References**
Alachlor, atrazine, benomyl, 2,4-D, diazinon, gossypol, 1,1-dichloroethane	Abnormal sperm (morphology, motility, and sperm count) Chloracne Leukemia, reproductive, immunological, and neurological cancers	(Fawell and Nieuwenhuijsen, 2003; Hughes, 2002; Papanikolaou, Hatzidaki, Belivanis, Tzanakakis and Tsatsakis, 2005)
Organochlorine pesticides		
Lead	Cataracts	(Fawell and Nieuwenhuijsen, 2003; Patra, Rautray and Swarup, 2011; Patrick, 2006; Sokol, Madding, and Swerdloff, 1985)
Mercury	Cerebral palsy	(Bernhoft, 2012; Berry and Ralston, 2008; Fawell and Nieuwenhuijsen, 2003)

Arsenic	Cancer of the skin, lung, bladder and probably liver, hyperkeratosis and peripheral vascular disease	(Fawell and Nieuwenhuijsen, 2003) and (Humans, Organization and Cancer, 2004) Ramesh, Aruna et al. 2014)
Fluoride	Dental fluorosis, an unsightly brown mottling of teeth, higher intakes result in skeletal fluorosis, a condition arising from increasing bone density and which can eventually lead to fractures and crippling skeletal deformity	(Fawell and Nieuwenhuijsen, 2003; Hughes, 2002; Humans et al., 2004; Ramesh, Aruna, Malathiand and Krishnan, 2014; Zahir, Rizwi, Haq, & Khan, 2005)
Selenium and uranium	loss of hair, weakened nails and skin lesions, and more seriously, changes in peripheral nerves and decreased prothrombin time	(Berry & Ralston, 2008; Buschmann et al., 2008; Fawell and Nieuwenhuijsen, 2003)
Nitrate	Stomach, esophagus, bladder, brain, colon, rectum, pancreas, ovarian, and kidney cancers, adverse pregnancy outcomes, diabetes and thyroid disorders	(Berry and Ralston, 2008; Hickeyand Martin, 2009; Lee and Beauchemin, 2014; Santamaria, 2006)

Biological contamination is either pathogenic or non-pathogenic. Pathogenicity of microorganisms contaminating food varies from morbidity to mortality. Diversified food safety measures are needed to prevent food borne illness because these organisms are well opted with the environmental conditions, multiple host range and rapid reproduction mechanism(Adamus-Bialek and Wawszczak, 2015). *Listeria monocytogenes, Escherichia coli, Salmonella spp., Clostridium botulinum* are the major bacterial contaminants. Potential measure to control their pathogenic impact need adoption of proper sanitary and hygienic conditions not only in processing premises but also in the production and consumption vicinities(Gorman, Bloomfield and Adley, 2002; Redmond, Griffith, Slader and Humphrey, 2004; Wright, Gundry and S Conroy, 2004).

Diarrhea and other systemic infections are caused primarily by the Salmonella affect immune deficient, infants and elder persons. *Salmonella enteridus* affected about 800,000 to four million peoples annually in USA only. Eggs are the most prominent food(Coker, Isokpehi, Thomas, Amisu and Obi, 2002) infecting people. Campylobacter is intestinal infecting bacteria. Pathogenicity may result in morbidity to mortality. *Campylobacter jejuni* and *C. coli* are food borne infectious agents causes Guillain-Barr syndrome. Raw milk and untreated water is major carrier of these bacteria.

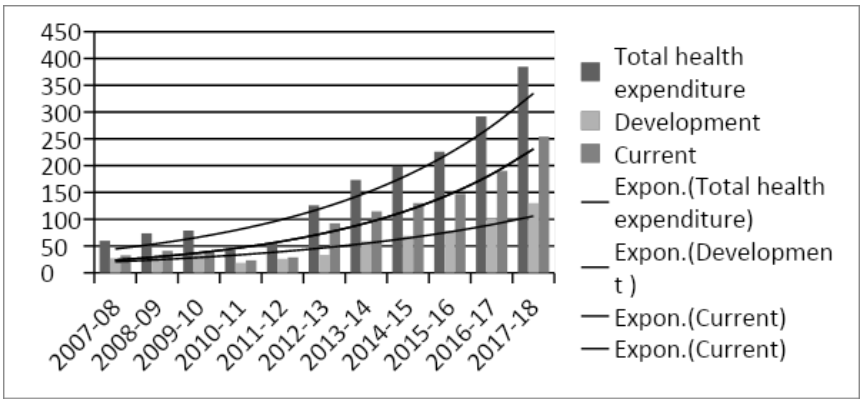
Table. 2. Microbial contamination health hazards

Toxicant	**Disorder/Disease**	**References**
Salmonella Species	Nausea, Vomiting, Abdominal, cramps, Diarrhea, Fever, Chills, Headache and Blood in the stool	(David et al., 2013; Finley, Reid-Smith, Weese and Angulo, 2006; Forshell and Wierup, 2006; Marzano, Mercogliano, Borghi, Facchetti and Caputo, 2003; Pui et al., 2011)
Eschersia Coli	Abdominal cramping, sudden, severe watery diarrhea that may change to bloody stools, gas, loss of appetite or nausea, vomiting, fatigue and fever.	(Bélanger et al., 2011; Harrington, Dudleyand Nataro, 2006; Johnson & Russo, 2002; Kaper, Nataro and Mobley, 2004)
Campylobacter	Belly cramps, Bloating and Fever	(Coker et al., 2002; Dasti, Tareen, Lugert, Zautner and Groß, 2010; Gilbert and Slavik, 2004; Nachamkin, Szymanskiand and Blaser, 2008)
Clostridium botulinum	Difficulty swallowing or speaking, Dry mouth, Facial weakness on both sides of the face, Blurred or double vision, drooping, abdominal cramps and Paralysis.	(Arnon et al., 2001; He et al., 2002; Savidge et al., 2003)
Vibrio cholera	Watery diarrhea, Rapid heart rate, Loss of skin elasticity, Dry mucous membranes (dry mouth) and Low blood pressure.	(Fasano, 2002; Sánchez and Holmgren, 2011)

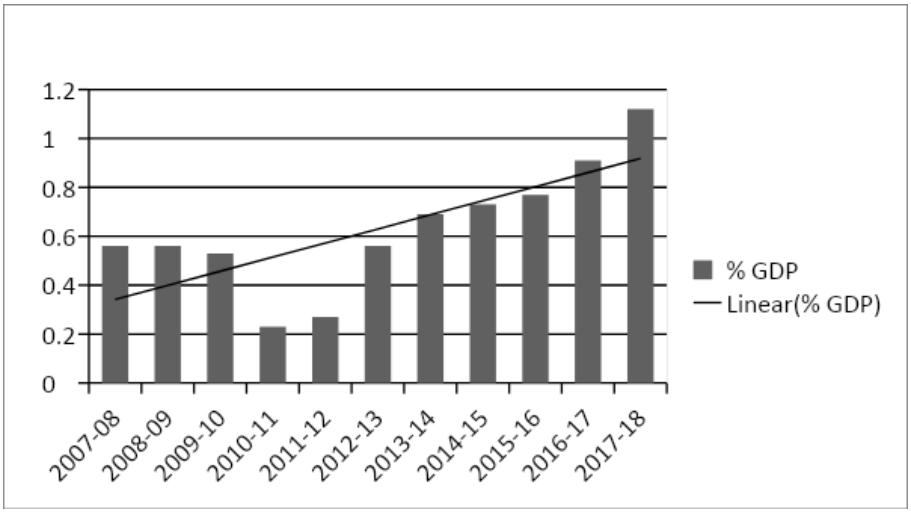
Some physical extraneous substance which don't induce pathogenicity but are not safe to be ingested with food stuff. Food containing these extraneous material iron pieces etc. can cause injury by mechanical abrasion in gastrointestinal track.

2. Global / Pakistan Health and Nutrition burden

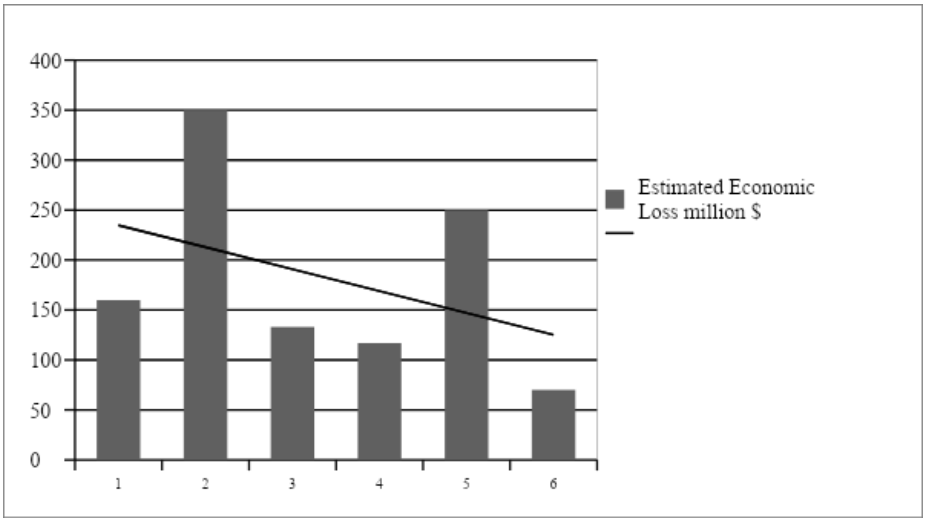
According to the economic survey there is consistent increase in the health budget each year. Data revealed in economic survey 2017 demands solid food safety policy which will be effective to decrease the public health burden of the country and will uplift the living standard of the people.



Graph. 1. Total health expenditure of Pakistan yearly comparison (Economic Survey of Pakistan, 2017-18)



Graph.2. Percentage GDP Share of Pakistan in health expenditure (Economic Survey of Pakistan, 2017-18)



Graph. 3. Global economic losses due to food borne illness (Hussain & Dawson, 2013)

A study was carried out to investigate the microbial load of fresh vegetable in Multan city. Vegetables are grown near the cities by irrigating with the sewage water. Sewage water is potential contaminator of these fruit and vegetables and contributing in outbreak of food borne illness. These vegetables contained higher microbial percentage of *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Enterobacter spp. and Salmonella spp*. to be 48%, 60%, 48%, 64% and 8% (Razzaq, Farzana, Mahmood and Murtaza, 2014). Another study indicated that eatables such as burger, chaana chart, and fruit chart and samosa has alarmingly high total coliform, *S.aureus* and *salmonella spp*. count. This microbial infestation has been propagated by the street vendor preparing these eatable in non-hygienic and unsanitary conditions. Street vendor are major contributory of increasing food borne illness (Afreen, Afreen, and Mustafa, 2016).

Water is the basic necessity and required to sustain life. Importance of water has been revealed in Holy Quran. Life has been created with water. Contaminated water is out breaking gastrointestinal as well as chronic liver disease. Situation is more miserable in urban population rather that the rural population. Water is being contaminated by various environmental and biological factors. Water horizon below the soil is being polluted by the synthetic fertilizers, pesticides and herbicides. Intake of contaminated water is causing foodborne illness. Among the food borne illness diarrheal problem is overwhelming and major contributor of infants' mortality. *E. coli, Citrobacter, Enterobacter and Kiebsiella species* are the major contaminator of water among bacteria. In Pakistan only 20 % population has access to the safe drinking water. Waterborne disease contributing 80% of all diseases and 33 % of all death(Hashml, Ghumman and Malik, 2005).

3. Pakistan: Present Scenario of Food safety

Globalization and urbanization has increased the food demand for the population. Ensuring the population health extensively needs the food safety measures. Street vendors involved in the eatable business and household has to be focused to create the mass awareness of food chain, food born contamination, ways to prevent/reduce instant food contamination, sanitation and hygienic conditions. Globally food safety has got attention in Uruguay round 1995 during international trade conference. Food regulations and standards are being observed throughout the production, processing and consumption chain to ensure the food safety. Punjab province has taken the initiative taking into consideration of food safety importance formulated Punjab food Authority Act, 2011 which has been followed by KPK in 2014. Two provinces of Pakistan has an organization for the standardization and regulation of food standards. Punjab Pure Food Rules covers about 104 food items with nine broader categories. Food safety is not only the national need now. International trade prerequisite the food safety measure for the export of the food items either raw or processed. ISO (The International Organization for Standardization) is an international standard setting body. It is headquartered in Geneva, Switzerland, with 162 member countries. USA, EU and majority of Gulf countries allow the import and export of eatables if have the food safety certification. Government of Pakistan has adopted 22070 ISO Standards and developed 8857 Pakistan Standards. Food Standards are aligned with Codex Alimentarius Commission and traceable with WHO.

4. Food Safety requirements for processing premises

Appropriate location and design

Equipment design

Pest control

Waste management

Sanitary measures

Personnel hygiene

Appropriate handling, packaging and storage

Training of staff

5. Food Safety requirements related to Processing

Food additives

Food preservatives

Flavoring

Antioxidants

6. Food safety certification in Pakistan

Following are the organization involved in the food safety certification

SGS Pakistan (Pvt.) Limited, Systems and Servicer Certification

Pakistan Systems Registrar

RICCI Pakistan

Bureau Veritas Certification (BV Certification)

CeSP (Certification Services Pakistan)

Moody International (Pvt.) Limited

CONCLUSION

National Food safety council is needed to be established under the umbrella of Federal Government. NFS will be the regulatory authority for the provincial bodies. Instead of imposing fines the food authorities should organize mass awareness campaign to give the understanding of safe food and food borne illness especially in the remote areas of Pakistan. Street vendor and household who are involved in food preparation should be educating regarding sanitation and hygienic conditions. This will be milestone in reduction of public health burden of the country and in term will ensure the prosperity of the people also.

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ANTIFUNGAL ACTIVITY AND INHIBITION MECHANISMS OF VARIOUS PLANT DERIVED NATURAL COMPOUNDS WITH THE EMPHASIS ON TERPENOIDS AGAINST YEAST CELLS

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ABSTRACT

Microorganisms develop resistance due to excessive and improper usage of synthetic chemicals as antimicrobials which have high toxic effects in food industry. Therefore, biological preservatives having antifungal properties began to take place of toxic chemicals. Essential oils (EOs) derived from plants which prevents the deterioration of biofilm formed by yeast and prolongs shelf life as protective agents have recently become important in food industry. However, there is a significant restriction on the usage of essential oils due to variations in their content depending on the extraction methods and storage time. Thus, direct usage of active substances involved in the plant extracts may be preferred for industrial production. On the other hand, knowledge about antifungal mechanisms of substance is an important factor to determine the areas where the components can be used effectively. Cell membrane has a vital role because of providing cellular integrity and homeostasis and carrying out molecular transport. Antifungal agents have activity at very low concentrations via disrupting cell membrane integrity. They can be used for preservation of foods, as well as for additives which are not toxic, and decreasing contamination and biofilm formation via coatings in food industry.

Since, EOs can be used as food preservatives and pharmaceutical agents, it is very important to understand their mode of action and their main target sites in the cell. Thus this research not only opens new perspectives to understand antifungal activity mechanisms of EOs, but also help widen their use.

Keywords: *Saccharomyces cerevisiae;* essential oils; antifungal activity; cell membrane; eugenol, thyme, carvacrol

INTRODUCTION

Essential oils (EOs) are complex mixtures of volatile compounds produced by plants and fruits. They are known to have antioxidant (Yang at al., 2010), antibacterial (Reichling at al., 2009), antifungal (Hammer at al., 2004), antiplasmid (Schelz et al., 2006), antiviral, antiparasitic and insecticidal properties (Unal et al., 2009). It is clear from different studies on EOs which are defined 'generally regarded as safe' (GRAS) by the FDA that they have potential uses in medicine and applications in the cosmetic, pharmaceutical and food industries and also in cleaning products (Souza et al., 2007; Van Vuuren et al., 2009; Lima de Sousa et al., 2013; Bialon et al., 2014; Rajkowska et al., 2014; Boire et al., 2016).

There are different antimicrobial mechanisms of EOs. Cell wall, cell membrane, intracellular proteins, enzymes and nucleic acids are significant target sites for EO contents (Helander et al.,

1998; Burt et al., 2004; Morten et al., 2012). Cell membrane is the first line of defense against environmental stresses. It was suggested that the lipophilic nature of EOs allows them to easily pass through cell membranes to change biological responses of cells (Wang et al., 2015). Especially phenolic compounds and terpenes may accumulate in the cell membrane and result in instant loss of membrane integrity, making it highly permeable to ions that might be responsible for the establishment of antimicrobial activity. It has also been shown that essential oil affects the membrane composition of *Yarrowia lipolytica* yeast and some bacteria (Di Pasqua et al., 2006; Papanikolaou et al., 2008). In other cases, changes in membrane fluidity and integrity of yeast cells were observed upon exposure to various stress conditions, by regulating the biosynthesis of fatty acids and sterols (Ding et al., 2009; Ta et al., 2010; Dupont et al., 2011; Turk et al., 2011).

Although antifungal activity of EOs was examined before, their effects on cytoplasmic membrane of *S. cerevisiae* have not been extensively studied (Tao et al., 2014a, 2014b). Due to our continuing interest on the mode of action of various chemicals on yeast membranes (Sezen, 2015), we set out to unearth the possible membrane dependent action of essential oils on yeast cells. In this study, we first examined the antifungal effects of *lemon peel, orange peel, tea tree, turpentine, rosemary, peppermint, thyme, oregano* and *clove oil* against *S. cerevisiae* by applying the measurement of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) techniques. Then we evaluated the membrane damage by measuring the extracellular conductivity of yeast cells after exposure to different concentrations of above EOs.

MATERIALS AND METHODS

Minimum Inhibitory Concentration (MIC) Measurement

The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the essential oil at which the yeasts did not demonstrate visible growth (Tao et al., 2009). S. *cerevisiae* strains were cultured overnight at 25˚C in YPD broth. The essential oils dissolved in DMSO were diluted to the mentioned concentrations in 24-well microtiter plate and then *S. cerevisiae* strain was added to each well. As controls, suspensions of yeasts in the medium without essential oils and yeasts in the medium with only DMSO were tested. Plates were incubated for 48 h at 25°C. After incubation, viability of yeasts was controlled based on turbidity clearance (Talebi et al., 2014).

Extracellular Conductivity Measurement

S. cerevisiae strain was cultured overnight at 25˚C in 50 mL of YPD broth. After incubation, the yeast cells were centrifuged at 3200 rpm for 5 min and pellet was washed twice with sterilized dH_2O. The pellet was then resuspended in sterilized dH_2O. About 200 mg wet weight of yeast cells were used for each experiment. Essenatial oils at mentioned concentrations prepared in DMSO were manually injected at zero point. Extracellular conductivity was recorded with an AD 31 Waterproof EC/TDS tester (Adwa, HUNGARY).

RESULTS AND DISCUSSION

The main purpose of this study was to unearth the dependence of antifungal activity of various essential oils on the integrity of cell membrane. After a thorough search of the literature we uncovered that the information on the antifungal activity against *S. cerevisiae* was limited. More importantly experimental data reported in the literature were based on studies with different experimental conditions preventing a simple comparison of the data with each other. Thus at the beginning of our studies we set out to determine the antifungal activity of *lemon peel, orange peel, tea tree, turpentine, rosemary, peppermint, thyme, oregano* and *clove oils* against *S. cerevisiae* via MIC and MFC measurements.

The MIC and MFC values are shown in Table 1. The most effective oil against yeast cells was turpentine oil (MIC: 0.01-0.04 μL/mL and MFC: 0.01-0.04 μL/mL). Orange peel oil (MIC: 0.04-0.08 μL/mL and MFC: 0.02-0.08 μL/mL), thyme oil (MIC: 0.2-0.3 μL/mL and MFC: 0.2-0.3 μL/mL) and oregano oil (MIC: 0.2-0.3 μL/mL and MFC: >0.3 μL/mL) were also effective. Lemon peel and tea tree oil had slight antifungal activity (MIC: >10 and MFC: >10, for both). The MIC and MFC values of rosemary, peppermint, and clove oil could not be measured due to solubility problems.

Table 2. Antifungal activity of essential oils presented as Zones of Inhibition. Essential oils were mixed with DMSO to increase solubility. The values are mean of four replicates ± standard deviation.

#	Essential Oil	%	Zones of Inhibition (cm)
1	lemon peel	100	1,3 ± 0,3
		20	<0,5
2	orange peel	20	3,3 ± 0,9
3	tea tree	100	<1
		20	<0,5
4	turpentine	20	2,5 ± 0,2
5	rosemary	100	<1
		20	<0,5
6	peppermint	100	1,7 ± 0,3
		20	<1
7	thyme	20	3,0 ± 0,5
8	oregano	20	3,6 ± 0,1
9	clove	20	2,2 ± 0,5

Later, extracellular pH and conductivity measurements were performed to determine their effect on membrane integrity and membrane permeability of yeast cells (Gaskova et al., 2013). It is well known that glucose-induced cells show a decrease in extracellular pH values (Souza et al., 2007). In order to reach a maximal pH gradient across the cell membrane, yeast cells were glucose-induced before extracellular pH measurement experiments. Figure 1 shows the changes in extracellular pH of orange peel, turpentine, thyme and oregano oil treated yeast cells

for 0-60 min. Upon addition of essential oils, an increase in extracellular pH was observed. Especially extracellular pH of yeast cells treated with various concentrations of turpentine oil dramatically increased in a concentration dependent-manner. Higher concentrations than 0.2 µL/mL could not be tested due to solubility problems. Addition of orange peel, thyme and oregano oils also caused an increase in the extracellular pH of yeast cells, possibly due to the neutralization of the glucose-induced pH gradient upon impairment of the cell membrane.

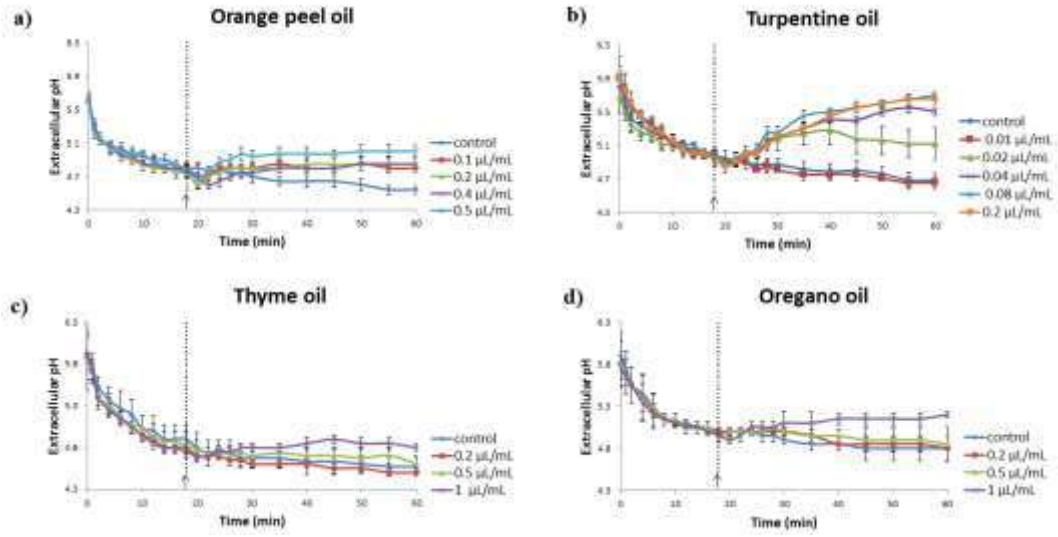

Figure 1. Effects of essential oils on the extracellular pH of *S. cerevisiae*. Concentration dependent effects of essential oils on yeast cells in glucose-induced medium are shown. The arrows indicate the time of addition of a) orange peel oil: 0.1; 0.2; 0.4; 0.5 µL/mL, b) turpentine oil: 0.01; 0.02; 0.04; 0.08; 0.2 µL/mL, c) thyme oil: 0.2; 0.5; 1 µL/mL, d) oregano oil: 0.2; 0.5; 1 µL/mL. The data represent the average of at least two independent experiments.

On the other hand, extracellular conductivity of *S. cerevisiae* cells treated with various concentrations of orange peel, turpentine, thyme and oregano oils for 0-120 min are demonstrated in Figure 2. Conductivity values increased clearly in cells exposed to oils, which indicates rapid leakage of ions to the extracellular medium due to loss of integrity of cellular membrane. Higher concentrations than 0.2 µL/mL could not be examined as the pH measurements due to solubility problems of EOs.

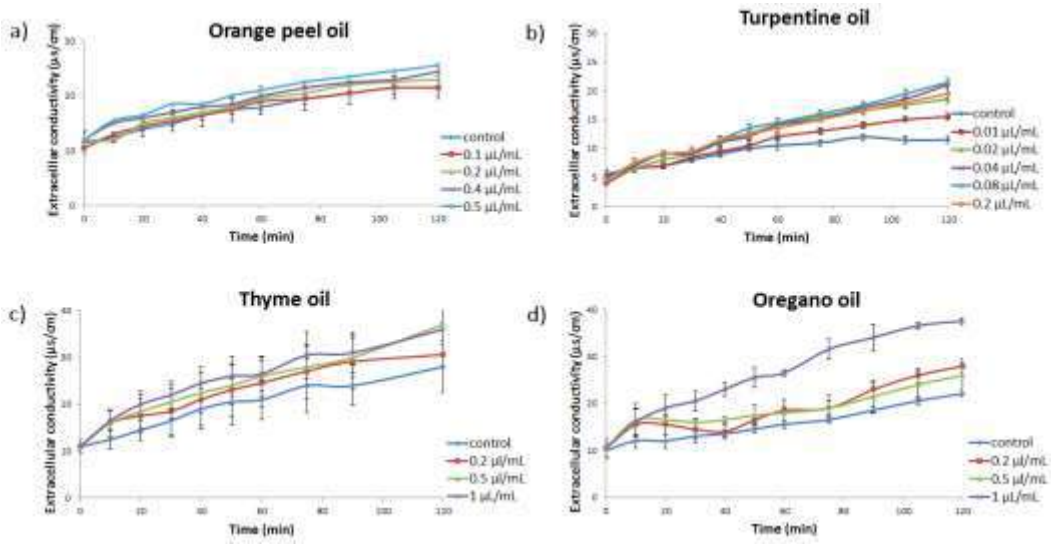

Figure 2. Effects of essential oils on the extracellular conductivity of *S. cerevisiae*. Concentration dependent effects of essential oils on yeast cells are shown. Point zero indicate the time of addition of a) orange peel oil: 0.1; 0.2; 0.4; 0.5 µL/mL, b) turpentine oil: 0.01; 0.02; 0.04; 0.08; 0.2 µL/mL, c) thyme oil: 0.2; 0.5; 1 µL/mL, d) oregano oil: 0.2; 0.5; 1 µL/mL. The data represent the average of at least two independent experiments.

The interest in EOs has significantly grown in recent years and there has been an increase in the number of scientific publications of essential oils. Our results demonstrate that essential oils extracted from different plants show wide spectrum of antifungal activity against *S. cerevisiae* and that the cell membrane is the main target for the antifungal agents in the content of EOs, while disruption of yeast cell membrane integrity is the basic mode of action of these agents. These results will augment our knowledge about the mechanism of action of EOs against *S. cerevisiae* cells and help us widen their usage in food, cosmetic and pharmaceutical industries.

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THE INFLUENCE OF SOIL CHARACTERISTICS ON ALBANIAN MERLOT WINE

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ABSTRACT

In this article it is presented the study of soil characteristics impact on the wine quality extracted from grapes grown in these vineyards. The studied lands are located in the southern area of the Albanian territory, which are known for the production of the Merlot variety. The study lands are rural areas away from the impact of the industrial activity. As shown by many authors, the quality of grape products is significantly related to the physicochemical characteristics of the soil, its geographical location, and the conditions in which it grows. Furthermore, physicochemical analyses of red wine are important in establishing their quality and authenticity. In this context, attention is paid to the mechanical and physicochemical characteristics of soils such as: granulometry, pH, electrical conductivity, and the content of humus, nitrogen, phosphorus, potassium and carbonate. While the wine was determined by density, pH, total acidity, volatile acidity, ethyl alcohol content, free and total SO_2, and reducing sugars following standard methods of the Albanian Food Law. The experimental results showed that the soils were characterized by low alkalinity pH reaction and high content of the macro elements, especially phosphorus. Meanwhile the wines produced from Merlot grapes cultivated in these lands resulted in very good quality, with an optimal pH value and qualified with high alcohol content.

Key words: Merlot wine, soil characteristics, wine quality

INTRODUCTION

The relationship soil characteristics-wine quality has been controversial for a long time [Lanyon D. M. et al., 2004]. Despite the opposite believes [Charters S, 2000], it is now clear that soil directly influences the quality of grapes and hence its products [Jackson, D.I. and Lombard P.B., 1993; Retallack G. J. and Burns S. F., 2016; van Leeuwen C. et al., 2018]. As it provides the medium for vineyard growth, its physicochemical characteristics, along with atmospheric and water characteristics, determine the wine physical, chemical, and sensory individuality. The study of all these parameters would lead to a comprehensive understanding on the optimal use of the land available for maximum and quality wine production.

As reported by many authors, quality wine could be produced by vine grown in many different soil types [van Leeuwen C. et al., 2018]. Consequently, it is not possible to specify just one ideally suitable soil for efficiently grape cultivation. In fact, any soil could be used; you need only to

understand its limits and to improve and alter the drawbacks. However, unfortunately, from the scientific point of view, this diversity leads to a high complexity degree of soil-wine interaction.

Due to the large number of the conditional parameters, mathematical models are needed. They usually are referred to as "terroir" and are able to clearly reflect the relation between the sensory attributes of a wine and its origin [van Leeuwen C. et al., 2018]. The "terroir" expression involves many interacting factors, such as cultivar, climate, soil, and human practices (agrotechnique, wine production technology) [Costantini E. A. C. and Bucelli P., 2014], whose optimum value could not be considered separately. However, based on the reports, a hierarchy of their influence on vine phenology could be proposed and it has been concluded that soil and climate are the most determinant ones [van Leeuwen C. et al., 2018].

High quality is obtained when the soil and climate do not limit the processes associated with the extraction from the soil of the nutrients beneficial to the vine. Optimal conditions are those that provide optimal amounts of nitrogen and water at the root zone. They are often related to the temperature, light, amount of available water, and nutrients. The later ones are a function of climate and soil characteristics. Highest possible quality potential is generally achieved when environmental conditions are moderately limiting [van Leeuwen C. et al., 2019]. For example, moderate temperature leads to ideal balance in grape composition with regard to sugar/acid ratio, color, and aromas [van Leeuwen C. et al., 2018; van Leeuwen C. et al., 2019]. Water deficits at specific stages of grape development are favorable for wine quality, because they reduce berry size and increase phenolic compounds in grape skins. Moderate nitrogen uptake induces similar effects on grape composition, reducing berry size, and increasing skin phenolics [van Leeuwen C. et al., 2019].

The main soil characteristics considered by viticulturalists are: the physical properties (soil texture, structure, color, temperature regime, water and air regime), the chemical properties (macro element content, microelement content, pH reaction), and soil type [de Andrés-de Prado R. et al., 2007]. They all influence the vegetative cycle of the vine and are considered essential factors of wine quality. But, it has been reported that two most important factors are nitrogen nutrition and water supply during certain phases of vine life cycle. This has been experienced in many wine-producing areas and with several varieties, such as in France, Australia, Hungary, USA, with Cabernet Sauvignon, Merlot, Sauvignon Blanc, Kékfrankos and Chardonnay [Costantini E. A. C. and Bucelli P., 2014].

The influence of physical properties depends on the relationship between the fractions of clay, sand gravel and stones. These properties are different in deltaic, humus, carbonic, sandy, clayey, quarry, etc. soils. To ensure good vine root development and consequently high quality wine grapes, soils should have something more sand than clay, be light, airy, hot, permeable, and moderately moist. Clay soils are heavy, not aerated, with poor water penetration, cold, where the root grows shallow. Thus they produce poor quality grapes and wine. Humic soils affect rapid vegetative growth, increase yield, but with poor quality. Sandy soils are more suitable for cultivating high quality varieties. To achieve high quality, they must be enriched with organic fertilizer. They successfully cultivate table grape varieties if properly irrigated.

Soil structure expresses the value of soil through the shape and size of grains, micro and macro aggregates. The best structure is the granular one, stable and with particle size of 1-10mm [Susaj L., 2012].

Heat regime is important in biochemical and physical processes. It affects soil moisture, aeration and root development. Sufficient heat affects the faster ripening of the grapes. Meanwhile, cold and wet soils enable later berries maturation. Ripening timing of grape is very important as it affects the organic compounds in wine. It should not be either too early or too late. If grapes ripen too early in the season in warm conditions, those grapes are high in sugar and low in organic acids. Wines produced from such grapes are unbalanced and lack freshness. Moreover, aromatic complexity is reduced in warm ripening conditions [Costantini E. A. C. and Bucelli P., 2014; van Leeuwen C. et al., 2018]. If grapes ripen too late in the season, they may not reach full ripeness, with the resulting wines tending to be acidic and showing an excess of green flavors. To be precise, the heat regime is not only a function of the soil characteristics but also to the climacteric conditions as well. This reinforces the importance of considering both factors for estimating optimal parameters for best quality wine.

Climatic conditions, such as rainfall, evaporation, water retention and soil structure, etc., affect also the water regime and its impact. As the water is stored in the soil porosity, soil texture has a major impact on soil water holding capacity [Saxton K., 1986]. It is also extremely dependent on rooting depth and percentage of coarse elements [van Leeuwen C. et al., 2018].

The air regime includes the aeration of the soil, respectively the exchange of gases (oxygen, carbon dioxide) between the soil and the air in the pores. It can be improved through tillage, plowing and drainage practices. Color has a specific effect. The dark colored soil increases the vigor of the vine and is suitable for dark wines, while red soils are suitable for rosé wines. The best quality of grapes and wine is given by light colored soils [Susaj L., 2012].

The influence of soil chemical composition in vine is debatable. The soil supplies the vine with major (Ca, N, P, Mg, etc.) and trace compounds (Mn, Zn, Fe, etc.). Beside nitrogen, these compounds seem to have a second hand impact to wine quality. Their impact is mainly attributed to their influence on the soil pH reaction. For example, excess calcium increases the soil pH deteriorating the optimal conditions for vine growth, as the most qualitative vines are settled in acidic soils. It also reduces soil organic matter, limiting the availability of mineral nitrogen. From the other side, high calcium content improves soil structure, which in turn improves root penetration, speeds up soil warming in the spring and improves internal drainage [van Leeuwen C. et al., 2018]. On the contrary, nitrogen is a highly important nutrient in grapevines as it influences all the vegetative stages and impacts all metabolites of the grape: sugar, organic acids, phenolic compounds, aroma precursors, etc. [Keller M., 2010]. For optimal performance there is needed moderate content of nitrogen. Especially at red grapes, low nitrogen supply limits berry size and berry malic acid content, and it increases sugar content and phenolic content. While high nitrogen supply increases the aroma precursors content [Helwi P. et al., 2015].

In terms of soil type, the vine can be cultivated in almost all types of soils. It adapts to a variety of soils from fertile and deep to poor and shallow. It can grow in sandy, stony and rocky soils, in which other crops can rarely grow [Susaj L., 2012].

In this paper it is presented a study of the influence of some soil characteristics on the merlot wine produced by the vineyard situated in the South Albanian land. In fact, grape cultivation and wine production is widespread in all districts of the Albanian country. Based on the geographical altitude, the country can be separated into four wine regions: the western lowland, rising to 300 m; the central hilly region, between 300 and 600 m altitude; the eastern sub-mountainous region, between 600 and 800 m; and the mountains, over 1,000 m altitude [Ilollari (Sotiri) P., 2010; Robinson J., 2015]. Because of the highly favorable conditions such as the geographical position, Mediterranean climate, terrain and soil, abundant sunlight, sufficient active temperatures, abundant rainfall in autumn, winter and spring, etc., it is suitable for the cultivation of many grape varieties, like: Merlot, Chardonnay, Muscat, Barbera, Cabernet Sauvignon. The main autochthonous grape varieties are: Shesh i Bardhe, Debine e bardhe, Pulez (White wine), Shesh i Zi, Kallmet, Vlosh, Serin, Debine e zeze [Edlira K. et al., 2017].

MATERIAL AND METHODS

The vineyards soils analyzed lay in the south of Albanian territory and specifically in Fier, Permet and Korça. They are used to cultivate the Merlot variety of grapes especially for wine purposes. In order to fulfill the goals of the study, soils and wines physicochemical characteristics were analyzed. The soil samples were taken and analyzed accordingly to the Albanian standards. Determination of mechanical composition was performed after SSH 1584/14:1990 standard. This standard provides the method of determining the fractions 1-0.002 and below 0.002 mm of the soil by the pipette method. It is based on the different sedimentation velocities of particles depending on their size, which is given theoretically by the Stokes formula. Aqueous pH was determined after SSH 1584/9:1989 and revised SSH ISO 10390:2001 standard. The method is based on colorimetric or potentiometric measurement of water pH in soil-water mixture. Specific electric conductivity was determined after SSH ISO 11265:2001 standard. The method is based on the determination of the electric conductivity on the soils water extract. The determination of Carbonates ($CaCO_3$) content was performed after SSH 1584/7:1989 standard. The method is based on measuring the volume of carbon dioxide released by the decomposition with hydrochloric acid of soil carbonates. The determination of humus content was performed after SSH 1584/8:1989 standard. The method is based on the oxidation of the organic carbon with chromium anhydride in the presence of H_2SO_4 and then by evaluation of CrO_3 consumed for oxidation (Tjurin method). The total nitrogen content was determined after SSH 1584/2:1989 and revised after SSH ISO 11261:2001 standard. The method is based on the transformation of organic nitrogen into ammoniacal nitrogen and its determination by Kjeldahl distillation. The total phosphorus determination is performed after SSH 1584/3:1989. This standard provides the method of determining the total phosphorus of the soil by colorimetry. The method is based on the colorimetric determination of phosphorus in the soil sample burned with concentrated sulfuric acid. The exchangeable potassium determination is

performed after SSH 1584/6:1989 standard. The method is based on the extraction with sodium bicarbonate and flame photometric evaluation of potassium at the extract.

The physicochemical characteristics of red wines analyzed are: density, pH, total acidity, volatile acidity, ethyl alcohol content, free and total SO_2, and reducing sugars. For the determination of the density, the pycnometric method was used. The pH measurement of red wine was obtained with a PHS-3CW Microprocessor pH Meter. The total acidity determination is performed after SSH 1446-3:1987 standard. The method is based on titrating with an alkali solution (usually NaOH 0.1N) to an endpoint using phenolphthalein indicator of the wine sample. Volatile acidity is performed after SSH 1446-4:1987 standard method. The method is based on steam distillation. The content of alcohol in wine is performed after SSH 1446-1:1987 method. This method is based on distillation method and then the resulting distillate can then be readily measured using a density meter (or alternatively, by using a pycnometer), although the temperature of the sample must be well controlled. The density reading can then be converted to alcohol content by reference to conversion scale. Free and total SO_2 are performed by titration with iodine standard solution, SSH 1446-7:1987 and SSH 1446-6:1987 respectively. The concentration of reduced sugars is performed with Fehling method, after SSH 1446-2:1987 standard.

RESULTS AND DISCUSSION

The aim of this work is to study the influence of the soil characteristics on the Merlot wine quality obtained from the vineyards grown in these soils. The soils taken in consideration are Ardenica (Fier), lying at the Western lowland wine region (rising to 300m), Maliq (Korça), lying at the Eastern sub-mountainous region (between 600 and 800m), and Permet, lying at the central hilly region (between 300 and 600m altitude) of Albania. They are famous for the production of the Merlot grape variety in Albania and have been used for decades for the production of Albanian Merlot wine, consumed mainly by the national market. The physicochemical characteristics of this wine are presented in Figure 1. They are important to establish the quality of the wine.

As can be seen, Figure 1 (a) and (b), these wines have a density range 0.992 to 0.997g/cm^3 and pH values 3.34 to 4.11 (typical for red wines). Red wines sometimes have a higher pH, in part, due to the longer contact time between the grape juice and the grape skins, hence it may be necessary to make acid additions in order to remain in the optimum pH range. The pH is closely related to the acidity and is important because it provides physico-chemical stability of the wine, gives color, brightness and a freshness taste. The acids are important in maintaining pH low enough to inhibit the growth of many undesirable bacteria, thus giving advantage to wine yeasts [Joshi V. et al, 2013]. The total acids in wine are grouped into two categories: the volatile acids and fixed acids. The volatile acids are used routinely as an indicator of wine spoilage. The production of acetic acid at highly levels can lead to an unpleasant, vinegar taste. Their values differ with respect to wines. According to Ribéreau and Traduction, the total acidity in wine ranges between 4 and 7.5g/l [Ribéreau-Gayon P. and Traduction A., 2003]. The total acidity determinations permit to make moderate corrections at harvest time, acid adjustments and treatments, and the overall stability or qualities of a wine (observe the progress of malo-lactic fermentations as well as spoilage by

vinegar production). The experimental values of the total acidity and volatile acidity of the Merlot wines taken in consideration are presented in Figure 1 (b). The total acidity ranges from 4.27 to 4.82 g/l tartaric acid.

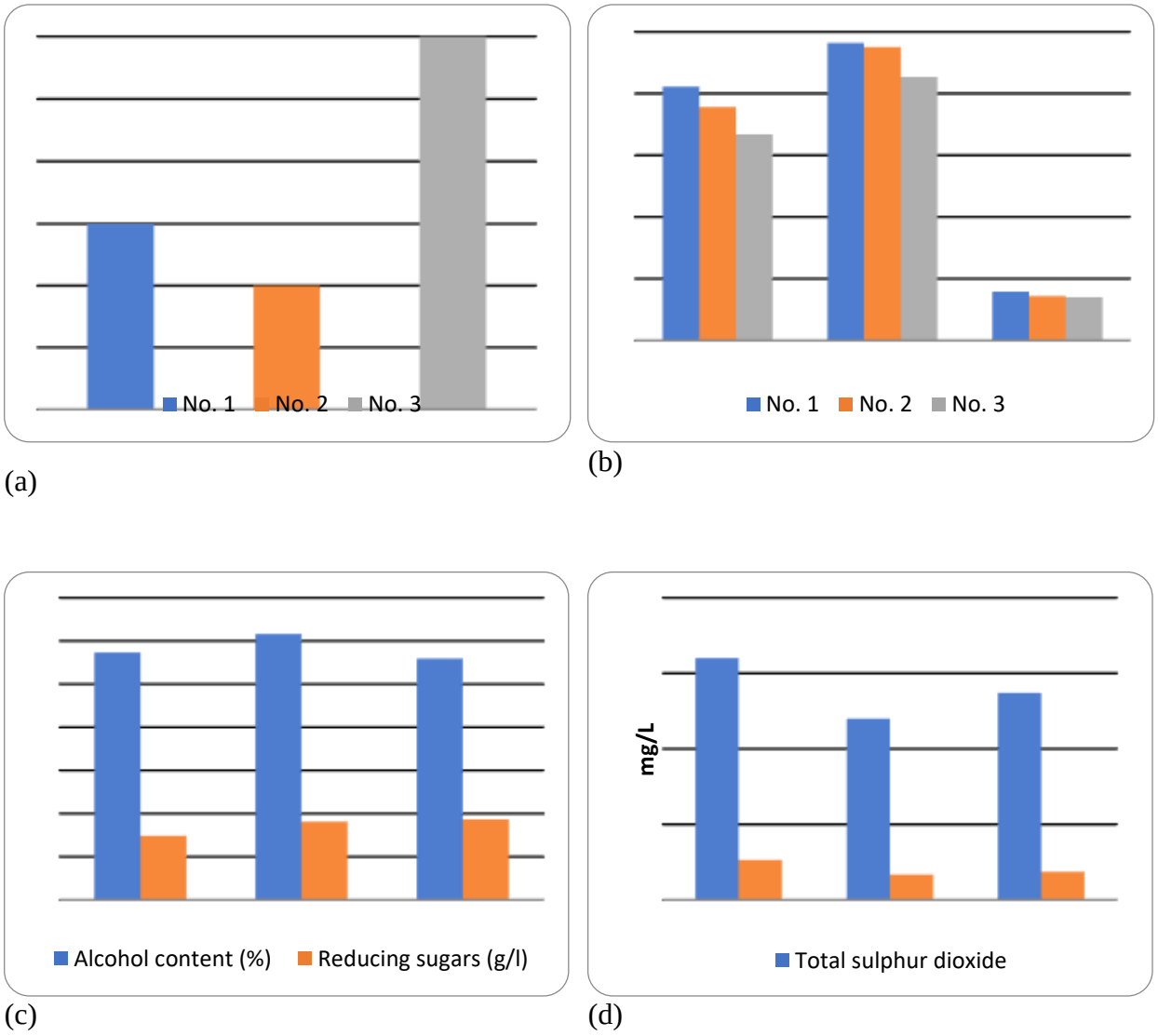

Figure 1. Physicochemical properties of different red wines: (a) density, b) pH, Total acidity and volatile acidity, (c) alcohol content and reducing sugars, and (d) total Sulphur dioxide and free Sulphur dioxide. No 1. Merlot wine from Ardenica, Fier; No 2. Merlot wine from Maliq, Korça and No 3. Merlot wine from Permet.

The alcohol content in wine is one of the most important parameters that determine wine quality. It is affected by many factors, such as ripeness of the grapes at the time of harvest, grape processing technology, and fermentation technology [Ribéreau-Gayon P. and Branco J.M., 2006]. The wines analyzed are characterized by an alcohol content from 11.46–12.32%, Figure 1 (c), of which, sample No.2 (Maliqi Merlot wine) has the maximum content, 12.32%. These values indicate that these wines are produced by low sweetness grapes and can be placed in the category of superior wines.

Another important parameter, which determines the product type, is the reducing sugar content. In accordance with European wine regulations, wines could be classified as: dry, medium dry, medium sweet and sweet. As seen from the experimental results of Figure 1(c), the reducing sugar content of the wines analyzed is not higher than 4 g/l, classifying them into the dry wines category. Reducing sugar content of wine was maximum in No. 3 (Permet Merlot wine) (3.73g/l), while No. 1 (Ardenica Merlot wine) has recorded minimum content (2.97g/l). Sulfur dioxide is the most common chemical compound used in winemaking. It is responsible for protecting wine against oxidation and microbial activity. The total Sulphur dioxide and free sulfur dioxide values for our wines range respectively from 120 to 160mg/l and 16.7 to 26.4mg/l, Figure 1 (d). None of the evaluated samples exceeded the maximum allowable limit, 160mg/l (total sulfur dioxide) and 30mg/l (free sulfur dioxide).

Concluding, the physicochemical parameters of the wines analyzed comply with the standards set by the national law of the Albanian Republic and European Union law indicating that they possess good quality of sensory properties.

Just like mentioned in the introduction, the quality of wine is a function of a number of factors such as climatic conditions, soil characteristics, and the human factor. In order to understand the impact of soil, its physicochemical characteristics are determined. The experimental results are summarized in Table 1 and 2.

Table 1. The physicochemical characteristics of the soil samples.

Sample	Depth cm	pH	EC dS/m	Humus %	N %	P ppm	K mg/100	$CaCO_3$ %
Ardenica, Fier	0-30	7.8	0.187	2.07	0.165	12.95	7.83	21.17
	30-60	7.9	0.172	1.76	0.141	35.5	6.39	19.07
Maliq, Korça	0-30	7.7	0.306	1.24	0.103	57.4	5.66	1.75
	30-60	7.8	0.163	1.29	0.107	54.9	5.79	1.5
Permet	0-30	7.7	0.105	1.71	0.137	8.25	6.39	0.59
	30-60	7.7	0.108	1.09	0.091	15.8	5.77	0.25

Table 2. The mechanical composition of the soil samples.

Sample	Mechanical composition (granulometry)			
	Sand, %	Silt, %	Clay, %	
Ardenica, Fier (30-60cm depth)	36.4	32.8	30.8	Clay-loam
Maliq, Korça (30-60cm depth)	34.2	33.8	32.0	Clay-loam
Permet (30-60cm depth)	31.8	35.4	32.8	Clay-loam

As can be seen, all the soils have the clay-loam texture and are characterized by a low alkalinity pH reaction. Soil pH is a suitable parameter that characterizes fertility. Thus, it plays a significant role in the growth, development and value of grape production. Vine species adapt to varying degrees to soil acidity, and the best conditions for growing the Merlot variety are pH 6.0-7.9. Being that the studied soils are characterized by a weak alkalinity, that positively affects the maturity of the vine, producing high quality wine. Regarding the granulometric composition, even though the vine can be cultivated in almost all types of soils, more suitable soils should have slightly more sand than clay, but still remaining around the values 30:30:30 of sand, clay and silt. In this way they are light, airy, with optimal temperature regime, permeable and with moderate humidity. In such soils the root of the vine develops well and thus, it could be cultivated high quality grapes and therefore could be obtained high quality wine [Pengili M., 1963]. In this context, our soils are suitable for the cultivation of the Merlot variety. However, because of their high water retainment characteristics (Clay-Loam soils), it could be necessary the application of good drainage practices and adding organic matter over time.

In relation to the macro-element content, nitrogen content seems to be the parameter that most affects the vegetative life of the vineyard and hence the red grape metabolites. Literature has shown that moderate nitrogen content values are the most significate because it decreases the berry malic acid and increases sugar and phenolic content [Helwi P., et al., 2015]. After the Albanian standard [Lushaj Sh. et al., 2005], nitrogen content of <0.1% is considered low, 0.1-0.15% medium, and >0.15% high. In this contest, the Ardenica soils seems to have moderate nitrogen content, while the two others low nitrogen content. Meanwhile, in terms of phosphorus and potassium, the soils are respectively very rich and moderately rich.

Nitrogen in the soil is usually found in the form of humus. The latter increases the fertility of the soil. The optimal amount of humus (degradable organic material) in the soil is 3%. The soils analyzed are moderately poor in the humus content as their values range between 1.5 and 3% (Albanian standard, [Lushaj Sh. et al., 2005]).

Analyzing the values of electrical conductivity, we find that they vary from 0.105 to 0.306dS/m. These values indicate that the lands have no salinization problems and favor the cultivation of

viticulture. In fact, the vineyard is considered a plant with moderate sensitivity to salting. According to the literature, the maximum electrical conductivity threshold allowable is 1.5dS/m. At this threshold vineyard production decreases by 9.6% [Maas E.V. and Hoffman G.J., 1977].

In terms of carbonate content, they are very important because affect the optimal temperature regime of the soil. Soils with carbonate salts produce grapes with high sugar content and aroma. The wine produced from these grapes is distinguished for its finesse and high alcohol content. The optimal range of carbonates is 2.5-15%. Soils with lower content need to be fertilized. Our samples appear to have different carbonate contents. Ardenica has the highest content (21.17 and 19.07%). While Maliqi and Permeti seem to have very low carbonate content respectively 1.75 and 1.5%, and 0.59 and 0.25 (for the two depth considered). Based on these results, Ardenica's wine should have the highest alcohol content. In fact, it does not. It is Maliqi's wine the one with the highest alcohol content. This is due to the influence of other factors such as nitrogen content in the soil, climatic conditions, wine production technology, etc.

CONCLUSIONS

The physicochemical parameters of red wines can be influenced by the plant's environment, different areas, varieties, viticulture and enological practices, and can be used as a way of characterizing the wine quality. The Merlot wines produced in different regions of Albania (Fier, Permet, Korça) were in accordance with the limits recommended by the national law of the Albanian Republic and the European Union law. They are categorized as superior dry wines. The soils have clay-loam texture and are characterized by low alkalinity reaction, moderate nitrogen content, and optimal electric conductivity values, suitable for the cultivation of the Merlot grape variety. Generally, they have good characteristics and no salinization problems but, in order to increase performance, it is necessary to employ good practices of soil drainage and fertilization with respect to organic material.

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CONTRIBUTION OF AMPELOGRAPHY FOR THE IDENTIFICATION OF AUTOCHTHONOUS GRAPEVINE VARIETIES IN NORTH-WESTERN OF MOROCCO

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ABSTRACT

The diversity of the autochthonous Moroccan varieties of grapevine is still not much studied, the big number of grape varieties local introducing fluctuations between them requires their characterization with a view to identifying them. Canvassing's were accomplished in the northwest of Morocco in stations of the region of Ouezzane with their coordinated GPS and the collection of the samples of leaves and bunches. Three varieties which carry very close nominations were studied by using quantitative and qualitative descriptors established by OIV: morphology of the leaf, size and weight of the bunch, forms size and weights of the berry, and the measure of the rate of sugar complete by method sulfuric phenol (DUBOIS 1956). The comparison of leaves shows a lot of similarities at the levels of theirs forms to know pentagonal form with three lightly opened lobes having teeth of two straight and on both sides convex sides with a light difference concerning anthocyanique pigmentation, while the color of berries, their sizes, number of pips reveals a very clear difference between the three varieties. For the rate of sugar, they can say that the content of sugar is medium (% in sugar ~18 %) compared with reference stocks, this rate which is in narrow relation with environmental conditions. Seen the pressure of the appellations which change a region in other one for the same variety, the ampelographic studies must be supplemented by the amperometry what encourages to deepen identifications to see even attaining molecular level in case of similarities between grape varieties.

Keywords: ampelography, characterization, autochthonous grapevines, Morocco.

INTRODUCTION

The grapevine is among the most ancient cultures, tradition takes back it up to the Cretaceous with the appearance of angiosperms (ARNOLD C. 2002). The most ancient vine stocks of grapevines existed in the close East, between Turkey, the Armenia and Iran, in southern Caucasus (BOTINEAU M. 2010). Fossilized kernels and traces of vine leaves being found in sediments to certify the presence of Vitaceaes in Service sector (BOTINEAU M. 2010). Movements of caravans have allow viticulture to be spread in two senses; eastward in India then in China and then in Mesopotamia and towards Egypt, from which the grapevine was transmitted to the Greeks and to the Romans before being cultivated in the Mediterranean basin (ARNOLD C. 2002). The history

of The grapevine in Morocco belongs in relation to the period of the Phoenician and to the Roman, it was in Marrakech, in the region of Nefis where there happens the vineyard of Oudaya with the foundation of the city in XIth century (EL FAÏZ M. 2000). The number of the varieties of grapevines is important with similarities between them.
The Moroccan diversity of vine stocks is not much studied. In this job we searched some examples of the autochthonous varieties of the North West region of Morocco first, and to try to identify them and the characterizing. In former days grape varieties were not represented enough in order to the bringing closer in our varieties actual (GALET P. 1998).
We tried to put in an obvious place some autochthonous varieties of grapevine of the region of Ouezzane in the West North of Morocco by canvassing's with coordinates GPS and the collection of the grown-up leaves (10 leaves) as well as of samples of bunches.
Among the pressures of identification of these varieties they pointed out nominations which can similar beings for different varieties while the same variety can have names different from a region in other one. were chosen to prove and search similarities or difference between them by being based on some characters ampelographic according to descriptors developed by OIV 2001 of the grown-up leaf, bunches, berries and measure it of complete sugars according to Method phenol - sulphuric. (DUBOIS. 1956)

MATERIAL AND METHODS

Several varieties were collected among them three varieties which introduce the same appellation have being compared, these three varieties are three types of Taferiyalte: Kahla, Hamra and Chahba
Variety 1: taferialte Kahla (V1) of Asjen
Variety 2: taferialte Hamra (V2) of ouezzane city.
Variety 3: taferialte Chahba (V3) of Mokrisset

THE LEAVES:

Leaves are collected, 10 leaves the sufficient number of repeat for this Type of studies (BOURSIQUOT, on 1989; MARTINEZ and al ., on 1997; MARTINEZ and al ., on 1999 and SANTIAGO and al ., 2005) for every variety for the preparation of a herbarium and description according to characters ampelographic grown-up Leaf following descript by OIV on 2001:

Codes	characters grown-up Leaf
067	Form of the limb
068	Number lobes
070	Contour
074	Pigmentation anthocyanique of the main nervures of the upper face of the limb

076	Form of teeth noticed on the lateral lobe
079	General form of sinus pétiolaire
081.1	Teeth on the edge of sinus pétiolaire
081.2	Sinus pétiolaire delimited by nervures
082	Form of the upper lateral sinus

The Grapes

The three varieties take a sample of samples of bunches with the aim of their to compare according to following criteria :

codes	Caracters of Grapes
U-39	Sharpen
O-204	Specific gravity
O-502	The weight of a bunch

The Berries

Codes	Caracters of berries
O-221	Size
O-223	Form
O-503	Weight of a berry
O-241	Presence of pips
O-233	Output in juice
O-244	Transverse flutes on sides of pips

Content of sugar of the must [% [O-505]]

We undertook the proportion of soluble sugars according to the method of Dubois (1956).

The method of DUBOIS and al (1956) allows to dose dare them by using phenol and concentrated sulphuric acid, in the presence of these two reactive, dare them give a color yellow-orange intensity of which is proportional to the concentration of carbohydrates.
These proportions are easily reproducible, and the precision of method is in the order of 3 % (BELGHITI ALAOUI A. 1990). Their diverting méthylés dare them and react with phenol and sulphuric acid concentrated to give a stable orange yellow product of coloring during several hours, the maximum of absorption is in 490 nm for hexoses and in 480 nm for pentoses. Use the technique as the Following :

Preparation of the range of calibration has:

A mother of glucose of concentration (0,9 g/l) has a leave a resolution, a range of calibration is prepared with dilutions of: S0, S0 / 2, S0 / 4, S0 / 8.

Table 1: Preparation of the range of calibration from a resolution mother of glucose (0,9 g/l).

	S1	**S2**	**S3**	**S4**
V resolution mother of glucose	8 ml	4ml	2ml	1ml
V distilled water	0ml	4ml	6ml	7ml

.**Extraction of sugars by ethyl alcohol 95%**

For the extraction of soluble sugars contained in the grapes 1ml of every sample of pure juice (diluted in the 1 tenth with some water distilled) is blended with 2ml of ethyl alcohol 95 %, an alcohol of lower title in 70° would risk entrained some's insolvable carbohydrates in alcohol (HANOVER P. 1964) then put in the bath marries to 70° during 30 min. About 64 % of sucrose gift in the plant cell are stocked in the vacuole, which is encircled with a protoplasm, broadcasting is performed in a temperature consisted of between 70°C and 80°C to be able to denature protoplasm (BELGHITI ALAOUI A. 1990). They undertake a filtering to eliminate the rubbish of mixture and have a completely homogeneous resolution before adding the others reactive (phenol and sulphuric acid).


Represent 1: Samples of juices diluted before extraction after extraction

Reading of optic specific gravity in the spectrophotometer in 485 nm:

In 2 ml of sample (range of calibration and samples of grape juice) in aqueous circles, they add 1 ml of resolution of phenol to 5 % (v / v) newly prepared then they agitate to blend well, they add fast to every tube 5 ml of concentrated H2SO4, they agitate reactional mixture then tubes are put in a bath marries the temperature of which is regulated in 95°C during 10 minutes. The formed colored product is stable during several hours (BELGHITI ALAOUI. 1990). The reading in the spectrophotometer is made in 485 nm with the water distilled as witness (the white). By using a range of calibration of a resolution of glucose pure, the concentration of soluble sugars; pure samples of grape juice; been determined via the acquired curve of calibration.

RESULTS AND DISCUSSION

Canvassing's

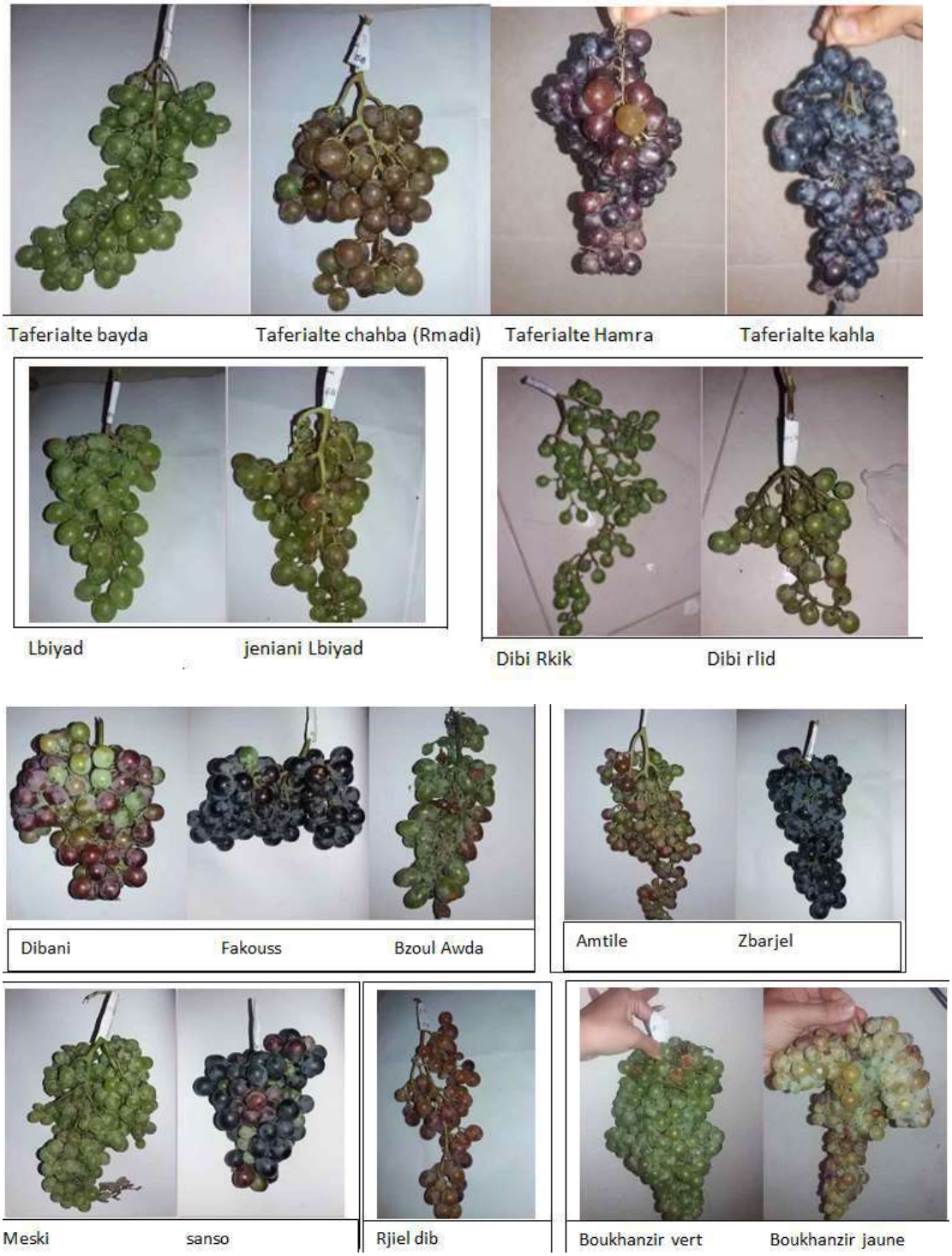
Canvassing's performed on the ground hereabouts by Ouezzane could put in an obvious place 28 varieties of autochthonous grapevines according to the local appellations, the picture so underneath introduces varieties canvassed with their locations GPS:

Table 2: The locations of the varieties of traditional grapevine of the region of Ouezzane.

Name of variety	Localizations (douar)	Localizations GPS
Dibani	Mokrisset (meskka, lbral, beriya)	Altitude 545m, E00504488, N00478508
Taferialte kahla		
Taferialte bayda		
Khodri		
Boukhanzir		Altitude 545m, E00504488, N00478508

Meski		
Bzoul awda		Altitude 556m, e00504757, n00479391
Sanso		Altitude 557m, E00504640, N00478735
Balaciana		Altitude 557m, E00504640, N00478735
Bourwiha		
Bouchikhi		Altitude 556m, E00504757, N00479391
Boukwaniss (bzoul awda)		
Boukhanzir jaun		
Boukhanzir vert		
Fakkouss	Souk had	Altitude 147m, E00499133, N00491854
Taferialte kahla	Zoumi (kchachda, kaleaa bni marchoud, marj, mraj, zawiya)	
Rjiel dib		Altitude 635m, e00510888, n00469763
Boukhanzir		Altitude 635m, E00510888, N00469763
Zbarjel		Altitude 526m, E00511823, N00466060
Bzoul awda		Altitude 526m, e00511823, n00466061
Jeniani biyad		Altitude 526m, e00511823, n00466062
Rmadi (taferialte chahba)	Fifi (bni jil)	Altitude 877m, E00526887, N00483985
Aberran		Altitude 596m, E00528537, N00485093
Taferialte kahla	Bab taza (dar laman, khzana, tanraya, bouztat, abrouj)	Altitude 993m, e00520575, n00488764
Dibi hmar asil		Altitude 953m, e00514463, n00493570
Lbiyad (sensla)		Altitude 953m, E00514463, N00493570
Mesca kahla		Altitude 971m, e00518797, n00491577

Mesca bayda		Altitude 971m, e00518797, n00491577
Dibi Rkik		Altitude 916m, E00513716, N00494993
Dibi rlid		Altitude 916m, e00513716, n00494993
Taferialte kahla	Ouezzane	Altitude : 333m,e 00483053, n00466049
Taferialte Hamra	Ouezzane	Altitude 309, E00482074, N00467140
	Guezrouf (asjen)	Altitude 153, E00478701, N00468407
	Taawonia tahrir	Altitude 194, E00478787, N00463145
	La gare	Altitude 168m, E00478884, N00465444
Boukhanzir	Taawonia tahrir	Altitude 191m, e00478738, n00463172
Rmadi (taferialte chahba)	Messoussa (asjen)	Altitude 210m, E00479308, N00471750
	Ouezzane	Altitude 287, E00482505, N00466428
Meski	Messoussa (asjen)	Altitude 218m, E00480238, N00471734
Amtile	Messoussa (asjen)	Altitude 210m, E00479308, N00471750



Represent 2: Photographs of some autochthonous varieties of grapevine canvassed in about of the region of Ouezzane.

They point out that the region is very rich in local varieties of grapevine, they are cultivated in small plots by the farmers with the aim of personal consumption as well as the supply of the home market by this fruit. You should not as neglect the making as this culture is a tradition to the population of the region many years what explains a knowledge deepened enough by techniques and requirements of this tree, that by experiments acquired since derivations, succeeded in watching and protecting several varieties during long period what proves the very ancient age of certain vine stocks which dated from a hundred of years. Among information to gather via these canvassing's, several operations are part of good practices of the culture of the vine stocks of grapevines by the farmers:

Size: all cultivators of grapevine play size towards the end of the year (December). Cuttings: following the practice of pruning, cuttings are shared between farmers in order to vary their crops.

Grafting: it is a technique very popular among the population to increase the life span of their crop. The grafting is carried out mainly between the desired variety is the graft and the male vine plants as a rootstock which they call "dalia harra". This operation to give very satisfactory results according to the farmers of the region.

Tillage such as ploughing during January-March and weed control.

Natural amendment is very occasional.

Irrigation is mainly indispensable for the home stock, while in the plot's irrigation is mainly by rain.

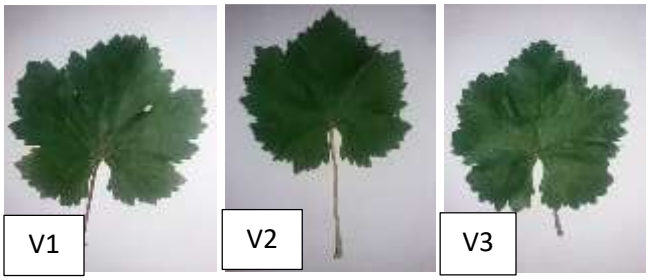
The treatment is mainly against fungal diseases such as Mildiou caused by *plasmopora viticola*.

This crop has been able to withstand high altitudes and dry climates (The climate is Mediterranean, subject to "chergui".

The different varieties cultivated differ in several characteristics including differences in the tree, fruit (taste, color, size and shape of the berries ...) as well as in the leaf.

The Comparison between the adult leaves of the three varieties

For all three varieties the leaves are pentagonal in shape with three slightly open lobes with teeth on two straight sides and two convex sides, the profile of the adult leaf is V-shaped, the petiolar sinus has no teeth on the edge and not delimited by a rib, on the lateral sinus the lobes are slightly overlapping. However, we notice that the length of the petiole is slightly shorter compared to the midrib in V1 and V3 while it is much longer in V2. An anthocyanin coloration of the main veins on the upper surface is very clear in V1, weak in V2, hardly distinct in V3.



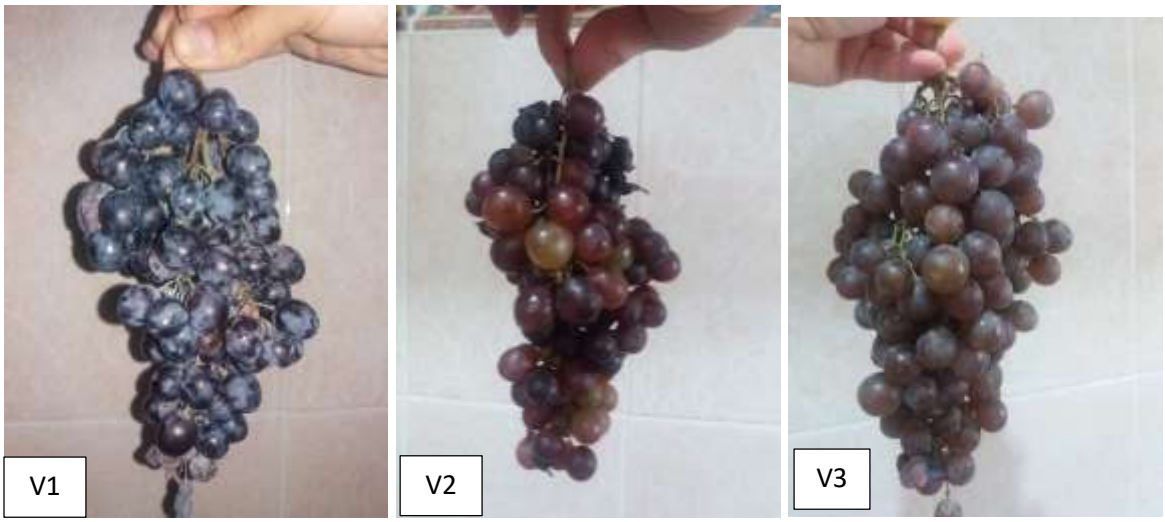
Represent 3: Photographs of the leaves of three vine varieties

Description of the bunch and berries:

the values below are the average of 10 measurements

Table 3: Some characteristics of clusters of local varieties

Varieties	Weight/cluster	Peduncle size	Number of berries/clusters	density	Pedicel detachment
V1	390	4 cm	140	Coward	Quite difficult
V2	335	4,5 cm	115	Coward	Quite difficult
V3	525	4,5 cm	160	Coward	Quite difficult



Represent 4: Photographs of the Graps of the three varieties

Bunches are cowardly, of a medium weight in matter according to the conditions of culture, principally irrigation.

By comparing the berries of the three varieties they can acquire following results:

Represent 5: Some characteristics of the berries of the three varieties

Varieties	Color	form of berries	Medium weight (30 berry)	Number of pips by berry (10)
V1	black	Ovary rounded	3,5 g	3 à 4 pips
V2	Red	Ovary rounded	2,7 g	2 à 3 pips
V3	Brown grey	Ovary rounded	3,5 g	1à 3 pips

Represent 6: photographs of the berries of the three varieties.

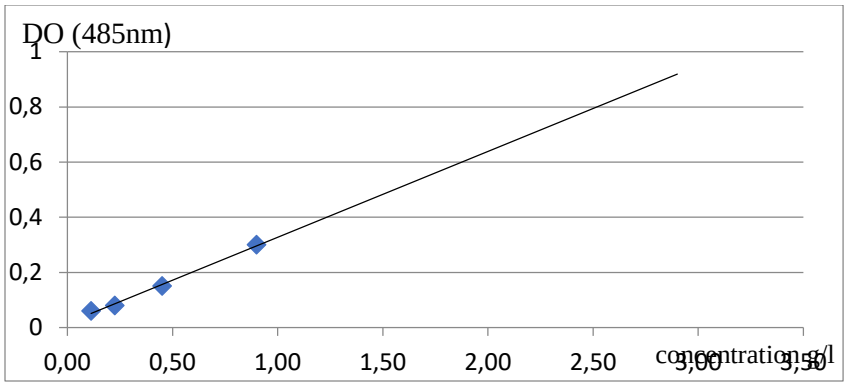
Berries are therefore rounded up having the same form to the three varieties but of different colors, weight is medium but variable, they can say that it is proportional to the content of water (output in different juice) in touch with the conditions of culture and the moisturizing of the plant, what influences weight and size of the berry. Among the three varieties berries the smallest are variety V2 (coming from the region of Zoumi), this known region a deficit in water to follow up the viticulture

The rate of sugar of the three varieties

reading of the optic specific gravity of the range of calibration has:

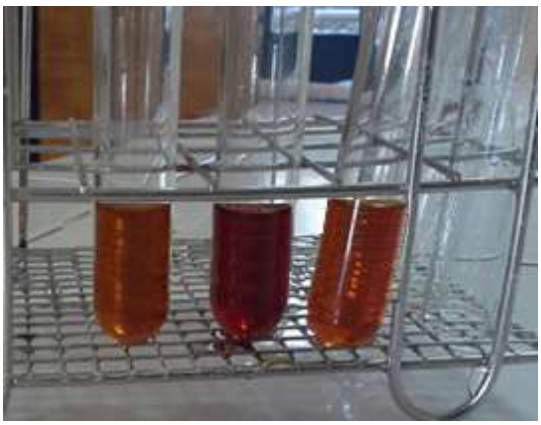
Table 4: Measure of the optic specific gravity of the range of calibration in 485nm.

Concentration g/l (Glucose)	0.9	0.45	0.225	0.112
DO_{485nm}	0.3	0.151	0.08	0.06



Represent 7: The curve of calibration of Glucose

b- Reading of the optic specific gravity of the varieties of grapevine



Represent 8: Photographs the tubes of the three varieties of grapevines. The range of calibration of glucose

Table 5: Optic specific gravity of the three varieties in 485nm

Varieties	**V1**	**V2**	**V3**
DO	0,735	0,888	0,729

These measurements are taken after a dilution of 10 because stocks acquired from absorption were superior to 1.

According to the curve of calibration concentration in sugars of the three varieties is:

c-Concentration in sugars of the varieties

Table 6: Concentration in sugars of the varieties of grapevines According to the curve of calibration.

Varieties	**V1**	**V2**	**V3**
Concentration (g/L)	2,25	2,75	2,20

They point out that V1 et V3 varieties have a content of very close complete sugars, however variety V2 has the biggest quantity of sugar

Concentration found according to the curve of calibration must be converted by holding dilutions in count.

Table 7: Concentration in sugars of every varietie.

varieties	**V1**	**V2**	**V3**
Concentrations (g/l)	2.25*10*10 225 g/l	2,75*10*10 275 g/l	2.20*10*10 220 g/l

This concentration is consulted in the weight of bunch (to acquire stocks in % of sugar)

Table 8: Calculate the percentage in sugar

varieties	Wight of 100 barries (g)	Output in juice (ml)	Content of sugars (%)
V1	110	80	16,2
V2	85	60	19,4
V3	105	90	18,8

They point out that content sweetens it for the three varieties am variable. Compared with reference stocks they can say that the content of sugar is medium (% in sugar
18 %)
The variety which has sparseness of sugar most it is V1, while V2 contains the strongest content.

variety (V2) has the weakest output in juice, in touch with the content of water they can deduct that these plants are in water stress what explains a strong synthesis of sugar.

CONCLUSION

The grapevine is a traditional agrarian production which occupies an important economic place. So, it exceeds its economic dimension, it is a cultural and social element of the population of the region.

In this study they could put in an obvious place some autochthonous varieties of grapevine of the region. Several characters one to be the object of a small comparison between most answered three varieties, morphological point of view, and biochemical. The three varieties even with difference at the level of the color of berries, the description of leaves and bunches shows the presence of strong similarities between them, what brings back to understand nomination very close to three varieties by being just based on the difference of color: Taferialte Kahla (black), Taferialte hamra (Red) and Taferialte Chahba (brown grey).

Of other one quoted it if proves that the descriptive classical ampelography owes be supplemented by one measure Ampélométrique as well as the use of other biochemical and molecular methods with microsatellites to identify and searches if there are family ties between these three varieties.

The content of sugar is in narrow relation with the environmental conditions of culture, principally the water and mineral nutrition.

A big competition with varieties imported outside as the Italian muscatel is in full development, it is for this reason additional studies on the local and autochthonous varieties can the promoting and will help to deepen knowledge better on their requirements and the good practices of their culture, so to develop techniques as grafting to resolve problems of output and diseases.

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THE CORRELATION OF MACRO AND MICRO NUTRIENT ELEMENTS AND ANTIBACTERIAL ACTIVITY OF THE BROCCOLI (*BRASSICA OLERACEA VAR. ITALICA)*

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ABSTRACT

In this study, the correlation of some macro and micro nutrient elements in the broccoli (*Brassica oleracea var. italica*) extract with the antibacterial activity of the extract on two Gram positive and two Gram negative bacteria was investigated. The extract of the fresh leaves of broccoli grown in pots in laboratory conditions was obtained with hexane. Some macro and micro nutrient element contents of the leaves were detected with ICP-OES after microwave burning. The results revealed that some macro nutrient elemental contents (P, K, Ca and Mg) of the extract were sufficient when compared to the limit values of Jones and Mills (1991). Among the micro nutrients Zn and B were sufficient, but Fe, Cu and Mn contents were insufficient. These values are thought to be related with the nutrient elements in the soil itself since no fertilizer was applied to the soil. The broccoli extract was effective on inhibition of *E. coli*, *P. aeruginosa*, *S. aureus* and *E. faecalis*. The P and K contents were positively correlated with antibacterial effect, while Ca and Mg were negatively correlated. The micro nutrient elements Fe, Mn, Zn, Cu and B were all positively correlated with the antibacterial effect of the extract. The results indicated the importance of the macro and micro nutrient elements on the antibacterial activity of the broccoli plant whose consumption increases day by day. Correct and conscious fertilization in order to increase the Fe, Cu and Mn contents of the plant is thought to enhance the antibacterial activity of the present plant and similar plants.

Keywords: *Brassica oleracea* var. *italica*, antibacterial activity, macro elements, micro elements, correlation

INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica*) is a cruciferous plant that belongs to Brassicaceae family. It is rich in bioactive phytochemicals which makes broccoli a health promoting vegetable. Broccoli contains high amounts of nutrients and minerals such as vitamin A, C, E, K, N, K, Ca, Fe etc. Moreover, the bioactive ingredients of broccoli takes attention. Thanks to the the high amount of phenolic compounds such as flavonoids, terpenoids, steroids, saponins, and nitrogen-sulfur compounds such as glucosinolates, broccoli is frequently suggested to consume for prevention of cancer, cardiovascular diseases and some chronic disorders (Vallejo et al. 2004; Domínguez-Perles et al. 2010; Chandekar 2018). Besides these health advantages, broccoli extracts have also

antibacterial effects. It was found to be effective on the skin infection causing *Pseudomonas aeruginosa* which was resistant to three antibiotic tested in the study by Chandekar (2018). In another study, broccoli extracts were tested on six different food borne pathogens, i.e. *E. coli*, *B. cereus*, *B. subtilis*, *S. aureus*, *S. typhimurium*, and *S. flexneri*. Among these bacteria, *B. subtilis* and *B. cereus* were found to be the most sensitive bacteria to broccoli obtained from a local market of India (Sibi et al. 2013). It is important to determine the antibacterial potential of vegetables and their nutrient contents because of their widespread use, especially for the underprivilaged communities where access to medications are limited. Moreover, antibiotic resistance is becoming a significant problem all over the world which obligates the search for new compunds with antibitoic effect (Laxminarayan et al. 2013). In addition to the antibiotic effects, it is important to deetermine the situation of the nutrient elements for the consumption of plant materials with promising source of bioactive ingredients. The miscellaneous studies about the plants, their nutrient contents and health aspects are therefore essential, as they will give insights for agricultural and medical applications in the future.

MATERIAL AND METHODS

The broccoli (*Brassica oleracea* var. *italica*) plants were grown in laboratory conditions in pots of 20 L in the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Tekirdağ Namık Kemal University. Plant seeds (Miracle, Turkey) were first germinated in viols filled with the production material peat (Klasmann-Deilmann, Potground H, Germany). Some specifications of the used peat are: 160-260 mg L^{-1} N, 180-280 mg L^{-1} P_2O5, 200-150 mg L^{-1} K_2O, 80-150 mg L^{-1} Mg, pH: 6, 70% organic matter, and 35% C. The plants were transferred to a pot after germination. The pot was filled with soil taken from the experimental field of the department. The plants were watered once a week and harvested after about four months. Freshly harvested aboveground parts of the plants were washed with pure water, dried as a whole at 65°C for 48 hours and then grinded. Elemental analyses were carried out on the dry grinded plant material by using ICP-OES device according to Kacar and İnal (2010) in Tekirdağ Namık Kemal University Central Laboratory (NABILTEM). Triplicate measurements were taken. Moreover, 50 g of fresh leaves and 50 g of stem were hand choped and mixed with 500 mL of hexane (Merck, Darmstadt, Germany) and the extract was obtained by mixing in a rotary shaker for 48 hours follwe3d by evaporation using a rotary evaporator. The extract of the leaves formed a two phase extract with an oily phase on top. The top and bottom phases and the extract of the stem were tested against four strains of bacteria. The reference strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* were obtained from Turkish Republic General Directorate of Public Health, Ankara, Turkey and Middle East Technical University, Ankara, Turkey. The bacteria were first grown on 5% sheep blood agar, then were adjusted to 0.5 McFarland in sterile saline solution and inoculated on Mueller Hinton agar plates using sterile cotton swabs under aseptic conditions. The antibacterial activity of *Brassica oleracea* var. *italica* extracts were tested using agar well diffusion method in triplicates (Balouiri et al., 2016) where wells of 8 mm size wells were formed with sterile pipette tips. 100µL of basil extracts were added to the wells. The plates were kept at 37 ± 0.1°C for 24 h in an incubator and afterwards the inhibition zones around the wells were measured in mm as an indicator of antibacterial activity.

The results of the experiment was analyzed using Minitab 13 software using Pearson correlation analysis, and ANOVA with a post hoc Tukey's Test.

RESULTS AND DISCUSSION

In this study, leaf and stem extracts of the broccoli plant (*Brassica oleracea* var. *italica*) grown in pots filled with soil from the experimental field of the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Tekirdağ Namık Kemal University. No herbicide and pesticide were used in this study. Some macro and micro elements of whole plant were measured and the amounts are given in Table 1. The P and Mg macronutrients are slightly lower than the values indicated in the literature, while K and Ca macronuterients are sufficient according to the literature. Fe, Cu and Mn micronutrients are lower than the values indicated in the mentioned literature, Zn is at the lowest limit. On the other hand, B amount is in the middle of the range (Jones J. B. Jr and Mills H. A. 1996).

Table 1. Some macro- and micro-nutrient elements of *Brassica oleracea* var. *italica*

	Nutrient Elements	Amount
Macro Nutrients	Mg (%)	0.199± 0.001
	K (%)	3.084 ± 0.010
	Ca (%)	2.002 ± 0.022
	P (%)	0.220 ± 0.000
Micro Nutrients	Fe (ppm)	51.450 ± 0.086
	Cu (ppm)	2.603 ± 0.006
	B (ppm)	38.293 ± 0.081
	Mn (ppm)	12.517 ± 0.174
	Zn (ppm)	20.123 ± 0.006

The correlations between the macro and micro nutritional elements of the basil extract are given in Table 2. The correlation between magnesium and calcium is strongly positive, but the correlation of Mg is strongly negative with potassium and phosphorus. The negative correlation of Ca with K is an expected result as K inhibits Ca absorption by the plant (Johansen et al. 1968). K is known as the quality element as it has crucial roles in quality parameters and it is vital for plant development and normal biochemical reactions (Çalişkan & Çalişkan 2018). The correlation of micro nutritional elements Cu, B, Mn and Zn are positively correlated with K, but it is negative between K and Fe. A similar relation between K and Fe with the other macro nutrients and was

found in maize where addition of K and Fe decreased Ca, P and Mg uptake of maize (Çelik et al. 2010).

Table 2. Corrrelation between the nutritional elements in brocoli

	Mg	**K**	**Ca**	**P**	**Fe**	**Cu**	**B**	**Mn**	**Zn**
Mg	1								
K	-0.983	1							
Ca	0.998*	-0.992	1						
P	-0.963	0.897	0.945	1					
Fe	0.911	-0.972	0.935	-0.768	1				
Cu	-0.989	0.945	-0.978	0.992	-0.842	1			
B	-0.943	0.988	-0.962	0.819	-0.996*	0.885	1		
Mn	-1.000**	0.984	-0.999*	0.961	-0.915	0.988	0.946	1	
Zn	-0.687	0.540	-0.640	0.857	-0.327	0.786	0.407	0.680	1

In order to assess the antibacterial activity of broccoli, extracts of the leaves and stem were obtained seperately and investigated on different bacteria with agar well method. The results of the antibacterial activity of leaf extracts are given in Figure 1. The stem extract did not have significant antibacterial inhibition, therefore the results are not shown here. The extract of the broccoli leaves contained 2 phases: an oily upper phase and an aqueous lowe phase. The three wells on the left hand side of the agar plates given in Figure 1, while the wells on the right hand side contained the upper phase. The oily upper pahse of the broccoli leaf extract was obviously more effective against all the bacteria tested in this study. The oily upper phase is estimated to include phytochemicals such as flavonoids, saponins etc., which are known to be the chemicals causing antibacterial activity in plant extracts. It was shown that broccoli extracts obtained with methanol consists of several phytochemicals, however the extracts obtained with distilled water contained none of the phytochemicals (Chandekar 2018).

The antibacterial activity of leaf extracts is significant (p=0.008). The greatest activiy was on *S. aureus*, and the least activity was on *E. coli*. (Table 3). This may indicate that brocoli extracts can be used for Gram positive bacterial infections more safely. Further studies are certainly of need, but in traditional medicine the organic solvent extracts of brocoli can be used.

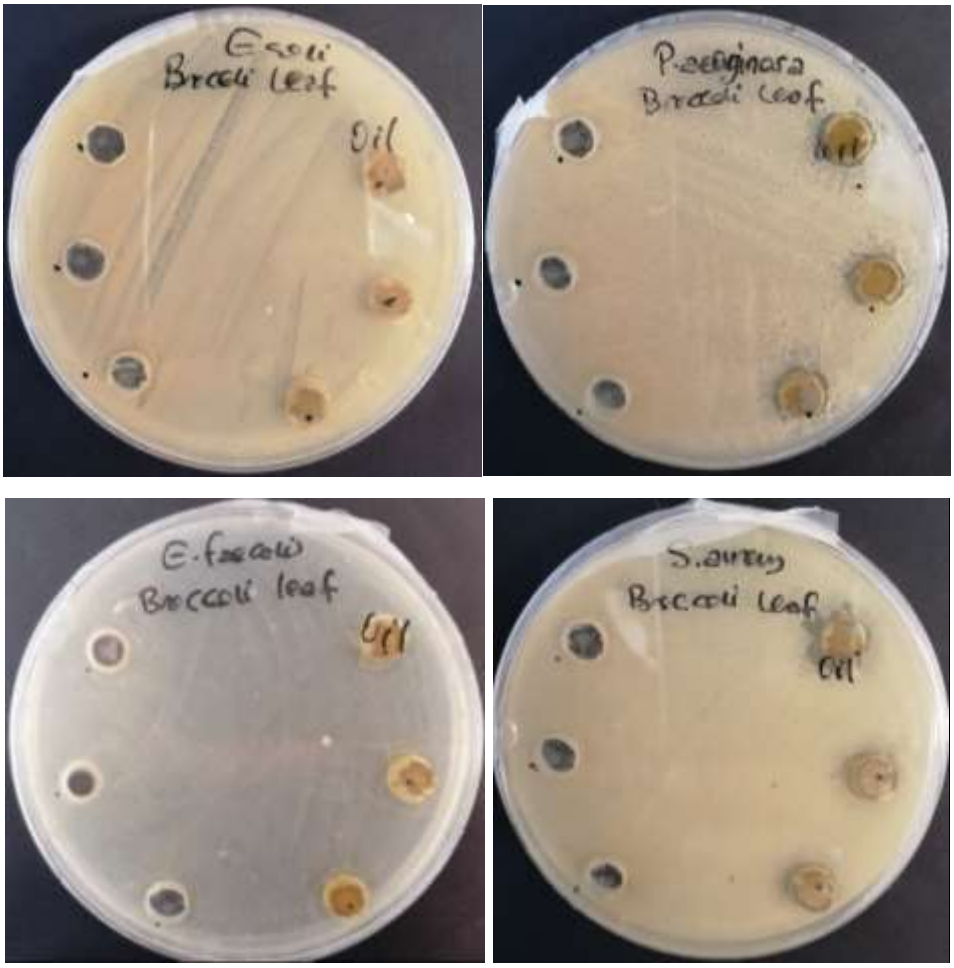

Figure 1. Agar plate images of broccoli leaf extracts (upper phase: right hand side; lower phase: left hand side) with different bacteria

Table 3. Antibacterial effect of *Brassica oleracea* var. *italica* on different bacterial strains

Bacterium	**Inhibition zone (mm)**
E. coli	9.333 ± 0.333
P. aeruginosa	10.333 ± 0.333
S. aureus	11.667 ± 0.333
E. faecalis	10.667 ± 0.333

The correlation between the nutritional elements of broccoli and the antibacterial activiy of the broccoli extract on different bacterial species are given in Table 4. Among the macro nutritonal elements, Ca and Mg have strong negative correlation, while P and K gave strong positive

correlation aith the antibacterial acitivity. As the P and K levels in the extract increase, the antibacterial activity of the extract also increases. In a similar way, as the Mg and Ca contents of the extract increases, the extract becomes less inhibitory against bacteria.

Among the micro nutritional elements, only Fe has a negative correlation with antibacterial activity of broccoli extract. Iron is a crucial element for bacterial metabolism and growth. Its uptake by bacteria is well controlled and the cellular concentration should be in a well balanced range for survival of bacteria (Symeonidis & Marangos 2012). Therefore, up to a point, the correlation of iron and antibacterial activity should be positive, but after a certain limit, which is to be determined by further studies, the correlation can be reversed.

Boron was found to be strongly and positively correlated with all bacteria, which suggests that B might inhibit bacteria, as suggested by Sayin et al. (2016). Boron is an important element, mostly found in Turkey. Besides many application areas of B, its antibacterial activiy has been demonstrated in several studies (Hernandez et al. 2013; Sopchenski et al. 2018). Our study also supports this fact. Soil zinc deficiency becomes a serious problem for both Turkey and in all the world (Adiloglu & Adiloglu 2006) and our results suggest that Zn and B should be high for higher antibacterial activity.

Table 4. Correlation between nutritional elements and bacteria

	Mg	**K**	**Ca**	**P**	**Fe**	**Cu**	**B**	**Mn**	**Zn**
E. coli	-0.887	0.786	-0.856	0.978	-0.619	0.945	0.683	0.882	0.945
P. aeruginosa	-0.843	0.928	-0.875	0.668	-0.990	0.756	0.974	0.849	0.189
S. aureus	-0.887	0.786	-0.856	0.978	-0.619	0.945	0.683	0.882	0.945
E. faecalis	-0.843	0.928	-0.875	0.668	-0.990	0.756	0.974	0.849	0.189

CONCLUSIONS

This study aimed to investigate the correlation between the macro and micro nutrient elements of the broccoli extract with antibacterial activity of them on four different bacterial strains.The results revealed that the broccoli grown in this study was sufficient in K and Ca, but contained P, Mg, Fe, Cu and Mn lower than the range indicated in the literature. On the other hand the Zn amount was on the lowest limit and B content was sufficient. The broccoli leaf extract was significantly effective on inhibiting pathogenic bacterial strains which are important as they cause nosocomial infections. The macro and micro nutrition elements might be the reason of antibacterial activity along with the phenolic content of this medicinal plant. It was found that Mg and Ca were negatively correlated with the antibacterial activity of broccoli extract, but K and P were positively correlated with bacterial inhibition. Among the micro nutrient elements Cu, Mn, Zn and B were positively correlated with bacterial inhibition, while Fe was negatively correlated. The investigation of the macro and micro nutrients with the antibiotic activity of broccoli is important as it is remarkably consumed in many countries. The nutrient element determination is therefore important for consumption of plant materials with promising source of bioactive ingredients for

human. The results of this study also gives an important insight about how the significance of contamination situations in the soil may affect the antibacterial activities of the plants.

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ANTIBACTERIAL ACTIVITIES OF DIFFERENT VARIETIES OF *BRASSICA OLERACEA*

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ABSTRACT

Various varieties of the *Brassica oleracea* species have been consumed all around the world as fresh, cooked, juice or pickles. The consumption of these popular vegetables are increasing day by day. The plants of this species contain numerous vitamins, phytochemicals, antioxidants and fiber, and hence suggested to be frequently eaten up for a healthy life. Different parts of these plants, such as sprouts, leaves, roots or stem contain different amounts of various bioactive ingredients, which are beneficial against disorders such as asthma, cancer, diabetes, or neural diseases. The anticancer features of these ingredients have been subject to many researches. In addition to prevent tumorigenesis, the varieties of *Brassica oleracea* species have antibacterial hallmarks, thanks to the phytochemicals they possess. The antibacterial activities of different varieties of the species *Brassica oleracea* were reviewed in this paper. The antibacterial activities of the plants extracts are attributed to secondary metabolites such as phenolic compounds, glycosides, terpenoids and alkaloids. The varieties of the *Brassica oleracea* contain different kinds of these secondary metabolites which can be extracted by a range of different solvents. The solvents used determine the active phytochemicals extracted, hence the antibacterial activity as different metabolites act on different groups of microorganisms. Therefore, it is important to determine the extraction method and solvents in a goal oriented manner. Moreover, the cultivation of the varieties of *Brassica oleracea* can be assorted accordingly, especially for use in traditional medicine.

INTRODUCTION

The *Brassicacea* family contains many species and varieties which are consumed as food all over the world. These vegetables can be cultivated in a very large range of area on earth. *Brassica oleracea* (wild cabbage) has almost 15 different varieties, namely *Brassica oleracea* var. *alboglabra* (Chinese kale or Chinese broccoli), *Brassica oleracea* var. *botrytis* (cauliflower), *Brassica oleracea* var. *capitata* (cabbage), *Brassica oleracea* var. *costata* (Tronchuda cabbage), *Brassica oleracea* var. *gemmifera* (Brussels sprouts), *Brassica oleracea* var. *gongylodes* (Kohlrabi), *Brassica oleracea* var. *italica* (broccoli), *Brassica oleracea* var. *medullosa* (marrow-stem kale), *Brassica oleracea* var. napobrassica (rutabaga), *Brassica oleracea* var. *palmifolia* (Jersey kale), *Brassica oleracea* var. *ramosa* (perennial kale), *Brassica oleracea* var. *sabauda* (savoy cabbage), and *Brassica oleracea* var. *acephala* (kale) (Schoch et al., 2020). They are suggested to be consumed as a part of healthy diet because of their antioxidant capacity, anti-carcinogenic features, anti-aggregation activity, and detoxification enzyme ingredients which prevent various health problems. These properties are the results of the bioactive compounds these plant possess (Da Silveira Vasconcelos et al., 2019).

The members of *Brassicacea* family contain many bioactive phytochemicals (Avato & Argentieri, 2015). Phenolic compounds are secondary metabolites such as quercetin, kaempferol, ferulic acid, T-resveretrol are important phytochemicals found in this plant group

(Ramirez, Abellán-Victorio, Beretta, Camargo, & Moreno, 2020). Phenolic compounds take important role in color and flavor of the plant, and contribute to the health benefits of the high nutritionally valued plants (Cheynier, 2012). Moreover, they are defense response chemicals of the plants, which become crucial in terms of antibacterial, antioxidant and anti-inflammatory characteristics (Lin et al., 2016). The composition and amount of the phenolic compounds in these plants are affected by some factors such as the plant species, plant organ consumed, the stress in the cultivation environment, or extraction method to derive the functional elements prior for usage (Nawaz, Shad, & Muzaffar, 2018). Besides phenolic compounds, there are around 96 different organosulfur compounds in the members of *Brassicacea* family. Their number of variety depend on the gender, species, and the condition of the plant, such as season and stress factors on the plant (Fallis, 2013; Fusari, Beretta, Locatelli, Nazareno, & Camargo, 2019). Other phytochemicals found in *Brassicacea* are carotenoids (such as β-carotene and α-carotene and β-cryptoxanthin, luteolin, zeaxanthin, cryptoxanthin, neoxanthin, and violaxanthin), terpenes, phytoalexins, and alkaloids (Ramirez et al., 2020).

There are many researches being conducted about these phytochemical bioactive compounds in terms of their different aspects. The healthy food markets making benefit of functional ingredients of the *Brassicaceae* are on the rise. Besides the puree, juices and soups of these vegetables, fibers and bioactive compound extracts are used to manufacture supplements (Ramirez et al., 2020). The antioxidant, anti-inflammatory and antifungal and antibacterial effects of the extracts of these plants are of interest. The antibacterial and antifungal activities of the extracts are especially important for the communities in developing countries where many people do not have the access to health services and medications easily. Antibiotics have been used immensely since the last 50-60 decades to save millions of life. However, the bacterial resistance gained against the existing synthetic antibiotics have become a major concern all over the world, and many recent researched focus on finding new antibacterial agents to be used against bacterial infections that people come across in daily life (Zaman et al., 2017). Therefore, in this paper, the antibacterial activities of various important varieties of the species *Brassica oleracea* are discussed.

Brassica oleracea* var. *alboglabra

Chinese kale (*Brassica oleracea* var. *alboglabra*), or Chinese broccoli, also called Gai-lan, is a leafy vegetable regularly consumed in South-East Asian countries, especially Thailand (Issarakraisila, Ma, & Turner, 2007). It is frequently consumed because of its high nutritional benefits such as antioxidants, vitamin C, glucosinolates and phenolic compounds (Sun, Yan, Zhang, & Wang, 2012). In a study by Hu et al. (2004), antibacterial activities of several cruciferous vegetables including Chinese kale were tested against the reference strains of common bacteria found in human body such as *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella enterica*, *Streptococcus faecalis*, *Staphylococcus aureus*, and the fungi *Candida albicans*. The extracts of the vegetables obtained from a local market in Taiwan were obtained using methanol. The results showed that the extracts were more effective on Gram negative than the Gram positive bacteria (Hu et al., 2004).

Brassica oleracea* var. *acephala

Kale (*Brassica oleracea* var. *acephala*) is recently a famous vegetable and it is on the top of the healthiest foods list, therefore referred as super food. It is also becoming more popular as a crop because it has an excellent resistance to changing temperature (Šamec, Urlić, & Salopek-

Sondi, 2019). It has dark green curled leaves (Figure 1) and it contains high amount of calcium, vitamin A and C (Becerra-Moreno et al., 2014; Kamchan, Puwastien, Sirichakwal, & Kongkachuichai, 2004).

Figure 1 *Brassica oleracea* var. *acephala* (Šamec et al., 2019)

Moreover, kale contain significant amounts of bioactive compounds such as phenolic compounds, carotenoids. The carotenoid ingredient is especially high, obvious from its dark green leaves. The carotenoid content was shown to be 40 times more than that of cabbage, and the highest among Brussels sprouts, broccoli, cauliflower, red cabbage, white cabbage, pak choi, and kohlrabi (Ramirez et al., 2020). These characteristics make this vegetable referred and suggested to be consumed for its antioxidant, anticarcinogenic activities, and for gastrointestinal system and cardiovascular system protections (Šamec et al., 2019). The leaves of kale were used to obtain different amounts of ethanol crude extracts and the extracts were tested against *S. aureus*, *E. coli*, and *B. cereus*. It was shown that the antibacterial activity was as a result of mutual actions of several compounds such as flavonoids, terpenoids, tannins, alkaloids, saponin and phytosterols. The highest antibacterial activity was observed against S. aureus, most probably due to the mobility of phytoconsitutents through the peptidoglycan layer of the mentioned bacterium (Mahadeva Rao, Shanmuga Sundaram, & Sivakumar, 2019).

Brassica oleracea* var. *italica

Broccoli (*Brassica oleracea* var. *italica*) is another popular vegetable from the Brassicaceae family. It is well known and commonly consumed all over the world. It is rich in many vitamins (Vitamins A, C and E), calcium and bioactive ingredients. The head and stem of the vegetable are consumed as food. Broccoli is suggested to be consumed against carcinogenesis, cardiovascular diseases and some chronic disorders (Chandekar, 2018; Domínguez-Perles et al., 2010; Vallejo, Tomás-Barberán, & Ferreres, 2004). The phenolic compounds such as flavonoids, terpenoids, steroids, saponins, and nitrogen-sulfur compounds such as glucosinolates make this food a functional food. Besides the mentioned effects, these bioactive compounds make the vegetable gain antibacterial characteristics. Sibi et al. (2013) obtained acetone and methanol extracts of broccoli and tested against *B. subtilis, B. cereus, E. coli, S.*

aureus, S. flexneri and *S. typhimurium*. The results showed that the mentioned extracts were most effective on B. cereus and B. subtilis. The results of the study also revealed that different solvents should be used for extraction of different phytochemicals. For example, chloroform can only extract glycosides while acetone can extract alkaloids, flavonoids, and steroids. Water can extract alkaloids, saponins and terpenoids, and ethanol can extract steroids, flavonoids and glycosides. Methanol id the solvent that can extract the most variable phytochemicals: flavonoids, steroids, saponins, terpenoids, and glycosides. This study therefore highlights the importance of the solvent. Moreover, the results suggest that bacteria have different levels of susceptibility to broccoli extracts obtained with different solvents. For instance, *B. subtilis* was inhibited even by 10 µg/mL broccoli extract obtained by petroleum ether, while *S. aureus* was not inhibited by any concentrations of extracts obtained by chloroform, acetone, methanol, and water, but was inhibited only by a concentration more than 160 µg/mL of extract obtained by petroleum ether. In another study, 70% methanol extracts of broccoli sprouts had higher total phenolic content, total flavonoid content and Vitamin C; and this extract was more effective on Gram positive bacteria than the Gram negative bacteria (Ninh Le et al., 2019).

Brassica oleracea* var. *botrytis

The cauliflower (*Brassica oleracea* var. *botrytis*) is a vegetable whose terminal inflorescence is mostly consumed by western nations (Grout, 1988). Similar to other members of *Brassicaceae* family, cauliflower has antioxidant activity thanks to the bioactive ingredients (Köksal & Gülçin, 2008). The antibacterial inhibitory effect of the extract of cauliflower was shown by (Prasad.M.P, 2014). The diethyl ether, chloroform and methanol extracts of the cauliflower obtained from markets and Botanical Garden of Bangalore, India, were tested against *E. coli*, *S. aureus* and *P. aeruginosa* bacteria. Methanol extract of cauliflower was effective only on E. coli, but not on the other two bacteria, but the ethanol extract was almost equally effective on *E. coli* and *P. aeruginosa*.

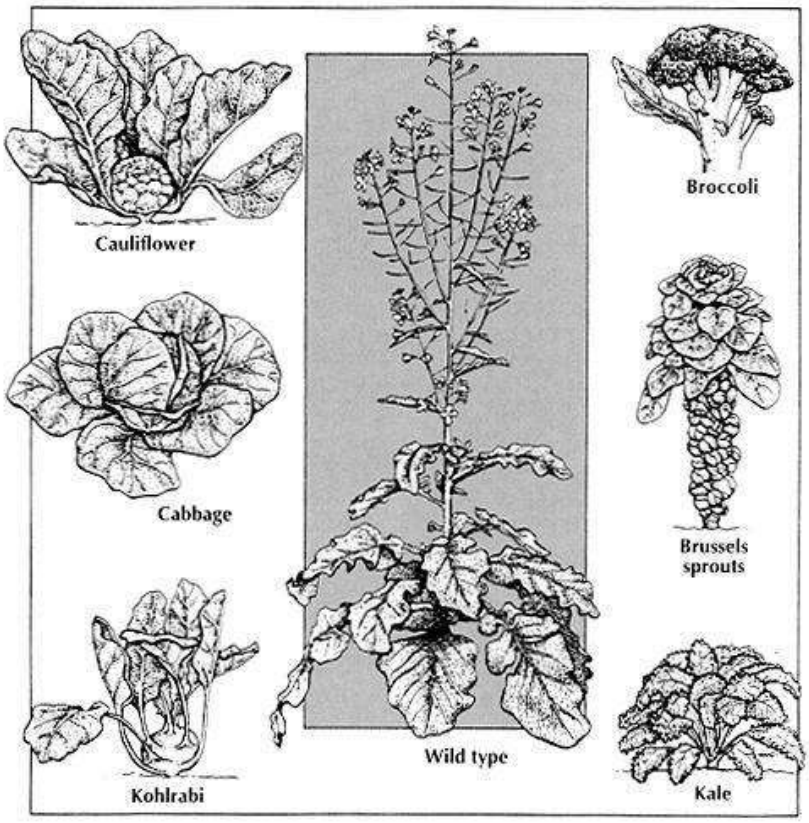

Figure 2 Some examples of *Brassica oleracea* varieties (Smyth, 1995)

Brassica oleracea* var. *capitata

The cabbage plant (*Brassica oleracea* var. *capitata)* is a vegetable whose leaves are consumed as food (Figure 2). It is mostly spread on the northern extratropical region (Leike, 1988). Similar to other *Brassicaceae* plants, cabbage contain bioactive compounds (Rokayya, Li, Zhao, Li, & Sun, 2013). In a study conducted in Ireland, cabbage, broccoli and Brussel sprouts were obtained from markets of Dublin and the extracts were obtained by different solvents. The 60% methanol extract yielded higher total phenolic content, flavonoid, hydroxybenzoic acid, hydroxycinnamic acid and polymethoxylated flavones than ethanol and acetone extracts (Jaiswal & Gupta, 2011). The antibacterial activity of the extract was moderate against *S. abony*, *L. monocytogenes*, *E. faecalis*, *P. aeuruginosa*. In another study, York cabbage extract was found to be the more effective against *L. monocytogenes* and *S. abony*, than Brussel sprouts *Brassica oleracea* var. *gemmifera*, broccoli and white cabbage; because the total phenolic content of York cabbage was higher than broccoli, which was followed by Brussel sprouts and then white cabbage (Kumar Jaiswal, Rajauria, Abu-Ghannam, & Gupta, 2011). In another study, four different cabbage varieties were tested: Green, Red, Savoy and Chinese cabbages. 80% methanol was used for the extraction. It was found that red cabbage had the lowest anti-inflammatory effect (Rokayya et al., 2013). However, the red cabbage was found to be rich in phenolic compounds and therefore a significant antimicrobial activity against Methicillin-resistant *S. aureus*, *E. coli* O157:H7, *P. aeruginosa*, *K. pneumoniae*, *Staphylococcus aureus*, and *S. enterica* (Hafidh, 2011). The phenolic content of shoots, roots and calli of another variety of cabbage, the Tronchuda cabbage (*Brassica oleracea* var. *costata)* were examined and the highest content was found to be in the shoots, therefore shoots should be consumed more to benefit from the health effects (Taveira et al., 2009).

Brassica oleracea* var. *gemmifera

A famous member of the *Brassicaceae* family is the Brussel sprouts (*Brassica oleracea* var. *gemmifera*). It is very popular because of the health benefits. It was revealed that the total phenolic content of Brussel sprouts were higher than the white cabbage but lower than broccoli and York cabbage, therefore the antibacterial activity of Brussel sprouts were moderate or weak againts *L. monocytogenes* and *S. abony* (Jaiswal & Gupta, 2011). Another study revealed that chloroform extract of Brussel sprouts was effective on *S. aureus*, but ethanol and methanol extracts were more effective on *E. coli* (Prasad.M.P, 2014). The antibacterial activity of Brussel sprouts, similar to other members of *Brassicaceae* family, is attributed to the glucosinolates they possess, as these kinds of compounds are naturally responsible for plant defense mechanisms against insects, bacteria, fungi, nematodes and viruses (Aires, 2015). The antibacterial activity studies of Brussel sprouts are very limited and many studies focus on the antioxidant effects of this plant.

CONCLUSION

The *Brassicacea* family contains many species and varieties which are consumed as food all over the world. There are many famous vegetables belonging to this family because of the health benefits they offer. These health benefits arise from the bioactive phytochemicals such as phenolic compounds, carotenoids, and organosulfur compounds. These bioactive compounds can be extracted by various solvents which determine the amount and the kind of the compound extracted. The antioxidant, anti-inflammatory and antifungal and antibacterial effects of the extracts of these plants are of interest. The extracts of broccoli, cauliflower, Brussel sprouts, kale, cabbage and similar plants were tested against many bacteria. The number of studies and the bacterial strains are still not enough. Further studies should be conducted to reveal the antibacterial activities of the members of *Brassicaceae* family.

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THE EFFECTS OF NITROGEN APPLICATIONS WITH PHOSPHORUS AND POTASSIUM ON YIELD AND ON SOME GROWTH PARAMETERS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) VARIETIES IN THRACE REGION

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ABSTRACT

This research was conducted to determine the effects of increasing doses of nitrogen (N) applications both with phosphorus (P) and potassium (K) on some growth parameters of sunflower (*Helianthus annuus* L.) varieties during a two year period in 2015-2016 vegetation seasons. Increasing doses of nitrogen (0, 4, 8, 12, and 16 kg da^{-1} N) and 8 kg da^{-1} P_2O_5 and 8 kg da^{-1} K_2O were applied to the soil under non-irrigated field conditions in Thrace region. It was observed that increasing N doses affected the yield, yield according to 10 % moisture, and oil yield statistically significant ($p<0.01$). The highest yield (279.81 kg da^{-1} and 332.64 kg da^{-1}), yield according to 10 % moisture (3995.70 kg da^{-1} and 3213 kg da^{-1}), and oil yield (128.64 kg da^{-1} and 152.42 kg da^{-1}) were found at 16 kg da^{-1} N dose in both two years. However the least amounts of these parameters were found at control plots where no fertilizer applied. An increase was also occurred with the application of only phosphorus and potassium income. Effects on the plant height, table diameter, thousand grain weight, day number of 50 % flowering and physiological maturity were not found statistically significant. There was no significant difference between years except yield according to 10 % moisture and day number of 50 % flowering. However plant height ($p<0.01$), table diameter ($p<0.05$), thousand grain weight ($p<0.05$), and physiological maturity ($p<0.05$) results of the varieties were found significant. In obtaining high yields in sunflower plants; besides the varieties, it was concluded that by taking into consideration the plant nutrient concentrations in the soil, the effect of phosphorus and potassium fertilizers, which will be applied in addition to nitrogen may be important.

Keywords: Yield, sunflower, nutrient elements, interaction

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oil plant and main raw material of the vegetable oil industry with high economic value (Kaya, 2014). It is cultivated in many countries of the world, and also is the most important oilseed crop in Turkey with a seed production of 1.9 million tonnes in an area of 6.5 million da, and Marmara region is known as the largest sunflower producing area (Sincik et al., 2013; Yağmur and Okur, 2017; FAOSTAT, 2018; TUİK, 2019). Furthermore, Thrace region which is the European part of Turkey, has large and fertile plains. This region is also the main sunflower cultivation areas of sunflower production more than 50% of the total area of Turkey (Kaya, 2014). It has been reported that average seed yield depends on regions, varieties, and irrigation conditions, and changes between 125-300 kg da^{-1} (Bozkurt and Karaçal, 2000, Kolsarıcı et al., 2005; Yağmur and Okur, 2017). Sunflower is generally cultivated in arid conditions in Turkey (Göksoy and Turan, 1999) and under these conditions it was reported that the yield has been a drop to 80 kg da^{-1} (Kolsarıcı et al., 2005). However, weather conditions are among the factors that limit seed yield and it has been emphasized in

many studies that insufficient precipitation causes low productivity and significant positive response can be taken when the crops were irrigated (Kolsarıcı et al., 2005; Demirtaş et al. 2010; Kaya, 2014).

In addition to irrigation, nitrogen fertilization of sunflower has also been reported to better yield results under irrigated conditions. Siddique et al. (2009) were reported that combined application of irrigation and nitrogen would be a better practice. In previous studies it has been indicated that the seed yield and its components are increased with increasing nitrogen rates and the highest yields were obtained from 8 and 12 kg da^{-1} nitrogen doses (Nawaz et al., 2003; Özer et al., 2004; De Giorgio et al., 2007; Oyinlola et al., 2010). It has been stated that the highest yield can be achieved with the 16 kg da^{-1} nitrogen dose is applied in conditions with irrigation possibilities. However, under conditions where semi-arid climatic conditions prevail and irrigation is limited, the excess nitrogen to be given for the purpose of increasing the yield will decrease the yield even more than the expectation of increasing the product. A result of a previous study reported that the rate of N uptake decreased by 50 % those grown in insufficient water conditions compared to those grown under sufficient water conditions and this situation also affected the yield (Alam, 1994).

In recent years, besides developing varieties that will be less affected by adverse environmental conditions or tolerant to these conditions, applications which will accelerate the development stages of plants and provide better development of root and shoots have gained great importance. Phosphorus and potassium have very important place in plant nutrition. The most important function of phosphorus in plant development and growth is the storage and transfer of energy. The energy stored in the phosphate compounds is then used in development and reproduction processes (Güneri et al., 2016). Potassium is also regarded as one of the major nutrient elements which plays an essential role in plant growth and metabolism and affects the yield and quality (Ören and Çelik, 2019). Many enzymes are activated when potassium present and had an important role on regulating the osmotic turgor pressure which regulates the opening and closing of the stomata (Mengel, 2007; Çelik et al., 2010). It has also been reported that sunflower needs potassium in excess, facilitates fat synthesis and significantly affects the rate of fat (Adiloğlu and Derin, 2019). However, in a study done in Uzunköprü district which was the most important sunflower production area of Thrace Region; it was emphasized that nitrogen in plant leaves is sufficient, but phosphorus is insufficient in 42%, and potassium in 20% of the study area (Adiloğlu and Derin, 2019). In conditions where water is limited; in order to increase the yield and quality; as well as nitrogen it may be important to have sufficient amount of phosphorus and potassium among other plant nutrients.

This research was conducted to determine the effects of increasing doses of nitrogen (N) applications both with phosphorus (P) and potassium (K) on some growth parameters, yield and oil yield of sunflower (*Helianthus annuus* L.) varieties during a two year period in 2015-2016 vegetation seasons.

MATERIALS AND METHODS

Two field trials were conducted during 2015 and 2016 seasons at Ballıhoca village of Muratlı, district, Tekirdağ city. The experimental site is located in the north east Marmara region (41°13'41.8"N 27°31'08.7"E) in Turkey. Average annual rainfall was 581,5 mm, annual humidity was 76% and mean monthly temperature was 14.0°C according to the climatic data obtained from the weather station. Total rainfall from March to August was 208,9 and 221,5 mm in 2015, 2016 and long term (1939-2019). Soil samples were collected from 0–30 and 30-60 cm depth of the field and analysed before the trial and the results were shown on Table 1. The soil had a loam texture and slightly alkaline pH. Additionally, it had low lime content and Electrical Conductivity (EC). The soil also had adequate concentrations of organic matter, N, and K. The amount of Ca was found high in both years but Mg was high at the second year.

The amount of Zn was found low in both years. However, the other nutrient element amounts were found sufficient according to the depth and years (Table 1).

The experimental plots were arranged in randomised block design with four replicates in both years. The varieties were the main plots, and six different fertilizer levels including 0, 4, 8, 12, and 16 kg da^{-1} N, 8 kg da^{-1} P_2O_5 and 8 kg da^{-1} K_2O [control (0-0-0 N-P_2O_5-K_2O), N0 (0-8-8), N1 (4-8-8), N2 (8-8-8), N3 (12-8-8) and N4 (16-8-8)] were the subplot treatments. The subplot size was 21.42 m^2 (5.1x4.2 m) and consisted of six rows, with the middle four rows designated as harvest rows.

The oleic type sunflower hybrids (ES Balistic CL and Oliva CL) obtained from "Euralis Semences S.A.S" (France) and "May Seed LTD. STI" (Turkey) were used as the planting material. The crop previously grown in the experimental area in both years was wheat (*Triticum aestivum L.*). The experimental area was cultivated by ploughing at a depth of 25-30 cm in the autumn and by disc-harrowing at 8-10 cm depth in the spring. Sunflower seeds were hand planted on 12 May 2015 and 13 May 2016 at 0.70 m between-row spacing and 0.30 m within-row spacing. Prior to planting various fertilizers one by one or combined (Monopotassium phosphate, potassium nitrate, and 15-15-15) were incorporated into the disc-harrowed soil due to the applications. Half the nitrogen rate was applied before planting, and the remaining N was top-dressed before the second hoeing. The nitrogen fertilizer was banded as ammonium nitrate, approximately 5 cm below and to the side of the seed row.

Table 1. Some soil properties of the trial field

		Amounts				Amounts	
Properties	**Soil**	**2015**	**2016**	**Properties**	**Soil**	**2015**	**2016**
pH (1:2.5 soil:water)	0-30 cm	7.88	6.65	Total nitrogen (N), %	0-30	0.101	0.106
	30-60	7.98	7.76		30-60	0.102	0.105
EC, µS cm^{-1}	0-30 cm	582	158	Available phosphorus (P), mg	0-30	13.21	11.02
	30-60	387	272		30-60	7.94	4.90
Lime, $CaCO_3$, %	0-30 cm	3.37	0.58	Extractable potassium (K), mg	0-30	228	141
	30-60	2.50	1.25		30-60	192	188
Texture	0-30 cm	Loam	Loam	Extractable calcium (Ca) mg kg^{-1}	0-30	8803	4449
	30-60	Loam	Loam		30-60	9105	7345
Sand, %	0-30 cm	46.84	39.84	Extractable magnesium,	0-30	313.7	1178
	30-60	40.84	33.84		30-60	321.3	833
Silt, %	0-30 cm	36.16	39.34	Extractable sodium (Na), mg kg^{-1}	0-30	119.7	106.5
	30-60	43.52	47.52		30-60	119.7	128.5
Clay, %	0-30 cm	17.00	20.82	Available Iron (Fe), mg kg^{-1}	0-30	6.20	26.27
	30-60	15.64	18.64		30-60	5.92	10.42
Organic matter, %	0-30 cm	1.92	2.02	Available Cupper (Cu), mg kg^{-1}	0-30	1.09	1.63
	30-60	1.94	2.01		30-60	1.11	1.18
Available Boron (B) mg kg^{-1}	0-30 cm	1.98	1.21	Available Zinc (Zn), mg kg^{-1}	0-30	0.22	0.18
	30-60	1.88	2.01		30-60	0.20	0.18
				Manganese (Mn), mg kg^{-1}	0-30	9.60	4.54
					30-60	9.47	7.30

During the growing season, all of the plots were cultivated once with a harrow (3 weeks after planting) and twice (5 and 8 weeks after planting) by hand. The plots were harvested by hand on 11 September 2015 and 19 September 2016 when the seed contained approximately 10% moisture content.

Data from all of the experiments were subjected to statistical analysis and the mean values were compared using the least significant difference (LSD) multiple range test with the computer program MINITAB 17.1.0.0 (Minitab Inc., State College, Pennsylvania, USA).

RESULTS and DISCUSSION

Effects on Seed Yield of Sunflower

According to the variance analysis results of seed yield, nitrogen rates and its interaction with years were found statistically significant ($p<0.01$). However, no significant difference was found between the sunflower varieties (Table 2 and 3). Increasing doses of nitrogen both with phosphorus and potassium elevated the seed yield. The highest seed yield was found at last dose (N4PK) both in two years (279.81 kg da^{-1}, and 332.64 kg da^{-1}) and also in combined year (306.22 kg da^{-1}) results. Because of its effective role on photosynthesis, and being an important component of chlorophyll and other bio-catalytic substances, nitrogen is known as the most yield limiting nutrient and partitioning into various parts of crops for growth and development (Škarpa, and Lošák, 2008; Banerjee et al., 2014). In accordance with the previous research literature (Sincik et al., 2013), in our research, application of the increasing doses of N elevated the seed yield.

At the first year of the field trial in 2015, the seed yield results were found at the same group. However in 2016, the least seed yield amount (194.48 kg da^{-1}) was found at control plots where no fertilization was done. In addition, the third (N3PK) and fourth (N4PK) dose of nitrogen had higher seed yield amounts (313.45 kg da^{-1} and 332.64 kg da^{-1}) than those of 2015. This situation was dedicated as a result of the phosphorus and potassium amounts of the field during the experiment. In the first year although the phosphorus and potassium amounts were in the sufficiency range, in the second year these amount were found below the range.

Table 2. Results of variance analysis and mean effects of nitrogen rate on yield, yield to 10 % moisture and oil yield of sunflower varieties in 2015, 2016 and combined years

	Seed Yield Kg da^{-1}			Seed Yield According to 10 % moisture Kg da^{-1}			Oil yield Kg da^{-1}				Seed Yield	Seed Yield According to 10 % moisture	Oil yield
	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years		*F* values	*F* values	*F* values
A	260.56	253.49	257.02	3720.85	2448.71	3084.78	118.39	112.41	115.40	Year (Y)	ns	**	ns
B	259.80	273.94	266.87	3710.04	2646.26	3178.15	118.67	120.53	119.60	Bloks x Years	ns	ns	ns
N rate kg da^{-1}										Varieties (V)	ns	ns	ns
Control	245.15 a A	194.48 d B	219.82 c	3500.77 ab A	1878.71 d B	2689.74 c	109.55 a A	83.05 c B	96.30 d	Y x V	ns	ns	ns
N0PK	265.84 a A	230.51 cd A	248.17 bc	3796.14 ab A	2226.70 cd B	3011.42 bc	120.62 a A	99.90 bc B	110.26 cd	Nitrogen rates (N)	**	**	**
N1PK	277.45 a A	240.02 cd A	258.74 b	3961.97 ab A	2318.61 cd B	3140.29 b	126.10 a A	102.38 bc B	114.24 bc	Y x N	**	**	**
N2PK	253.38 a A	271.19 bc A	262.28 b	3618.21 ab A	2619.69 bc B	3118.95 b	115.79 a A	119.31 b A	117.55 bc	V x N	ns	ns	ns
N3PK	239.49 a B	313.45 ab A	276.47 ab	3419.87 b A	3027.92 ab A	3223.90 ab	110.48 a B	139.76 a A	125.12 b	Y x V x N	ns	ns	ns
N4PK	279.81 a B	332.64 a A	306.22 a	3995.70 a A	3213.28 a B	3604.49 a	128.64 a B	154.42 a A	141.53 a				
Mean (year)	**260.19**	**263.72**		**3715.44 A**	**2547.48 B**		**118.53**	**116.47**					

The differences between values by different letters are significant. Capital letters are for each row and small letters are for each column. *P < 0.05, ** P< 0.01, ns not significant. A: ES Balistic CL B: Oliva CL

Table 3. Effects of increasing doses of nitrogen on yield, yield to 10 % moisture and oil yield of sunflower varieties.

	N rate kg da^{-1}												
	Control		N0PK		N1PK		N2PK		N3PK		N4PK		Mean
Yield													
Varietie	2015												
A	249.56		267.99		269.75		249.98		230.93		295.17		260.56
B	240.74		263.69		285.15		256.78		248.05		264.45		259.80
Mean	**245.15**	**A**	**265.84**	**A**	**277.45**	**A**	**253.38**	**A**	**239.49**	**A**	**279.81**	**A**	
	2016												
A	188.48		236.89		241.78		254.16		296.91		302.73		253.49
B	200.49		224.12		238.27		288.23		329.99		362.55		273.94
Mean	**194.48**	**D**	**230.51**	**C**	**240.02**	**C**	**271.19**	**B**	**313.45**	**A**	**332.64**	**A**	
	Combined Years												
A	219.02		252.44		255.77		252.07		263.92		298.95		257.02
B	220.62		243.90		261.71		272.50		289.02		313.50		266.87
Mean	**219.82**	**C**	**248.17**	**BC**	**258.74**	**B**	**262.28**	**B**	**276.47**	**A**	**306.22**	**A**	
Yield to 10 % moisture													
Varietie	2015												
A	3563.7		3826.8		3852.0		3569.7		3297.6		4215.0		3720.8
B	3437.7		3765.4		4071.8		3666.7		3542.1		3776.3		3710.0
Mean	**3500.7**	**A**	**3796.1**	**AB**	**3961.9**	**AB**	**3618.2**	**A**	**3419.8**	**B**	**3995.7**	**A**	
	2016												
A	1820.6		2288.3		2335.6		2455.1		2868.1		2924.3		2448.7
B	1936.7		2165.0		2301.6		2784.2		3187.7		3502.1		2646.2
Mean	**1878.7**	**D**	**2226.7**	**C**	**2318.6**	**C**	**2619.6**	**B**	**3027.9**	**A**	**3213.2**	**A**	
	Combined Years												
A	2692.2		3057.6		3093.8		3012.4		3082.8		3569.7		3084.7
B	2687.2		2965.2		3186.7		3225.4		3364.9		3639.2		3178.1
Mean	**2689.7**	**C**	**3011.4**	**BC**	**3140.2**	**B**	**3118.9**	**B**	**3223.9**	**A**	**3604.4**	**A**	
Oil yield													
Varietie	2015												
A	111.40		121.84		122.65		113.60		105.73		135.12		118.39
B	107.70		119.39		129.55		117.98		115.22		122.16		118.67
Mean	**109.55**	**A**	**120.62**	**A**	**126.10**	**A**	**115.79**	**A**	**110.48**	**A**	**128.64**	**A**	
	2016												
A	80.95		104.22		103.28		111.38		133.94		140.70		112.41
B	85.16		95.58		101.49		127.25		145.59		168.14		120.53
Mean	**83.05**	**C**	**99.90**	**BC**	**102.38**	**BC**	**119.31**	**B**	**139.76**	**A**	**154.42**	**A**	
	Combined Years												
A	96.18		113.03		112..96		112.49		119.83		137.91		115.40
B	96.43		107.49		115.52		122.61		130.40		145.15		119.60
Mean	**96.30**	**D**	**110.26**	**C**	**114.24**	**BC**	**117.55**	**B**	**125.12**	**B**	**141.53**	**A**	

Therefore, the effect of especially phosphorus and potassium fertilizers in the second year was found much more distinctive than in the first year. In a past research of Sincik et al. (2013), 0, 4, 8, 12, 16, and 20 kg da^{-1} N, 8 kg da^{-1} P_2O_5, and 5 kg da^{-1} K_2O were applied and based on the two year average, they reported the highest seed yield at 16 kg da^{-1} N dose as 271.36 kg da^{-1}. Karasu et al. (2006) in Bursa ecological conditions, they reported the highest seed yield as 223.5 kg da^{-1} at 12 kg da^{-1} N dose which were found lower than the findings of us. This differences is thought to be coming from the agronomic performances of the varieties used in the trial and

also from the ecological factors. However, we have also to take account the properties of the soil, its element contents and also their rates to each other. Zheljazkov et al. (2011) and Tan (2014) reported that the different cultivars grown on different cultures may have different agronomic performances. In order to determine the performances of the varieties, lots of research results which were conducted at different ecological locations with different varieties, indicated different values of grain yield, nutrient uptake and agronomic characters (Tan et al, 2000; Kaya et al., 2003; Tozlu et al., 2008; Tan et al., 2013; Tan, 2014). Abdel-Motagally and Osman (2010) reported that sunflower varieties react differently to nitrogen and potassium applications as a result of the study. Similar results were also obtained by Basha (2000). In various field researches, applied nitrogen doses to sunflower were reported between 4 to 24 kg da^{-1} N and the consequences were found different depending to the differences on ecological conditions, managements, and plant varieties (Gül and Kara, 2015; Sheoran et al., 2016; Nasim et al., 2016; Nasim et al., 2017; Yağmur and Okur, 2017).

Effects on Seed Yield of Sunflower According to 10 % moisture

The variance analysis results of seed yield according to 10 % moisture, showed statistically significant differences ($p<0.01$) on year, nitrogen doses and its interaction with years (Table 2 and 3). Seed yield amounts according to 10 % moisture were found high in the first year (3715.44 kg da^{-1}) than second year (2547.48 kg da^{-1}). The highest seed yield according to 10 % moisture was found at the last dose (N4PK) both in two years (3995.70 kg da^{-1}, and 3213.28 kg da^{-1}) and also in combined years (3604.49 kg da^{-1}) result. The least seed yield amount according to 10 % moisture (1878.71 kg da^{-1}) was also found at control plots where no fertilization was done in 2016.

Effects on Oil Yield of Sunflower

According to the variance analysis results of oil yield, nitrogen rates and its interaction with years were found statistically significant ($p<0.01$). However, there were no significant differences between the sunflower varieties (Table 2 and 3). Increasing doses of nitrogen both with phosphorus and potassium elevated the oil yield when compared to control. The highest seed yield was found at last dose (N4PK) both in two years (128.64 kg da^{-1}, and 154.42 kg da^{-1}) and also in combined years (141.53 kg da^{-1}) result. At the first year of the field trial in 2015, oil yield results were found at the same group. However in 2016, the least seed yield amount (83.05 kg da^{-1}) was found at control plots where no fertilization was done. In addition, the third (N3PK) and fourth (N4PK) dose of nitrogen had higher oil yield amounts (139.76 kg da^{-1} and 154.42 kg da^{-1}) than those of 2015. In a past research of Sincik et al. (2013), 0, 4, 8, 12, 16, and 20 kg da^{-1} N, 8 kg da^{-1} P_2O_5, and 5 kg da^{-1} K_2O were applied and based on the two year average, they reported the highest crude oil yield at 16 kg da^{-1} N dose as 101.27 kg da^{-1}. Karasu et al. (2006) in Bursa ecological conditions, they reported the highest oil yield as 96.9 kg da^{-1} at 12 kg da^{-1} N dose which were found lower than the findings of us. This differences is also not only coming from the agronomic performances of the varieties used in the trial and the ecological factors but also it is the result of the differences of the soil and its element contents and also their rates to each other.

Effects on Some Growth Parameters of Sunflower

According to the variance analysis results of plant height, table diameter, thousand grain weight, and physiological maturity; nitrogen rates and its interaction with years were not found statistically significant except day number of 50 % flowering ($p<0.01$) (Table 4 and 5).

Table 4. Results of variance analysis and mean effects of nitrogen rate on plant height, table diameter, thousand grain weight, day number of 50 % flowering and physiological maturity of sunflower varieties in 2015, 2016 and combined years

	Plant height			Table diameter			Thousand grain weight			Day number of 50 % flowering			Physiological maturity				plant height	table diameter	thousand grain weight	day number of 50 % [illegible]	Physiological maturity
	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years		*F*	*F*	*F*	*F*	*F*
A	137.49	139.49	138.4 b	17.52	17.49	17.5 a	51.69	50.42	51.05 a	63.98	62.88	63.43	104.91	103.91	104.4 b	Year (Y)	ns	ns	ns	**	ns
B	146.47	148.47	147.4 a	16.97	16.97	16.9 b	49.19	49.45	49.32 b	63.96	63.00	63.48	106.58	105.58	106.0 a	Bloks x Years	*	ns	*	ns	*
N rate kg da^{-1}																Varieties (V)	**	*	*	ns	*
Control	142.48	144.48	143.48	17.55	17.55	17.55	49.31	49.56	49.44	63.97	63.13	63.55 ab	105.58	104.58	105.08	Y x V	ns	ns	ns	ns	ns
N0PK	141.63	143.63	142.63	16.84	16.75	16.80	51.31	49.88	50.59	63.53	62.63	63.08 b	105.91	104.91	105.41	Nitrogen (N)	ns	ns	ns	**	ns
N1PK	143.29	145.29	144.29	17.42	17.42	17.42	50.88	50.00	50.44	64.50	63.50	64.00 a	106.94	105.94	106.44	Y x N	ns	ns	ns	ns	ns
N2PK	141.59	143.59	142.59	17.65	17.65	17.65	50.69	50.31	50.50	64.13	62.88	63.50 ab	107.16	106.16	106.66	V x N	ns	ns	ns	ns	ns
N3PK	144.91	146.91	145.91	17.01	17.01	17.01	48.94	50.59	49.77	63.56	62.63	63.09 b	104.00	103.00	103.50	Y x V x N	ns	ns	ns	ns	ns
N4PK	137.97	139.97	138.97	17.00	17.00	17.00	51.50	49.25	50.38	64.13	62.88	63.50 ab	104.91	103.91	104.41						
Mean (year)	**141.98**	**143.98**		**17.25**	**17.23**		**50.44**	**49.93**		**63.97 A**	**62.94 B**		**105.75**	**104.75**							

The differences between values by different letters are significant. Capital letters are for each row and small letters are for each column. *P < 0.05, ** P< 0.01, ns not significant. A: ES Balistic CL B: Oliva CL

Table 5. Effects of increasing doses of nitrogen on plant height, table diameter, thousand grain weight, day number of 50 % flowering and physiological maturity of sunflower varieties.

	N rate kg da^{-1}						
	Control	N0PK	N1PK	N2PK	N3PK	N4PK	Mean
Plant height							
Varieties	2015						
A	134.50	135.25	138.46	140.00	140.20	136.50	137.49
B	150.45	148.00	148.13	143.19	149.61	139.44	146.47
Mean	**142.48**	**141.63**	**143.29**	**141.59**	**144.91**	**137.97**	
	2016						
A	136.50	137.25	140.46	142.00	142.20	138.50	139.49
B	152.45	150.00	150.13	145.19	151.61	141.44	148.47
Mean	**144.48**	**143.63**	**145.29**	**143.59**	**146.91**	**139.97**	
	Combined Years						
A	135.50	136.25	139.46	141.00	141.20	137.50	138.49 b
B	151.45	149.00	149.13	144.19	150.61	140.44	147.47 a
Mean (Varieties)	**143.48**	**142.63**	**144.29**	**142.59**	**145.91**	**138.97**	
	Table diameter						
Varieties	2015						
A	17.67	17.19	17.78	18.19	17.27	17.00	17.52
B	17.42	16.50	17.06	17.11	16.75	17.00	16.97
Mean	**17.55**	**16.84**	**17.42**	**17.65**	**17.01**	**17.00**	
	2016						
A	17.67	17.00	17.78	18.19	17.27	17.00	17.49
B	17.42	16.50	17.06	17.11	16.75	17.00	16.97
Mean	**17.55**	**16.75**	**17.42**	**17.65**	**17.01**	**17.00**	
	Combined Years						
A	17.67	17.09	17.78	18.19	17.27	17.00	17.50 a
B	17.42	16.50	17.06	17.11	16.75	17.00	16.97 b
Mean (Varieties)	**17.55**	**16.80**	**17.42**	**17.65**	**17.01**	**17.00**	
	Thousand grain weight						
Varieties	2015						
A	50.13	51.38	51.75	52.38	49.50	55.00	51.69
B	48.50	51.25	50.00	49.00	48.38	48.00	49.19
Mean	**49.31**	**51.31**	**50.88**	**50.69**	**48.94**	**51.50**	
	2016						
A	49.13	50.50	50.00	51.13	50.25	51.50	50.42
B	50.00	49.25	50.00	49.50	50.94	47.00	49.45
Mean	**49.56**	**49.88**	**50.00**	**50.31**	**50.59**	**49.25**	
	Combined Years						
A	49.63	50.94	50.88	51.75	49.88	53.25	51.05 a
B	49.25	50.25	50.00	49.25	49.66	47.50	49.32 b
Mean (Varieties)	**49.44**	**50.59**	**50.44**	**50.50**	**49.77**	**50.38**	
	Day number of 50 % flowering						
Varieties	2015						
A	63.94	63.56	64.25	64.31	63.56	64.25	63.98
B	64.00	63.50	64.75	63.94	63.56	64. 00	63.96
Mean	**63.97**	**63.53**	**64.50**	**64.13**	**63.56**	**64.13**	
	2016						
A	63.25	62.50	63.25	62.75	62.75	62.75	62.88
B	63.00	62.75	63.75	63.00	62.50	63.00	63.00
Mean	**63.13**	**62.63**	**63.50**	**62.88**	**62.63**	**62.88**	
	Combined Years						

A	63.59	63.03	63.75	63.53	63.16	63.50	63.43
B	63.50	63.13	64.25	63.47	63.03	63.50	63.48
Mean (Varieties)	**63.55 AB**	**63.08 B**	**64.00 A**	**63.50 AB**	**63.09 B**	**63.50 AB**	
	Physiological maturity						
Varieties	2015						
A	104.35	105.06	105.63	108.75	101.69	104.00	104.91
B	106.81	106.75	108.25	105.56	106.31	105.81	106.58
Mean	**105.58**	**105.91**	**106.94**	**107.16**	**104.00**	**104.91**	
	2016						
A	103.35	104.06	104.63	107.75	100.69	103.00	103.91
B	105.81	105.75	107.25	104.56	105.31	104.81	105.58
Mean	**104.58**	**104.91**	**105.94**	**106.16**	**103.00**	**103.91**	
	Combined Years						
A	103.85	104.56	105.13	108.25	101.19	103.50	104.41 b
B	106.31	106.25	107.75	105.06	105.81	105.31	106.08 a
Mean (Varieties)	**105.08**	**105.41**	**106.44**	**106.66**	**103.50**	**104.41**	

Increasing rates of nitrogen both with phosphorus and potassium elevated the day number of 50 % flowering and the highest value (64.00) was determined at N1PK dose. There was also difference between the years and the highest mean (63.97) was determined at 2015. However, between varieties; the plant height values ($p<0.01$), table diameter values, thousand grain weight, and physiological maturity values ($p<0.05$) were found statistically significant. Although the highest plant height (147.47 cm) and physiological maturity (106.08 days) values were determined at variety B, table diameter (17.50 cm), thousand grain weight (51.05 g) were determined high at variety A. Similar to the findings of us, in a past research of Karasu et al. (2006) in Bursa ecological conditions, they reported the highest plant height, thousand grain weight and table diameter values of sunflower as 159.7 cm, 54.7 g, 16.9 cm respectively.

CONCLUSION

Increasing doses of nitrogen both with phosphorus and potassium elevated the yield and oil yield of the sunflower seeds. Differences were also found between the varieties. In addition to the amount of plant nutrients in the soil, their ratios among each other affected the growth and inspected parameters. Although the varieties were grown under same ecological conditions in each year, the differences between the amounts of the elements in the soil effected the amounts. The differences between the concentrations of the elements had effected the photosynthesis rate and the yield directly. In obtaining high yields in sunflower plants; besides selecting the cultivars, it was concluded that by taking into consideration the plant nutrient concentrations in the soil, especially the effect of phosphorus and potassium fertilizers, which will be applied in addition to nitrogen may be important.

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GREEK NATIVE FOREST FRUIT TREES AND SHRUBS: COLLECTION, DOCUMENTATION AND *EX SITU* CONSERVATION FOR SUSTAINABLE UTILIZATION

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ABSTRACT

Forest trees and shrubs fruits have a high nutritional value and therefore assure a present and future growing market potential. Although many such speciesare native in Greece, so far there has not been any documentation or any effort to commercially propagate and cultivate them. The Eco Variety research project focussed on 8 different fruit tree/shrub species:*Amelanchier ovalis*, *Cornus mas*, *Prunus spinosa*, *Rhuscoriaria*,*Rosa canina*, *Rubusidaeus*, *Sambucus nigra* and *Vaccinium myrtillus*. The project aims are to document these species in their habitat, collect several samples from different sites, evaluate their fruit characteristics, begin asexual propagation trials, develop propagation protocols and ultimatelyto apply this knowledge to pilot crop cultivations. The results are expected to set the basis for future sustainable utilization of these genetic resources through commercialization and breeding programs.

Keywords: *native plants, small fruits, neglected and underutilized crops, cuttings, rooting*

INTRODUCTION

Wild fruits and berries make up a specific category of fruits with well-known value for human health as they are sources of primary and secondary metabolites, which provide excellent antioxidant activity for humans (Cosmulescuet al., 2017). Small fruit trees and shrubs belong to plant families with several members that are both commercially cultivated and naturally occurring across Greece. Particularly for Greece, in addition to the nutritional value, the native forest fruit trees and shrub species are associated with significant natural and cultural heritage that has been neglected over the years and has not been properly utilised. Additionally, native plant species present innate high adaptability to domestic environmental conditions which may enhance fruit quality (Kozlowski et al., 1991; Milla et al., 2005; Gacheru et al., 2017).

In this framework, this study presents the national Greek research project 'Eco-Variety' (T1EDK-05434) which is being implemented across four large areas of high ecological importance of northern Greece. The project focuses on two plant groups: Group A - wild-growing Greek native forest fruit trees and shrubs with potential to become minor crops (which are presented herein), and Group B - local varieties of fruit trees that are traditionally cultivated or are neglected/abandoned. For all these, the project's aims are their survey through targeted collection and documentation (taxonomic identification, accession numbering, DNA barcoding

and species-specific geographical and ecological information) as well as their *in-situ* and *ex-situ* conservation in order to facilitate their pilot propagation, evaluation and sustainable utilization. To date, no documented propagation material of Greek origin exists regarding these focal plants, thus limiting their possible use either as new crops, or as valuable phytogenetic resources for future plant breeding.

MATERIALS AND METHODS

Selected focal wild-growing species

The wild-growing native forest trees and shrubs species selected (Group A) are: *Amelanchier ovalis*, *Prunus spinosa, Rosa canina* and *Rubus idaeus* (all in Rosaceae family), *Cornus mas* (Cornaceae), *Rhus coriaria* (Anacardiaceae), *Sambucus nigra* (Caprifoliaceae) and *Vaccinium myrtillus* (Ericaceae). All wild focal species of Group A develop nutritious fruits (food for human consumption) with beneficial properties or can be used as spices (*R. coriaria*) or for infusion-decoctions and are associated with significant natural and cultural heritage in Greece and elsewhere.

Selection of study areas

The field research was conducted from October 2018 until October 2019, including a complete plant growth cycle. The work included multi-day visits to selectedsites, demarcation via remote sensing, species tracking, identification and description, collection of samples and collection of additional information from local authorities.The research was focused on fourlarge mountainous and ecologically important areas of Northern Greece (Table 1), and the fieldwork was divided between the different partners of the project. Sistada partner undertook five geographical areas of Eastern Macedonia and Thrace, Central and Western Macedonia (Rodopi mountain range, Almopia mountain range, Mt. Pieria, Mt. Grammos, Mt. Vitsi and Prespes lakes), HAO Demeter undertook Central and Western Macedonia (Almopia mountain range, Mt. Vermio, Mt. Paiko, Mt. Kroussia, Mt. Lakmos and Mt. Pieria) and the University of Ioannina focused on the mountain ranges of the Epirus area. In the context of the project, literature review was conducted in the first place, aiming at the organization and realization of the field expeditions in the focal areas that were set for the collection of propagation material and fruit samples. Additionally, species-specific information was sought from the online site of the botanical museum of Lund, Sweden (http://herbarium.emg.umu.se/index.html), and from the botanical museum of Berlin, Germany (http://ww2.bgbm.org/herbarium). These sources were particularly useful since they included specific location and habitat details as well as geographical coordinates for the target plants. According to the literature review results, the aim set for the project partners was to collectively undertake field research work for each of the eight selected taxa (Group A) in at least three different sites of at least three distinct geographical areas (see Table 1).

Table 1. Focal wild-growing plants with potential to become minor crops that were prioritised by the Eco-Variety project and number of collection sites per Greek prefecture as implemented by the project partners during the period of 10/2018 – 11/2019

Species	Area	Number of collection sites
Rhus coriaria	Pella Prefecture	7
	Imathia Prefecture	2
	Ioannina Prefecture	5

Rubus idaeus	Kastoria Prefecture (Mt Grammos)	4
	Grevena Prefecture	2
	Pella Prefecture	4
	Kozani Prefecture	3
	Kilkis Prefecture	3
	Trikala Prefecture	1
Vaccinium myrtillus	Pella Prefecture	9
	Florina Prefecture	1
	Kozani Prefecture	4
	Ioannina Prefecture	1
	Kilkis Prefecture	1
Cornus mas	Pella Prefecture	3
	Ioannina Prefecture	12
	Kilkis Prefecture	3
	Kozani Prefectures and Mt Pieria	2
	Kastoria Prefecture (Mt Grammos)	1
	of Drama Prefecture (Mt Rodopi)	1
	Preveza Prefecture	1
	Xanthi Prefecture	2
Amelanchier ovalis	Trikala Prefecture	2
	Kilkis Prefecture (Mt Paiko)	1
	Florina Prefecture	3
	Pella Prefectures	2
Rosa canina	Grevena Prefecture	1
	Kilkis Prefecture	4
	Ioannina Prefecture	2
	Pella Prefecture	4
	Imathia Prefecture (Mt Vermio)	1
	Preveza Prefecture	1
	Trikala Prefecture	1
Sambucus nigra	Preveza Prefecture	1
	Ioannina Prefecture	5
	Pella Prefecture	3
	Kilkis Prefecture (Mt Paiko)	3
	Imathia Prefecture (Mt Vermio)	1
	Trikala Prefecture	1
Prunus spinosa	Preveza Prefecture	1
	Ioannina Prefecture	2
	Pella Prefecture	3
	Kilkis Prefecture (Mt Paiko)	1
	Imathia Prefecture (Mt Vermio)	1
	Trikala Prefecture	1
	Florina Prefecture	3

Documentation of collected samples

Each of the eight selected species was effectively documented in at least three different sites of at least three distinct geographical areas, thus resulting in a total of 70 surveyed sites from which sufficient plant material was collected (at least 20 cuttings and 10 g of fruits per taxon population from each site). The collection protocol included collection of suitable propagation material (cuttings) and/or live plants, leaf samples for DNA analysis, original soil samples, fruit samples and photographic documentation, all taken *in-situ*. The collection of plant material was conducted with the aid of a special collection permit issued yearly by the Greek Ministry of Environment and Energy. Additionally, botanic, geographical and ecological data were recorded for each site and for each focal species (codes and accession numbers, habitat characteristics, geographical coordinates, altitude, vegetative and fruit morphological traits). All this valuable information serve documentation purposes ('passport info') and is incorporated in the project's database (https://ecovariety.gr/).

Propagation trials

Consequently, propagation trials for each population of each species have been conducted in the laboratory of the Institute of Plant Breeding & Genetic Resources of HAO Demeter, using the collected material. The objective of the trials was the development of a successful propagation protocol prior to *ex situ* pilot cultivation trials. The collected material was handled on the day of its arrival. The cuttings were prepared, were treated with rooting hormone and were set for rooting in 3 perlite:1 peat substrate under mist (70 – 80 % of Relative Humidity). The rooting hormone used was Indole-3-butyric acid (IBA) in a range of concentrations from 1000 ppm to 10000 ppm dissolved in 50% ethanol and it was applied through a quick dip (5-7 sec) of the cutting's incision.

Statistical analysis of the data

In order to evaluate the effect of the hormonal treatments applied to the cuttings, the results of the rooting trials were analysed through ANOVA using the Tukey HSD for mean comparison ($P<0.05$) provided that the data satisfied the assumptions of the ANOVA. Otherwise, non-parametric tests were used as appropriate. The statistical software used was IBM-SPSS 20.

RESULTS

The number of population samples that were collected for the eight eligible taxa of the project were 124 in total. Collected from four designated areas of the project were 23 population samples of *C. mas*, 20 samples of *R. idaeus*,15 samples of *P. spinosa*, and 9 population samples of *A. ovalis*. Similarly, collected from three designated areas of the project were 16 population samples of *V. myrtillus*, 14 samples of *R. canina*, 14 samples of *S. nigra*, and 12 population samples of *R. coriaria*. The highest number of population samples were those of *C. mas* (23) due to great variability of fruits detected *in-situ*, whereas the lowest number was those of *A. ovalis* (9) due to the unforeseen rarity of this taxon in the wild habitats (Table 2). As far as leaf samples are concerned, 115 composite samples were collected in total, each including leaves from 20 individuals. Most of the samples came from *C. mas* (21) and *R. idaeus* (20) whereas the lowest number of samples came from *A. ovalis* (7) and *R. coriaria* (11) (Table 2). Soil samples proved to be more difficult to be collected due to the ruggy-rocky and inhospitable terrain of many surveyed areas. As a result, 93 soil samples were collected in total, most of them being from habitats of *C. mas* populations (18), *R. idaeus* (18), *V. myrtillus* (15), *S. nigra* (11) and *R. coriaria* (10). Comparatively fewer soil samples were collected for *P. spinosa* (9), *R. canina* (6) and *A. ovalis* (6) (Table 2). Regarding fruit samples, already 45 collections have

been made and much more are going to be implemented during the 2020 fruiting season (autumn).

During the propagation trials, 108 population samples were successfully propagated via cuttings (at least 20 cuttings per population) with rooting patterns ranging from 17.5% to 100%, which resulted in the production of 1,128 new plants raised *ex-situ* from seven focal species (Table 3). *S. nigra, C. mas, R. idaeus* and *R. canina* have shown high rooting rates of cuttings (>75% - 100%) which is commercially acceptable; *P. spinosa* showed intermediate rooting rates (45.65%), whereas *R. coriaria* and *A. ovalis* presented lower rates and further research is undertaken (Table 3). All produced plants have presented good adaptability at the *ex-situ* environment of the Balkan Botanic Garden of Kroussia in Thermi, Thessaloniki (sea level), where currently they are being cultivated successfully in pilot cultivation trials.

Table 2. Number of documented population sampling per focal taxon (alphabetically) of the Eco-Variety project (cuttings from different populations, leaf samples of 20 individuals, soil samples and fruit samples) collected *in-situ* from the designated focal areas of the project (Table 1), as stemmed from the field work of all partners of the project for the period of 10/2018 – 11/2019

Focal species	Population samples	Focal areas	Leaf samples	Soil samples	Fruit samples
Amelanchier ovalis	**9**	3	7	6	2
Cornus mas	**23**	4	21	18	9
Prunus spinosa	**15**	4	14	9	11
Rhus coriaria	**12**	3	11	10	4
Rosa canina	**14**	3	14	6	6
Rubus idaeus	**20**	4	20	18	5
Sambucus nigra	**15**	3	14	11	3
Vaccinium myrtillus	**16**	3	14	15	5
TOTAL	**124**	**8**	**115**	**93**	**45**

Table 3. Number of propagated clones (cuttings' groups from distant populations) for each focal species of the project Eco-Variety with corresponding rooting capacity and number of produced plants to date

Species	Propagated clones	Rooting (%) (in progress)	Produced plant individuals
Cornus mas	23	85,0	217
Rosa canina	14	75,7	172
Rubus idaeus	20	100	229
Sambucus nigra	15	100	454
Prunus spinosa	15	45,6	25
Rhus coriaria	12	18,5	17
Amelanchier ovalis	9	17,5	14

DISCUSSION

None of the targeted wild fruit species can be considered as ubiquitous (commonplace), at least in Greece. Some of them, like *C. mas* and *P. spinosa*, are more common, while others, like *A. ovalis* and *V. myrtillus*, are fairly uncommon and are rather difficult to encounter in the wild.

Consequently, although the literature sources were theoretically a reliable indication for the existence of the eight focal taxa in the wild habitats of Greece (which determined the actual on-site visits), in practice, the focal species were not always easily or successfully located. Moreover, even when the target species were successfully located, ripe fruit samples were not always available during the on-site visits (in many cases fruits were absent or unripen, even in other subsequent efforts).

Concerning the propagation trials, the results showed that *S. nigra, C. mas, R. canina* and *R. idaeus* responded well to the application of the selected rooting hormone (IBA) and the tested treatments presented higher rooting rates compared to the control ($P<0.05$). On the other hand, *P. spinosa, R. coriaria* and *A.ovalis* presented comparatively lower rooting rates. However, the produced plants of all focal species showed high survival rates and good adaptability to the human-made *ex-situ* environment. The external application of auxin proved to be essential for high rooting rates, whereas the quick dip application method coupled with the least amount of time from excision of cuttings till quick dip (Blythe et al., 2007) also seemed to affect the rooting patterns detected. In addition, our results and observations suggest that the physiological condition and the developmental stage of the mother plants might have also affected the observed rooting of cuttings, as expected (see Da Costa et al., 2013).

The above work has resulted in the documentation of valuable Greek native population samples of wild-growing focal fruit species with potential to become minor crops which provides the basis for their sustainable utilisation. In addition, species-specific asexual propagation protocols have been developed in order to pave the way for their commercialisation and future breeding programmes. The work of the Eco-Variety project is in full development through pilot cultivation trials and targeted phytochemical analysis of the selected focal taxa in order to further document their respective potentials as new minor crops.

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THE EFFECTS OF NITROGEN APPLICATIONS WITH PHOSPHORUS AND POTASSIUM ON SOME NUTRIENT ELEMENT AMOUNTS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) VARIETIES IN THRACE REGION

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ABSTRACT

This research was conducted to determine the effects of increasing doses of nitrogen (N) applications both with phosphorus (P) and potassium (K) on some nutrient element amounts of sunflower (*Helianthus annuus* L.) varieties during a two year period in 2015-2016 vegetation seasons. Increasing doses of nitrogen (0, 4, 8, 12, and 16 kg da^{-1} N) and 8 kg da^{-1} P_2O_5 and 8 kg da^{-1} K_2O were applied to the soil under non-irrigated field conditions in Thrace region. It was observed that increasing the doses of N had statistically significant effect ($p<0.05$) on the nitrogen amounts of sunflower cultivars in both years. The highest nitrogen amount in the first year (3.02 %) and also combined of the two years results (2.92%) were found at 12 kg da^{-1} dose, however in the second year (2.96 %) it was determined at 4 kg da^{-1} N dose. Increasing the nitrogen did not affect the other nutrient concentrations significantly. But the results taken in two years period were found statistically significant. The result of P, K, Mg, Cu, Zn, Mn and B were found higher in the first year than the second year. Statistically significant differences were also determined between the varieties of the sunflower on K, Ca and B amounts. In obtaining high yields in sunflower plants; besides the cultivars, it was concluded that by taking into consideration the plant nutrient concentrations in the soil, the effect of phosphorus and potassium fertilizers, which will be applied in addition to nitrogen may be important.

Keywords: Yield, sunflower, nutrient elements, interaction

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oil plant and main raw material of the vegetable oil industry with high economic value (Awatif and Shaker, 2014; Kaya, 2014). It is cultivated in many countries of the world, and also is the most important oilseed crop in Turkey with a seed production of 1.9 million tonnes in an area of more than 6.5 million da, and Marmara region is known as the largest sunflower producing area (Sincik et al., 2013; Yağmur and Okur, 2017; FAOSTAT, 2018; TUİK, 2019). Furthermore, Thrace region which is the European part of Turkey, has large and fertile plains. This region is also the main sunflower cultivation areas of sunflower production more than 50% of the total area of Turkey (Kaya, 2014). It has been reported that average seed yield depends on regions, varieties, and irrigation conditions, and changes between 125-300 kg da^{-1} (Bozkurt and Karaçal, 2000, Kolsarıcı et al., 2005; Yağmur and Okur, 2017).

In addition to irrigation, nitrogen fertilization of sunflower has also been reported to better yield results under irrigated conditions. Siddique et al. (2009) were reported that combined application of irrigation and nitrogen would be a better practice. In previous studies it has been indicated that the seed yield and its components are increased with increasing nitrogen rates

and the highest yields were obtained from 8 and 12 kg da^{-1} nitrogen doses (Nawaz et al., 2003; Özer et al., 2004; De Giorgio et al., 2007; Oyinlola et al., 2010). It has been stated that the highest yield can be achieved with the 16 kg da^{-1} nitrogen dose is applied in conditions with irrigation possibilities. However, under conditions where semi-arid climatic conditions prevail and irrigation is limited, the excess nitrogen to be given for the purpose of increasing the yield will decrease the yield even more than the expectation of increasing the product. A result of a previous study reported that the rate of N uptake decreased by 50 % those grown in insufficient water conditions compared to those grown under sufficient water conditions and this situation also affected the yield (Alam, 1994).

In recent years, besides developing varieties that will be less affected by adverse environmental conditions or tolerant to these conditions, applications which will accelerate the development stages of plants and provide better development of root and shoots have gained great importance. Adequate amounts of nutrient elements in the soil will rise the yield and its quality (Gül and Kara, 2015). Phosphorus and potassium have very important place in plant nutrition. The most important function of phosphorus in plant development and growth is the storage and transfer of energy. The energy stored in the phosphate compounds is then used in development and reproduction processes (Güneri et al., 2016). Potassium is also regarded as one of the major nutrient elements which plays an essential role in plant growth and metabolism and affects the yield and quality (Ören and Çelik, 2019). Many enzymes are activated when potassium present and had an important role on regulating the osmotic turgor pressure which regulates the opening and closing of the stomata (Mengel, 2007; Çelik et al., 2010). It has also been reported that sunflower needs potassium in excess, facilitates fat synthesis and significantly affects the rate of fat (Adiloğlu and Derin, 2019). However, in a study done in Uzunköprü district which was the most important sunflower production area of Thrace region; it was emphasized that nitrogen in plant leaves is sufficient, but phosphorus is insufficient in 42%, and potassium in 20% of the study area (Adiloğlu and Derin, 2019). In conditions where water is limited; in order to increase the yield and quality; as well as nitrogen it may be important to have sufficient amount of phosphorus and potassium among other plant nutrients.

The interaction between nutrients affects the amount and cost of fertilizers applied by farmers to obtain the highest quality and quantity of crops. As agriculture intensifies and the severity and amount of nutrient deficiencies increase, the importance of interactions between nutrients increases. Due to the use of fertilizers that are not based on soil and plant analysis, Turkish agriculture has entered the stage of multiple nutrient deficiency and at least 4 to 6 plant nutrient deficiencies can be seen in some agricultural soils (Gezgin and Hamurcu 2006). Fertilizer requirements, seed and oil yields of sunflower plants vary significantly depending on the region, rainfall or irrigation and plant type. New research is needed to determine the most appropriate fertilization and other cultural practices in our country (Bozkurt and Karaçal, 2000).

This research was conducted to determine the effects of increasing doses of nitrogen (N) applications both with phosphorus (P) and potassium (K) on some nutrient element amounts of sunflower (*Helianthus annuus* L.) varieties during a two years period in 2015-2016 vegetation seasons.

MATERIALS AND METHODS

Two field trials were conducted during 2015 and 2016 seasons at Ballıhoca village of Muratlı district, Tekirdağ city. The experimental site is located in the north east Marmara region (41°13'41.8"N 27°31'08.7"E) in Turkey. Average annual rainfall was 581,5 mm, annual humidity was 76% and mean monthly temperature was 14.0°C according to the climatic data obtained from the weather station. Total rainfall from March to August was 208,9 and 221,5 mm in 2015, 2016 and long term (1939-2019). Soil samples were collected from 0–30 and 30-60 cm depth of the field and analysed before the trial and the results were shown on Table 1.

The soil had a loam texture and slightly alkaline pH. Additionally, it had low lime content and Electrical Conductivity (EC). The soil also had adequate concentrations of organic matter, N, and K. The amount of Ca was found high in both years but Mg was high at the second year. The amount of Zn was found low in both years. However, the other nutrient element amounts were found sufficient according to the depth and years (Table 1).

The experimental plots were arranged in randomised block design with four replicates in both years. The varieties were the main plots, and six different fertilizer levels including 0, 4, 8, 12, and 16 kg da^{-1} N, 8 kg da^{-1} P_2O_5 and 8 kg da^{-1} K_2O [control (0-0-0 N-P_2O_5-K_2O), N0 (0-8-8), N1 (4-8-8), N2 (8-8-8), N3 (12-8-8) and N4 (16-8-8)] were the subplot treatments. The subplot size was 21.42 m^2 (5.1x4.2 m) and consisted of six rows, with the middle four rows designated as harvest rows.

Table 1. Some soil properties of the trial field

		Amounts				Amounts	
Properties	**Soil**	**2015**	**2016**	**Properties**	**Soil**	**2015**	**2016**
pH (1:2.5 soil:water)	0-30 cm	7.88	6.65	Total nitrogen (N), %	0-30	0.101	0.106
	30-60	7.98	7.76		30-60	0.102	0.105
EC, µS cm^{-1}	0-30 cm	582	158	Available phosphorus (P), mg	0-30	13.21	11.02
	30-60	387	272		30-60	7.94	4.90
Lime, $CaCO_3$, %	0-30 cm	3.37	0.58	Extractable potassium (K), mg	0-30	228	141
	30-60	2.50	1.25		30-60	192	188
Texture	0-30 cm	Loam	Loam	Extractable calcium (Ca) mg kg^{-1}	0-30	8803	4449
	30-60	Loam	Loam		30-60	9105	7345
Sand, %	0-30 cm	46.84	39.84	Extractable magnesium,	0-30	313.7	1178
	30-60	40.84	33.84		30-60	321.3	833
Silt, %	0-30 cm	36.16	39.34	Extractable sodium (Na), mg kg^{-1}	0-30	119.7	106.5
	30-60	43.52	47.52		30-60	119.7	128.5
Clay, %	0-30 cm	17.00	20.82	Available Iron (Fe), mg kg^{-1}	0-30	6.20	26.27
	30-60	15.64	18.64		30-60	5.92	10.42
Organic matter, %	0-30 cm	1.92	2.02	Available Cupper (Cu), mg kg^{-1}	0-30	1.09	1.63
	30-60	1.94	2.01		30-60	1.11	1.18
Available Boron (B) mg kg^{-1}	0-30 cm	1.98	1.21	Available Zinc (Zn), mg kg^{-1}	0-30	0.22	0.18
	30-60	1.88	2.01		30-60	0.20	0.18
				Manganese (Mn), mg kg^{-1}	0-30	9.60	4.54
					30-60	9.47	7.30

The oleic type sunflower hybrids (ES Balistic CL and Oliva CL) obtained from "Euralis Semences S.A.S" (France) and "May Seed LTD. STI" (Turkey) were used as the planting material. The crop previously grown in the experimental area in both years was wheat (*Triticum aestivum L.*). The experimental area was cultivated by ploughing at a depth of 25-30 cm in the autumn and by disc-harrowing at 8-10 cm depth in the spring. Sunflower seeds were hand planted on 12 May 2015 and 13 May 2016 at 0.70 m between-row spacing and 0.30 m within-row spacing. Prior to planting various fertilizers one by one or combined (Monopotassium phosphate, potassium nitrate, and 15-15-15) were incorporated into the disc-harrowed soil due to the applications. Half the nitrogen rate was applied before planting, and the remaining N was top-dressed before the second hoeing. The nitrogen fertilizer was banded as ammonium nitrate, approximately 5 cm below and to the side of the seed row.

During the growing season, all of the plots were cultivated once with a harrow (3 weeks after planting) and twice (5 and 8 weeks after planting) by hand. The plots were harvested by

hand on 11 September 2015 and 19 September 2016 when the seed contained approximately 10% moisture content.

Data from all of the experiments were subjected to statistical analysis and the mean values were compared using the least significant difference (LSD) multiple range test with the computer program MINITAB 17.1.0.0 (Minitab Inc., State College, Pennsylvania, USA).

RESULTS and DISCUSSION

Effects on N, P, and K Concentrations of Sunflower Seeds

According to the variance analysis results of N, P and K concentrations, years were found statistically significant ($p<0.01$). No significant difference was found between the sunflower varieties except at K concentrations, however its interaction between years was found significant at N and P (Table 2 and 3). Nitrogen rates ($p<0.05$) and its interaction with years ($p<0.01$) were also found significant at N. Interactions between varieties and nitrogen rates was only found significant ($p<0.05$) at K.

Table 2. Results of variance analysis and mean effects of nitrogen rate on N, P and K concentrations of sunflower varieties in 2015, 2016 and combined years

	N			**P**			**K**				**N**	**P**	**K**
Varieties	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years		*F*	*F*	*F*
A	3.00 a A	2.61 b B	2.81	0.65 a A	0.36 b B	0.51	0.66	0.38	0.52 a	Year (Y)	**	**	**
B	2.54 b B	3.15 a A	2.85	0.60 b A	0.42 a B	0.51	0.62	0.37	0.50 b	Bloks x Year	**	**	**
N rate kg da^{-1}										Varieties (V)	ns	ns	**
Control	2.62 b B	2.88 a A	2.75 c	0.62	0.39	0.51	0.62	0.38	0.50	Y x V	**	**	ns
N0PK	2.70 b B	2.92 a A	2.81 bc	0.61	0.40	0.50	0.62	0.39	0.50	Nitrogen (N)	*	ns	ns
N1PK	2.80 b A	2.96 a A	2.88 ab	0.63	0.41	0.52	0.61	0.37	0.49	Y x N	**	ns	ns
N2PK	2.70 b A	2.81 a A	2.76 c	0.64	0.38	0.51	0.66	0.40	0.53	V x N	ns	ns	*
N3PK	3.02 a A	2.81 a B	2.92 a	0.63	0.39	0.51	0.67	0.37	0.52	Y x V x N	ns	ns	ns
N4PK	2.79 b A	2.91 a A	2.85 ab c	0.63	0.38	0.50	0.65	0.36	0.51				
Mean	**2.77 B**	**2.88 A**		**0.62 A**	**0.39 B**		**0.64 A**	**0.38 B**					

The differences between values by different letters are significant. Capital letters are for each row and small letters are for each column. *P < 0.05, ** P< 0.01, ns not significant. A: ES Balistic CL B: Oliva CL

Table 3. Effects of increasing doses of nitrogen on N, P and K concentrations of sunflower varieties.

	N rate kg da^{-1}						
	N0	N1	N2	N3	N4	N5	Mean
				N			
Varieties				2015			
A	2.84	3.01	3.03	2.93	3.18	3.01	3.00 a
B	2.39	2.39	2.57	2.48	2.87	2.56	2.54 b
Mean	**2.62 B**	**2.70 B**	**2.80 B**	**2.70 B**	**3.02 A**	**2.79 B**	
				2016			
A	2.63	2.59	2.70	2.50	2.60	2.66	2.61 b
B	3.14	3.26	3.22	3.12	3.03	3.16	3.15 a
Mean	**2.88 A**	**2.92 A**	**2.96 A**	**2.81 A**	**2.81 A**	**2.91 A**	
				Combined Years			
A	2.73	2.80	2.86	2.72	2.89	2.84	2.81
B	2.76	2.83	2.89	2.80	2.95	2.86	2.85
Mean	**2.75 C**	**2.81 BC**	**2.88 AB**	**2.76 C**	**2.92 A**	**2.85 ABC**	
				P			
Varieties				2015			
A	0.64	0.62	0.67	0.67	0.65	0.65	0.65 a
B	0.60	0.59	0.58	0.61	0.60	0.61	0.60 b
Mean	**0.62**	**0.61**	**0.63**	**0.64**	**0.63**	**0.63**	
				2016			
A	0.37	0.37	0.38	0.34	0.38	0.35	0.36 b
B	0.42	0.44	0.45	0.42	0.41	0.41	0.42 a
Mean	**0.39**	**0.40**	**0.41**	**0.38**	**0.39**	**0.38**	
				Combined Years			
A	0.50	0.49	0.53	0.50	0.52	0.50	0.51
B	0.51	0.52	0.51	0.52	0.51	0.51	0.51
Mean	**0.51**	**0.50**	**0.52**	**0.51**	**0.51**	**0.50**	
				K			
Varieties				2015			
A	0.64	0.62	0.70	0.67	0.67	0.67	0.66
B	0.61	0.61	0.53	0.66	0.67	0.63	0.62
Mean	**0.62**	**0.62**	**0.61**	**0.66**	**0.67**	**0.65**	
				2016			
A	0.37	0.40	0.38	0.41	0.36	0.37	0.38
B	0.38	0.39	0.36	0.39	0.38	0.36	0.37
Mean	**0.38**	**039**	**0.37**	**0.40**	**0.37**	**0.36**	
				Combined Years			
A	0.50 a A	0.51 a A	0.54 a A	0.54 a A	0.51 a A	0.52 a A	0.52 a
B	0.49 a A	0.50 a A	0.44 b B	0.52 a A	0.52 a A	0.49 a A	0.50 b
Mean	**0.50**	**0.50**	**0.49**	**0.53**	**0.52**	**0.51**	

Increasing doses of nitrogen both with phosphorus and potassium elevated the nitrogen concentrations. The highest nitrogen in 2015 (3.02 %) and in combined years (2.92 %) were found at N3PK but in 2016 (2.96 %) it was found at N1PK. Nitrogen concentration of the sunflower was found high in 2016 (2.88 %) than 2015 (2.77 %) according to the mean results of the years. Because of its effective role on photosynthesis, and being an important component of chlorophyll and other bio-catalytic substances, nitrogen is known as the most yield limiting nutrient and partitioning into various parts of crops for growth and development (Škarpa, and

Lošák, 2008; Banerjee et al., 2014). In accordance with the previous research literatures, in our research, application of the increasing doses of N elevated the nitrogen concentrations of the seeds as well as the seed yield. In a past research of Bozkurt and Karaçal (2000), 0, 4, 8 and 12 kg da^{-1} N, 7 kg da^{-1} P_2O_5, and 5 kg da^{-1} K_2O were applied and they reported the highest seed N concentration at 12 kg da^{-1} N dose as 2.99 % and was found similar to the findings of us.

When we compared the varieties, although the variety A had the highest nitrogen amount (3.00 %) in 2015, it was found high (3.15 %) for variety B in 2016. Variety B was seemed strong and adaptive to nutrient deficient conditions. Increased nitrogen applications had also influenced the nutrients uptake of sunflower varieties. Different cultivars grown on different cultures may have different agronomic performances (Zheljazkov et al., 2011; Tan, 2014). In order to determine the performances of the varieties, lots of research results which were conducted at different ecological locations with different varieties, indicated different values of grain yield, nutrient uptake and agronomic characters (Tan et al, 2000; Kaya et al., 2003; Tozlu et al., 2008; Tan et al., 2013; Tan, 2014). Abdel-Motagally and Osman (2010) reported that sunflower varieties react differently to nitrogen and potassium applications as a result of the study. Similar results were also obtained by Basha (2000).

Increasing doses of nitrogen both with phosphorus and potassium had no statistically significant effect on the phosphorus concentrations (Table 2 and 3). However the concentrations were found significant between years. In 2015 phosphorus concentrations (0.62 %) were found higher than in 2016 (0.39 %). This situation was dedicated as a result of the phosphorus amounts of the field during the experiment. In the first year although the phosphorus amount was in the sufficiency range, in the second year these amount were found below the range and it affected the translocation of phosphorus to the roots and to the seeds in the second year. Sunflower is a deep rooted and fast growing plant so it responds to fertilization applications under low nutrient soil conditions. Bozkurt and Karaçal (2000), were reported the seeds P concentrations between 0.44 % and 0.64% in support of our findings. They also reported the highest P amount at N0 applied plots because of the insufficient amounts of P in the soil and were not found enough for the elevated nitrogen. It was reported that 8.8 kg da^{-1} N, 1 kg da^{-1} P, and 5.4 kg da^{-1} K removes for 180 kg da^{-1} seed production (Shyamkiran, 2000; Banerjee et al., 2014). In another research this amounts were found as 6.3 kg da^{-1} N, 1.9 kg da^{-1} P_2O_5, and 12.6 kg da^{-1} K_2O for 100 kg da^{-1} seed production (Hegde and Sudhakarababu, 2009). In our research results at combined years, up taken N, P, K amounts from seeds were calculated as 8.73 kg da^{-1} N, 1,53 kg da^{-1} P, and 1,56 kg da^{-1} K for 306 kg da^{-1} seed production (data not shown). In various field researches, applied nitrogen doses to sunflower were reported between 4 to 24 kg da^{-1} N and the consequences were found different depending to the differences on ecological conditions, managements, and plant varieties (Gül and Kara, 2015; Sheoran et al., 2016; Nasim et al., 2016; Nasim et al., 2017; Yağmur and Okur, 2017).

The variety A had the highest phosphorus amount (0.65 %) in 2015, however it was found high (0.42 %) for variety B in 2016. Similar to nitrogen results, Variety B was found strong and adaptive to phosphorus deficient conditions.

Increasing doses of nitrogen both with phosphorus and potassium had no statistically significant effect on the potassium concentrations (Table 2 and 3). However the concentrations were found significant between years as found at phosphorus. In 2015 potassium concentrations (0.64 %) were found higher than in 2016 (0.38 %). This situation was dedicated as a result of the potassium amounts of the field during the experiment. In the first year although the potassium amount was in the sufficiency range, in the second year these amount were found below the first year and it may affected the translocation of potassium to the roots and to the seeds in the second year. Bozkurt and Karaçal (2000), were reported the seeds K concentrations as 0.56-0.57 % in support of our findings. According to the literatures, higher dry matter was recorded due to the increased photosynthesis in relation with sufficient nutrient conditions. However

decreased dry weight yield and nutrient uptake were also reported with the excess amounts (Škarpa, and Lošák, 2008; Banerjee et al., 2014; Ravishankar and Malligaward, 2017). Gerendás et al., 2008 reported a fall in productivity of sunflower due to the high potassium levels. Gheorghe et al. (2011) reported an increase in the yield with 8 kg da^{-1} K. However because of having sufficient amounts of potassium in the soil may decrease the effects. Sağlam et al. (1992) applied 5 kg of N and P_2O_5, and 2.5, 5, 7.5, 10, and 12.5 kg da^{-1} K_2O to an experimental area containing medium potassium and they found 2.5 kg da^{-1} K_2O dose as sufficient for the maximum product. In another study with sunflower Ciobanu et al. (2008), applied 0, 8, 16 kg N da^{-1}; 0, 4, 8 kg of P_2O_5 da^{-1}; and 0, 4, 8, 12 kg K_2O da^{-1} to the soil, and reported the highest yield at 8 kg K_2O da^{-1} fertilizer dose. They pointed out that in the case of further application of potassium, the current increase is not economical and that the benefit from potassium applications is related to the doses of nitrogen and phosphorus. The results in the literature have been found to support the results we have experimentally obtained.
Variety A had the highest potassium amount (0.52 %) than variety B in combined years result. It may also being as a consequence of the seasonal weather changes.

Effects on Ca, Mg, and Fe Concentrations of Sunflower Seeds

According to the variance analysis results of Ca and Mg concentrations, years were found statistically significant ($p<0.01$). Ca concentrations of the sunflower varieties, and its interaction between years were found significant ($p<0.01$) (Table 4 and 5). Interactions between varieties and years were also found significant at Mg concentrations ($p<0.05$).

Table 4. Results of variance analysis and mean effects of nitrogen rate on Ca, Mg and Fe concentrations of sunflower varieties in 2015, 2016 and combined years

	Ca			**Mg**			**Fe**				**Ca**	**Mg**	**Fe**
Varieties	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years		*F*	*F*	*F*
A	0.15 b B	0.23 a A	0.19 b	0.29 a A	0.18 b B	0.24	42.48	35.23	38.85	Year (Y)	**	**	ns
B	0.20 a B	0.23 a A	0.22 a	0.28 b A	0.20 a B	0.24	59.85	42.54	51.20	Bloks x Year	*	**	ns
N rate kg da^{-1}										Varieties (V)	**	ns	ns
Control	0.17	0.23	0.20	0.29	0.19	0.24	39.76	37.77	38,76	Y x V	**	**	ns
N0PK	0.18	0.23	0.21	0.27	0.19	0.23	37.49	39.03	38.26	Nitrogen (N)	ns	ns	ns
N1PK	0.17	0.23	0.20	0.28	0.20	0.24	39.22	38.15	38.69	Y x N	ns	ns	ns
N2PK	0.18	0.24	0.21	0.29	0.19	0.24	39.69	39.88	39.78	V x N	ns	ns	ns
N3PK	0.17	0.22	0.20	0.28	0.20	0.24	40.68	39.14	39.91	Y x V x N	ns	ns	ns
N4PK	0.18	0.22	0.20	0.28	0.18	0.23	39.81	39.35	39.58				
Mean	**0.18 B**	**0.23 A**		**0.28 A**	**0.19 B**		**39.44**	**38.88**					

The differences between values by different letters are significant. Capital letters are for each row and small letters are for each column. *P < 0.05, ** P< 0.01, ns not significant. A: ES Balistic CL B: Oliva CL

Increasing doses of nitrogen both with phosphorus and potassium had no statistically significant effect at any of the elements concentrations. However the concentrations were found significant between years. In 2016 season, concentrations of calcium (0.23 %) were found higher than that of 2015 (0.18 %). Bozkurt and Karaçal (2000), were reported the seeds Ca concentrations between 0.075 and 0.093 % which were lower than our findings. Although the variety B had the highest calcium amount (0.20 %) in 2015, it was found similar (0.23 %) for the varieties in 2016. Variety B was found dominant for Ca concentrations due to the combined year results.

Table 5. Effects of increasing doses of nitrogen on Ca, Mg and Fe concentrations of sunflower varieties.

	N rate kg da^{-1}						
	N0	N1	N2	N3	N4	N5	Mean
				Ca			
Varieties				2015			
A	0.15	0.16	0.15	0.16	0.15	0.15	0.15 b
B	0.20	0.20	0.19	0.21	0.20	0.20	0.20 a
Mean	**0.17**	**0.18**	**0.17**	0.18	**0.17**	**0.18**	
				2016			
A	0.23	0.23	0.23	0.25	0.23	0.22	0.23 a
B	0.23	0.24	0.24	0.23	0.22	0.23	0.23 a
Mean	**0.23**	**0.23**	**0.23**	**0.24**	**0.22**	**0.22**	
				Combined Years			
A	0.19	0.20	0.19	0.20	0.19	0.19	0.19 b
B	0.21	0.22	0.21	0.22	0.21	0.22	0.22 a
Mean	**0.20**	**0.21**	**0.20**	**0.21**	**0.20**	**0.20**	
				Mg			
Varieties				2015			
A	0.29	0.28	0.30	0.29	0.29	0.29	0.29 a
B	0.28	0.27	0.27	0.28	0.28	0.28	0.28 b
Mean	**0.29**	**0.27**	**0.28**	**0.29**	**0.28**	**0.28**	
				2016			
A	0,19	0.18	0.19	0.17	0.19	0.18	0.18 b
B	0.20	0.20	0.21	0.20	0.20	0.19	0.20 a
Mean	**0.19**	**0.19**	**0.20**	**0.19**	**0.20**	**0.18**	
				Combined Years			
A	0.24	0.23	0.25	0.23	0.24	0.23	0.24
B	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Mean	**0.24**	**0.23**	**0.24**	**0.24**	**0.24**	**0.23**	
				Fe			
Varieties				2015			
A	40.69	39.48	44.01	42.66	44.33	43.70	42.48
B	38.82	35.50	34.43	36.72	37.04	35.92	59.85
Mean	**39,76**	**37.49**	**39.22**	**39.69**	**40.68**	**39.81**	
				2016			
A	33.23	35.91	36.05	35.90	35.78	34.51	35.23
B	42.30	42.15	40.26	43.86	42.50	44.19	42.54
Mean	**37,77**	**39.03**	**38.15**	**39.88**	**39.14**	**39.35**	
				Combined Years			
A	36.96	37.69	40.03	39.28	40.05	39.10	38.85
B	40.56	38.83	37.34	40.29	39.77	40.05	51.20
Mean	**38,76**	**38.26**	**38.69**	**39.78**	**39.91**	**39.58**	

Mg concentrations were found higher (0.28 %) in 2015 season than 2016 (0.19 %). Variety A had higher Mg (0.29 %), but in 2016 it was found high (0.20 %) for variety B. Bozkurt and Karaçal (2000), were reported the seeds Mg concentrations between 0.073 and 0.099 % which were lower than our findings. Increasing doses of nitrogen both with phosphorus and potassium had no statistically significant effect on Fe concentrations. Bozkurt and Karaçal (2000), were also reported no significant difference and found the seeds Fe concentrations between 50.4 and 57.5 mg kg^{-1} which were higher than our findings. This situation is dependent on the amounts of the elements in the soil and antagonistic relations of the cations especially Ca, and Mg, and also the other micronutrients.

Effects on Cu, Zn, Mn, and B Concentrations of Sunflower Seeds

According to the variance analysis results of Cu, Zn, Mn, and B concentrations, years and its interaction between varieties were found statistically significant ($p<0.01$). B concentrations of the sunflower varieties, and its interaction between years were found significant ($p<0.05$) (Table 6 and 7). Interactions between varieties and nitrogen rates were only found significant at Cu ($p<0.01$) and Mn concentrations ($p<0.05$).

Table 6. Results of variance analysis and mean effects of nitrogen rate on on Cu, Zn, Mn and B concentrations of sunflower varieties in 2015, 2016 and combined years

	Cu			Zn			Mn			B						
													Cu	Zn	Mn	B
Varieties	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years	2015	2016	Combined Years	*F*	*F*	*F*	*F*
A	17.39 a	A 15.45 b	B 16.42	45.86 a	A 38.06 a	B 41.96	17.31 a	A 10.84 b	B 14.07	11.22 a	A 8.85 b	B 10.04b	Year (Y)	**	**	**	**
B	16.39 b	A 16.89 a	A 16.64	42.59 b	A 40.61 a	A 41.60	14.65 b	A 13.71 a	B 14.18	11.23 a	A 10.47 a	B 10.85a	Bloks x Year	**			**
N rate kg da^{-1}													Varieties (V)	ns	ns	ns	*
Control	17.31	16.03	16.67	44.00	38.93	41.47	17.03	12.01	14.52	11.53	9.78	10.66	Y x V	**	**	**	*
N0PK	16.32	16.09	16.20	43.21	38.99	41.10	15.24	12.47	13.86	10.73	9.48	10.11	Nitrogen (N)	ns	ns	ns	ns
N1PK	16.57	16.05	16.31	43.55	41.77	42.66	15.75	12.45	14.10	11.19	9.26	10.23	Y x N	ns	ns	ns	ns
N2PK	16.90	16.33	16.61	43.46	40.01	41.73	15.58	12.02	13.80	11.45	10.13	10.79	V x N	**	ns	*	ns
N3PK	16.69	16.39	16.54	45.84	38.92	42.38	16.02	12.24	14.13	10.86	10.33	10.59	Y x V x N	ns	ns	ns	ns
N4PK	17.55	16.14	16.84	45.30	37.37	41.33	16.25	12.44	14.35	11.61	8.99	10.30					
Mean	**16.89 A**	**16.17 B**		**44.23 A**	**39.33 B**		**15.98 A**	**12.27 B**		**11.23 A**	**9.66 B**					

The differences between values by different letters are significant. Capital letters are for each row and small letters are for each column. *P < 0.05, ** P< 0.01, ns not

Table 7. Effects of increasing doses of nitrogen on Cu, Zn, Mn and B concentrations of sunflower varieties.

	N rate kg da^{-1}						
	N0	N1	N2	N3	N4	N5	Mean
				Cu			
Varieties				2015			
A	17.49	16.68	18.32	17.01	17.55	17.30	17.39 a
B	17.13	15.96	14.83	16.80	15.83	17.80	16.39 b
Mean	**17.31**	**16.32**	**16.57**	16.90	**16.69**	**17.55**	
				2016			
A	15.24	15.39	15.83	15.19	15.75	15.30	15.45 b
B	16.82	16.79	16.26	17.47	17.03	16.97	16.89 a
Mean	**16.03**	**16.09**	**16.05**	**16.33**	**16.39**	**16.14**	
				Combined Years			
A	16.37 a A	16.03 a	17.07 a A	16.10 a A	16.65 a A	16.30 a A	16.42
B	16.97 a A	16.38 a AB	15.55 b B	17.13 a A	16.43 a AB	17.39 a A	16.64
Mean	**16.67**	**16.20**	**16.31**	**16.31**	**16.54**	**16.84**	
				Zn			
Varieties				2015			
A	44.07	43.76	47.03	45.76	49.04	45.49	45.86 a
B	43.93	42.66	40.07	41.15	42.64	45.10	42.59 b
Mean	**44.00**	**43.21**	**43.55**	**43.46**	**45.84**	**45.30**	
				2016			
A	37.50	37.88	42.02	37.84	37.03	36.09	38.06 a
B	40.37	40.11	41.53	42.17	40.82	38.66	40.61 a
Mean	**38.93**	**38.99**	**41.77**	**40.01**	**38.92**	**37.37**	
				Combined Years			
A	40.78	40.82	44.52	41.80	43.03	40.79	41.96
B	42.15	41.38	40.80	41.66	41.73	41.88	41.60
Mean	**41.47**	**41.10**	**42.66**	**41.73**	**42.38**	**41.33**	
				Mn			
Varieties				2015			
A	16.99	16.70	17.57	16.82	18.22	17.54	17.31 a
B	17.07	13.79	13.92	14.35	13.82	14.96	14.65 b
Mean	**17.03**	**15.24**	**15.75**	**15.38**	**16.02**	**16.25**	
				2016			
A	10.79	10.92	10.96	10.01	11.25	11.22	10.84 b
B	13.23	14.02	14.05	14.04	13.23	13.67	13.71 a
Mean	**12.01**	**12.47**	**12.45**	**12.02**	**12.24**	**12.44**	
				Combined Years			
A	13.89 b AB	13.81 a AB	14.21 a AB	13.41 a B	14.74 a A	14.38 a AB	14.07

B	15.15 a A	13.90 a B	13.99 a B	14.20 a AB	13.52 b B	14.31 a AB	14.18
Mean	**14.52**	**13.86**	**14.10**	**13.80**	**14.13**	**14.35**	
				B			
Varieties				2015			
A	11.99	9.88	12.32	11.16	10.37	11.63	11.22 a
B	11.07	11.59	10.07	11.73	11.35	11.59	11.23 a
Mean	**11.53**	**10.73**	**11.19**	**11.45**	**10.86**	**11.61**	
				2016			
A	9.35	8.29	8.21	9.29	9.61	8.38	8.85 b
B	10.21	10.68	10.32	10.96	11.06	9.61	10.47 a
Mean	**9.78**	**9.48**	**9.26**	**10.13**	**10.33**	**8.99**	
				Combined Years			
A	10.67	9.08	10.26	10.23	9.99	10.00	10.04 b
B	10.64	11.14	10.19	11.35	11.20	10.60	10.85 a
Mean	**10.66**	**10.11**	**10.23**	**10.79**	**10.59**	**10.30**	

Concentrations of Cu, Zn Mn, and B were found high in 2015 season and found as 16.89, 44.23, 15.89 and 11.23 mg kg^{-1} respectively. Bozkurt and Karaçal (2000), were reported the seeds Mn concentrations as 9.2 mg kg^{-1} and Zn as 39.7 mg kg^{-1} which were lower than our findings. It depends on pH, lime, and the amounts of the elements in the soil and antagonistic relations of the micronutrients. This is also related with the sunflower varieties which react differently to fertilizers under different ecological conditions. Although variety A had the highest Cu (17.39 mg kg^{-1}), Zn (45.86 mg kg^{-1}), Mn (17.31 mg kg^{-1}) in 2015 season, variety B had higher Cu (16.89 mg kg^{-1}), Zn (40.61 mg kg^{-1}), Mn (13.71 mg kg^{-1}) and B (10.47 mg kg^{-1}) concentrations in 2016 season. Variety B had the highest boron amount (10.85 mg kg^{-1}) than variety A in combined years result.

CONCLUSION

Increasing doses of nitrogen both with phosphorus and potassium elevated the nitrogen concentrations of the sunflower seeds. Nitrogen applications had also influenced the nutrients uptake of sunflower varieties and differences were found between the varieties nutrient concentrations. In addition to the amount of plant nutrients in the soil, their ratios among each other affected the growth and nutrient content of the plants. Although the varieties were grown under same ecological conditions in each year, the differences between the amounts of the elements in the soil effected their concentrations in the plants. The differences between the concentrations of the elements had effected the photosynthesis rate and the yield directly. In obtaining high yields in sunflower plants; besides selecting the cultivars, it was concluded that by taking into consideration the plant nutrient concentrations in the soil, especially the effect of phosphorus and potassium fertilizers, which will be applied in addition to nitrogen may be important.

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IMPACT OF CLIMATE VARIATIONS ON THE FLORISTIC DIVERSITY OF PROTECTED STEPPIC RANGELANDS - THE CASE OF THE ALGERIAN STEPPE

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ABSTRACT

The primary purpose of the present work consists of evaluating the floristic diversity of the protected *Stipa tenacissima* rangelands under the conditions of climatic disturbances that have affected the Algerian steppe. The floristic inventory was carried out in stations which represent rehabilitated and restored areas. We took reference stations near of this rangelands. The number of phytoecological surveys carried out was 195 surveys, with 135 species registered. All phytoecological surveys were performed subjectively, while respecting the station scale, and taking in to account the criteria relating to the structural, floristic and ecological homogeneity. The recorded statements were subjected to a Factorial Analysis of Correspondence (FAC) and a Hierarchical Ascending Classification (HAC). The interpretation of the results of the factorial analysis of correspondence have allowed to highlight the main groupings currently characterizing the *Stipa tenacissima* steppes. The results obtained helped to show the changes that occurred in the floristic composition of the vegetation in the protected rangelands. The environmental disturbances that the Algerian steppe is experiencing have led to a regressive dynamic of the *Stipa tenacissima* rangelands.

Keywords: Floristic diversity; Protected Steppic rangelands; Climatic disturbances; Regressive dynamic; *Stipa tenacissima*

INTRODUCTION

The Intergovernmental Panel on Climate Change (IPCC) under the auspices of the World Meteorological Organization (WMO) has reported that our planet's climate has undergone changes. Based on documents published by the IPCC and recent publications, the Mediterranean region is presented as very vulnerable to climate change (Folland, C. K., et al. 2001, Lionello, 2006a). The simulations proposed for the end of this century show that there is an increase in drought (duration, intensity and frequency) and the decrease in precipitation around 30% but varying according to the authors. As is the case for many Mediterranean countries, Algeria could not avoid these changes. These climatic fluctuations could jeopardize the conservation of Algerian steppic ecosystems and their sustainability.

The Algerian steppe extends over an area of 32 million hectares. This territory constitutes a vital space for a population of more than 9 million inhabitants, the majority of which derives its income through the practice of breeding a sheep herd estimated at more than 25 million heads. These last years, the synergistic combination of climate change and too intense anthropogenic pressures has engendered irreversible changes (loss of vegetation, alteration of soil by silting up caused by wind, etc.), as well as the impoverishment of the natural and social

heritage of the Algerian steppes (Haddouche and al., 2008; Benabadji and al., 2009; Nedjimi, 2012). It is worth noting that several studies have confirmed this problem (Floret and Pontanier, 1982; Le Houerou, 1985; Aidoud, 1994; Bedrani, 1999; Le Houerou, 2001 and 2002; Ferchichi and al., 2003; Aidoud and al., 2006; Nedjraoui and al., 2008). The cartographic assessment, carried out within the framework of the Roselt program in the southwest of Oran in 1978 (Roselt, 2003), allowed highlighting the regressive dynamics of the vegetation of all steppe formations. Indeed, this study reported the disappearance of facies, which were mapped in 1978, and were replaced by others, such as *Atractilys serratuloides* and *Peganum harmala*, which are indicators of degradation (Nedjraoui and Bedrani, 2008).

Being aware of the extent of threat and the importance of preserving steppe ecosystems against the desertification phenomenon, the Algerian Government has initiated programs aimed at restoring disturbed and degraded ecosystems (Nedjimi and Guit, 2012). Currently, increasing temperatures and precipitation alterations are significantly affecting the distribution and functioning of steppe ecosystems. The main purpose of the present work is to study the impact of these climatic changes on the floristic diversity of protected steppe ecosystems.

MATERIALS AND METHODS

Study zone

The area under study is located in the central part of Algeria; it is equidistant from the eastern and western borders of the country. The Wilaya (Province) of Djelfa occupies a strategic geographical position which makes it a real crossroads of exchange between different regions of the country (Figure 1). It lies between the geographic coordinates of 2° and 5° East longitude, and 33° and 35° North latitude. This is mainly a pastoral region with 95.74% of rangelands (including alfa) with respect to the total agricultural area. It covers a total area of 3 225 635 hectares. It has a Mediterranean climate that is characterized by cold winters and hot and dry summers, and low and irregular rainfall. Note also that the temperatures are low in winter and very high in summer. The flora is dominated by steppe vegetation such as Alfa, mugwort, drinn, etc. The present study focuses on four (04) stations, which represent defenses and pastoral plantations, in the southern and northern parts of the Wilaya of Djelfa, as shown on figure 1.

Figure 1. Location of the zone and the study stations

METHODOLOGY OF WORK

Data collection

Climate data collection

The climate data were provided by the meteorological station of the Wilaya of Djelfa. Our climate study was based on the exploitation of the data series collected over a period of 42 years, which extends from 1975 to 2016. The climatic data recorded in our country during the 20th century indicate an estimated warming of 1 ° C with an accentuated trend during the 21st century (UNDP, 2001). In this context, a comparative climate study was established between two periods (1975 - 1996) and (1997 - 2016). Then, two factors, namely precipitation (annual and monthly) and temperature (m, M and T), were addressed in this climate study. Aidoud Lounis, 1997 in his work considers the parameters (precipitation and temperature) as significant because they condition the distribution of plant communities in arid zones.

Collection of floristic and ecological data

The realization of the phytoecological survey was carried out in a subjective way, while respecting the scale of the station, and the criteria related to the structural, floristic and ecological homogeneity (Gehu and Rivas Martinez, 1981; Gehu, 1987 and Kadi Hanifi, 1998). For our study zone, the minimum area was determined according to the method of Braun-Blanquet and De Bolos (1957), depending on the shape of the area-species curve; the area retained was 100 m^2. This surface corresponds to the area previously determined by Djebaili (1978) for the whole of the Steppe. The surveys were carried out during the spring periods 2014-2015-2016 in plots corresponding perfectly to the homogeneity and representativeness of alfa facies. The nomenclature retained for our study is that of Quezel and Santa (1962 - 1963) and Ozenda (1977). All data are gathered in a double entry table, in which the columns correspond to the surveys and the rows represent the species.

Analysis of collected data

Analysis of climate data:

The collected data were entered in an Excel spreadsheet. The calculations performed are presented in tables which allow getting graphical representations of the results obtained. In the present work, the climate study was based on the calculation of the averages of temperature, and the totals of rainfall. The totals were obtained by cumulating the values obtained (Arlery and al., 1973).

Floristic data analysis

Numerical analysis

The first technique applied in this study is the Factorial Correspondence Analysis (FCA) which has long been used in phytosociology and phytoecology, and described by Guinochet (1952), Charles and Chevassut (1957), Pouget (1980), etc. This method makes it possible to extract the main environmental factors that organize the distribution of vegetation. This method was followed by the ascending hierarchical classification (CHA) which intervenes as an aid to factorial correspondence analysis (CFA).

From the data collected, an overall abundance-dominance matrix, containing 195 surveys and 135 species, was produced. This matrix was processed by the STATISTICA software, version 8.0.

Abundance-dominance matrix

Abundance is defined as the relative proportion of individuals of a given species, while *Dominance* is the area covered by that species. It was then decided to establish the correspondences between the percentages and Braun-Blanquet index: + < 1% (very low recovery), 1 ≥ 1% (weak but abundant recovery), 2 ≥ 5% (recovery greater than 5%), 3 ≥ 25% (25 to 50% recovery), 4 ≥ 50% (50 to 75% recovery), 5 ≥ 80% (recovery greater than 75%).

Characterization of groups

The different groups, which were individualized by the Factorial Correspondence Analysis (FCA) and Ascending Hierarchical Classification (AHC) were characterized on the plan: biological, phytogeographic and by:

Floristic richness (**S**): it is the simplest expression of biological diversity; it represents the number of species populating a given space.

Shannon index (H): It is expressed by the following formula:

H'= - $\sum \boldsymbol{Pi\ log2\ Pi}$ (Pi: Specific contribution)

This index varies from [0 to 5], it is maximum when the species have identical abundances in the stand and it is minimum when only one species dominates.

Regularity index (E): this index has for formula:
E = H '/ Hmax E = H' / log2 S

The regularity (E) varies between [0 to 1]; it tends towards (0) when almost all the numbers correspond to a single species in the population, and tends towards (1) when each of the species is represented by the same number of individuals (Ramade, 1984)

RESULTS AND DISCUSSIONS

Climate variability in the study area

Precipitations

Annual and monthly precipitations

Table 1. Monthly and annual precipitation in mm for the two periods (1975-1996) and (1997-2016): Wilaya (Province) of Djelfa

Periods	J	F	M	A	M	J	J	A	S	O	N	D	Cumulative
1975-1996	37,12	33,47	35,87	29,35	40,13	23,81	10,11	19,07	27, 1	28,57	32,93	28, 8	346,3
1997-2016	23,2	23,9	21,8	28,6	25,9	14,5	8,6	21,9	35,8	25,6	25,8	25,9	281,4

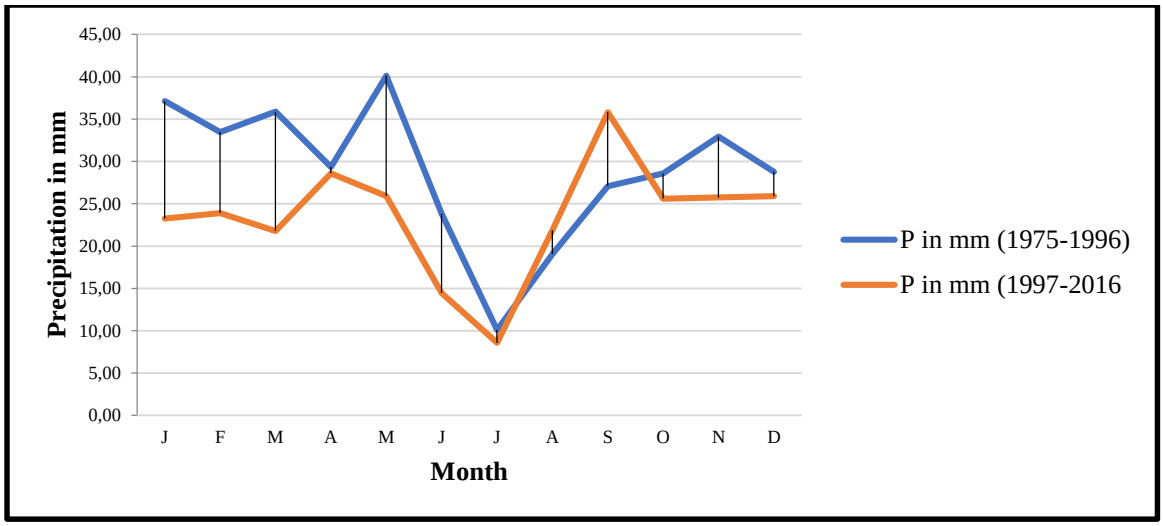

Figure 2. Monthly precipitation (in mm) between the periods (1975-1996) and (1997-2016): Wilaya (province) of Djelfa

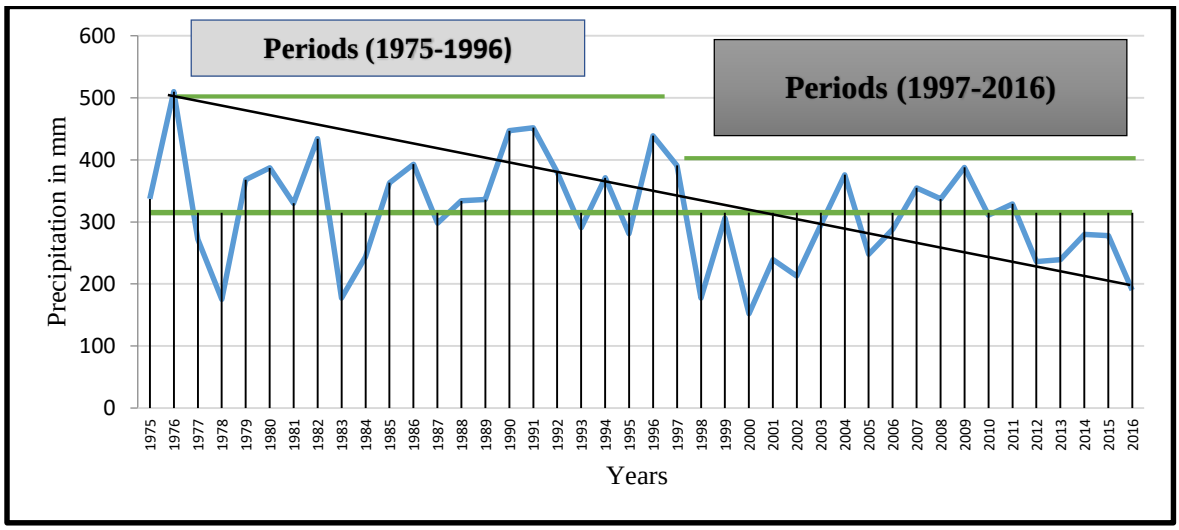

Figure 3. Annual precipitation in mm from 1975 to 2016 (comparative study between two periods (1975-1996) and (1997-2016)): Wilaya (province) of Djelfa

It was noticed, in the area under study, that precipitation was characterized by annual and inter-annual variability. A remarkable decrease in annual rainfall (a decrease rate of 19%) was observed during the last decades in study area. (Figures 2 and 3). This general downward trend in precipitation had a negative influence on the diversity of steppe ecosystems. The Algerian steppes were marked by a significant interannual variability of rainfall, showing a tendency to aridity and drought. A remarkable decrease in annual rainfall (decrease from 17 to 28%) was observed during the last decades, sometimes with several successive years of permanent drought (Djellouli, Nedjraoui, 1995; Hirche and al., 2007)

Temperatures

Annual and monthly temperatures:

Temperature is an essential meteorological factor that conditions the climate of any region. It is expressed as the monthly average temperature. It is also expressed as the monthly average of the minimums of the coldest month (m) and the monthly average of the maximums

of the hottest month (M); these averages represent, according to Sauvage (1963), the two thermal extremes between which plant life takes place. The analysis of Table 2 indicates that the monthly average of the minimums of the coldest month (m), which is the month of January, exhibits a positive trend, with an average temperature of 0.6 °C for the period 1997 - 2016, while the hottest month remains July for both periods, with a higher temperature value for the period 1997-2016. (Table 2, Figure 4). Average minimum and maximum temperatures increased over the entire study area; this same trend continued. Over the past two decades, the maximum temperatures have risen faster than the minimum temperatures, and the temperature elevation reached 2 °C. The rate of change in seasonal precipitation and the minimum temperature increase do have an impact on the distribution of species (IPCC, 2007; Hannah and al., 2005).

Table 2. Monthly minimum (m), maximum (M) and average (T) temperatures of the DJELFA station for the two periods (1975-1996) and (1997-2016)

Periods	Month Parameters	p	F	M	A	M	J	J	A	S	O	N	D	Annual
1975-1996	m en °C	0,34	1,85	3,04	5,61	9,7	14,77	17,5	17,45	13,63	8,75	4,72	2,09	8,29
	M en °C	9,83	11,97	14,67	17,8	22,96	29,33	33	32,86	27,48	20,78	14,65	11,02	20,54
	T en °C	5,09	6,91	8,86	11,7	16,33	22,05	25,25	25,16	20,55	14,77	9,69	6,56	14,41
1997-2016	m en °C	0,6	1,18	3,56	6,66	10,81	15,56	19,17	18,62	14,76	10,39	4,67	1,42	8,95
	M en °C	10,04	11,19	15,25	18,5	24,19	30,33	34,53	33,45	27,23	22,11	14,33	10,47	20,96
	T en °C	5,32	6,19	9,41	12,6	17,50	22,95	26,85	26,04	21,00	16,25	9,50	5,945	14,96

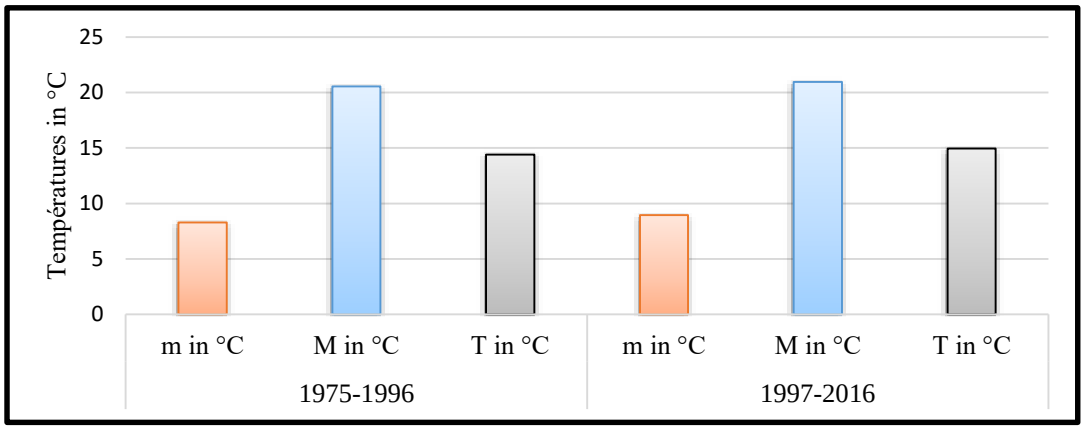

Figure 4. Minimum, maximum and average temperatures during the two periods 1975-1996 and 1997-2016

It is worth noting that in arid and semi-arid zones, ecosystems are subject to all types of disturbances (irreversible losses of biological diversity, modification of natural habitats, etc.).

Note that if the temperature rise exceeds 2 °C (IPCC, 2007), then between 20 and 30% of plant and animal species could be threatened with extinction. Under these conditions, important

changes in the structure and function of ecosystems could occur, which can certainly engender bad consequences on biodiversity.

Global numerical analysis of flora

A matrix of 195 surveys and 135 species was subjected to factorial correspondence analysis (FCA), while taking into account the abundance-dominance coefficients of the species.

Identification of the different plant groups and diversity

The factorial map, resulting from the factorial correspondence analysis (FCA), depicts the distribution of plant groups as a function of the environmental variables (Figure 5).

The percentages of inertia explained by each of the axes have been taken into consideration.

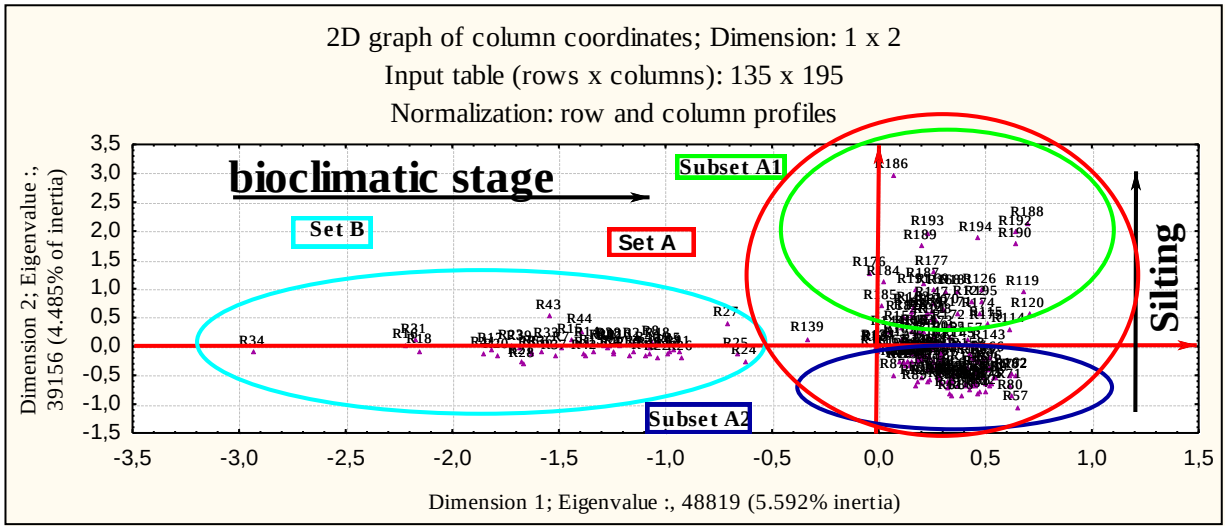

Figure 5. Situation of Set B and A of the CHA on plan 1 and 2 of the AFC

Negative side of axis 1

Set B - This set gathers the surveys that are strictly related to the arid climate, grouping all *chamaephytic* formations of *xerophytic* character.

Positive side of axis 1

Set A - This set is made up of surveys relating to steppes linked to the semi-arid bioclimate.

This set is subdivided into two subsets:

Negative side of axis 2

Subset A2 : This subset consists of surveys from more or less open matorral groupings and syrveys of steppes often wooded with *Stipa tenacissima*; this stony steppe is located on the glacis of the ancient Quaternary where the presence of a shallow, very hard soil (limestone accumulation) conditions vegetation adapted to this environment such as: *Noaea mucronata, Herniaria fontanesii, Helianthemum virgatum*

Positive side of axis 2

Subset A1 : brings together the surveys relating mainly to the Alfa steppes on sand, these mixed steppes are found on encrusted glacis of the Middle Quaternary covered by a more or less thick sandy Aeolian veil favoring the presence of numerous perennial and annual

psammophiles such as : *Thymelaea microphylla , Calendula aegyptiaca, Bromus rubens, Stipa tortilis*.

Then, an Ascending Hierarchical Classification (AHC) was used for the purpose of classifying the surveys into homogeneous groups, according to Ward's method, using a similarity index of Euclidean distance.

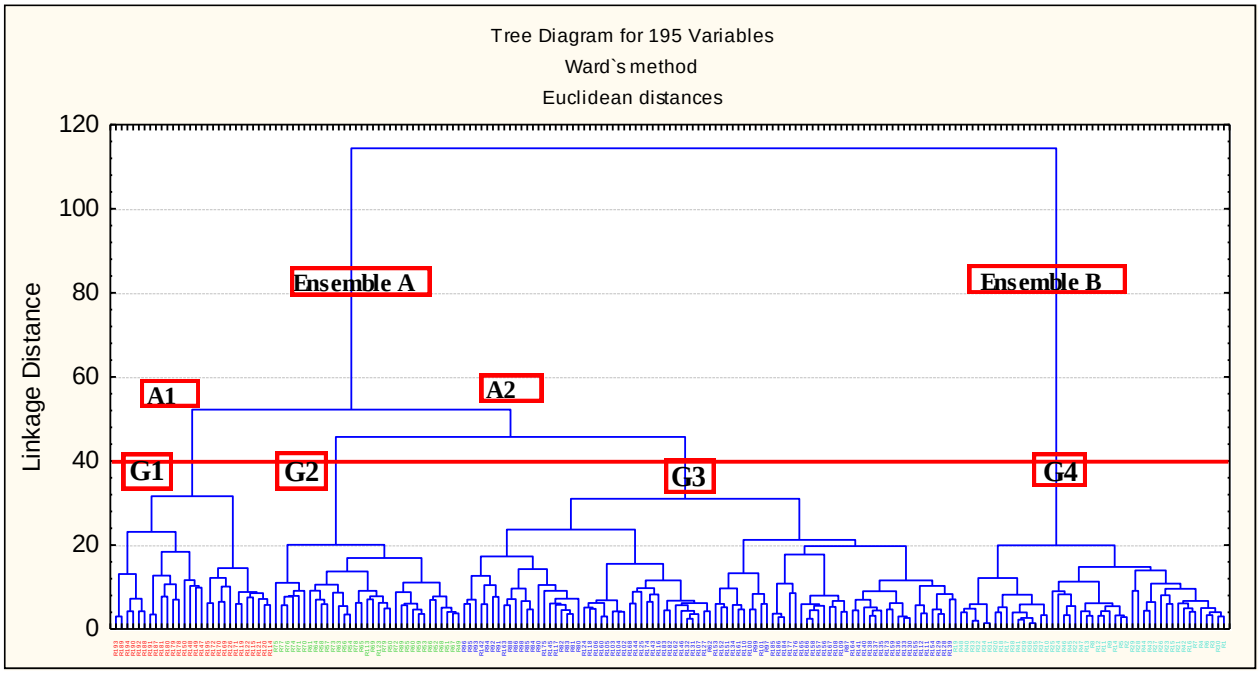

Figure 6. Ascending hierarchical classification of 195 surveys

The results of the factorial correspondence analysis and ascending hierarchical classification made it possible to distinguish four (04) groups: (Figure 7)

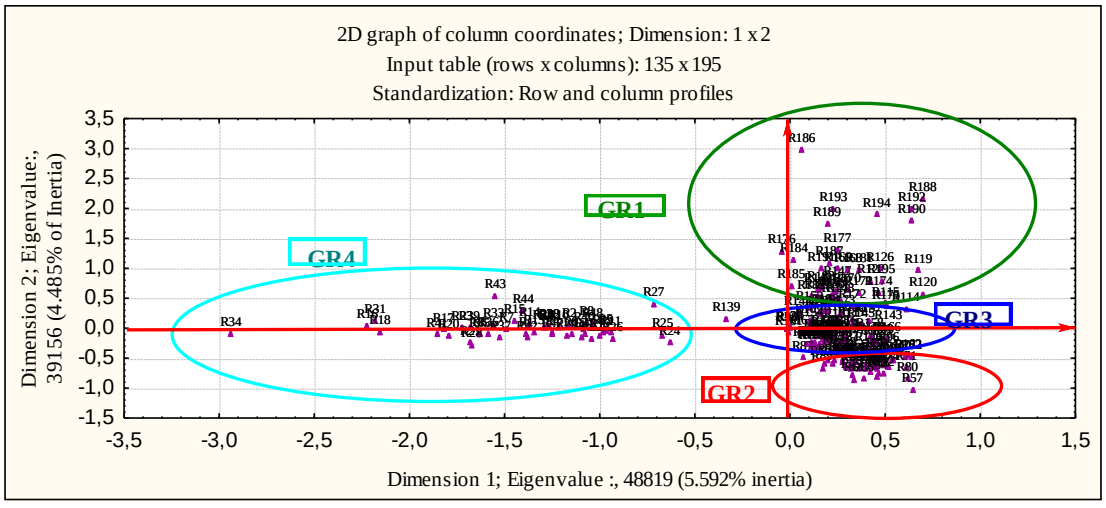

Figure 7. Situation of CHA groups on AFC plan 1-2

Group of *Stipa tenacissima* on sand (G1) - This grouping represents the regressive evolution of *Stipa tenacissima* formations, previously reported by several authors, in particular Pouget (1980), Aidoud-Lounis (1997), and Kadi-Hanifi (1998)

Group of *Stipa tenacissima, Helianthemum virgatum* and *Atractylis serratuloides* (G2) - It was observed that although the surveys were carried out in an area under deferred grazing, it suffered

intense degradation due to overgrazing. This is confirmed by the presence of *Atractylis serratuloides* and *Noaea mucronata*.

Group of *Stipa tenacissima*, *Stipa parviflora* and *Atractylis serratuloides* (G3) - In this group, there are mainly species that are linked to soils of degraded forest environments with various textures (rockery, crust), such as *Noaea mucronata*, *Herniaria fontanesii*, *Teucrium polium* , and *Thymus algeriensis* (Quezel and Santa, 1962-1963; Le Houerou, 1969; Pouget, 1980). It is also interesting to note the abundance of annual species such as *Plantago Albicans*, *Malvaa egyptiaca*, *Shismus barbatus*, *Herniaria fontanesii*, *Artemisia campestris*, encountered in pastures and sandy environments (Quezel and Santa, 1962-1963 ; Pouget, 1980).

Group of *Stipa tenacissima*, *Arthrophytum scoparium* and *Astragalus armatus* (G4) - Several researchers (Ozenda, 1954 ; Boughani, 1987 and 2014) have reported the existence of Saharan species in alfa rangelands. A very clear change has been noted in recent years; indeed, *Arthrophytum scoparium* has invaded the land and alfa was replaced with remth (Boughani, 2014). From a physiognomic point of view, this set corresponds to pre-Saharan steppe formations of *Stipa tenacissima* and *Arthrophytum scoparium*. This set is characterized by the lowest overall vegetation cover (32%) of all groups. From a floristic point of view, the most characteristic species are *Aristida obtusa*, *Argerolobium uniflorum*, *Medicago laciniata*, *Atractylis cancellata*, *Erodium glaucophyllum*, *Anabasis oropediorum and Fagonia microphylla.*

Qualitative assessment of biodiversity

Biological spectra

The biological spectrum is a graphic representation of the spatial distribution of different biological types over a given area. It takes into account the position of the renovationl bud of the plant relative to the soil during the cold period. (Daget, 1980)

Real biological characterization of individualized groups

The weighting by species recovery makes it possible to highlight three situations, shown in Figure 8, namely: CH> GE> HE> TH> PH, then CH> HE> GE> TH and TH> HE> CH > GE

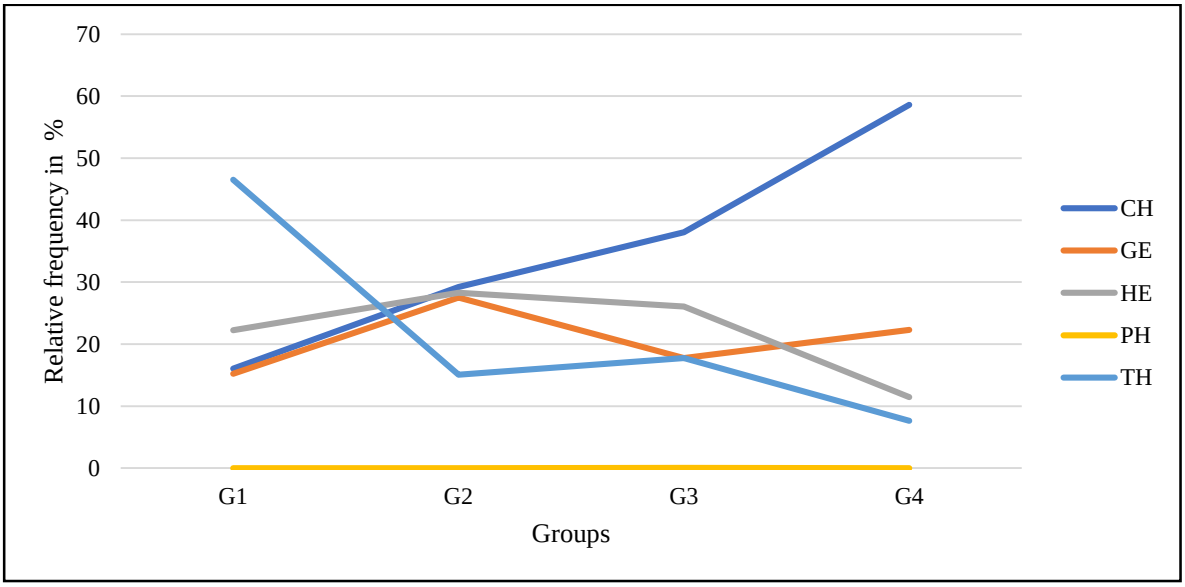

Figure 8. Real biological spectra of the different groups

(CH: Chamaephyte, HE: Hemicryptophyte, PH: Phanerophytes: GE: Geophyte, TH: Therophyte)

The distribution of the real biological types (Figure 8) shows the strong contribution of the *chamaephytes* which occupy the first position in the three groups G2, G3 and G4. This not insignificant representation may be explained by their good adaptation to environmental conditions. Indeed, according to Benabadji and Bouazza (2002), chamaephytes adapt better to drought because they are more xerophilic. As previously stated by Kadi-Hanifi (1998), the intensification of the anthropozoic action, associated with the aridification of the climate, would favor the dominance of chamaephytes such as *Astragalus armatus*, *Arthrophytum scoparium*, *Atractylis serratuloides* and *Noaea mucronata*. The relatively high rate of therophytes in group 1 may be assigned to the self-mulching phenomena which are mechanisms mainly encountered in silted areas. These phenomena reflect, at the same time, the effects of successions of dry years and overgrazing (Le Houerou, 2001).

Phytogeogrphic Types

Real phytogeogrphic characterization of individualized groups

Figure 9 shows the overlapping dominance of Mediterranean elements in all groupings except for group G4 in which the Mediterranean-Saharo-Arabian elements dominate. A negative correlation was observed between the Mediterranean and the Mediterranean-Saharo-Arabian elements (Amghar, 2002). The Saharo-Arab elements come second in all the groups, which testifies to the impact of climate aridity on the structure of the steppe flora. (Figure 9)

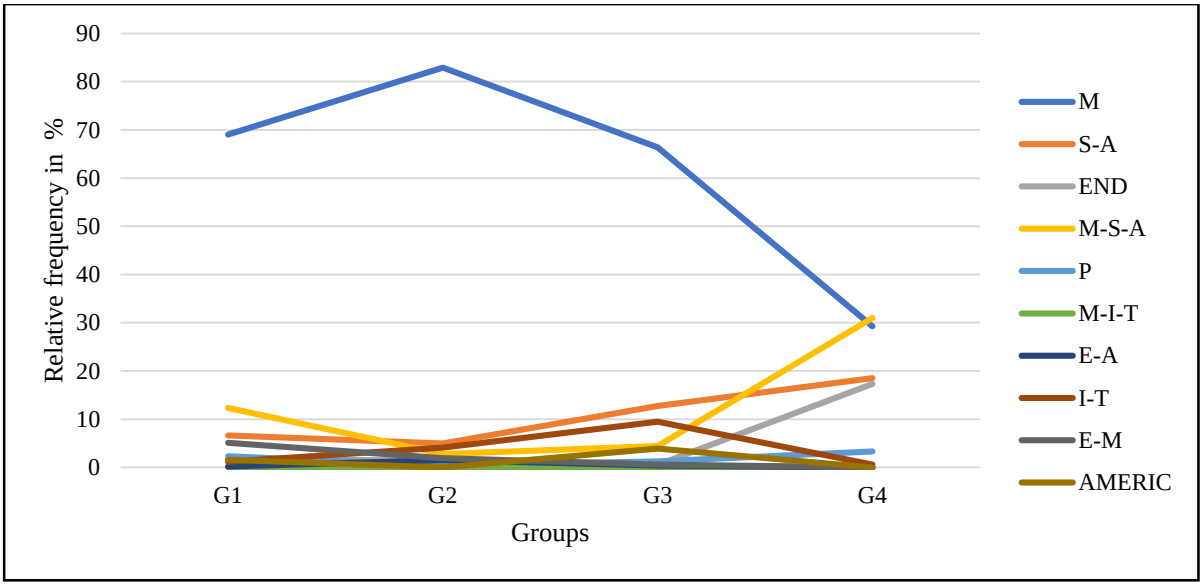

Figure 9. Real phytogeographic types of the different groups

(AMERIC: American, E: European, EA :, Eurasatic, EM: Ero-Mediterranean, M Mediterranean, MIT: Mediterranean-Iranian-Turanian, IT: Irano-Turanian, MSA: Mediterranean-Saharo-Arabian, SA: Saharo-Arabian , END: Endemic, P: Pluriregional

Quantitative assessment of biodiversity

Table 3. Distribution of diversity indices by groups

Ensembles	Groupements	S	H'	E
Ensemble A	G1	74	0,22	0,04
	G2	77	0,3	0,1
	G3	83	4,52	0,69
Ensemble B	G4	62	3,59	0,6

S: specific richness, H': Shannon index, E: regularity or equitability index

It is worth noting that with aridity, the floristic richness decreases (Amghar, 2002); it goes from 83 species (found in a semi-arid bioclimatic stage) in group G3, to 62 species (found in an arid bioclimatic stage) in group G4 (Table 3). With excessive exploitation of a deferred area and prolonged drought, only chamaephytes can resist and persist with high recovery, which gave a very low diversity (H') and regularity (E) indices for group G2. It is worth indicating that silting as well as excessive exploitation have a negative impact on floristic diversity, which is justified by the very low Shannon and regularity indices for group G1. (Table 3)

CONCLUSION

The present study allowed showing that, during the last two decades, the amount of rainfall has exhibited a tendency to decrease, while the average minimum and maximum temperatures have increased over the entire study area. This climate variability accentuated the degradation of steppe ecosystems and therefore engendered modifications in the composition of vegetation, even that of the protected areas. The excessive exploitation of these perimeters in recent years has led to a regressive dynamic of the vegetation and a very appreciable decrease in the productivity of the rangelands, resulting from the disappearance of palatable species and the development of species with little or no appetite. Faced with this situation, adaptation proved necessary, in order to cope with climatic variations, by implementing an adaptive management strategy in this circumstance of rapid change. This would certainly help to minimize the negative impacts on the economy of the steppe region and on the local populations too.

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PSYCHOSOCIAL PROPERTIES OF CLINICAL LEARNING ENVIRONMENT FROM THE PERSPECTIVE OF STUDENTS IN HEALTH LICENSE EDUCATION

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ABSTRACT

Health science undergraduate education has long been an important requirement in providing human resources to the health sector. In order to become a good clinical professional, it's necessary to have in-depth knowledge in medicine. Besides; it needs to have great clinical experience. To become professionals, the students must go through challenging learning processes. Health professionals must practice in clinical areas to develop their basic skills. The purpose of our study; to present a comprehensive picture of students' perceptions about the obstacles that students may encounter in clinical settings. Our study was carried out with Trakya University School of Health Science undergraduate students in November 2019- May 2020. The Clinical Learning Environment Inventory (CLEI) was used. This scale consists of 42 items. It has two versions; 1. "Real learning environment"; 2. "Preferred learning environment". It examines students' perception of psychosocial features of real learning environments. At the same time, the scale; evaluate how they want the ideal learning environment to be. It consists of six subscales. The sub-scales are: personalization, student participation, satisfaction, task orientation, teaching innovation, individualization. 280 undergraduate students participated in our study. The average age was 19.7. The number of girls in our study was 159; men was 121. After the students were asked about their sociodemographic characteristics, the scale was used. It asks students to perceive the psychosocial characteristics of real learning environments and how they think of the preferred or ideal learning environment. In terms of gender; there was no statistical difference for the scale. In the study, participants; scores from personalization and student participation subscales were higher in the real learning environment. The students; scores from satisfaction, teaching innovation, individualization subscales; it was higher in idealized clinical settings. The total scale scores of the 4th grades; were higher than the scores of students in the 1st, 2nd and 3rd grades. The students may have to deal with patients with different physical and psychological problems and contribute to their care during clinical practice. However, with systematic home visits, public health interventions, rehabilitation center project studies, they can gain experience outside of a more medically oriented environment. Thus, it will be easier for students to have a wide perspective and their self-confidence will be increased. Undergraduate programs should be developed with a proactive and holistic approach in order to create a sensitive workforce in the future.

Keywords: Health science undergraduate education, clinical learning environment, Clinical Learning Environment Inventory (CLEI)

INTRODUCTION

Health sciences undergraduate education has long been an important requirement in providing human resources to the health sector. Good clinicians are professionals with both in-depth knowledge of medicine and great clinical experience (D. S. Chan, 2003). In addition, they must go through challenging learning processes in order to be valuable and skilled healthcare professionals. Practice in clinical fields is essential to develop basic skills professionally (Papathanasiou, Tsaras, & Sarafis, 2014). For this reason, the mission of health sciences undergraduate and graduate education becomes more important than ever. Clinical learning

environment is an important factor affecting the teaching and learning process (D. Chan, 2002). This clinical environment is a challenging area as it is completely different from the classroom (Papathanasiou et al., 2014). In practice environments for students; clinical technology, all interactions with other people, physicians, patients, relatives and other healthcare professionals may be potential concerns.

Health sciences are a practice-based profession. Professional disciplines such as health sciences require a clinical learning environment that will prepare students to become well-equipped practitioners (Chang et al., 2013). Therefore, clinical experience is essential when preparing health sciences students for their professional roles. The role of clinical learning in health sciences undergraduate education has been proven. It is emphasized that health sciences students should benefit from the training in clinics (D. Chan, 2002; Chang et al., 2013).

Cooperation between educational institutions and health institutions is essential. By examining the findings of this study; Special recommendations can be made regarding educators, clinical staff, quality of clinical settings, and health sciences students in the field of practice.

Aims and Scope

Purpose of the study; to present a comprehensive picture of health science students' perceptions of the barriers they may face in clinical settings.

MATERIAL AND METHODS

Our study was conducted with Trakya University Health Sciences Undergraduate School, health sciences students between November 2019 and March 2020. Clinical Learning Environment Inventory (CLEI) consists of 42 items. The scale has two versions; "Real learning environment" and "preferred learning environment". The validity and reliability of the scale has been proven (D. S. Chan, 2003). The scale; asks health undergraduate students to perceive the psychosocial characteristics of real learning environments and how they think of the preferred or ideal learning environment.

The scale consists of six subscales: Personalization (emphasis on the student's individual opportunities to interact with faculty members and concern for the student's personal well-being), Student Participation (degree of active and attentive participation of students in practical activities), Satisfaction (the scope of which is the pleasure of clinical placement), Task Orientation (how open and well organized the practice activities are), Teaching Innovation (The instructor's new, interesting and productive internship experiences, teaching techniques, learning activities and patient care planning degree) and Individualization (different application based on students' decision-making authority and ability or interest). skill). It is a five-point Likert type scale (Strongly Agree = 5, Agree = 4, Undecided = 3, Disagree = 2, and Strongly Disagree = 1).

Ethical consideration

The study was approved by the Ethics Committee of Scientific Research of Trakya University Faculty of Medicine

Statical evaluation

All statistical analyses were performed using the SPSS 20.0 package program. Normal distribution of the data was tested using the Shapiro-Wilk test. Bivariate group comparisons were performed using Student’s t-test and the Mann-Whitney U test. The data are summarized with appropriate descriptive statistics. Mean and standard deviation were evaluated for

numerical variables, and frequency and percentage were evaluated for categorical variables. Level of significance for all statistical analyses was accepted as 5%.

RESULTS

Our study was conducted with Trakya University Health Science School undergraduate students in November 2019- March 2020. The students were asked to sign informed consent forms for the study. 280 undergraduate health science students participated in our study. In our study, the average age of the students was 19.7.

The number of girls in our study is 159; men were 121. After asking the students about their sociodemographic characteristics, the Clinical Learning Environment Inventory (CLEI) was used. The CLEI; It consists of 42 items. The scale has two versions; "Real learning environment"; "Preferred learning environment". It asks students to perceive the psychosocial characteristics of real learning environments and how they think of the preferred or ideal learning environment. The CLEI consists of six subscales: personalization, student engagement, satisfaction, task orientation, teaching innovation, individualization. In terms of gender; there was no statistical difference for the scale ($p = 0.232$). When the participants are evaluated according to their country of origin; There was no statistical significance for the scale ($p = 0.171$). In our study, while the scores they got from personalization and student participation subscales were high in the real learning environment; satisfaction, teaching innovation, individualization; the preferred environment was high. The total scale scores of the 4th grade students were higher than the first, second, and third grade students for both environments.

DISCUSSION

The results of this study show a number of important results for health university education. Participants had low overall scores on all subscales. This means that health science students may not have an awareness of the importance of the psychosocial features of clinical settings.

Health science students may not fully understand the social, health and economic impacts at the individual and societal level. Participants in our study did not have enough awareness and knowledge. As a result, they may miss out on adequate screening and recognition of patients they will care for in the future. This may result in less detection of health problems both in health centers and at the community level. In addition, more attention needs to be paid to health science interventions that address patient's emotional needs. For university students to play an important role in this at a wider level, students must understand and develop many different approaches (such as preventive strategies) beyond what they perceive as the main clinical roles of patient supportive care.

Good practice clinical settings and experiences for health sciences university students who will become future health professionals; It can be established with good cooperation between educators and clinical staff (Papp, Markkanen, & von Bonsdorff, 2003). Cooperation between educational institutions and health institutions is essential. Considering the findings of this study, there are some specific recommendations regarding universities, academic lecturers, clinical staff and health science students. The aim of the undergraduate health sciences program is to train students who are well-equipped and able to provide safe, competent and compassionate care in professional business life in the future. It is important to provide health sciences undergraduate students with a constructive and positive clinical learning experience. How this can be achieved is frequently discussed in the literature. For example, Chen, Brown, Groves, and Spezia (2007) suggest that self-care homes (also called elderly rehabilitation homes) offer a better opportunity for undergraduate students to learn acute psychiatric and

communication skills at their beginner level than hospitals (Chen, Brown, Groves, & Spezia, 2007).

Some researchers; suggests practice in hospital for beginners (D. S. Chan, 2003). One of the best definitions of clinical teaching in hospitals was made by McCabe as "the heart of vocational education". Common denominator; Clinical practice experience in hospitals and other healthcare services is an important part of health science education.

CONCLUSION

Health sciences students may have to deal with patients with different physical and psychological problems during their clinical practice. However, in addition to practices in clinics, they can also gain experience outside of a more medically oriented environment with systematic home visits, public health interventions, rehabilitation center project studies. Thus, it will be easier for students to have a broad perspective and their self-confidence will be increased. Undergraduate education programs should be developed with a proactive and holistic approach to create a sensitive health sciences workforce in the future.

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LIMITATIONS

Our study is an example of Trakya University School of Health. Further studies can be carried out in the following years, with the participation of more students or by taking samples from health sciences undergraduate students from all over the country.

CONFLICT OF INTEREST

There is no conflict of interest.

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EFFECTS OF FENUGREEK SEED (*TRIGONELLA FOENUM GRAECUM*) SUPPLEMENTATION ON PERFORMANCE OF MID LOCATIONAL GOATS

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ABSTRACT

Galactogenic plants are used to increase milk production both in human and lactating animals. There are a limited numbers of studies in the literature about the use of fenugreek seeds for increasing ruminant animal's milk production. This study was aims to determine the effects of fenugreek seeds on performance of mid lactation goats. For this purpose 20 heads of Turkish Saanen goats were used as an animal material of the study. The experiment animals kept in individual cages during the study. The study was carried out on the basis of two group Control (CON; n=10) was fed with total mixed rations (TMR) while the Fenugreek group (FG; n=10) was fed with total mixed rations (TMR) and 100 g/day fenugreek seeds. In the experiment feed intake was recorded daily, body weight, milk yield and milk nutrient compositions were determined weekly interval. There were no differences between the groups about daily dry matter, crude protein and metabolizable energy intake ($P>0.05$). The fenugreek supplementation did not change live weight ($P=0.4940$) and milk production ($P=0.9251$). But increased 3.5% fat corrected milk yield (FCM). The fenugreek supplementation was increased milk fat ($P<0.0001$) and total solids ($P=0.0002$) concentration. In conclusion fenugreek seeds supplementation level at 100 g/day was significantly increase fat concentration in goat's milk. There is a need for future studies to determine the effects of supplementing different level of fenugreek seeds and it is effects on milk fatty acid profile.

Keywords: Galactogen, lactation, milk production, milk fat, Turkish Saanen goat

INTRODUCTION

Milk production and quality can be altered by feed additives. Probiotics, enzymes and some plants have some special effects (Mishra et al., 2006). It is reported that plants such as *Trigonella foenum graecum, Foeniculum vulgare, Galega officinalis, Asparagus racemosus, pimpinella anisum, Milk thistle* are increase milk production (Westfall, 2003; Mohanty et al., 2014; Tabares et al., 2014). Fenugreek seeds (Trigonella foenum graecum) have long been use for increase breast milk production by Arabian and Indian Women (Al-Shaikh et al., 1999; Tiran, 2003). In addition used as a feed for support lactation and lactation performance in ruminats (Kheder et al., 2012; Balgees et al., 2013; Değirmencioğlu et al., 2016).

Fenugreek seeds are have aromatic, bitter taste, strong odor (Losso et al., 2009) and have antibacterial (Haouala et al., 2008) and galactogoue activity. Seeds contain high protein (20-30%), high fat (5-10%) and certain amount of saponins (0.6-1.7%) (Mehrafarin et al., 2010). Plants which contain saponin can stimulate feed consumption (Kamel, 2000), nutrient metabolism and hormon secretion (prolactin, TSH and T_4) (Kheder et al., 2012).

The objective of this study was to investigate the effects of fenugreek seeds on performance of mid lactation goats.

MATERIAL AND METHODS

The research protocol of this study was approved by the Animal Care and Use Committee (2010/4-03) of Çanakkale Onsekiz Mart University. Twenty Turkish Saanen goats 39.54±0.3 kg of LW were selected from the Faculty of Agriculture Research and Application Farm Animal Production Unit of Canakkale Onsekiz Mart University. The study was conducted for a period of 8 weeks. The selected goats were at the mid stage of lactation (110±7 DIM; 1.7 ±0.03 L/d). Goats were kept and fed in individual cages (1.5X1.5 m). Goats were randomly assigned to two equal groups (10 animals in each group). The Control (CON) goats were fed with a total mixed ration (TMR) without fenugreek seeds. The Fenugreek (FG) group was fed with a total mixed ration (TMR) and fenugreek seeds. The basal diet was in a form of total mixed rations (TMR) which obtained from feed factory (Rasyonel Agriculture food industry/ Turkey) in a semi vacuum packs (40 kg/pack). Daily amount of TMR offered to meet the energy and protein requirements of experiment goats averaging 40 kg of live weight and 2 kg daily milk production according to NRC (2007). FG group was fed with the TMR and 100 g /day fenugreek seeds per head. Individually, FG group animals were fed with fenugreek before morning and evening meal equal weight (50 g/meal). The feeds offer to the goats twice a day (08:30 am in the morning and 16:30 pm in the afternoon). The goats allowed free access water and limestone through the study. The goats feed intakes were monitored on a daily basis for this purpose feeds refusal from previous day were collected and recorded individually. Ration samples were collected weekly to determine the chemical composition. The goat's daily nutrient intake was calculated by the difference between the analyzed chemical content of TMR and fenugreek seed in the offered and refusals feed weights.

Table 1. Chemical composition of total mixed ration and fenugreek seeds

Feeds	DM	CP	CF	NDF	ADF	ADL	Ash
TMR	51.00	16.81	3.5	47.98	39.84	7.28	8.99
Fenugreek	91.00	29.18	3.91	13.75	10.55	2.26	3.94

DM: dry matter, %; CP: crude protein, %DM; CF: crude fat, %DM; NDF: neutral detergent fiber, %DM; ADF: acid detergent fiber, %DM; ADL: acid detergent lignin, %DM

Live weight (LW) changes of goats were determined weekly basis. Milk yield of individual animals were determined by weekly interval (18:00 pm-09:00 am) with milking machine and milk samples taken (50 ml/goat; mixed equal volume of evening and morning milk) for chemical composition. Milk was analyzed for fat, protein, solids-non-fat (SNF) and lactose by an ultrasonic milk analyzer (Milk Lab Minor Milk Analyzer). Dry matter content of milk samples calculated by multiple solids-non-fat and fat content.

Statistical Analysis

Statistical analyses were conducted using the SAS (1999) program. The repeated measured data (feed intake, live weight, milk yield and composition parameters) were analyzed by the GLM procedures. The least squares means given in the tables LSMEANS options.

RESULTS

Goats dry matter, crude protein and metabolizable energy intake did not affected by Fenugreek seed supplementation (P>0.05). Live weight (P=0.4940) and milk yield (P=0.9251) of goats did not changed by Fenugreek seed supplementation. On the other hand fenugreek supplementation was increased fat corrected milk (3.5 % FCM) yield (P=0.0082).

Table 2. Feed intake, milk yield and milk composition of experimental goats least square means (LSM), standard error of means (SEM) and P values

Item	CON		FG		P
	LSM	SEM	LSM	SEM	
DMI, kg/d	1.64	0.076	1.55	0.073	0.4054
CPI, g/d	277.69	20.991	262.39	12.502	0.4054
MEI, Mcal ME/d	4.27	0.199	4.04	0.192	0.4047
LW, kg	39.83	0.499	39.32	0.534	0.4940
Milk Yield, kg/d	1.37	0.051	1.36	0.058	0.9251
3.5% Fat corrected milk (FCM)	1.27	0.034	1.41	0.038	0.0082

Milk compositions of experimental goats are presented in Table 3. Milk fat concentration were higher in FG group than CON group due to milk yield and fat concentration (P<.0001). Protein, lactose and non- fat solids were found similar between the groups (P>0.05). The highest total solids (P=0.0002) content was found in FG group (Table 3). The fenugreek supplementation did not affect feed efficiency in the experiment (P=0.1605).

Table 3. Milk chemical composition of experiment goat's least square means (LSM), standard error of means (SEM) and P values

Item	CON		FG		P
	LSM	SEM	LSM	SEM	
Fat, %	3.74	0.089	4.46	0.093	<.0001
Protein, %	2.88	0.018	2.91	0.019	0.2886
Lactose, %	4.33	0.027	4.38	0.029	0.1741
Total solids, %	11.79	0.114	12.41	0.120	0.0002
Non –fat solids, %	7.87	0.049	7.95	0.051	0.2552
Feed efficiency	0.77	0.032	0.84	0.035	0.1605

DISCUSSION

The fenugreek seeds supplementation increase dry matter consumption of dairy goats (Smit, 2014). Due to fenugreek seeds contain certain amount of saponins (Mehrafarin et al., 2010) and Plants which contain saponin can stimulate feed consumption (Kamel, 2000). Şahin et al. (2003) reported that feeding fenugreek seed at a level of 0, 2, 4 and 8 % did not affect feed intake. In this study there were no differences between the CON and FG groups about dry matter, crude protein and energy consumption. The fenugreek supplementation did not change the goats live weight (P=0.4940). On the contrary Kheder et al. (2012) reported that fenugreek seeds supplementation (2.5 and 5 g/d kg of LW) for 7 weeks were enhancing the live weight of Sudanese desert ewes. The authors conclude that live weight change of ewes was associated with the increase of feed consumption.

Fenugreek seed supplementation at the level of 10% and 15% were significantly increase milk yield in Sudanese Nubian goats after two months postpartum (Balgees et al. 2013). Kheder et al. (2012) reported that administration of fenugreek seed (2.5-5 g/kg LW) for 7 weeks to mid lactating Sudanese desert ewes increased daily milk yield. Kheder et al. (2012) reported that fenugreek supplementation increased blood prolactin level which has detrimentally effect on milk production. In this study fenugreek seed supplementation did not increased milk yield (P=0.9251). It may due to study was conducted in mid lactation period (110±7 DIM) and supplementation level of fenugreek seed (100 g/d) was not adequate to increase milk yield of goats.

The 3.5% FCM (P=0.0082) value was increased by fenugreek seed supplementation. Because of FG group have the highest milk fat (P<.0001) concentration. Likewise the total solids percentage of FG group higher than CON group (P=0.0002). El-Alamy et al., (2001) reported that fenugreek supplementation to buffaloes increased milk yield but decreased milk fat percentage. Abo El-Nor et al., (2007) reported that fenugreek supplementation increased milk lactose content of buffaloes in their study. However milk fat content did not change in their study. Feed efficiency did not affected by fenugreek supplementation (P=0.1605). It was reported that fenugreek seed supplementation was increased feed efficiency in lactating buffaloes (Abo El-Nor et al., 2007).

CONCLUSIONS

Supplementing fenugreeks seeds to mid lactation dairy goats ration at 100 g/head day did not increase milk production, dry matter consumption and live weight. Fenugreek seed supplementation was led to increase milk fat and total solid percentage. In future work the effects of fenugreek seed supplementation on milk fatty acid profile and milk aroma compound could be investigated.

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SURVEY ON THE PREPARATION OF A TRADITIONAL ROASTED MULTIGRAIN FLOUR: BSISSA

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ABSTRACT

Bsissa is one of the oldest traditional breakfasts in North Africa. It is made of roasted multigrain flour based on cereals, legumes, spices, and aromatic herbs. Bsissa is usually consumed mixed with water, milk, or oil. This traditional meal plays an important role in heritage food in Tunisia, and we can find many recipes differing from region to another. So that investigate the processes of Bsissa preparation and to determine its common recipe, a survey was conducted in the city of Lamta. This city, located on the central coast of Tunisia, is known for its culinary heritage and especially by its festival of Bsissa organized annually in April-May. In order to determine the know-how of artisan women, fifteen of them were interviewed. The results showed that the average age of producers is 49 years old and that the majority of them are artisans who sell their goods at fairs or on order. The majority of them inherited the formula from their antecedents (73 %) and the rest have followed of training.

The investigation shows that 67% of producers buy their raw materials from supermarkets. After purchasing the ingredients, the preparation of Bsissa begins with the cleaning of the grains, followed by roasting, grinding, and finally sieving to preserve them in the form of a homogeneous powder. They claimed that Bsissa flour can be stored for 3 to 12 months, at room temperature, in a non-humid place.

Then, 103 types of Bsissa were collected and their recipes were characterized. These Bsissa were prepared mostly from cereal 74.75% (durum wheat (26), sorghum (34), barley (13), oat (4)) and legumes 18.44% (fenugreek (6), lentil (13)) and 6.79% Bsissa of sesame (7). Chickpea, anise seed, fennel seed, and coriander were the most common ingredients in all the different Bsissa formulas, respectively16.38%,12.56%,11.32%, and 11.08%. This study indicates that the ingredients of Bsissa flour range from 1 to 9 ingredients and this diversity of composition can satisfy the preferences of a wide range of consumers.

Finally, the estimation of the nutritional composition of these different recipes confirmed their high nutritional value and the role that this traditional food can play in the diet of consumers, as well as on the economic development of the rural community.

Keywords: Bsissa, Heritage food, Multigrain, Recipe, Survey, Artisan women.

INTRODUCTION

Traditional foods are based usually on old recipes identified in specific regions or countries. They are prepared with local products, traditional manufacturing processes, and have been, mostly, inherited between generations. Heritage foods are part of people's way of life, culture, and identity (Guerrero et al., 2009; Al-Habsi et al., 2019; Al-Khusaibi et al., 2019). Rural communities contribute to the preservation and the protection of traditional foods and play a role in economic development through traditional foods, like Bsissa, Keddid, or Harissa. For example, the traditional Tunisian red pepper paste called "Harissa" is marketed in many countries around the world, from Africa to Europe, Asia, and America (GICA(Groupement des Industries de Conserves Alimentaires)). It has become the ambassador of Tunisian cuisine around the world. Indeed, many visitors travel to visit different regions just to taste the local products known as "food tourism", which has benefited the socio-economic sector of these regions (Everett & Aitchison, 2008).

Due to its long history, North Africa is characterized by a great diversity of common traditional foods, like Bsissa, also named Tamina, Sullu, and Zemita in some Mediterranean countries (Smati, 2006) or Gofio in the Canary Islands (Caballero et al., 2003). Bsissa is a multigrain blend generally composed of cereals and legumes that are prepared by dry roasting. Spices, herbs, dried fruit, or other ingredients are then added to the Bsissa flours, mixed with water, oil, or milk and consumed as a liquid, sub-liquid, or paste. The formula of the flour also varies from region to region depending on the type/proportion of ingredients used (GharbiYahyaoui et al., 2017). Its long history dates back to Roman and Byzantine times when it was prepared with cereals, but when it arrived in North Africa, the Aborigines added legumes (Zaroual et al., 2019).

Despite the scarcity of studies on Bsissa, some research treated detection of mycotoxins (Maaroufi et al., 1995), analysis of aromatic profiles (Khiari et al., 2012), isolation of probiotic bacteria(Gharbi Yahyaoui et al., 2017), or formulation of Bsissa using sprouting durum wheat (Jrıbı & Debbabi, 2019).

In Tunisia, Bsissa occupies an important place in the food heritage. In this context, the Ministry of Cultural Affairs and the Association for the Safeguarding of the Medina of Lamta, organize the festival of local products devoted to the Bsissa fair («Patrimoine Culinaire de Lamta», n.d.). This annual festival of traditional products brings together the best producers from all over Tunisia. It is an opportunity to collect information, take surveys, and take samples of Bsissa.

The objective of this work is to characterize the different types and ingredients of Bsissa flour in order to find the common and most preferable recipe among Tunisians, taking into account the nutritional values of Bsissa.

Material and methods

Description of the study area

The study was conducted in the city of Lamta (Fig. 1), which is part of the city of Monastir, located on the east-central coast of Tunisia, where the twentieth edition of the festival of local products was held in April-May 2019. This festival was an opportunity for producers of bsissa, from Tunisia and even from neighboring countries such as Algeria and Morocco, to meet and sell their products. The best Bsissa producers from all regions have been invited to present their Bsissa to visitors and participate in the competition for the best Bsissa.

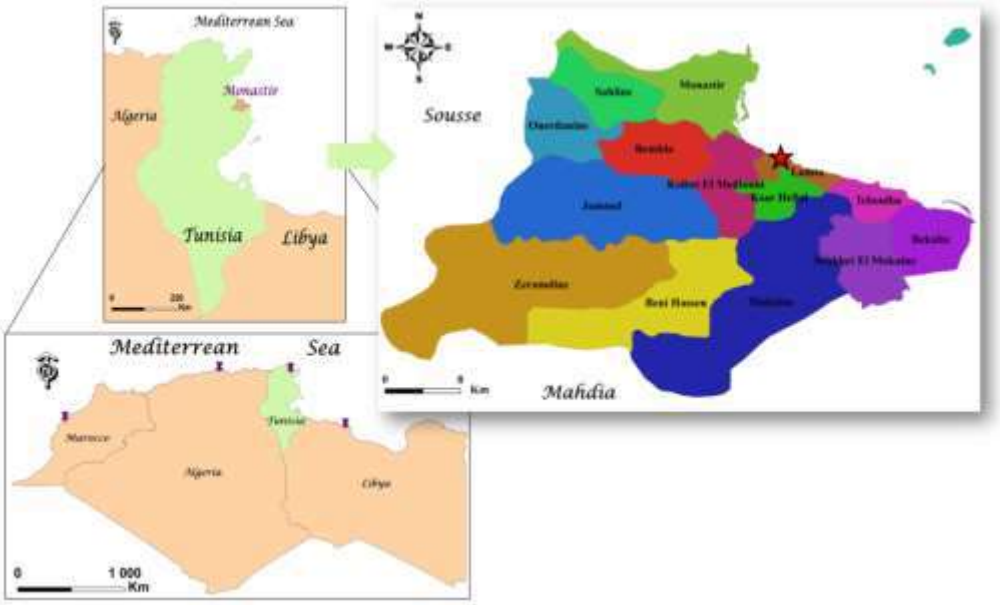

Fig. 1 :The study area of the survey "Lamta, Monastir".

Survey on the characterization of Bsissa

The survey was conducted using a questionnaire based on interviews conducted in Arabic (Tunisian dialect). The survey was divided into three parts:

- The first part covered socio-demographic characteristics (gender, age, personal experience, and occupation);
- The second part was a questionnaire on information related to the Bsissa recipe (origin of the formula, shelf life, origin of ingredients, type of sale, and flour analysis);
- The last part focused on collecting several types of Bsissa from participants from different regions of Tunisia. For this purpose, 103 types of Bsissa were collected and characterized so that determine the main ingredients present and the most common ones used in the preparation of all Bsissa formulas.

Results

Socio-demographic characteristics of the producers

All the producers interviewed in the Festival of Lamta were rural women, 80% were 40 years old or more, 66.66% of them had less than 10 years of professional experience in the field of Bsissa manufacturing and 86.66% are artisans (Fig. 2). Jabeen et al. (2020) affirm that artisan women in the rural area have a dominant role in the economy through traditional activities. Furthermore, (Tellstrom et al., 2005)indicated that there is an important relationship between rural areas, traditional food, and its marketing in the economic development of countries. Daglioğlu. (2000) confirmed also the major role of the rural community in the economic progress of the countries.

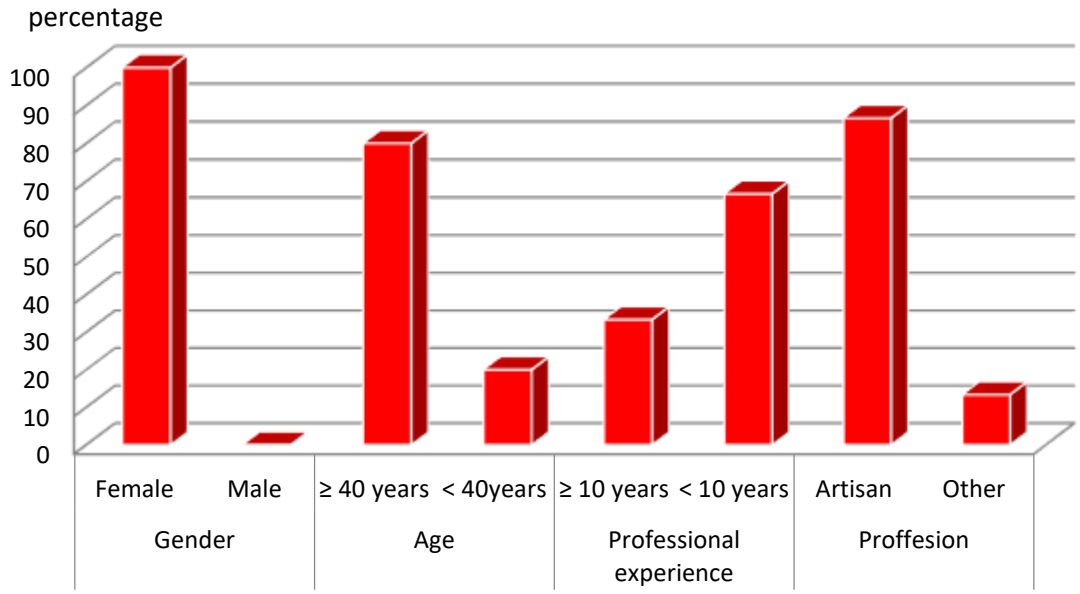

Fig. 2 :Bsissa producers profiles (n= 15)

Preparation of Bsissa

When asked about the recipe of Bsissa, the majority of producers said that they inherited the formula from their background (73%) and the remainder had taken training (Figure 2). In 2019, a study of high-end restaurants in Jakarta showed that the original recipes used by these top chefs are inherited from their own families, parents, and grandmothers, to maintain the ethnicity and originality of traditional recipes (Tambunan et al., 2019). In another hand, 67% of them buy their raw materials at the supermarket. Finally, producers are used to selling 86.66% of Bsissa flour to order via social networks, to simplify the task of producers and consumers.

Concerning Bsissa flour preparation, women producers explained that it is based on the following steps: raw material cleaning, grain roasting, sifting, then adding spices or sugar and finally mixing it with water, oil, or milk. It is prepared for celebrations such as the birth of a prophet (mouloud), during Ramadan, or it is consumed daily at breakfast, as it was indicated by Zaroual et al. (2019).

According to the respondents, about 60% Bsissa flour can be stored for up to 12 months at room temperature in anon-humid place (Fig. 3).In order to determine the effect of storage on the nutritional quality of blended flours, a study published by Rehman et al. (2017) on cereal-legume blended flours during the storage period, showed a loss of nutrients and an increase in the number of molds with increasing storage days. The researchers explain this by the hygroscopic characteristic of the flour which can absorb moisture and deteriorate quality. Accordingly, prolonged storage can lead to an environment rich in toxic substances especially mycotoxins. Hence, Oueslati et al. (2018) detected mycotoxins in Bsissa samples, that can, unfortunately, cause serious health problems (cancers, kidney disease or immune system problems)(Bennett & Klich, 2003), but through heat treatments such as roasting; the shelf life of flour can be extended (Prabakaran et al., 2019).

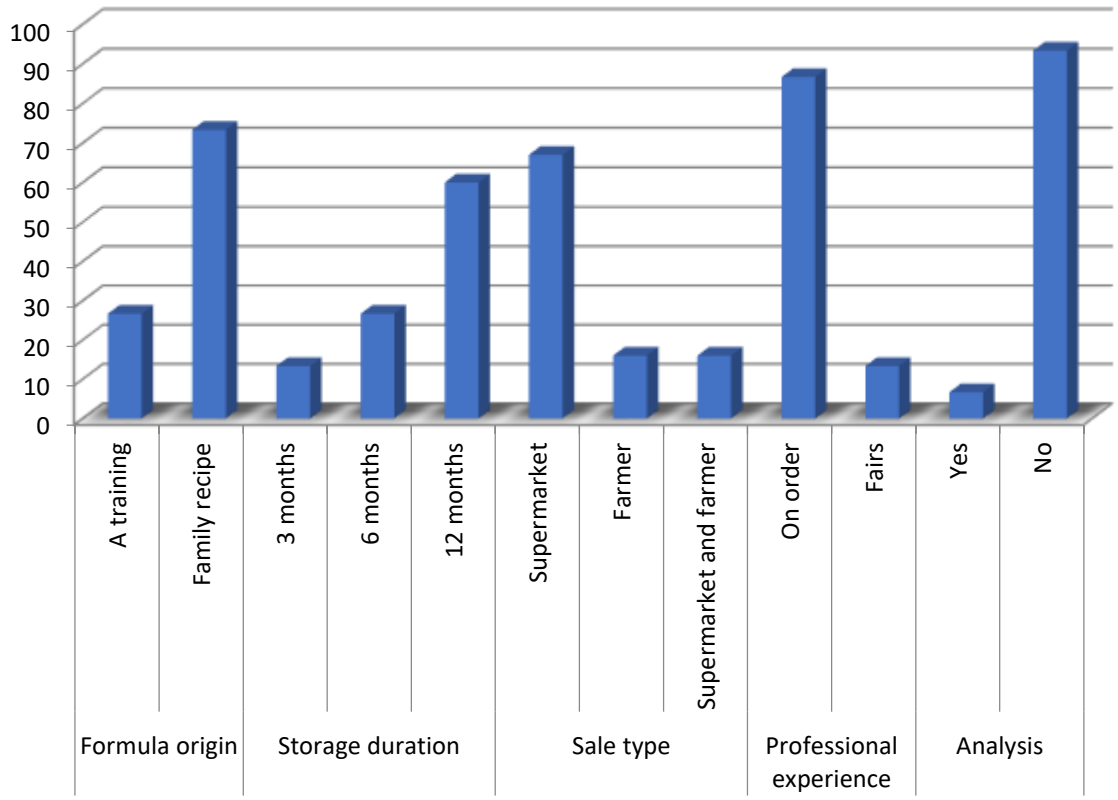

Fig. 3 :Information about recipe, storage sale and preparation of the Bsissa

Types of Bsissa and common ingredients

Our survey showed that, in Tunisia, Bsissa is obtained from 1 to 9 ingredients (and even more): a combination of roasted cereals, legumes mixed with spices in varying proportions depending on the region and family recipes. The name Bsissa comes from the ingredient with the highest proportion in the flour formula.

The 103 types of Bsissa collected (fig. 4) show that 74.75%were composed mainly of cereals (especially sorghum and durum wheat), 18.44% of legumes (lentils were the most present), and 6.79% of sesame. It was pointed out that this multigrain flour varies in number and quantity of ingredients, proportion, and type of consumption (liquid or semi-liquid) (Gharbi Yahyaoui et al., 2017). This diversity can satisfy the preferences of a wide range of consumers.

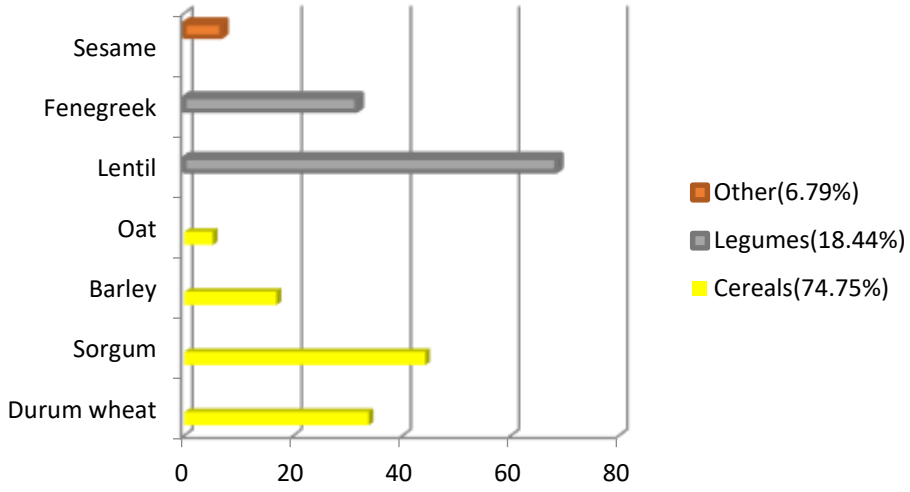

Fig. 4 : Main ingredients of Bsissa (n = 103)

In order to identify the main ingredients in Bsissa recipes, the percentage of each ingredient was calculated.Results (Fig. 4) showed that chickpea was the commonest ingredient (16.38%) followed by anise (12.56%), fennel seed (11.32%) and Coriander (11.08%).Chickpea is added to North African meals because of its availability, low cost, and main richness in nutrients such as fibers, proteins and amino acids. Roasting chickpea has been led to an increase in lysine content, and when mixed with wheat showed good techno-functional properties and considering the decrease of the trypsin inhibitor compared to the raw control(Barron et al., 1992).Since ancient times, spices and aromatic herbs have been used for medicinal purposes, in particular for obesity, cancer, and cardiovascular disease effects (Anderson et al., 1999) due to their richness in bioactive components (Yashin et al., 2017). Many medicinal properties of certain herbs and spices have been discussed by Kurian. (2012); such as the diuretic, tonic, analgesic, and anti-inflammatory properties of coriander. Fennel seeds were known for their gastric benefits. Anise is considered antiemetic, anthelmintic, mild expectorant, diaphoretic and carminative.

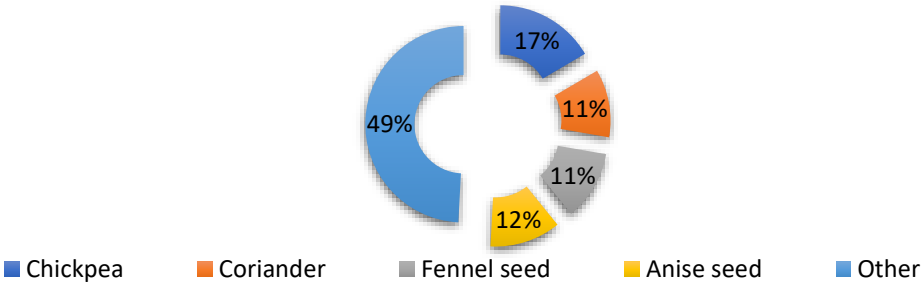

Fig. 4: Percentage ratio of common ingredients used in the preparation of Bsissa.

DISCUSSION

Cereals and legumes are the main constituents of Bsissa. Their roasting has beneficial effects on nutritional quality and shelf-life. Several studies have shown some benefits of cereals and legumes roasting process on nutritional composition and technological and sensory characteristics (Karaoglu & Kotancilar, 2006; Kavitha &Parimalavalli, 2014; Wronkowska et al., 2019). In a study conducted by Febles et al. (2000) on the phytic acid content of two types of traditional roasted cereal-based desserts (gofio and frangollo) in the Canary Islands, it seems that there is a difference between corn gofio (5.92 mg/g) compared to frangollo (6.54 mg/g), The authors explained this difference by the grinding and roasting process of gofio. Also, significant reductions in oxalate, tannins, phytate, hydrogen cyanide, and trypsin inhibitors activities were found after roasting Bambara groundnut kernels (Ndidi et al., 2014). During roasting, new products are formed by the Maillard reaction, there is also the loss of certain components of unlikely flavors (off-flavor) (Coşkuner & Karababa, 2004; Sila Bhattacharya, 2014). Thus, it is recommended to better control the roasting process (time and/or temperature) in order to avoid the risk of nutrient loss during excessive roasting (Nout, 1993).

In the other side, The cereal-legume mix has a high nutritional value especially a balance of amino acids (lysine in legumes, methionine in cereals) (Duodu & Minnaar, 2011). Besides, fortified food by spices and aromatic herbs have a major role in improving the organoleptic quality (color, flavor, and taste) and can also act as food preservatives (El-Sayed & Youssef, 2019). So, the use of short-term heat treatment (Rose & Pike, 2006)or spices (Yashin et al., 2017) enriched flour and prolong their shelf-life during storage.

This diversity in terms of combinations and ingredients makes Bsissa a product with high nutritional value. Bsissa is an excellent source of macro and micronutrients such as vitamins, fibers, and antioxidants, and is also an energy-rich food. It may be involved in the prevention of many diseases such as heart disease, cancer, and diabetes (Zaroual et al., 2019). Mixing cereals, legumes, spices, and other ingredients (sugar, dried fruits, oilseeds, and dried herbs) can help reducing malnutrition and provides a much higher intake of nutrients.

CONCLUSION

According to our survey, women artisans play a major role in the preservation of traditional foods and lead to the economic development of rural areas. Bsissa is a leguminous and cereal powder enriched with spices and aromatic herbs or other ingredients. Its preparation goes through different steps, including roasting and milling, and usedifferent ingredients and formulas.Our findings suggest that the use of roasted premixed fortified with spices and culinary herbs has a higher positive value on the quality of the product but also on human health and can reduce problems of malnutrition.Future studies may allow better control of the preparation factors of Bsissa flour: quantity and type of ingredients, roasting temperature and time and milling process.

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NATIONAL INVENTORY AND PLANT GENETIC RESOURCES DOCUMENTATION IN BULGARIA

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ABSTRACT

Conservation of agricultural biodiversity is a complex interdisciplinary process, which is the object of different national and international initiatives. The European countries unite their efforts in this direction by organizing the European Cooperative Programme for Plant Genetic Resources (ECPGR). One of the priorities of the programme is adapting uniform mechanisms for registration and description of their seed accessions stored. The National *ex situ* collection in the Genebank of IPGR Sadovo is part of the European Search Catalogue for Plant Genetic Resources, EURISCO (*http://eurisco.ecpgr.org*). The database (Bulgarian National Inventory) includes passport information about 69,412 accessions, registered according to FAO/Bioversity Multi-Crop Passport Descriptors. Based on cooperation in A European Genebank Integrated System (AEGIS), 261 Bulgarian genotypes, identified as "unique germplasm" of local origin, are registered with AEGIS status. A condition for inclusion of plant genetic resources into the AEGIS collection (*http://aegis.cgiar.org*) is safety duplication of accessions in another genebank, or in the Global Seed Vault in Svalbard. European Information Systems provide free access for potential users to conserved genotypes according to the principles of the International Treaty on Plant Genetic Resources for Food and Agriculture and the implementation of the Nagoya Protocol on equitable distribution of their benefits. Electronic portals eliminate the restrictions associated with sterically distant locations of genebanks and as a result, improve coordination between organizations and researchers in the area of plant genetic resources and have an active impact on preservation of natural ecosystems. The present study provides an overview of plant genetic resources of the Bulgarian National Inventory, based on EURISCO, as well as a documentation process of plant genetic resources in Bulgaria.

Keywords: *plant genetic resources, databases, free access, EURISCO, AEGIS, Bulgaria*.

INTRODUCTION

Plant genetic resources comprise of crop plants and their wild/weedy related species of actual or potential use. The development of improved types used today and those that would be cultivated in the future are essentially based on the effective utilization of plant biodiversity. These have helped in broadening the genetic base of crop plants within species and among species and in the diversification of cropping and farming systems through stability and sustainability. Plant genetic resources need to be exchanged and selected continuously for specific traits, to improve crops in terms of yield and nutritional value. Every nation is concerned with the acquisition of diverse and superior germplasm for conservation and utilization (FAO, 1992).

Conservation of agricultural biodiversity is a complex interdisciplinary process, which is the object of different national and international initiatives (FAO, 2008).

The European countries unite their efforts in this direction by organizing the European Cooperative Programme for Plant Genetic Resources (ECPGR).

ECPGR aims at contributing to national, sub-regional and regional programmes in Europe to rationally and effectively conserve natural in situ, genebank ex situ and agricultural resources on farm and increase their utilization. The programme, which is entirely financed by the

member countries, is coordinated by a secretariat hosted by Bioversity International, Rome, Italy. ECPGR operates through broadly focused networks dealing with groups of crops or general themes related to plant genetic resources, such as documentation (*http://www.ecpgr.cgiar.org/*).
One of the priorities of the programme is adapting uniform mechanisms for registration, for specific crop conservation, implementation of standards for germplasm characterization and documentation (Engels et al., 2008; FAO, 2014).
ECPGR developed an effective platform for exchange of information related to plant genetic resources (PGR) – the European search catalogue for plant genetic resources (EURISCO). EURISCO is a system providing information about ex situ plant collections maintained in Europe (Dias et al., 2012; Weise et al., 2017). It is based on a European network of PGR National Inventories, from which it receives data regularly. EURISCO provides information at accession level about more than 1,9 million PGR accessions conserved in European genebanks. It is maintained on behalf of the Secretariat of ECPGR, in collaboration with and on behalf of the National Focal Points for the National Inventories (Weise et al., 2017).
As a result, numerous of ECPGR Central Crop Databases are on line available, as well as knowledge on the conserved material and its potential for use.
ECPGR has embarked on an initiative to establish A European Genebank Integrated System (AEGIS) in 2009 with the aim of rationalizing the European germplasm collections, to improve the quality management of the conservation and utilization efforts, ensuring the availability of the unique and important material that makes up the European Collection. These efforts are based on formal agreements with countries and provide the foundation for sharing conservation and use responsibilities (Engels et al., 2012).
ECPGR builds constructive cooperation between partners (different stakeholders, including genebanks, non-governmental organizations, research and technical institutes, breeders, farmers, etc.) and rationalizes conservation activities, documentation and sustainable use of plant germplasm (ITPGRFA, 2009; CBD, 2011).
The aim of this study is to provide an an overview of Bulgarian National Inventory, as well as a documentation process of plant genetic resources.

MATERIAL AND METHODS

Conservation of plant biodiversity is a priority activity at the IPGR "K. Malkov" – Sadovo, which is the National Coordinator on Plant Genetic Resources in Bulgaria.
Annually, accessions from foreign PGR centres are introduced, registered, studied and maintained. Expeditions in different geographical regions of the country are conducted and valuable local varieties are collected. In the National Genebank, new improved cultivars of Bulgarian origin from breeding programmes are stored.
All accessions are registered with their passport data in the national electronic register, according to the international standards of the FAO/Bioversity International (2017).
The scientific names of crops are checked using the taxonomic system of GRIN (USDA Genetic Resources Information Network – Taxonomy for Plants).

The National *ex situ* collection is part of the European Search Catalogue for Plant Genetic Resources – EURISCO (*http://eurisco.ecpgr.org*).
Based on cooperation in AEGIS, genotypes identified as "unique germplasm" of local origin selected by the curators are registered as AEGIS accessions. A prerequisite for inclusion of plant genetic resources into AEGIS (*http://aegis.cgiar.org/*) is safety duplication of accessions in another genebank, or in the Global Seed Vault in Svalbard.
The survey of the Bulgarian National Inventory and of Bulgarian material in European genebanks is based on EURISCO.

RESULTS

Bulgarian National Inventory

The database (Bulgarian National Inventory) includes passport information about 69,412 accessions. The associated dataset comprises information about holding genebank, genus, species, country of origin, status of the sample, source of the material, date of collection, type of storage, whether the genotype has safety duplication in other genebanks, etc.

Holding institutes

There are three Bulgarian collections documented as holding institutes in EURISCO (Table 1). Holding institutions of the Bulgarian National Inventory are coded by FAO institution codes, available from the FAO WIEWS website (*www.fao.org/wiews/*).

Table 1. Current status of Bulgarian National Inventory

FAO Institution Code	Holding Institution	Number of accessions	Accessions of Bulgarian origin
BGR001	Institute for Plant Genetic Resources, Sadovo	64,992	16,009
BGR005	Institute of Rose and Essential-oil Plants, Kazanlak	563	4
BGR029	Dobrudja Agricultural Institute, General Toshevo	3,857	1,829
	Total number	69,412	17,842

Taxonomic composition

The taxonomic description of accessions is a problematic issue in genebanks and their databases, especially when the accessions are compiled from a large number of different origins and sources of material (Hintum and Knüpffer, 2010).

With respect to the taxonomic composition, the Bulgarian National Inventory consists of accessions belonging to 520 genera and 1,903 species (including 1,794 genus-species combinations and 109 names consisting of genus only), after correcting of some spelling errors and removing of synonyms (especially between *Triticum* and *Aegilops*). The material of Bulgarian origin belongs to 204 genera and 401 species (all having genus-species combination). The genera of germplasm with more than 400 accessions are presented in Table 2. The species with more than 1,000 accessions are shown in Table 3.

Table 2. Genera from the Bulgarian National Inventory with more than 400 accessions

Genus	Crop name	Number of accessions	Accessions of Bulgarian origin
Triticum	Wheat	16,054	4,022
Hordeum	Barley	7,189	291
Zea	Maize	4,773	1,892
Phaseolus	Garden bean	3,507	1,461
Avena	Oat	2,640	147
Vicia	Faba bean, vetches	2,150	573
Pisum	Pea	1,633	244
Linum	Flax	1,461	78

Genus	Crop name	Number of accessions	Accessions of Bulgarian origin
×Triticosecale	Triticale	1,459	533
Cucumis	Cucumber, melon	1,411	211
Arachis	Groundnut	1,325	428
Brassica	Cabbage	1,320	53
Capsicum	Vegetable pepper	1,319	815
Secale	Rye	1,283	810
Lycopersicon	Tomato	1,270	370
Sorghum	Sorghum	1,032	21
Lactuca	Lettuce	928	143
Oryza	Rice	917	106
Allium	Onion, leek, etc.	819	247
Glycine	Soybean	758	13
Medicago	Clover	722	63
Aegilops	Goatgrass	662	314
Trifolium	Medic	561	152
Helianthus	Sunflower	549	69
Solanum	Potato, eggplant, etc.	515	61
Lens	Lentil	512	42
Panicum	Panicgrass	501	21
Cucurbita	Pumpkin, squash	434	281
Cicer	Chickpea	419	44
Lolium	Ryegrass	413	125

Table 3. Species from the Bulgarian National Inventory with more than 1,000 accessions

Species	Crop name	Number of accessions	Accessions of Bulgarian origin
Triticum aestivum	Common wheat	12,886	2,784
Hordeum vulgare	Barley	6,205	287
Zea mays	Maize	4,770	1,892
Phaseolus vulgaris	Garden bean	3,135	1,345
Avena sativa	Oat	2,452	142
Triticum durum	Durum wheat	2,367	1,193
Pisum sativum	Pea	1,628	240
×Triticosecale	Triticale	1,459	533
Linum usitatissimum	Flax	1,442	77
Arachis hypogaea	Groundnut	1,325	428
Capsicum annuum	Vegetable pepper	1,286	815
Secale cereale	Rye	1,258	808
Lycopersicon esculentum	Tomato	1,168	336

Countries of origin

The Bulgarian National Inventory consists of plant genetic resources of different geographical origin. There are 17,842 accessions with Bulgarian origin, i.e. 25,7 % of the National collection. The country of origin of 29,593 accessions is unknown. Besides Bulgaria, the material comes from 115 different countries, including the U.S. (2,840 accessions), the Russian Federation

(2,574), Germany (1,828), France (1,326), Ethiopia (1,114), Hungary (990), Japan (858), Italy (704) and The Netherlands (701) with more than 700 accessions each.

Status of the samples

According to the sample status, the germplasm from Bulgaria can be divided into various categories, including wild, weedy forms, landraces, advanced cultivars, and others.
The largest proportion of the genotypes belongs to traditional local cultivars and landraces, followed by breeding or research material with several subcategories (Table 4). A large number of accessions have not yet been classified according to sample status.

Table 4. Bulgarian National Inventory classified by sample status

Status of sample	Number of accessions	Accessions of Bulgarian origin
100 – wild	3,799	1,230
200 – weedy	19	3
300 – traditional cultivar/landrace	12,174	6,017
400 – breeding/research material	5,466	2,534
410 – breeders' line	2,404	1,201
411 – synthetic population	40	22
412 – hybrid	326	48
420 – genetic stock	266	212
500 – advanced or improved cultivar	6,118	600
unknown	46,265	5,975

Type of germplasm storage

According to EURISCO 43,534 accessions from Bulgarian National Inventory are stored in the base collection under long-term conditions, 7,649 are in the medium term and 2,989 genotypes are free for exchange. The other accessions are included in short term storage, field and in vitro collection.

Bulgarian National Inventory in AEGIS

Based on cooperation in A European Genebank Integrated System (AEGIS), 261 Bulgarian genotypes, identified as "unique germplasm" of local origin, are registered with AEGIS status. A condition for inclusion of plant genetic resources into the AEGIS collection is a safety duplication of accessions in another genebank. Seeds samples from 930 Bulgarian local accessions were sent for safety duplication in Global Seed Vault in Svalbard, Norway.

DISCUSSION

Documentation is essential for a genebank management to allow efficient and effective use of germplasm. Information plays a significant role in biodiversity conservation. Accurate information about conserved materials is essential for greater use. Computerized documentation systems enable rapid dissemination of information to users as well as assist curators to manage the collections more efficiently.
The passport information on the species and variety names, place of origin, etc. is a part from germplasm sustainable maintenance. Catalogues of the collected germplasm for various crops are published by the gene banks` focal points and provided free access to conserved plant diversity.

According to EURISCO (August, 2020), the Bulgarian *ex situ* collection, documented by 36 descriptors is the seventh-largest National Inventory in Europe, comprising 3,4 % of the whole European collections.

There are 123 European genebanks (except Bulgaria) holding germplasm accessions of Bulgarian origin with a total of 11,607 accessions, taking into consideration the FAO WIEWS database and Genesys (a worldwide database on plant genetic resources), provided data also for non-European genebanks holding Bulgarian material(Knüpffer, 2016).

The Treaty (IPGRFA, 2009) provides for facilitated access to Contracting Parties for the crops of Annex I under Multilateral System (MS) solely for the purpose of utilization and conservation for research, breeding and training for food and agriculture and purpose that does not include chemical, pharmaceutical and/or other non-food/feed industrial uses. In case of multiple-use, their importance for food security should be the determinant for their inclusion in the MS and availability. It is also ensured that passport data information is to be made free available.

Perhaps the most important part of the genetic resources use process is the provision of information obtained through collecting, characterization and evaluation – it may be argued that this information is as much a public good as the genetic resource itself, in particular when one realizes that the biological material without the information on how to utilize it can hardly be regarded as a genetic resource. It is the experience and knowledge of farmers collected and developed over centuries by growing and improving and adjusting the crop that has resulted in the genetic resource as we know it today. Therefore, the raw material in germplasm collections cannot be used effectively without information on agronomic practices and the genetic traits of value to users including the harvested produce for food preparation and/or other uses. This information is obtained from farmers during the collecting of the germplasm material as well as through characterization and evaluation of the genetic material, often using molecular methods. The free exchange of information, like the flow of germplasm, can be impeded or facilitated by policies and legal instruments, so that provision of information on genebank holdings by information networks such as EURISCO, are crucial to management and use of the collections.

CONCLUSIONS

The overview of Bulgarian plant genetic resources presented the wealth of crop plant species, and it may assist in identifying gaps and needs for further collecting. It may also be a good starting point for compiling checklists of cultivated plant species and future research activities. European Information Systems provide free access for potential users to conserved genotypes according to the principles of the International Treaty on Plant Genetic Resources for Food and Agriculture and the implementation of the Nagoya Protocol on equitable distribution of their benefits. Electronic portals eliminate the restrictions associated with sterically distant locations of genebanks and as a result, improve coordination between organizations and researchers in the area of plant genetic resources and have an active impact on preservation of natural ecosystems.

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PROPAGATION THE TAHAR APPLES (*MALUS SYLVESTRIS SPP. ORIENTALIS*) BY SEED

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ABSTRACT

In this study, it was carried out on seeds taken from Tahar apples selected from Nevşehir province Ürgüp district and its surroundings in order to determine the propagation ability of Tahar apple, which has the potential to be rootstock for cultivated apples. After the seeds obtained were soaked in water for 24 hours, they were stratification in sand at + 4^{o}C. The seeds, which remained in 60 days of stratification, were removed from the stratification when the signs of germination were seen from the ends, and planted in the peat + sand medium. The germination rate of apple seeds in stratification was determined to be 90%. The seeds planted in the peat + sand medium were transferred to the land when they reached 15 cm in length, and the retention rates were 100%. The vegetations transferred to the land have been observed in a vegetation period and have reached 30 cm in length and 0.5 cm in diameter. Consequently, due to the dwarf feature of Tahar apple, it was concluded that under normal care conditions, the slow growing of the seeds, and propagation of Tahar apple from seed was important for creating variation although it is a time consuming job.

Keywords: Tahar apples, seed, propagation, stratification

INTRODUCTION

The Tahar apple, which grows in the Ürgüp region of Nevşehir Province in the Central Anatolia Region, is a local apple variety that is about to disappear like our other local varieties. The Tahar apple (*Malus sylvestris* spp. *orientalis*) is actually a local variety whose fruits are consumed for summer. This local apple variety consists of small shrubs that develop very healthy without irrigation and have genetic dwarf characteristics in ecology where annual precipitation is below 400 mm (Çağlar, 1997). However, this genotype has begun to disappear as a result of the expansion of settlements and the reduction of agricultural land.

In recent years, dwarfing apple rootstocks have become increasingly widespread all over the world, allowing more economical apple cultivation in industrial sense. On the other hand, there are no perfect rootstocks that meet the desired criteria and demands for new breeding techniques. Therefore, apple rootstock breeding studies continue in countries that are leading in apple cultivation. As the world's natural flora in many regions of Turkey it is also included apples genetic resources. Davis (1972) defined by specifying different *Malus* species in Turkey. However, studies of developing new rootstocks and varieties from these apple gene sources have been very limited. Apple breeding activities in Turkey, where only limited by the kinds of selection and despite the determination of the characteristics of the type that was often selection has been reported that the conservation of these materials (Akgül et al., 2011). Tóth et al. (2004) pointed out that local old apple varieties that try to survive in their original growing places could be an important gene source for apple breeding programs, and reported that since 1997, they have collected and evaluated local apple varieties in Ukraine in a collection area and put them into the service of breeders and geneticists.

In our country, which has a great material richness, the breeding activities first aimed to select the most valuable ones among the existing ones. Today, studies for breeding in our country are limited to selection breeding. In addition, since the studies were not carried out within a program, they did not show continuity and only the characteristics of the types were determined and the material obtained was not preserved most of the time (Atay et al., 2010). Ülkümen (1973) pointed out the importance of this variety in terms of breeding by stating that it is our national and humanitarian duty to protect these local varieties, which have miraculous breeding material such as the 'Tahar Apple', from disappearance.

In order to breed a species, it is necessary to know the propagation ability of that species. Plants are propagated by generative (seed) and vegetative (asexual) ways (Ağaoğlu et al 2019). One of the natural ways to propagation plants is by seed propagation. In this context, it was carried out in order to determine the propagation capability of Tahar apples (*Malus sylvestris* spp. *orientalis*) by seed, which has the potential to become rootstock for cultivated apples grown in Ürgüp District (Nevşehir) and about to disappear.

Material and Methods

In the study, the seeds of Tahar apples, a summer variety grown in the Ürgüp region of Nevşehir Province in the Central Anatolia Region (Figure 1), were used. Fruits were cut in half with a knife in the laboratory and seeds were removed from core. After the extracted seeds are washed with tap water, they are placed in a way that they do not touch each other and left to dry at room temperature. The dried seeds were stored in cloth bags until stratification. To make stratification in January, apple seeds were first soaked in water for 24 hours, then stratification in sand at 4 ° C. The seeds, which remained in stratification for 60 days, were removed from the ends as soon as the signs of germination were seen, and planted in plastic containers (25 x 10 cm in size) in peat sand environment. The seeds were kept at laboratory temperature (21-25 ° C) and in semi-shade conditions for 15 days for germination.

Figure 1. Geographical location and map of Nevşehir Province (Anonymous, 2020)

Results and Discussion

The germination rate of the seeds that were remained in stratification for 60 days was determined as 90%. It has been observed that the embryos of non-germinating seeds are underdeveloped. The seeds, which were removed without folding, grew in a healthy way in the

plastic containers they were planted in, and when they reached a height of 15 cm, they were transferred to pots and grown successfully at room temperature. A homogeneous growth was observed in the seedling, and then they were planted in the field. There was no loss in transferring the seedling to the land. The retention rate of the seedling in the field was 100 % and reached a length of 25 cm and a diameter of 0.5 mm.

Similarly, Aysabar (2017) in his study carried out in order to propagation Tahar apple clones by seed, cuttings and layering and to determine the effect of a cultivated apple grafted on rooted rootstocks on sapling development; he reported that the germination rate of Tahar apples was 78.06% after the seeds were folded in moist sand for 60 days.

In addition, Boyacı (2014) determined some basic morphological features on Tahar apples that should be taken into consideration in dwarf apple rootstocks such as plant vigor, growth habit, ramification, internode length and root suckering status between 2010 and 2013 and evaluated them with Weighted Grading Method. Within the scope of the study, three of the 17 genotypes with the highest score (50 TE001, 50TE002 and 50TE 012) were determined as rootstock candidates. The propagation status of Tahar apples was investigated with green cutting, semi-wood cutting and wood cutting methods in all genotypes. Since the annual number of shoots on Tahar apples was extremely limited, they took cuttings from them and multiplied the material by grafting it on the seedling rootstock for nursery conditions. He reported that the rate of rooting of genotypes with cuttings was very low and no rooting occurred. He reported that he successfully propagated by means of stool bed layering method, made morphological characterization on plants according to UPOV criteria, and molecular analysis was performed to determine the genetic affinity of genotypes to each other. He concluded that the three selected genotypes (50 TE001, 50TE002 and 50TE012) would be promising materials for the development of new dwarf apple rootstocks, since the genotypes in his experiment had different genetic structures.

Büyükyılmaz et al. (1995) *Malus sylvestris* Mill, which was found to be widely grown in the Marmara Region. The seeds of 13 types, which are determined to be suitable for the seed rootstock among the wild trees belonging to their species, have been stratification in moist perlite at 4ºC for 60, 70, 80 and 90 days. In their studies, in which germination values were determined based on Czabator's method, which combines germination speed and duration and which is calculated, and by evaluating the germination rates together with the germination rates; they found that the stratification time of seeds of wild apples (*Malus sylvestris* Mill.) at 4ºC was around 80 days.

Bozhüyük and Güleryüz (2014) in their studies conducted in order to determine the relationship between the endogenous plant growth regulation substances during the stratification in the dormant seeds of apple. Changes of the endogenous plant growth regulation substances were determined by using oat coleoptil tests.The results showed that growth inhibitors decreased periodically during stratification. It was also found that growth promoters significantly increased towards the last weeks of stratification. In the study, while growth promoters were found in the sections of Rf 0,2–0,3 and Rf 0,8–1,0, growth inhibitors were found in the sections of Rf 0,4–0,7 in the oat coloeoptil tests.

Conclusion

At the end of the study, it was determined that Tahar apple, which is a local apple variety, which is about to disappear like our other local varieties grown in Nevşehir Province Ürgüp region in

the Central Anatolia Region, can be easily propagated with seeds. However, the growth of seedlings in the field was very slow according to the measurements made at the end of the year. Under normal maintenance conditions, Tahar apple seedlings should be expected to grow slowly, and propagation by seedlings will last up to 2 years. For this reason, it will be possible to create variation from the fruits on the trees that will be obtained by growing these plants, although it is a time-consuming task to reproduce the Tahar apple from the seed. This slow growth feature may be due to the fact that the Tahar apple is not a tree but a dwarf shrub.

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DETERMINATION OF HEAVY METALS IN MILK COLLECTED FROM SMALL FARMS IN THREE REGIONS OF ALBANIA

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ABSTRACT

In Albania, the issue of monitoring the quality and safety of food products remains an important concern. The aim of this research was to evaluate the possible heavy metals contamination of milk from small farms in Lushnja, Kavaja and Fieri Regions. A total of 120 milk samples from 12 dairy farms (n = 10 per farm) were collected and analyzed with AAS Thermo-SOLAAR for the presence of Cd and Pb. The determination of these heavy metals in according with EC No 1881/2006 was performed. Each sample, was homogenized, powdered and mineralized in a microwave oven. Quantitative analyses of Cd and Pb were performed using an atomic absorption spectrophotometer with graphite furnace. The data of this study showed that the two heavy metals (Pb and Cd) were below their respective MRLs in all analyzed samples. Despite the results described above, the possibility that milk and dairy products exceeding the standards for these indicators cannot be excluded. Since environmental pollution due to these metals increases every year, this can lead to the appearance of these contaminants in the dairy industry at some point.

Keywords: *Milk, pollution, heavy metals, food safety, Cd, Pb*

INTRODUCTION

Increased awareness of the influence of diets on human health has prompted producers to produce food of higher quality, rich in nutrients and nutraceuticals, in environmentally friendly manner. (Sikirici et al., 2003). Cow milk and its products are basic foods and constitute an important source of nutrients in human diet. Their content of protein, fat, carbohydrates, vitamins and minerals determine their biological and technological properties (Pérez et al., 2016). In fact, milk is an ideal source of macroelements, such as Ca, potassium (K), phosphorus (P). Moreover, microelements and even heavy metals can be found in milk. Microelements, also called trace elements such as copper (Cu), iron (Fe), selenium (Se) and zinc (Zn) are known to be essential for normal growth. However, heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) have no beneficial effects on human (Li et al, 2009). As milk is widely used in the human diet, it needs to be collected from healthy animals that are contamination free. In Albania, the dairy sector is very important for agribusiness. According to data from the Albanian Institute of Statistics (INSTAT), milk production for consumption in Albania in 2015 was about 11.9 thousand tons, in 2016 it was 11 thousand and in 2017 it was 13 thousand tons. In Lushnja, Fieri and Kavaja Region are located the largest number of farms with cow breeding for milk and consequently are the largest producers of milk in Albania. Also in these areas is located the largest number of milk processing factories. Based on this fact, the determination of trace metals such as lead and cadmium is important (Rroko et al., 2016)

Heavy metal toxicity is linked with a number of diseases but the severity of situation multiplies too many folds if these heavy metals are found in milk, which is the basic food item of vulnerable age group of people. The presence of heavy metals in dairy products may be due to

contamination of the primary milk of the cow, which may be due to exposure to the environment or the consumption of food and water due to exposure to cow's livestock. In addition, raw milk may be contaminated during its production (Parisa et al., 2018)

Generally, animals reduce human exposure to trace metals; for instance, levels present in different environmental matrices are higher than those found in food. However, some trace elements have been found in cattle diet at levels tolerated by the animals that could be transferred to their tissues at concentrations not acceptable for human consumption. The elements identified are As, Cd, Cr, Cu, Pb, and Se (NRC 2005, Ayar et al., 2009). The presence of heavy metals and trace elements in milk and dairy products has been reported in different countries (Maas et al., 2011; Bilandzic et al ., 2011). Cadmium, lead and mercury are very dangerous to humans and are considered as a major threat to food in terms of industrial use. Lead and cadmium are considered potential carcinogens and are associated with etiology of a number of diseases in the cardiovascular system, kidneys, nervous system, blood and skeletal system (Zhuang et al., 2009). Animals use metals when grazing in the pasture and feeding with contaminated concentrations. However, in the case of cows, the transfer of minerals to milk is very variable. Pollutants are transferred to the air as a result of various industrial activities. Pollution of various industrial environments in the soil, water, food and heavy metals causes them to join the food chain and create a great threat to human and animal health (Licata et al., 2014).

This study aimed to evaluate the Pb and Cd contamination in raw bovine milk samples from the Fieri, Lushnja and Kavaja region, with Atomic Absorption Spectrometry Thermo-SOLAAR.

MATERİALS AND METHODS

In the period 2015– 2017, a total of 120 milk samples were analysed with AAS Thermo-SOLAAR for two heavy metals (Cd and Pb), The trace metal determination was carried out following standardized methods for the analysis of cadmium and lead such as SSH EN 13804:2003, SSH EN 14084:2003, CE 1881/2006

Area included in the study

Lushnja, Fieri and Kavaja Region (Locations of the farms selected in the study) is one of the richest regions in Albania with cattle farms bred for milk production. Also in this region there is a high concentration of factories collecting, processing, and distributing milk and its products. The distribution market of the products of these factories covers the territory of Albania as a whole.

Sampling

Sampling was conducted in two periods, in a summer and winter. Samples were taken from cow's milk, mixed of morning and evening milking. A total of 120 raw milk samples (500 mL) were collected from 12 small farms (n = 10 per farm), and were placed into polyethylene bottles that were rinsed with 10% nitric acid in deionized water prior to collection. The samples were immediately transported to the laboratory in a cooler with ice packs and were stored at -20 ^{0} C until analysis. Sampling of milk were realized in accordance with EC No 333/2007.

Preparation of samples

Before analytical determination the milk samples were cleaned from fat and homogenized using Ultra Turrax homogenizer. Two parallels for each sample were prepared according to the

following procedure. Samples (1.0 ±0.2 mL) were weighed into Teflon digestion vessels; 4.0 ml nitric acid (69.5% m/V) and 1.0 ml hydrogen peroxide (30% m/V) were subsequently added. The sealed containers were placed in a microwave oven (Microwave Digestion BERGHOF) for 17 min and the samples were heated according to the temperature program. After complete digestion the sample solutions were cooled for 20 min to the room temperature. The sample solutions were diluted with ultra-pure water to the total volume of 20 ml and transferred into volumetric flasks, capped and stored at room temperature (Pecnikaj et al., 2016, Patra et al., 2008).

Measurement of elemental concentration in milk samples

Atomic Absorption Spectrometer (Thermo SOLAAR GF 95/FS 95), equipped with graphite furnace system, was used to determine the concentration of cadmium and lead in milk samples. The quantitative determination of Pb and Cd were performed by using a four different point calibration curve for lead and three different point calibration curve for cadmium. For both the element were used matrix modifiers. Each sample was repeated several times for each element and the average was recorded. The concentrations of Cd and Pb were determined for each sample of milk. (Pecnikaj et al., 2016)

RESULTS

The determination of two heavy metals (Cd and Pb), was performed using AAS Thermo-SOLAAR, in according with EC No 1881/2006. According to obtained results we can conclude, that all samples of milk contain heavy metals under the permissible limits. In all milk samples that underwent laboratory testing, the cadmium and lead content was below the quantification threshold (LOQ), referring to Commission Regulation (EC) no. 1095/2010. 1881/2006 of 19 December 2006 on certain contaminants in foodstuffs. 2006R1881-EN-01.07.2014. (for metals and PCBs)

DISCUSSION

Taking into account the data obtained in this work and considering it from a toxicological point of view, trace elements content in milk samples do not represent a risk for human health
Livestock is a very important agro-food sector in Albania about 1/2 of farmers are engaged in livestock, including the dairy sector. Dairy products occupy an important part in the consumer basket of Albanian households. Milk production is dominated mainly by cow's milk (more than 4/5). In general, dairy products are targeted at the domestic market, so the increase in production is mainly driven by the increase in domestic demand. while the balance of international trade in dairy products shows a structural deficit.

CONCLUSION

Significantly lower level of cadmium and lead was observed compare with other European countries. The results suggest that the procedure described here is simple, low risk of contamination and particularly suitable for testing a large number of raw milk samples, as also results in other studies. Moreover, the very low concentrations of (lead and cadmium) in raw milk indicate that exposure to these two elements by people consuming milk is negligible. In conclusion, we can claim that control over environmental pollutants in milk can take control of the environmental situation, hygienic-sanitary verification of food and preventive actions on the person, in the broadest sense of the word. Further studies are necessary to evaluate the contents of heavy metals on a greater number of milk samples from various farms in Kavaja, Fieri and Lushnja region to confirm the absence of possible toxicological risks in this region.

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SOME SYSTEMATICS DATA FOR SPECIES MIRIDAE – PLANT BUGS (HEMIPTERA) IN HABITATS OF COASTAL REGION (KAVAJA)

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ABSTRACT

Systematic data on plant bugs Miridae (Hemiptera) collected during 2017 and 2019 from coastal habitats near Kavaja, were compiled. From this study we determined 15 genus and 21 species. From our taxonomic analysis, genus *Deraeocoris* was the most represented with 3 species (frequency, 14.29%). Most of the species, 15 species and 71.42% we have found in habitats of Spille (Kavaj coastal region). Based on the Jaccard Index of Similarity coefficient, habitats of Spille and M.Robit showed higher similarity (42.10%) than the other stations that means similarity with each-other in ecological factors and habitats.

Keywords: *Miridae*, Albanian Hemiptera, coastal habitats, Kavaja

INTRODUCTION

The family Miridae Hahn, 1831 (plant bugs) comprises a considerable proportion of species in the order Hemiptera (Schuh, 1995), though estimates reach 20,000 (Henry and Wheeler, 1988). Members of this family possess a number of consistent characteristics. The body length ranges from 2–11 mm. Distinguishing features include an oval, extended, ellipsoidal body, which is often colourful, from dark to bright vermilion (Gavin, 2000). One useful feature in identifying species of the family is the presence of a cuneus; it is the triangular tip of the corium, the firm, horny part of the forewing, the hemelytron. The cuneus is visible in nearly all Miridae, and only in a few other Hemiptera. The tarsi almost always have three segments (Richards, 1977). These insects can cause substantial damage to agricultural crops (Silvestri, 1939; Servadei, 1972). The family is dominated by phytophagous species, though some predators also are present (Carvalho *et al*, 1968; Tremblay, 1981; 1990). Due to some of the their features they have been described as integrated biological weapons (Miller, 1971; Gennaro, 1977; Tremblay, 1990; Pollini, 2002). In this study we present some data for family Miridae collected in coastal habitats in Kavaja (Halimi, 2013).

MATERIAL AND METHODS

Biological material was collected from field trips undertaken between 2017 and 2019 in different habitats near Kavaja, at Spille, Mali i Robit, and Golem. Sampling was carried out from May to September, from 9.00–15.00 hrs. In the present investigation, entomological mowing nets of diameter 80cm, aspirators and Pitt's traps were employed. Mowing with entomological nets was undertaken using diagonals across surfaces of $100m^2$ (10m x 10m), passing five times across each square's diagonal (Colas, 1969).

Once collected, the individuals were placed in plastic bottles and labelled with the date and name of station. Once placed in 150–200 cm^3plastic flacons, the fine biological material was sent to the laboratory and preserved in bottles containing 95 per cent ethanol, acetic acid, distilled water in a ratio of 80:5:20 ml (v:v:v) with a few drops of ether (Colas, 1969; Chapman, 1985). Taxonomic determination is done with stereomicroscope Trinocular Stero Microscope (*with still camera model 50240003 n/s C88794*) in the MSN lab employing standard classification keys for this family (Drake, 1965; Servadei, 1967; Dolling, 1991; Aukema et al.,

1999;). *Jaccard similarity* coefficient (Jaccard, 1901) was used to assess the species similarity at the different stations.

In the present investigation, efforts were made to record the characteristics of the different sites and thus to assess any impact the different habitats have on the distribution of the species (Halimi, 2016).

RESULTS AND DISCUSSION

Data on the species collected at each station are reported in Table 1. From our analysis results 21species and 15 genera at the stations of Kavaja (Table 2).

Table 1. List of Miridae species collected in: S, Spille; MR, Mali i Robit; G, Golem

Species	S	M	G
Adelphocoris			
* *lineolatus* Goeze, 1778	+		+
**vandalicus* Rossi, 1790	+	+	
Anapomella			
**arnoldii* V.G. Putshkov, 1961	+	+	
Capsus			
**wagneri* Remane, 1950	+		
Deraeocoris			
**lutescens* Schilling, 1837	+	+	+
**schach* Fabricius, 1781	+	+	
**serenus* Douglas& Scott, 1868			+
Halticidae			
**punctulata* Reuter, 1901	+		+
Heterocapillus			
**tigripes* Mulsant, 1852		+	+
Lopus			
**decolor* Fallén, 1807	+	+	
Lygus			
**pratensis* Linnaeus, 1758	+	+	
**punctatus* Zetterstedt, 1839			+
Macrolophus			
* *pygmaeus* Herrich-Schâffer, 1835	+		

**costalis* Fieber, 1858	+		
**atricapillus Scott, 1872*		+	+
Orthocephalus			
**brevis* Panzer, 1798	+	+	
Orthops			
**kalmi* Linnaeus, 1758		+	
Phytocoris			
**insignis* Reuter, 1876			+
**pini* Kirschbaum, 1856	+		+
Piezocranum			
**medvedevi* V.G. Putshkov, 1961		+	
Polymerus			
* *cognatus* Fieber, 1858	+		+
**vulneratus* Panzer, 1806	+	+	

Table 2. Number and frequency of species per genus

Genus	Number of species	Species frequency (%)
Adelphocoris	2	9.52
Anapomella	1	4.76
Capsus	1	4.76
Deraeocoris	3	14.29
Halticidae	1	4.76
Heterocapillus	1	4.76
Lopus	1	4.76
Lygus	2	9.52
Macrolophus	2	9.52
Macrotylus	1	4.76
Orthocephalus	1	4.76
Orthops	1	4.76
Phytocoris	1	4.76
Piezocranum	1	4.76
Polymerus	2	9.52

By our analysis *Deraeocoris* was the most represented genus, with three species (frequency, 14%), followed by *Adelphocoris, Lygus, Macrolophus* and *Polymerus* with two species each (frequency, 9.52%) and *Anapomella, Capsus, Halticidae, Heterocapillus, Lopus, Macrotylus, Orthocephalus, Orthops, Phytocoris, Piezocranum and Polymerus* with a single species each (frequency, 4.76%).

From our analysis habitats of Spille has the largest number of species, 15 species and 71.42%, followed by M.Robi, and then Golem (Table 3).

Table 3. Number and frequency of species by sampling location

Station	Species number	Frequency
Spille	15	71.42
Mali Robit	12	57.114
Golem	10	47.61

Our analysis reports data onthe number of species the different station have in common (C), along with the coefficient of similarity C_J.The stations here reported are ecologically diverse (Table 4).

Table 4. Number of common species (C) and coefficient of similarity (C_J) between sample sites

	Spille	Mali i Robit	Golem
Spille		C = 8 C_J = 42.10%	C = 2 C_J= 8%
Mali i Robit			C = 3 C_J= 15.78%

Analysis of the data give indication that the highest similarity coefficient (Jaccard index) between Spille and M.Robit (42.10%), followed by M.Robit and Golem (15.78%). *Similarity* coefficient tells us the impact of ecological anthropogenic factors on their distribution belongs especially to sites Spille and Mali Robit.

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PHYTOESTROGENS AND THE IMPORTANCE OF PHYTOESTROGENS AS FUNCTIONAL FOOD INGREDIENTS

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ABSTRACT

Depending on today's living and working conditions, consumers have focused on the efforts to protect or improve their health status, especially due to intense work pace and increased stress level. Consequently, awareness and demand for functional food is increasing day by day. Functional foods are defined as food or components of it that provide important health benefits on human metabolism and physiology, protect the individual against diseases or contribute to its recovery, as well as it contains the basic nutrients that the body needs. These foods can be obtained by applying various technological or enrichment methods, as well as in the natural form of food. Foods can be made functional by adding various functional food ingredients. Food supplements produced for this purpose are also available on the market. Many plants have positive effects on human health due to the functional food components they contain. For this reason, studies on functional food components are increasing day by day. The most studied functional food ingredients are phenolic substances, antioxidants, dietary fibers, oligosaccharides, probiotics, prebiotics, vitamins, polyunsaturated fatty acids, sulfur-containing components, phytosterols and phytoestrogens. One of the plant-derived phenolic compounds is phytoestrogens. Also, phytoestrogens are in the class of naturally occurring esterogens. They have antioxidant properties as they ensure the protection of DNA by binding metals with the hydroxyl group in their structure. In addition, they can change the effectiveness of some enzymes that play a role in body estrogen metabolism. These components are important because of their positive effects on some types of cancer, heart disease, menopausal symptoms, and treatment of osteoporosis. In this review, the structure of the phytoestrogens as a functional food component, its types, general effects on health, the foods it contains, its bioavailability, the effects of food processing methods on these components and analysis methods are examined.

Key words: *Phytoestrogens, functional food, health.*

INTRODUCTION

There is an increasing demand for functional foods. The main reason for this can be thought to be the efforts of individuals to protect and improve their health, as living conditions become difficult and stress levels increase. (Antmen and Ögenler, 2018)

Functional foods are briefly defined as "foods that have positive effects on health as well as containing essential nutrients". The most studied functional food ingredients are phenolic substances, antioxidants, dietary fibers, oligosaccharides, probiotics, prebiotics, vitamins, polyunsaturated fatty acids, sulfur-containing ingredients, phytoestrogens and phytosterols. By adding these ingredients, foods can be made functional (Meral et al., 2012). There are also food supplements produced for this purpose on the market. In this review, information is given about the structure of phytoestrogens, their types, general effects on health, the foods they contain,

their bioavailability, the effects of food processing methods on these components and their analysis methods.

Phytoestrogens

Phytoestrogens are nonsteroidal compounds produced by plants with chemical structures similar to natural or artificial estrogenic compounds (Kurzer, 1997). As a molecular structure, phytoestrogens contain phenol and hydroxyl groups. Functionally, they activate estrogenic activity by binding to receptors instead of natural estrogens in mammals. Phytoestrogens have important functions in the prevention of cancer, heart diseases, menopausal symptoms and osteoporosis (Yıldız and Fracis 2006; Soldamli and Arslanoglu, 2019). They have antioxidant properties as they protect the DNA by binding metals with the hydroxyl group in their structure. Additionally, they may change the activity of some enzymes that play a role in estrogen metabolism (Dean, 2005; İnanç and Tuna, 2005).
Phytoestrogens are included in the class of naturally occurring estrogens as shown in Figure 1 and are divided into different subclasses in terms of their chemical structures. The most studied group of phytoestrogens are flavonids and lignans (Kahraman, 2002; Shi et al., 2002; Büyüktuncer and Başaran, 2005; Andersen et al., 2006; Özer and Konuklugil, 2007; Meral et al., 2012; Soldamli and Arslanoglu, 2019).

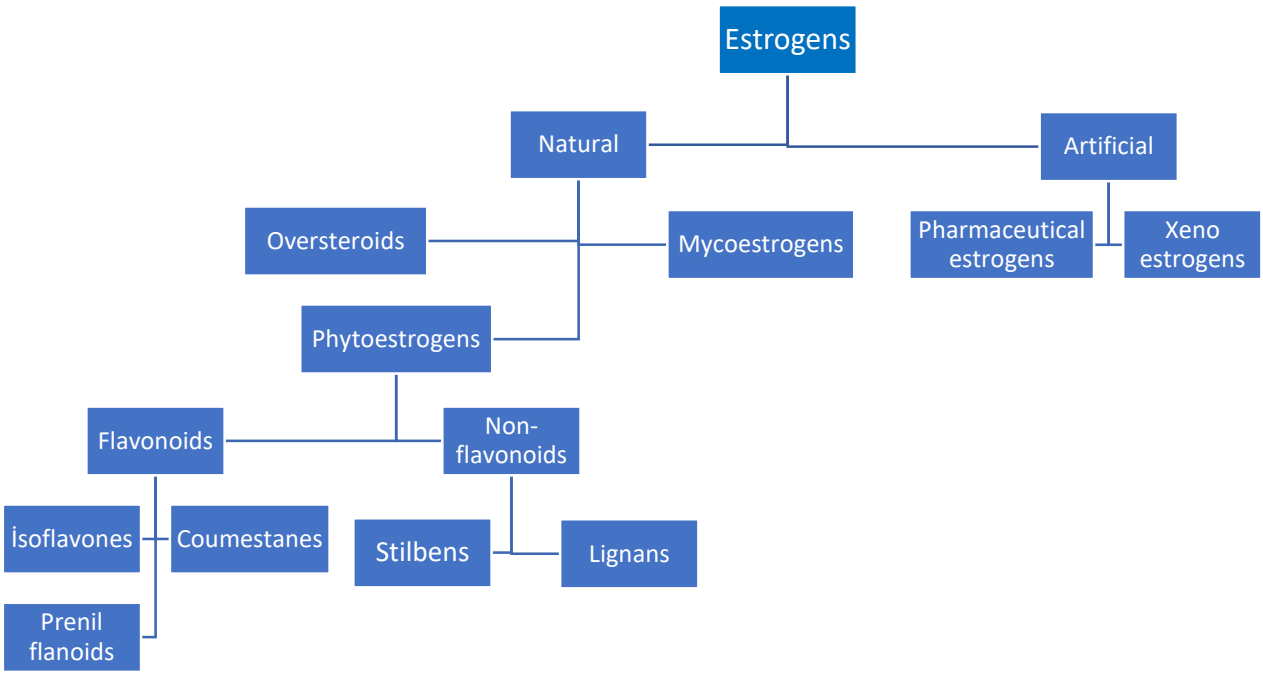

Figure 1: Classification of phytoestrogens

Although plants rich in phytoestrogens are mostly included in the *Fagales*, *Cucurbitales*, *Fabales and Malpighiales* families, they are also found in some different families. Pumpkin, hemp, soy, licorice root, red clover and flaxseed, evening glory, dong quai, ginseng, chaste tree, hops, and flax are known as plants with estrogenic activity (Shi et al., 2002; Patisaul and Jefferson, 2010; Soldamli and Arslanoglu, 2019).
The most research on phytoestrogen sources is on isoflovanes and non-flavonoid lignans included in the flavonoid group (Büyüktuncer and Başaran, 2005; Soldamlı and Arslanoglu, 2019). For this reason, in this review, the subject of isoflavones and lignans is given in more detail.

Flavonoid phytoestrogens

The term flavonid is derived from the word "flavus" which means "yellow" in Latin (Shi et al., 2002). Flavonoids are grouped in 3 separate classes as isoflavones, prenyl flanoids and cumestanes (Antmen and Ögenler, 2018). The most important group of flavonoids are isoflavones.

Isoflavones

The most important group of flavonoids are isoflavones. The main isoflavones are Daidzein (4′,7- dihydroxy isoflavone), Genistein (4′,5,7-trihydroxy isoflavone), Glycitin, Biochanin A and Formononetin (Özer and Konuklugil, 2007). Isoflavones have a colorless and crystalline phenolic ketone structure. Genistein and Daidzein are the two main isoflavone groups. Their main sources are soy, clover, lentils, beans and chickpeas. Soybean kernel is a good source of farmononetin and biochanin A. Apart from these, sunflower seeds, hazelnuts and walnuts contain a significant amount of isoflavones (Antmen ve Ögenler, 2018).

The positive effects of genistein and daidzein on breast cancer have been determined by many studies. It has been determined that genistein given in low doses causes an increase in cancerous cells and a decrease in high doses. It is stated that when used regularly, it has reducing effects in the formation of prostate cancer. Also the positive effects mentioned in the prevention of postmenopausal increasing adiposity. In addition, they have been found to be effective in the regulation of the immune system (Özer ve Konuklugil, 2007). Daidzein is important in the prevention of bone loss as it is transformed into ecole with a more estrogenic effect than itself in the intestinal microflora (Özer and Konuklugil, 2007; Branco and Hidalgo, 2011). The isoflavone content of some foods is indicated in Table 1 (Bhagwat et al., 2008).

Table 1: The isoflavone content of some foods

Food	**Total isoflavones (mg/100 g food)**
Soy protein isolate	91.05
Soy protein concentrate	94.65
Soy flour (defatted)	150.94
Soybeans (mature seeds, canned)	52.82
Soymilk (made from soy isolate)	5.90
Tofu yogurt	16.30
Tempeh	60.61
Tea, green (Japan)	0.02
Clover sprouts (raw)	0.25
Nuts, pistachio nuts (raw)	3.63

Soy-based products (soy flavonoids) used as isoflavone source in food formulations are classified into three main forms as can be seen in Table 2 (Nilüfer and Boyacıoğlu, 2008).

Table 2. Classification of soy-based products

Oil products	**Whole soy products**	**Soy protein products**
Gliserol Rafinated soy oil Soy lesitin	Soy Soybean sprout Soy milk Soy flour Soy cheese Tempeh, tofu vb.	Soya flour Soy protein concentrate Soy protein isolates

Soy protein is the only vegetable protein that is equivalent to animal protein. The FDA (Food and Drug Administration) stated that intake of 25 grams of soy protein in a low-saturated fat and cholesterol-based daily diet reduces the risk of heart diseases by lowering blood cholesterol levels. The nutritional properties of soy proteins have also been declared by the USDA (United States Department of Agriculture). Since soy proteins do not contain cholesterol, they regulates blood cholesterol. Also they have preventive effects on colon cancer as they contain dietary fiber. In addition, they also contain other important nutrients such as phytic acid, saponins and peptides (Nilüfer and Boyacıoğlu, 2008; Perez and Majem, 2012; Natalia and Samuel, 2014).
Soy isoflavones are known to reduce the risk of colon, prostate and breast cancer by removing free radicals from metabolism. It has been reported in various studies that they increase the level of estrogen hormone in postmenopause, reduce the troubles such as hot flushes and sweating, increase bone density, lower cholesterol and reduce the risk of heart diseases by preventing the development of cells that cause plaque formation in the vessels. In addition, soy protein intake of vegetable origin reduces urinary calcium excretion caused by high animal protein intake. It has been stated that soybeans have positive effects on the prevention of dementia and hair loss as well as reducing the risks of some cancer. However, there are not enough studies yet on some thyroid and liver diseases and infertility risks in men (Shi et al., 2002; Nilüfer and Boyacıoglu, 2008; Perez and Majem, 2012; Branco and Hidalgo, 2011; Anon, 2014)

Prenil flanoids

Some plants have flavones containing 1,1-dimethylalll and 3,3 dimethylalll allyl groups in their structure. Flavonols containing these allyls are generally referred to as prenylated flavones (Kaouadji and Ravanal, 1990). Prenyl flanoids are composed of 8-Prenilnaringenin, 6-Prenilnaringenin, Xsanthumol and Isoxanthohumol subgroups (Soldamlı and Arslanoğlu, 2019). The best known prenyl flanoit is hops that is also used in brewing and contains 8-prenilnaringenin in its structure. It is used in traditional medicine to prevent excitement, edema and sleeping problems (Umland et al, 2000; Antmen and Ogenler, 2018).

Coumestane

Since coumestanes are less present in the diet, fewer studies have been done on them (Dixon, 2004). In the group of coumestanes, only coumestrol shows estrogenic activity. The main source of coumestrols, which are similar in structure to isoflavones, are legumes and clover. found in small amounts in sunflower seeds, spinach, lima beans and Brussel sprouts (İnanç and Tuna, 2005; Özer and Konuklugil, 2007, Kocaadam and Akdevelioğlı, 2018).

Non-flavonoid isoflavones

Lignans

Lignans, which are the most important component of the non-flavonoid phytoestrogen group, contribute to the formation of lignin in plant cell and have very important functions in protecting the plant from environmental stress factors and in human nutrition. It has been reported to reduce the risk of heart disease, breast cancer, menopausal symptoms, and osteoporosis (Rodríguez-García et al., 2019). Lignans, whose main source is fiber foods, can be found more than isoflavones in Western diets due to their nutritional culture (Büyüktuncer and Başaran, 2005; Özer and Konuklugil, 2007; Aehle et al., 2011)
Basically non-estrogenic base lignans such as secoisolarisiresinol and metharinol have been detected in biological fluids of mammals such as faeces, urine and plasma, as well as plants. However, these two compounds are transformed into enterodiol and enterolactane as a result of

the reactions in the intestinal flora (İnanç ve Tuna, 2005; Özer and Konuklugil, 2007; Aehle ve ark., 2011).
The main sources of lignans are oilseeds, flaxseeds, wheat, rye and oatmeal (Adlercreutz, 2007). The lignan contents of some foods are shown in Table 3 (Cassidy, 2000).

Table 3: The lignan contents of some foods

Food	Lignan (µg/g)
Flax seeds	675
Lentil	17.9
Soy bean	8.6
Dried beans	5.6
Wheat bran	5.7
Oat bran	6.5
Carrot	3.5
Potato	3.0
Broccoli	2.3
Pear	1.8
Plum	1.5

Lignan can inhibit the proliferation of tumor cells by suppressing membrane ATPase activity and inhibiting the enzymes involved in cell proliferation. It has been demonstrated that dietary intake of 5% flaxseed reduces the amount of dimethylbenzantracin which causes tumor formation in mammals.

SDG (secoisolarikirsinol diglucoside), which is a type of lignan and found in flaxseed, has antiviral, antifungal, antibacterial, anticarcinogenic and antioxidant properties. Flaxseed extracts, and especially purified lignans, can reduce oxidative stress and are known to be protective against skin and colon cancer. Lignans help to lower serum cholesterol by regulating the activity of 7α-hydroxylase and acyl CoA cholesterol transferase enzymes involved in cholesterol metabolism (İşleroğlu et al., 2005).

Stilbens

Stilbens are phenolic antioxidant compounds. Resveratrol is the most important molecule in stilbens. It is stated that resveratrol prevents cancer formation and protects heart health by lowering LDL (low-density lipoprotein). Resveratrol is found in foods as cis and trans isomers. However, it is only the trans form that has estrogenic properties (Özer and Konuklugil, 2007). Resveratrol has been detected in 72 plant species. Although its main source is grapes (Bavaresco et al., 2002; Çetin, 2012), it is also available in peanuts and pineapple. Also, red wine is a very good source of resveratrol, and the longer the fermentation period, the higher the resveratrol content. Resveratrol is mostly concentrated in the skin of the grape fruit. It showed that the amount of trans-resveratrol in red wine produced from grape fermented with its skin can be as high as 14.5 mg per liter. In addition, studies have shown that as peanuts mature, the amount of resveratrol decreases. Therefore, small peanuts have higher resveratrol content (Özer and Konuklugil, 2007).

General Health Effects of Phytoestrogens

Premenopause and postmenopause

It is stated that phytoestrogens affect the distribution of estradiol to target organs and the length of the menstrual cycle by regulating the activity of pre-menopausal sex hormone-binding

globulin (SHBG) in women (Brzezinski and Debi, 1999).It has been stated that it is possible to prevent post-menopausal disorders such as night sweats and sleep disorders by increasing soy consumption in the diet (Burke et al. 2000).

Osteoporosis

It has been shown in various studies that osteoporosis, which occurs with estrogen deficiency, can be reduced by soy isoflavones. Isoflavone phytoestrogens act by stimulating the proliferation of osteoblast cells responsible for bone formation, protecting some cells against oxidation-related damage, and increasing the apoptosis of osteoclast cells that are effective in bone destruction (Dixon 2004).

Cardiovascular diseases

Phytoestrogens show a protective effect against coronary heart disease by reducing lipid oxidation and membrane lipid peroxidation. The FDA stated that consumption of 25 grams of soy protein a day together with a diet restricted in terms of saturated fat and cholesterol can reduce the risk of heart disease (Erçetin, 2007) by reducing LDL and triglyceride levels (Antmen and Ögenler, 2018)

Anticarcinogenic activity

It has been determined that phytorogens suppress the activities of DNA topoisomerase I and II, tyrosine kinase, ribosomal S6 kinase and 5α-reductase enzymes that play an important role in tumor formation. In addition, the antiproliferative properties of phytoestrogens prevent the proliferation of cells by dividing them, and they reduce the metastasis of tumor cells with their antiangiogenetic effect. It has been shown by epidemiological, in vivo, in vitro animal and human studies that phytoestrogens can be protective against hormonal cancers such as breast, prostate, endometrium and other cancers such as colon, stomach, and pancreas (Erçetin, 2007).

Antioxidant activity

By affecting free radicals, isoflavones can prevent DNA from being damaged by oxidants in cells. Dietary isoflavones decrease lipid oxidation by increasing resistance to LDL oxidation (Djuric et al., 2001).
If we evaluate the above in general, the positive effects of phytoestrogens on the prevention of cardiovascular diseases, cancer and menopausal symptoms, providing bone health and antioxidant activity have been revealed by many studies (Büyüktuncer and Başaran, 2005; İnanç and Tuna, 2005; Patisaul and Jefferson, 2010; Avcı, 2014). Apart from these, phytoestrogens are thought to reduce the risk of obesity, loss of cognitive ability, diabetes and kidney diseases, so research on these subjects is increasing (Büyüktuncer and Başaran, 2005).

Bioavailability of phytoestrogens

Phytoestrogen consumption is 2 mg/day in Western countries and 150-250 mg/day in far Asian countries such as China, Japan and Korea. It has been determined that phytoestrogens should be taken at least 50 mg/day in order to have a physiological effect (Yıldız and Fracis, 2006).
The phytoestrogen content of a food may vary according to the genetic structure of the plant, the geographical region and seasonal characteristics of the plant, the state of infection with microorganisms and the processing methods applied in food production processes (Büyüktuncer and Başaran, 2005; Erçetin, 2007).
The bioavailability of flavonoids depends on a variety of factors such as the type, structure and sugar groups of the flavonoid, intake dose and shape in the diet, nutritional habits, gender,

hereditary characteristics, intestinal microflora and absorption of other nutrients may also affect bioavailability (Güven et al, 2010).

There are many studies on the bioavailability of soy flavonoids in particular. Consumption amount, isoflavone content, type of food affect the bioavailability of soy isoflavones. For example, isoflavones taken with protein and fat-rich foods are thought to have higher bioavailability than their tableted forms due to their ability to increase bile secretion (Nilüfer and Boyacıoğlu, 2008).

The production of various soy products, processes applied to soy may reduce the bioavailability of isoflavones. Isoflavone losses occur in processes using water. For example, in the cooking process, a transition to cooking water may occur. It is also affected by pH changes. In the production of products such as tofu and tempeh, there is a decrease in the amount of isoflavones in the coagulation, immersion and heating stages. Processes such as extraction, oil removal, fermentation and peeling can also cause isoflavone loss. Soy protein concentrate production is carried out by its alcoholic or aqueous extraction from defatted soy flour. Since isoflavones are soluble in alcohol, the solvent used during extraction may cause losses (Shi et al., 2002; Nilüfer and Boyacıoğlu, 2008).

The recommended daily dose of isoflavone is approximately 40-50 mg. Soy absorption can be affected by factors such as antibiotic use, alcohol intake, and intestinal disorder. Isoflavones, which are fat-soluble substances, are absorbed from the intestines and pass into the tissues. Absorption rate is between 20-55% (Antmen and Ögenler, 2018)

There are not many studies on the intake of phytoestrogens, especially flavonoids, with food other than soy products. It is stated that daily flavonoid intake in the United States is 1.0-1.1 g/day, depending on the season. Based on the flavonoid content of foods, the daily intake can be estimated. The total phenolic amounts in 1 portion of some foods were calculated as it shown in Table 3 in some studies (Güven et al, 2010).

Table 3. Total phenolic amounts of some foods in 1 serving

Food	Total amount of phenolic in 1 serving (mg)
Apple	400
Pear and grape	300
Blueberries and cherries	200-400
Black tea, coffee, red wine, white wine and beer (200 ml) respectively	150-250, 150-180, 200-500, 40-60 ve 50-100
Bitter chocolate (40 g)	340

Based on the data in Table 3, it is estimated that consumption of 9 portions of fruits and vegetables and consumption of tea, coffee, wine, beer or chocolate in adequate amounts daily, phenolic substance intake is 1 g or more (Güven et al., 2010).

Phytotoxicity / side effects of phytoestrogens

Since the potential and mechanism of phytoestrogens are not fully determined, caution should be considered when consuming (Ibarreta et al, 2001). In some animal studies, it has been reported that reproductive dysfunction and infertility are observed in animals fed heavily with foods that are sources of phytoestrogens (Büyüktuncer and Başaran, 2005)

There are also concerns in humans that Phytoestrogens can cause infertility and stimulate breast cancer. In addition, the use of isoflavones in soy-based neonatal foods is also a concern.

In some studies, it has been stated that isoflavones can cause thyroid diseases by inhibiting the thyroid peroxidase (TPO) enzyme, which plays a key role in the production of thyroid

hormones. However, as there is no evidence of these concerns, it does not appear to support these concerns in populations where these compounds are consumed regularly and in high concentrations.

In animal studies, ıt ıs determıned that it is not possible to reach toxic doses humans with consumption of phytoestrogen sources. In addition, no cases of phytoestrogen toxicity have been reported in any country in the world, especially in societies that consume foods rich in these compounds. However, despite all these, it is not known what the results of long-term or high doses of isoflavone supplements, especially in pill, powder or gel form, will be. Hence, the use of these compounds should be kept under control until it is supported by new study results because of possible dangerous effects due to overdose (Büyüktuncer and Başaran, 2005).

Analysis methods of phytoestrogens

The main methods used for the determination of phytoestrogens, especially isoflavones, are: Chromotagophic methods (Paper and Thin Layer Chromatography, Counter Current Chromatography (CCC), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC)), spectroscopic methods (Nuclear Magnetic Resonance (NMR) Spectroscopy, Ultraviolet and Visible Spectroscopy (UV-Vis), Proton Magnetic Resonance (1H-NMR) Spectroscopy, Carbon-13 Magnetic Resonance (13C-NMR) Spectroscopy, Mass Spectroscopy (MS) and capillary electrophoresis. Today, analyzes are performed mostly by HPLC, GS gas chromatography-mass spectroscopy (GC-MS) (Oomah, 2002; Shi et al., 2002; Andersen et al., 2006; Valls et al., 2009; Yerramsetty et al., 2011; Çabaş, 2017).

CONCLUSION

A lot of studies have been conducted over the years on the functional food ingredient phytoestrogens. Although there are many research results showing the positive effects of these compounds on health, the effects of long-term and high-dose use should be clarified. There are various concerns in this regard. Therefore, it is necessary to adjust the dosage and the term of use of phytoestrogens with food and especially in the form of food supplements. Planning and conducting new clinical trials on this topic will help clarify concerns about the use of phytoestrogens.

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HEAVY METAL TOXICITY ASSESSMENT IN *LEPIDIUM SATIVUM*

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ABSTRACT

Growing industrial activities and uncontrolled agronomic practices lead to excessive accumulation of harmful substances such as heavy metals in water and soil matrixes. In recent decades, a great concern has been dedicated to remediating contaminated sites using plants. Various plants have been used to assess the toxic effect of toxic compounds. *Lepidium sativum* L. is one of the most useful standardized test withs its high sensitivity to the pollutants and its ability to germinate in a short period (3 days). The endpoints of this method are reported to be the percentage of seed germination and the length of the root. In this study, the literature on phytotoxicity of heavy metals on *L. sativum* is revised. Environmental risk assessment of various metals is given through data on *L. sativum*. According to the bioavailability data of metals, phytoremediation technology is evaluated for abatement of metals.

Keywords: *Heavy metal toxicity, Lepidium sativum, Toxicity test, Remediation*

INTRODUCTION

Heavy metal is a term used to describe metals and metalloids with atomic mass greater than 20 and a specific gravity greater than 5 (Hedayatzadeh, Banaee, & Shayesteh, 2020). Heavy metals pose a significant threat to both water and terrestrial ecosystems. They deteriorate natural ecosystems of water sources, sediments, and soils after release from natural and anthropogenic sources (Ali, Khan, & Ilahi, 2019; Hedayatzadeh et al., 2020). Heavy metals that are released into the atmosphere because of volcanic eruptions and different industrial emissions return to the land and cause soil and water pollution (Ali et al., 2019). Having an important role in organisms due to their toxic effects, accumulation and high stability, these metals enter the food chain by being absorbed by the air, absorbed by the soil and mixed with groundwater. Heavy metal pollution is different from organic pollutants in that it is a latent, permanent and irreversible pollution. This pollution not only impairs the quality of water resources, food crops and atmosphere and but also becomes an essential threat to the health of organisms and people by the reason of its accumulation in the food chain (Li et al., 2019).

Lepidium sativum L. is a useful model to represent environmental stress under standardized conditions as indicated by OECD (OECD, 1984). Studies with this strain were conducted to evaluate the soil phytotoxicity caused by heavy metals, polycyclic aromatic hydrocarbons, petroleum derivatives and in the not too distant past, zinc, and nickel nanoparticles. It has been proven by studies that *L. sativum* is also useful in remediation processes (Pignattelli, Broccoli, & Renzi, 2020).

In this study, the literature on the phytotoxicity of some heavy metals on *L. sativum* was reviewed and the environmental risk assessment of some heavy metals was investigated through *L. sativum* data.

PHYTOTOXICITY OF METALS

Even though some heavy metals (Cr, Ni, Co, Cu, Zn, Fe, Mn etc.) have a key role in controlling osmotic balance, metabolic activity and enzymatic reactions some metals such as Cd, Pb, Cr, Ni, Hg, have toxic effects and may inhibit the growth of plants even at low concentrations. Negative phytotoxic effects such as excessive heavy metal accumulation in water and soil environments, slowing of growth, photosynthesis impairment, biomass decrease and nutrient intake can be observed (Diaconu et al., 2020). And also, some heavy metals are known to cause teratogenic, mutagenic or carcinogenic effects on different species, according to the exposure dose and period (Ali et al., 2019). Hence, it is important to observe these contaminants in the food chain of living things and to reduce their side effects (Hedayatzadeh et al., 2020).

Every year, new pollutants are being known to cause a negative impact or may harm living organisms (Oleszczuk, 2008). Although it is well known that high total heavy metal concentrations observed in areas contaminated with metals released from past industrial activities may not always become too toxicity, bioavailability and high mobility, the risks in these areas are generally evaluated based on total concentration analysis (Hagner et al., 2018).

Biological tests used in this process are used to determine the negative effects of pollutants and possible interactions between them (Oleszczuk, 2008). Organisms are sensitive only to the bioavailable true metal fraction. When chemical analyzes are completed with biotests, the environmental hazard potential of metals is better understood. Biological tests can integrate the effects and bioavailability of mixtures. Therefore, it is extremely useful in the site-specific assessment of real ecological risks. (Hagner et al., 2018). Plants, for example, are the main primary producers in terrestrial ecosystems. It is important to determine the potential of plants and phytotoxins and to comprehend the magnitude of their impact on various terrestrial ecosystems.

Recent reports support the usefulness of phytotoxicity testing in assessing environmental toxicity such as soils and sediment and anthropogenic toxicity such as compost and sewage sludge (Oleszczuk, 2008). The basic principles for evaluating phytotoxicity are the same as fungicide, pesticide, herbicide, heavy metal, or other toxic compounds.

Acute and chronic phytotoxicity tests have been used the toxicity assessment of chemicals in the soil that based on ISO guidelines have been used for many years. Germination and root growth tests can be used for different herbaceous plant species. Trees, which are a long-lived species and can be exposed to small amounts of pollution for a long time, have also been used as test organisms by some researchers. The use of plants in these tests is advantageous over other organisms because they are more sensitive to environmental stress and are easy to handle and store (Visioli, Menta, Gardi, & Conti, 2013).

Lepidium sativum is a sensitive plant species. It is mostly used in toxicity tests due to its rapid growth, cheapness, and ease of analysis. By evaluating the seed germination, root length and dry biomass of the plants, it is observed whether the metal ions have an inhibitory effect on the seed germination process of *L. sativum* and the toxic value of the metal is obtained by evaluating the obtained data (Pavel, Sobariu, Diaconu, Stătescu, & Gavrilescu, 2013).

EFFECTS OF HEAVY METALS ON *LEPIDIUM SATIVUM* GERMINATION AND GROWTH

Lepidium sativum L., also known as garden cress, is an edible plant belonging to the Brassicaceae family, rich in vitamins as well as macro and microelements, fast-growing and low nutrition requirement (Mojiri et al., 2013; Smolinska & Leszczynska, 2015).

Lepidium sativum L. is a plant that stands out with its short vegetation period and its ability to grow in any climate, soil type or season. This plant, which is extremely sensitive to environmental pollution, is used for biological tests evaluating water and soil quality (Shulaev et al., 2020). It is widely grown in the world in Europe, Asia, and Egypt because it has medicinal and culinary values (Mojiri et al., 2013).

Lepidium sativum L. is a sensitive test type that evaluates seed germination, root length and dry biomass of plants. The test is to investigate whether the metal ions have an inhibitory effect on the seed germination process of *Lepidium sativum*. In the *L. sativum* toxicity test, the per cent inhibition of seed germination (GI), root growth inhibition (RI) and biomass inhibition (BI) is calculated by the formula (Oleszczuk, 2008):

$$GI; RI; BI = \frac{A-B}{A}\ x\ 100 \qquad (1)$$

A refers to root length, seed germination and biomass in the control soil and B refers to the root length, seed germination and biomass in the test soil.

The study by Montvydiene & Marčiulioniene (2004) aimed to evaluate the toxicity of heavy metals (Ni, Mn, Cr, Cu, Zn, Pb and Cd) and their mixtures in different concentrations on *Lepidium sativum* in root growth inhibition test in 2 days. At the end of the test, the germination capacity of the seeds and, the length of roots in distilled water were found as 96% ± 4% and 36.0% ± 2.8 mm, respectively. Collecting data are analyzed and calculated by one-way analysis of variance (ANOVA) program and Dunnett's multiple comparison test at p 0.05. Standard prediction errors were not exceeding 8%. The linear regression analysis of *L. sativum* was calculated and EC50 values (toxic concentration inducing 50% growth inhibition) were estimated according to this analysis. EC50 values were determined to be for Ni (II), Cu (II), Cr (VI), Mn (II), Pb (II), Cd (II), and Zn (II) and the results respectively were found; 26.5 mg / L, 7.6 mg / L, 1.8 mg / L, 300 mg / L, 73.7 mg / L, 77.8 mg / L and 149 mg / L. These results showed that Cr was the most toxic elements for *L. sativum*. Seed germination was not significantly different from the control at the tested concentrations. On the other hand, the highest copper concentration (10 mg / L) was reduced seed germination by 20%.

Fuentes et al. (2006) reported that heavy metals content in sludge generated in wastewater treatment plants limits the use of these resources for agricultural aims. In this study, different types of sludge such as aerobic, unstabilised, anaerobic and sludge from a waste stabilization pond were studied to investigate heavy metal toxicity using simple extraction, DTPA and water. Treatment sludges were depended on chemical characterization and toxicity tests (ecotoxicity and phytotoxicity tests) without substrate, and initial assessment of their suitability for the land practice was made. As a result of ecotoxicity analyzes, it was observed that no sludge produced hazardous waste, but it was reported that sludge extracts had a significant negative effect on the germination index (GI) of barley (*Hordeum vulgare* L.) and cress (*Lepidium sativum* L.). This result showed that some traits affect root growth.

Oleszczuk (2008) investigated the phytotoxicity of composted sewage sludge and its relationship with heavy metals and polycyclic aromatic hydrocarbon contents. In the study, the toxicity of four different municipal sludge tested with *Lepidium sativum* after 76 days of composting was evaluated. It has been observed that the whole PAH amount in the sludge's change from 3674.1 to 11236.3 mg/kg, while heavy metal contents are between 156-335 mg/kg for Cu, 37.5-59.5 mg/kg for Pb, 1.9-76 mg/kg for Cd, 27.6-120 mg/kg for Cr, 1015-1385 mg/kg

for Zn and 21.7-155 mg/kg for Ni. It was observed that seed germination was inhibited by 100% in both tested sludge samples. Also, in the evaluation of root length, it was observed that sludge damaged *L. sativum* and in three of these four sludge samples, root growth was largely inhibited.

As a result of increased cadmium (Cd) intake in contaminated soils, plant metabolism changes and crop production efficiency are limited. *Lepidium sativum* L. toxicity was studied at different Cd concentrations (0, 25, 50 or 100 mg/kg soil) by Gill, Khan, & Tuteja, (2012). They observed that Cd accumulation in roots and leaves increased with increasing Cd concentration in the soil. The leaf area was inhibited by high Cd concentration and other features such as a plant dry mass, and the net photosynthetic rate, stoma conductivity, intercellular CO2, and chlorophyll (Chlorophyll a, Chlorophyll b also total Chlorophyll) content is have been decreased. Particularly, the reduction in nitrogen content showed that cadmium negatively affected N metabolism. The high concentration of cadmium inhibited the growth of *L. sativum* by interfering with the photosynthetic pathway and altering the balance between carbon, N and S metabolism. Besides, the coordination of S and N metabolism has completed the antioxidant mechanism so that the growth and photosynthesis of plants are not adversely affected at low cadmium concentration (25mg Cd/kg soil).

Pavel et al. (2013) investigated the phytotoxic effects of heavy metals chrome (VI) and cadmium (II) on plant germination and growth. Stock solutions of Cr (VI) and Cd (II) were prepared in distilled water at a concentration of 1000 mg / L. Diluted working solutions were prepared as 30, 60, 90, 120, 150 and 300 mg/L. In phytotoxicity tests, Whatman filter papers placed in petri dishes were dipped in 3 mL heavy metal solution for each metal ion. The seed germination, root length, and dry biomass of *Lepidium sativum* were evaluated and as a result metal ion was found to inhibit the seed germination process of *L. sativum*. Root growth has been reported to be affected by both the metal ion test, and its concentration. The data obtained from the measurements to determine the growth (root length, stem length, stem wet material mass and dry mass) has been compared for the test group, and the control group. Metal ions were observed to have an inhibitory effect on the seed germination process of *L. sativum*. The degree of inhibition has been varying depending on the metal ion, and their concentration in aqueous solution. While the inhibition effect on germination decreased at low concentrations up to 20 -25 mg/L, approximately 50% inhibition effect on germination was observed at samples of 25-300 mg / L compared to the control. Both Cr (VI) and Cd (II) at concentrations of 300 mg/L caused a reduction in root length of the seedlings by about 61-68% compared to the control.

The effect of different concentrations of chrome and cadmium metal ions has been evaluated in comparing to a similar group grown non-toxic (control sample) on *Lepidium sativum* biomass, stem length, seed germination and root development by Diaconu et al. (2020). They observed that Cr (VI) affected plant growth through seed germination. Depending on the heavy metal content of the environment, the germination degree varied between 50-60%. As regards the control sample, the lowest germination degree was observed to be 300 mg Cr (VI)/ L. This result showed that *L. sativum* was particularly impressed by chrome at high metal ion concentration (900 μg germination medium) in the medium. However, *L. sativum* was found to be tolerant to chrome at the root in consequence of ion immobilization where it can be decreased to the fewer toxic state of Cr (III).

ESTIMATION OF RISK/HAZARD QUOTIENTS FOR HEAVY METALS

The risk quotient (RQ) or Hazard Quotient (HQ) is the basic guideline internationally admitted and adopted the measure in the improvement of international guidelines (Hernando, Mezcua, Fernández-Alba, & Barceló, 2006). The risk to target organisms is found by calculating the

ratio between the measured environmental concentration (MEC) and predicted non-observed environmental concentration (PNEC) data sets. Accordingly, the RQ or HQ ratio calculation is given below:

$$HQ = \frac{MEC}{PNEC} \quad (2)$$

Table 1. Estimation of Risk/Hazard Quotients for Heavy Metals

Risk quotient (RQ) or Hazard Quotient (HQ) values	**Risk assessment**
‹ 0.1	no risk
between 0.1 and ≤ 1	low risk
>1 and ≤ 10	moderate risk
> 10	high risk

As described above, EC_{50} values of heavy metals obtained for *Lepidium sativum* can be given in Table 2. considering average values.

Table 2. *EC_{50} values (mg/L)* and risk quotients of heavy metals for *Lepidium sativum*

	Conditions	*Cd (II)*	*Cu (II)*	*Cr (VI)*	*Mn (II)*	*Ni (II)*	*Pb (II)*	*Zn (II)*
Pavel et al. (2013) *	Distilled water, 3-d seed germination	150		275				
Montvydiene& Marčiulioniene (2004)	Distilled water, 2-d root length	26.5	7.6	1.8	300	73.7	77.8	149
MEC (mg/L) (WHO, 1993)	Drinking water (mg/L)	0.01	2	0.05	0.4	0.02	0.05	3
PNEC values (root length) **	Montvydiene& Marčiulioniene (2004)	0.265	0.076	0.018	3	0.737	0.778	1.49
PNEC values (seed germination) **	Pavel et al. (2013) *	*1.5*	-	*2.75*	-	-	-	-
RQ/HQ root length	Montvydiene& Marčiulioniene (2004)	*0.0377*	*26.316*	-	*1.333*	*0.027*	*0.064*	*2.013*
RQ/HQ seed germination	Pavel et al. (2013) *	*0.006*	-	*0.018*	-	-	-	-

*A 300 mg/L concentration caused a reduction in root length of the seedlings by about 61-68% compared to the control. **(EC50/AF); AF is taken to be 100 as only one specie is taken into account.

CONCLUSION

Heavy metals coming from industry, traffic and agricultural activities have significant effects on human health, plants and animals. In general, these pollution sources directly affect water, air and soil quality. Therefore, the detection and removal of these heavy metals are important for researchers. Toxicity tests are used for the analysis and classification of these heavy metals. Different toxicity tests are being applied today, but *Lepidium sativum* toxicity test is mostly preferred because of its easy growth, not being too selective in ambient conditions and being less affected by toxicity. In this article, different toxicity studies on *Lepidium sativum* plant were examined and risk assessment was calculated according to the data. When we examined the studies, it was seen that different concentrations of heavy metals affect root length and seed germination. According to Pavel et al., high Cr and Cd concentration negatively affect root length and seedling. Also, the EC50 values (EC50 = 150 mg / L for Cd (II) and EC50 = 275 mg / L for Cr (IV)) show the negative effect of Cd (II) and Cr (IV) on *Lepidium sativum.* Plant growth and germination showed negative effects on development by being affected by the presence of heavy metals. In another study on heavy metal toxicity related to *Lepidium sativum*, it was reported that only one metal affected the plant less, but plant growth was more inhibited in the presence of more than one metal. When the RQ or HQ values found according to Table 2. and Pavel et al. are examined, Cd (II) and Cr (IV) metals have no risks (0.06 <1; 0.018 <1) for the plant at the concentrations studied. Also, in another study by Montvydiene & Marčiulioniene, Cd was risk-free (0.0377 <1), Cu was high risk (26.316 > 10), Mn was moderately risky (1.333> 1), Ni (0.027 <1) and Pb is not at risk (0.064 <1) and finally Zn is moderately risky (2.013> 1).
The studies show that the *Lepidium sativum* is a useful model to represent environmental stress under standardized conditions and useful in remediation processes due to less effect by heavy metals.

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DIMENSIONS OF MIDDLE-BELT LEAVES IN BASMA TOBACCO VARIETIES

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ABSTRACT

Macedonian tobacco production is recognizable around the world by oriental types of tobacco, represented by a large number of varieties. The morphological properties of tobacco are a very important factor in determining primarily the type of tobacco, and then the individual varieties within the species. These traits of each species or variety are more or less different and are genetically controlled. Experience to date in this area shows that tobacco plants of a certain type under the influence of agro-ecological conditions and agro-technical measures in vegetation can also show strong variations in terms of bio-morphological properties, especially leaf dimensions. Important indicators for determining the quality of raw tobacco are: insertion, leaf size, content, elasticity, color, degree of damage. Therefore, the aim of the study was to present the characteristics of Basma's varieties from the middle belt insertion: length, width and angle of attachment to the lower middle, right middle and upper middle leaf, in the stage of technological maturity. The study was conducted in the field of the Tobacco Institute in Prilep; the trial was set up in 5 replications, using a random block system, including 4 oriental varieties of tobacco: three Basma's: MK-1, MB-2 and MB-3, and Yaka 7-4/2 as a control (ø). Basma's varieties were created by the Scientific Institute for Tobacco - Prilep, and the State Commission for Variety Recognition recognized them in 2010. The obtained data were statistically processed and tested by LSD test. In terms of leaf dimensions, the length of the lower middle leaf for the MB-3 variety showed statistically significant differences compared to the control variant. On average, all Basma's varieties have slightly larger leaf dimensions compared to Yaka 7-4/2 (ø), but the dimensions of the middle belt leaves (MB-3), are important for achieving higher green mass yield per plant/per hectare and obtaining quality raw tobacco. Macedonian tobacco production is recognizable around the world by oriental types of tobacco, represented by a large number of varieties. The morphological properties of tobacco are a very important factor in determining primarily the type/variety of tobacco, and they differ to a greater or lesser extent and are genetically controlled. Experience to date in this area shows that tobacco plants under the influence of agro-ecological conditions and agro-technical measures can also show strong variations in terms of bio-morphological properties, especially leaf dimensions. Important indicators for determining the quality of tobacco are: insertion, leaf size, content, elasticity, color, degree of damage. Therefore, the aim of the study was to present the characteristics of Basma's varieties from the middle belt insertion: length, width and angle of attachment to the lower middle, right middle and upper middle leaf, in the stage of technological maturity.

Keywords: *oriental tobacco, basma, middle-belt leaves, dimensions*

INTRODUCTION

Oriental tobaccos account for almost 95% of the total tobacco production in Republic of North Macedonia. In recent years this stock has been enriched with Basma's tobacco, which yield

and quality meets the criteria and standards of manufacturers and companies that purchase tobacco from this region. The varieties of the type Basma are ranked in the high-quality group of oriental aromatic tobaccos. This type was Introduced and spread in Macedonia and it consists of the newly created varieties (MK-1, MB-2, MB-3). From total annual tobacco production (25-30000 tons) in Republic of North Macedonia, over 90% are intended for exports. Republic of North Macedonia is recognized as tobacco country, small in size and population but one of the biggest in production of good quality oriental tobacco per capita. With the change of smoker's taste preference, the manufacturers requirements for particular raw materials of oriental tobacco used in cigarette blends also change. For these reasons, each year foreign buyers are offered raw materials of various types and varieties to satisfy their requirements in terms of flavor, taste and other properties of tobacco. The Basma's tobacco is one of the most highly valued oriental tobaccos in the world. It is mainly grown in Greece and Turkey, but after the dramatic decline in these two countries (especially in Greece), tobacco companies see a chance to relocate a part of the production in areas where soil and climate conditions favor the production of this type in order to make its production more attractive to manufacturers. Due to its quality, there is no problem with the export of this type and it gives incentive for production of newly created varieties which would be interesting for farmers, manufacturers, traders etc. Korubin-Aleksoska (2018). The highest length of leaves from the middle belt, in control variants has YK-48 (21.65 cm. in 2016 and 21.35 cm in 2017), while in the oriental line of the Prilep type L7 the dimensions of the leaves are (31.5 cm in 2016 and 30.9 cm in 2017). The author concludes in her research that the dimensions for the length of the leaves in the control and the Jaka type lines are quite similar, but of the newly created of the Prilep type has significantly longer leaves. Dimitrieski & Miceska (2015) in their research state have been a new prospective variety of the oriental Yaka tobacco of inheritance of the characteristic and absence of interallelic interaction. Miceska (2017), investigated some morphological, productional and quality characteristics in four new lines the type Prilep obtained by generative hibridization. Regarding the morphological properties (plant height, leaf number, largest leaf size), all lines showed very low variability, which is an indication of morfological uniformity and stability.The subject of our research is to study the major quantitative properties of different oriental tobacco varieties, to make a comparison between them and to estimate their variability.

MATERIAL AND METHODS

As material for this research are used the following 4 varieties: YK 7 – 4/2 (control), and Basmak tobacco, varieties MK – 1, MB -2 and MB – 3. The tobacco seedling was produced with the usual traditional method in cold beds covered with polyethylene at the nursery for tobacco seedlings at the Scientific Tobacco Institute in Prilep.During the investigation, we used elite seed material with quantity of 5 g per 10 m^2. All necessary agro technical measures were applied.The soil was prepared with one autumn and three spring ploughings before tobacco planting in the field.The manuring of the soil during the three years of investigation was done with 300 kg/ha mineral manure NPK (8:22:20).The transplantation at open field was done in the beginning of June.

The trial was set up in the Experimental field of Tobacco Institute–Prilep, in randomized block design with five replications, using traditional agricultural practices, and planting density of 45×12 cm on a previously prepared soil. The useful area of the experiment was 214 m^2. During the growing season we monitored the length of the growing period of tobacco in the field and conducted morphological measurements of the following parameters on 50 plants per variety:length and width of upper leaves per plant, and we checked the variability of morphological characters of tobacco varieties using the basic statistical parameters:standard deviations, degree of variability and LSD test method (Najceska, 2002).

RESULTS AND DISCUSSION

Morphological properties are one of the most important indicators to determine the type (variety), harvest belt and insertion of the tobacco. Depending on the agro-ecological conditions and applied agro-technique, the tobacco can be developed with a bigger or smaller habitus, but still in certain hereditary limits that are characteristic for the tobacco type or variety. Only fully and morphologically formed stalks can be subject to assessment according to their characteristics of witch tobacco type or variety they belong, and even to predict with high accuracy the yield (number and dimensions of leaves). According to Atanasov (1972), the number of leaves is a variety feature and represents highly consistent quantitative property. On the other hand, dimensions of leaves by insertion mostly depend on the type–variety of the tobacco and the growing conditions. Karajankov at al. (2007) divided all types and varieties of tobacco into three basic groups: small-leaf (up to 18 cm), additional-semi-oriental (19-30 cm) and large-leaf (over 30 cm). In Basma tobacco types, the leaves on the stem are arranged in a spiral and have smaller dimensions, and with slightly larger dimensions are the leaves from the middle harvest belt at the insertion - true middle leaf. Going up in the insertions, their dimensions decrease and in the top belt they are the smallest. In the examined varieties, measurements were made of the leaf dimensions of the following insertions: lower middle leaf, true middle leaf, upper middle leaf.

According the size of the middle insert leaves of Basma tobacco in different research years with different rain capacity have shown to be typical for oriental type of tobacco. Mitreski (2012) state that According to the Rulebook on assessment and purchases of oriental types of tobacco, depending on their quality, the inserts from the middle belt leaves, are in the second and third class.

Photo 1. JK 7-4/2 Photo 2. MK-1 Photo 3. MB-2 Photo 4. MB-3

Dimensions of the lower middle green leaf and angle of attachment

This leaf has an ovoid, elliptical, oval or other shape, which depends primarily on the variety, and represents a transition from the lower to the middle belt. The tip of this leaf is sharper and the base is slightly wider compared to the upper primings. The largest width is in the middle of the leaf in the upper half. It has larger dimensions of the leaves from the upper primings, and participates in the total yield of the stalk with about 15%.

According to the data in Table 1, the length of the lower middle leaf ranges from 17.6 cm in the variety MK-1 in the dry year 2011, to 21.5 cm in the variety MB-3 in 2010. On average, the smallest length of the lower middle leaf is characterized by the variety MK-1 with 18.3 cm, and the largest variety MB-3 with 20.2 cm, which is 10.4% higher relative difference than the control variety.

Table 1. Dimensions of the lower middle green leaf and angle of attachment

Variety	Year	Length, cm	3 years Average	Differences of relative %	Width, cm.	3 years Average	Differences of relative %	Leaf attachment angle	3 years Average	Differences of relative %	Ratio L: W	3 years Average	Differences of relative %
YK 7-4/2 ∅	1	18.7	18.7	100.00	9.0	9.5	100.00	27.3	27.8	100.00	2.1	2.0	100.00
	2	19.6			10.7			28.2			1.8		
	3	17.7			8.7			28.0			2.0		
3MK-1	1	18.1	18.3	98.0	8.3	8.4	88.42	25.6	25.8	92.81	2.2	2.2	110.00
	2	19.3			8.7			25.7			2.2		
	3	17.6			8.3			26.2			2.1		
MB-2	1	19.0	19.3	103.21	8.6	9.1	95.79	24.8	24.8	89.21	2.2	2.1	105.00
	2	20.4			9.8			24.8			2.1		
	3	18.4			8.8			25.0			2.1		
MB-3	1	19.1	20.2	108.02	8.4	9.4	98.95	25,4	25.6	92.09	2.2	2.1	105.00
	2	21.5^{+}			10.4			25.0			2.1		
	3	19.6^{++}			9.4			26.5			2.1		

1st year, leaf length
LSD 5% 1.29 $^{+}$
1 % 1.82 $^{++}$

2nd year, leaf length
LSD 5% 1.58 $^{+}$
1% 2.22 $^{++}$

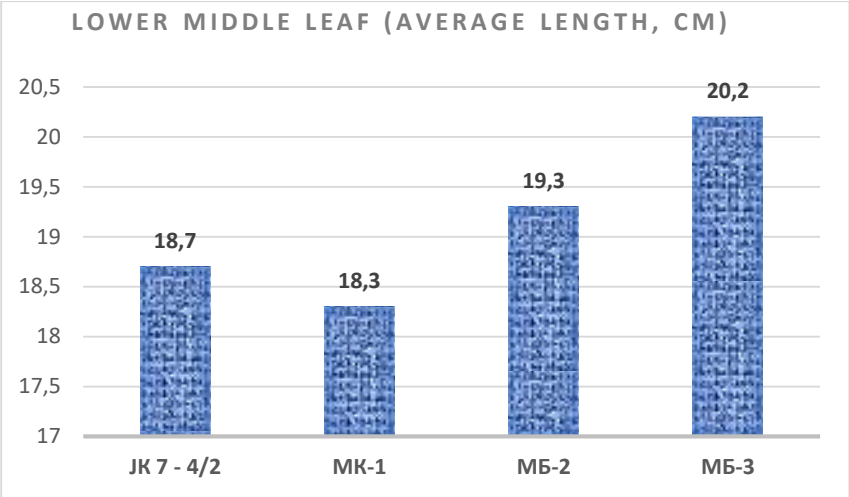
3rd year, leaf length
LSD 5% 1.26 $^{+}$
1% 1.78 $^{++}$

1st year, leaf width
LSD 5% 0.82 $^{+}$
1% 1.16 $^{++}$

2nd year, leaf width
LSD 5% 0.84 $^{+}$
1% 1.17 $^{++}$

3rd year, leaf width
LSD 5% 0.80 $^{+}$
1% 1.13

Only the MB-3 variety showed statistically significant differences of 1% in 2010 and 5% in 2011. The other varieties did not show significance compared to the control one. The leaf width of the varieties by years ranges from 8.3 cm in MK-1 (2009 and 2011) to 10.7 cm in the control variety JK 7-4 / 2 (2010). On average, the width of the lower middle leaf ranges from 8.4 cm in MK-1 to 9.5 cm in the variety JK 7-4 / 2. The angle of attachment on average ranges from 24.8˚ in MB-2 to 27.8˚ in the control variety JK 7-4 / 2. The ratio length: width on average ranges from 2.0 : 1 for the variety JK 7-4 / 2 to 2.2 : 1 for MK-1. This data shows that the shape of the leaves of the examined varieties of this insertion are approximately the same. The average length of the lower middle leaves, according to the variants, are shown in the Graph 1.



Graph 1. Lower middle leaf

Dimensions of true middle green leaf and angle of attachment

The real middle leaf is the largest and its widest width is in the middle of the leaf. In the total yield per stalk this leaf participates with about 25% (Photo 2). According to the data in Table 2, the smallest length of the true middle leaf (18.1 cm) has the control variety JK 7-4 / 2 in the dry year 2011, and the biggest length was registered with the variety MK-1 in 2009 (22.6 cm). On average for the three years, the smallest length of these leaves has the control variety JK 7-4 / 2 (19.5 cm), and the biggest (21.0 cm) variety MB-3, which is 7.69% more compared to the control one. Statistically, for this attribute significant differences of 5% were shown in MB-2 in 2010 and MB-3 varieties in 2011, and a difference of 1% was shown in MK-1 and MB-3 varieties in 2009.

The width of the leaves by years ranges from 9.4 cm for JK 7-4 / 2 and MK-1 (2011) to 11.7 cm in the variety MK-1 (2009). On average, the width ranges from 10.1 cm in the control variety JK 7-4 / 2 to 10.9 cm in MB-2, which is 7.92% more compared to the control one. Statistically significant differences of 5% were shown by the varieties MK-1 and MB-2 (2009) and MB-3 in 2011. On average, the angle of attachment ranges from 23.4˚ in MB-2 to 27.2˚ in the control variety JK 7-4/2. Length: width ratio ranges from 1.9 : 1 for JK 7-4/2, MK-1 and MB-2 varieties and to 2.0 : 1 for MB-3. The average length of the true middle leaves, according to the variants, are shown in the Graph 2.

Table 2. Dimensions of true middle green leaf and angle of attachment

Variety	Year	Length, cm	3 years Average	Differences of relative %	Width, cm	3 years Average	Differences of relative %	Leaf attachment angle	3 years Average	Differences	Ratio L: W	3 years Average	Differences of
ЖК 7-4/2	1	20.8	19.5	100.00	10.8	10.1	100.00	25,8	27.2	100.00	1.9	2.0	100.00
	2	19.7			10.1			30.0			1.9		
	3	18.1			9.4			25.7			1.9		
МК-1	1	22.6^{++}	20.4	104.61	11.7^{+}	10.5	103.96	22.8	23.5	86.40	1.9	2.2	100.0
	2	20.0			10.5			25.0			1.9		
	3	18.5			9.4			22.8			2.0		
МВ-2	1	21.8	20.7	106.15	11.6^{+}	10.9	107.92	23.1	23.4	86.03	1,9	2.1	100.00
	2	21.1^{+}			11.0			24.0			1,9		
	3	19.1			10.1			23.1			1,9		
МВ-3	1	20.4	21.0	107.69	10.2	10.4	102.97	24.3	24.5	90.10	2,0	2.1	105.26
	2	22.2^{++}			10.7			25.0			2.1		
	3	20.2^{+}			10.4^{+}			24.3			1.9		

1st year, leaf length
LSD 5% 1.06 $^{+}$
1% 1.49 $^{++}$

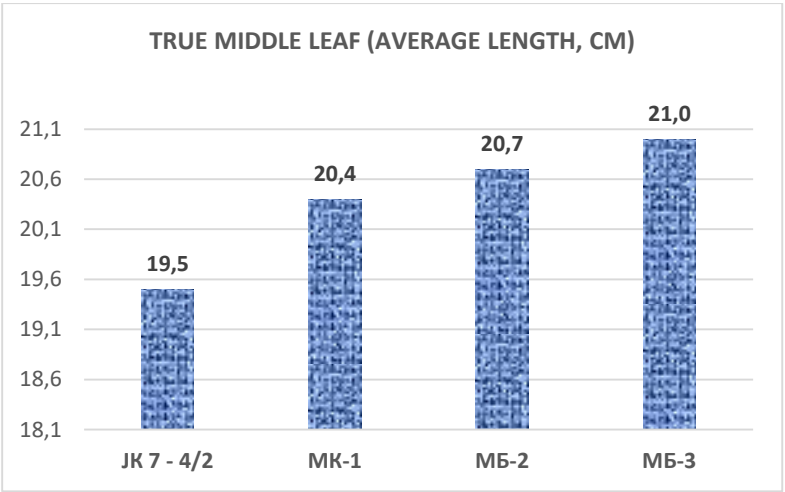
2nd year, leaf length
LSD 5% 1.22 $^{+}$
1% 1.72 $^{++}$

3rd year, leaf length
LSD 5% 1.58 $^{+}$
1% 2.21 $^{++}$

1st year, leaf width
LSD 5% 0.67 $^{+}$
1% 0.95 $^{++}$

2nd year, leaf width
LSD 5% 0.94 $^{+}$
1% 1.35 $^{++}$

3rd year, leaf width
LSD 5% 0.83 $^{+}$
1% 1.17^{++}



Graph 2. True middle leaf

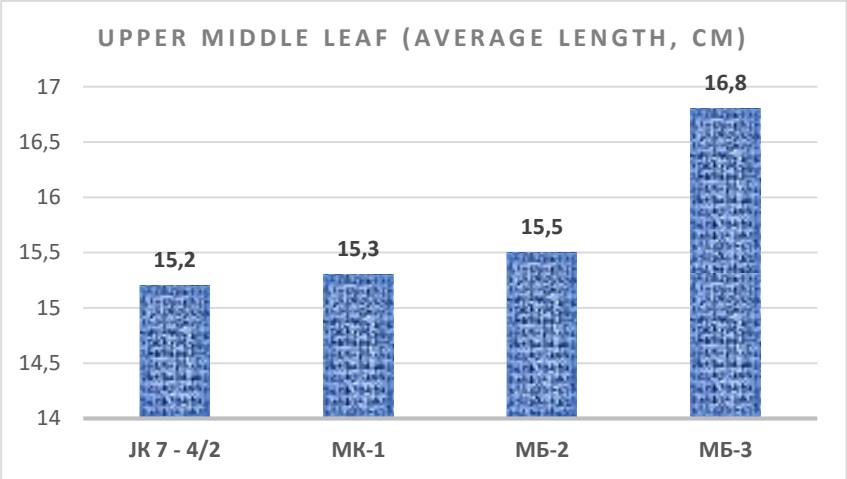
Dimensions of upper middle green leaf and angle of attachment

The upper middle leaves have an expressed tip and the base of the leaf is slightly wider. The surface of the leaf is flat, and the biggest width is just below the middle of the leaf. In the total yield of the stalk this insertion participates with about 20%. The leaves of the examined varieties of this insertion are shown in photo 3. From the data in Table 3 it can be seen that the smallest length of these leaves (13.8 cm) is characterized by the control variety JK 7-4/2 in the dry year 2011, and the biggest length of the leaf is registered in the variety MB-3 (18.0 cm) in 2010. The control variety JK7-4/2 is characterized with lowest average leaf length (15.2 cm) and the largest (16.8 cm) variety MB-3, which is 10.53% more compared to the control one. For this attribute, statistically significant differences of 1% was shown by the varieties MB-2 in 2011 and MB-3 in 2011. The statistically significant difference of 5% was shown by the variety MB-3 in 2010. The varieties MK-1 and MB-2 in 2009 and 2010 did not show statistically differences compared to the control one. The width of the leaves by years ranges from 6.9 cm in the variety MK-1 (2009) to 9.0 cm in the variety MB-3 (2010). On average, the width of these leaves ranges from 7.4 cm in the variety MK-1 to 8.1 cm in MB-3, or 5.19% more compared to the control one.

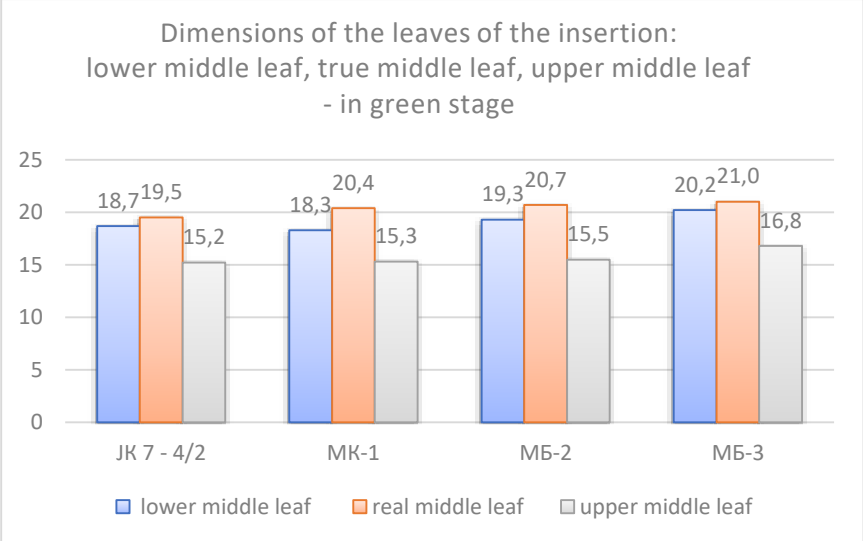
Table 3. Dimensions of upper middle green leaf and angle of attachment

Variety	Year	Length, cm	3 years Average	Differences of relative %	Width, cm	3 years Average	Differences of relative %	Leaf attachment angle	3 years Average	Differences of relative	Ratio L: W	3 years Average	Differences of relative %
JK 7-4/2 ∅	1	15.6	15.2	100.00	7.3	7.7	100.00	30.7	31.1	100.00	2.1	2.0	100.00
	2	16.1			8.3			30.7			1.9		
	3	13.8			7.4			32.0			1.9		
MK-1	1	14.7	15.3	100.66	6.9	7.4	96.10	30.3	29.7	95.50	2.1	2.1	105.00
	2	16.8			8.3			30.3			2.1		
	3	14.4			7.0			28.5			2.0		
MB-2	1	15.8	15.5	101.97	7.3	7.5	97.40	30.9	31.5	101.29	2.1	2.1	105.00
	2	16.1			8.1			31.5			2.1		
	3	14.7 ++			7.2			32.0			2.0		
MB-3	1	15.3	16.8	110.53	7.1	8.1	105.19	32.6	32.3	103.86	2.2	2.1	105.00
	2	18.0 +			9.0			32.8			2.1		
	3	17.2 ++			8.3			31.5			2.0		
1st year, leaf length LSD 5% 1.60 + 1 % 2.25 ++					2nd year , leaf length LSD 5% 1.40 + 1% 1.97 ++						3rd year , leaf length LSD 5% 0.57 + 1% 0.81 ++		
1st year, leaf width LSD 5% 0.50 + 1% 0.70 ++					2nd year, leaf width LSD 5% 0.77 + 1% 1.09 ++						3rd year, leaf width LSD 5% 0.57 + 1% 0.81 ++		

The examined varieties did not show statistical differences in terms of this property. On average, the attachment angle ranges from 29.7˚ on the MK-1 to 32.3 ˚ on the MB-3 and is slightly larger compared to the true middle leaf-range. On average, the length: width ratio ranges from 2.0 in JK 7-4/ 2 to 2.1 in MK-1, MB-2 and MB-3. The average length of the upper middle leaves, according to the variants, are shown in the Graph 3.



Graph 3. Upper middle leaf



Graph 4. Dimensions of the leaves of the insertion lower middle leaf, true middle leaf, upper middle leaf (average length, cm)

CONCLUSION

On average, the smallest length of the lower middle leaf is characterized by the MK-1 variety with 18.3 cm, and the biggest variety MB-3 with 20.2 cm, which is 10.4% higher relative difference than the control variety. Only the MB-3 variety showed statistically significant differences of 1% in 2010 and 5% in 2011.

On average, the width of the lower middle leaf ranges from 8.4 cm in MK-1 to 9.5 cm in the JK 7-4/2 variety. The angle of attachment on average ranges from 24.8˚ in MB-2 to 27.8˚ in the

control variety JK 7-4/2. On average for the three years (Graph. 4), the smallest length of these leaves has the control variety JK 7-4/2 (19.5 cm), and the biggest length (21.0 cm) MB-3 variety, which is 7.69% more compared to the control one (Graph 4). Statistically significant differences of 5% for this attribute were shown by the varieties MB-2 in 2010 and MB-3 in 2011, and a difference of 1% was shown by the varieties MK-1 and MB-3 in 2009. On average, the width ranges from 10.1 cm in the control variety JK 7-4 / 2 to 10.9 cm in MB-2, which is 7.92% more compared to the control one. Statistically significant differences of 5% were shown by the varieties MK-1 and MB-2 (2009), and MB-3 in 2011. On average, the angle of attachment ranges from 23.40˚ in MB-2 to 27.17˚ in the control variety JK 7-4/2. The smallest average leaf length (15.2 cm) is characterized by the control variety JK 7-4/2, and with biggest length (16.8 cm) variety MB-3, which is 10.53% more compared to the control one. For this attribute, statistically significant differences of 1% were shown by the varieties MB-2 in 2011 and MB-3 in 2011. The statistically significant difference of 5% was shown by the variety MB-3 in 2010. The varieties MK-1 and MB-2 in 2009 and 2010 did not show statistically differences compared to the control one. On average, the width of these leaves ranges from 7.4 cm in the MK-1 variety to 8.1 cm in MB-3, or 5.19% more compared to the control one. The examined varieties did not show statistical differences in terms of this attribute. On average, the angle of attachment ranges from 29.7˚ on the MK-1 to 32.3˚ on the MB-3 and is slightly larger compared to the true middle leaf -range. Summarizing all the results, we concluded that the examined varieties according to the size of the leaves from the middle belt are characterized by the appropriate size of the leaves, which is characteristic and typical for the oriental type of tobacco.

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UNDERGRADUATE STUDENT NURSES' PERCEPTIONS OF INTIMATE PARTNER VIOLENCE, AWARENESS AND FREEDOM LEVEL IN NURSING CARE

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ABSTRACT

Intimate Partner Violence (IPV); Is an important public health problem in Turkey. Nurses play an important role in the evaluation, intervention and support of women who are exposed to violence. It is reported by the World Health Organization that women below the age of 50 are 61% physically abused by their partners. In universities, nursing undergraduate education; student nurses should be ensured to be ready for these future roles. The knowledge and attitudes of undergraduate students about IPV should be evaluated. It's important in terms of making the necessary interventions into the undergraduate education processes. Our study was conducted September 2019- January 2020 with 276 volunteer students in Trakya University School of Health Sciences undergraduate program. After asking their sociodemographic characteristics, IPV questions were asked to evaluate the level of knowledge, attitude and awareness about IPV. This questionnaire consisted of 3 subscales. These are; " Nursing self-efficacy", " nursing roles and values ", " preparation for education ".

The results of the study; showed that the awareness level of IPV of students is low. The scores of the students from three subscales were low. Their self-efficacy perceptions were low in recognizing a woman who was subjected to violence and creating necessary care and support interventions. The issue of informing the victim about their legal rights was a concern for students. They didn't fully know the role of nurses in preventing abuse. The area where students had the best level of self-confidence was their ability to communicate.
The knowledge, attitude of being aware of IPV and providing effective interventions are directly related to the self-efficacy of the nurses. This will increase the quality of social health service. The students aren't fully aware of the importance and impact on the individual, social level. In order to prevent IPV, one of the duties of healthcare professionals is; to realize violence, to apply care interventions of physical violence, to provide psychosocial support. This social problem is less detected; it can reduce the benefit to women. They had low confidence in understanding women who had been subjected to violence. In order for nurses to take a more active role in preventing violence against women in society, it's not only individual patient care; it's important that they have preventive approach skills.

Keywords: *Intimate Partner Violence (IPV), University Students, Women's Health*

INTRODUCTION

Intimate Partner Violence (IPV), is a key determinant of health and well-being for women from lifelong and diverse social, cultural and economic backgrounds. IPV can be defined as physical or emotional violence that occurs between people who are or have been in a close relationship (Smith et al., 2018). Most of the literature on IPV deals with violence between heterosexual partners, and women are more likely to experience it (Organization, 2010; Smith et al., 2018). IPV, apart from physical and sexual violence; It can also occur in the continuity of economic, psychological and emotional abuse.

The World Health Organization states that; Women who are exposed to the physical and emotional effects of violence apply to 'primary care' health professionals such as nurses and family health centers for support and intervention (Organization, 2010). Previous studies indicate that healthcare professionals, including nurses, often do not readily recognize exposure to adverse events. The studies state that healthcare professionals sometimes have sloppy attitudes and can negatively affect victims of violence by leaving them more vulnerable to adverse situations (Organization, 2010). Using inadequate screening procedures, such cases can inadvertently be overlooked. Health professionals; are in a unique position to identify, be able to support and care for women who have experienced intimate partner violence.

University students do not fully know the effects of violence on social and health at individual and social levels (Beccaria et al., 2013). It is thought that the results of this study may provide suggestions for undergraduate health education curriculum in universities. In particular, students' self-confidence should be evaluated in terms of "understanding women who have been subjected to violence but who do not directly express". In order for undergraduate students to take a more active role in preventing violence against women in society; not just patient care and treatment; It is important that they have many different preventive, protective approach skills.

It should be ensured that students are ready for future professional roles in health undergraduate education at universities. It is important to evaluate the knowledge and attitudes of health undergraduate students regarding intimate partner violence in terms of making the necessary interventions in their education processes. It is essential that the student nurse graduates ready to provide health care services to the victim. Being ready to manage your intimate partner website will increase the quality of social health service. In the management of intimate partner violence; is important to be able to recognize the victim and to provide effective responses to the victim. Their knowledge, attitude and awareness of IPV is directly related to the self-efficacy of health professionals.

MATERIALS AND METHOD

In our study, "intimate partner violence scale" was used (Beccaria et al., 2013). This scale provides information on the students' demographic profile (gender, age, and year of education), and descriptions of intimate partner violence's perceptions and understanding of healthcare workers and students.

Participants and Settings

Our study was conducted with 280 students in the nursing undergraduate program of Trakya University School of Health between February 2020 and February 2021. After the sociodemographic characteristics of the students were asked, "Intimate Partner Violence Scale" questions were asked to evaluate the level of knowledge, attitude and awareness about intimate partner violence. The validity and reliability of the scale has been proven (Beccaria et al., 2013).

IPVS consists of 3 subscales. These subscales; "Self-efficacy in combating intimate partner violence"; "Perceptions of nurses' role values"; and; and "training preparation to manage your intimate partner violently clinically" (Beccaria et al., 2013). Participants will state their perceptions and understanding on a five-point Likert-type scale ranging from 1 (strongly disagree) to 5 (strongly agree).

In this study, known causes of abuse of women; The status of university students to identify women who were subjected to violence and their perpetrators was examined. IPV beliefs in health undergraduate education; perceived nursing roles; and training readiness was evaluated.

Undergraduate students; were examined whether women knew the reasons for abuse or not. Students' ability to identify women and perpetrators of violence was evaluated. Nursing students' beliefs; perceived nursing roles; and whether they have any preparations in this regard during their training processes.

Data Analysis

All statistical analyzes evaluating the collected data were carried out using the SPSS 21.0 Package Program. Data were summarized with appropriate descriptive statistics. Mean, standard deviation for numerical variables; Frequency and percentage were evaluated for categorical variables. The normal distribution control of the data was controlled with the Shapiro-Wilk test. Paired group comparisons were made by Student's t test. Chi-square test was used for relationships between categorical variables. Descriptive statistics for numerical variables were given as mean and standard deviation. Descriptive statistics for categorical variables were given as percentage and frequency. The significance level was determined as 5% in all statistical analyzes.

RESULTS

Our study was conducted with Trakya University Health School nursing undergraduate students. 72.2% of the participants were women and 28.8% were men. Average age was 21.5.

The relationship between the perceptions of being prepared for nursing education, self-efficacy, and perceptions of nursing roles to manage IPV in the clinical field were evaluated. Those who got high scores from the self-efficacy subscale also had higher education readiness subscale scores.

According to the classes, all 3 subscales and total scale scores were compared.

The results obtained showed that; The students are “of the opinion that nurses have no place in IPV management”. Participants in the study did not have enough awareness and knowledge of a woman exposed to IPV.

Overall scores were low in all subscales. This means that nursing students may not have an awareness of the importance of IPV.

DISCUSSION

Intimate partner violence is a major social health problem around the world. It is reported by the World Health Organization that among women under the age of 50, the rate of physical abuse by their partners is 61% (Organization, 2010). To identify this social problem; increases the benefit to women.

The World Health Organization estimates that 10-69% of women worldwide are physically attacked by a male partner. Intimate partner violence throughout the life of the woman; it is well known that it can cause long-term effects physically, socially and economically (Beccaria et al., 2013; Organization, 2010).

In terms of mental health, women exposed to violence experience higher rates of depression and eating disorders, increased stress, suicidal thoughts and post-traumatic stress disorder. (Beccaria et al., 2013; Organization, 2010). Healthcare professionals, women subjected to intimate partner violence; they should be able to recognize as they are often the 'first point of contact' in the healthcare system (especially in emergency rooms) (Sundborg, Törnkvist, Saleh-Stattin, Wändell, & Hylander, 2017). Healthcare professionals; It is in a unique position to identify, support and care for the victim. They play an important role in providing assistance and support to responding to the cycle of violence by empowering women, connecting with and advocating support services. The nurses have a role in evaluating, intervening and supporting those who are exposed to violence.

The readiness of a healthcare professional to manage IPV can be greatly influenced by their knowledge, attitudes and beliefs resulting from their self-efficacy in identifying women experiencing violence (through new screening procedures) and providing effective responses (Beccaria et al., 2013).

In some studies, professional nurses stated that the lack of violence education during undergraduate education negatively affected their confidence / self-efficacy about violence in their professional life (Sundborg et al., 2017). Negative attitudes about the severity of violence, those who were exposed to it, and their reluctance to intervene are seen in healthcare workers. Among the reasons why nurses cannot make this management optimally; lack of knowledge and educational preparation about the causes, symptoms, and screening procedures of violence; and a lack of self-efficacy or confidence in screening for violence and effectively communicating with survivors may also be important factors.

Although some training programs are successful in developing knowledge, nurses stated that skills and professional self-efficacy / confidence in violence should be gained during undergraduate education. Healthcare professionals may not receive sufficient training in IPV at the undergraduate level to define it and implement effective strategies (Beccaria et al., 2013; Davila, 2006). In order to develop positive attitudes and skills required to intervene in IPV issues; undergraduate nursing education is recognized as a critical time (Sundborg et al., 2017).

Health undergraduate students do not fully understand the importance of IPV issues and their impact at the individual and societal level (Beccaria et al., 2013; Sundborg et al., 2017). In undergraduate health professional education programs; The social, economic and health effects of violence should be addressed. In order to prevent intimate partner violation, healthcare workers are at the beginning of their duties; to recognize the violence, to apply care interventions to the effects of physical violence, to provide the necessary psychosocial support to women. It is important to consider and support the development of knowledge, skills and positive attitudes in the health undergraduate curriculum.

The World Health Organization (2010) suggests using an ecological model that addresses the issue from a public health perspective, focusing on the role of health professionals in protection and public change through legislation and advocacy (Organization, 2010). An example of this would be a more comprehensive approach to facilitate women's empowerment by enhancing women's self-esteem and self-efficacy, ensuring routine screening, and supporting and contributing to social discourse.

CONCLUSION

The results of this study show some important results for health undergraduate education. University students may not fully understand the social, health and economic impacts at the individual and societal level. As a result, they may miss opportunities to screen and recognize women adequately. This may cause the social problem to be less identified.In addition, more attention needs to be paid to nursing interventions that address the emotional needs of patients. For caring to play an important role in this at a wider level, students must also understand and develop many different approaches (such as preventive strategies) beyond what they perceive as the main clinical roles of supportive care.

Health undergraduate students can contact women exposed to IPV during their clinical practice. However, systematic home visits will provide a different perspective outside of a more medically oriented setting, with public health interventions. In order to create a sensitive professional health workforce in the future, undergraduate education programs should be developed with a proactive and holistic approach.

Conflict of Interest

The authors declare that; there is no conflict of interest.

Ethical consideration

The study was approved by the Ethics Committee of Scientific Research of Trakya University Faculty of Medicine

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SOLUTIONS FOR PROBLEMS VIA KAIZEN APPLICATION AS LEAN MANAGEMENT IN PRODUCTION – CASE STUDY

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ABSTRACT

In recent years, Lean philosophy gained more popularity among other kinds of production strategies. Via this study, it is aimed to improve production lines by means of lean methods for an exclusive company that is situated in İstanbul/TURKEY. There occurred some problems as a nature of production and in order to diminish these, the problems of production were controlled via Lean Management methods, especially via Kaizen, and new solutions for developing production were done and applied. The solutions for problems were defined via lean system which targets at improving efficiency in recent industry world such as KAIZEN. Kaizen philosophy was effectively applied by means of Why-why analyse and PDCA (Plan-Do-Check-Act) methods. The main aim was to make an end for customer complaints and have more products which have required quality and minimum problem. Three of the applied KAIZEN methods were explained with details in this study.

Keywords: *Lean Management, Kaizen, Solution*.

INTRODUCTION

The materials which store product placed inside, protect it according to its size and structure, ensure that it is sterile, carry it easily and advertise with the visuals on it are called package. Packaging is very important for purchasing and sales strategies. Indeed, the packaging plays a role in all areas, from the initial product development and improvement process to the production, storage, transportation, storage, advertising and sales to the end consumer, which are then designed to suit market needs. In case of looking into sector deeply, it is seen that competition is very high and demanding. So as to deal with this competition, many companies have started to adopt lean philosopy. Especially, manufacturing companies are able to gain lean methods. Kaizen is one of the methods of lean management. In 2016, Maarof examined Kaizen's practices and challenges in small and medium-sized enterprises in Malaysia (Maarof, 2016). Communication between senior management and employees has shown that the organizational staff's attitude towards Kaizen, good management knowledge and workforce are effective in successful Kaizen practice. The aim of Kaizen applications is to reduce costs, increase quality, flexibility and at the same time increase productivity. With the combination of Japanese words Kai (change) and Zen (Better), Kaizen (Continuous Improvement) is the primary objective of the method to improve production processes with small but effective and efficient changes (Tsao, 2015). Kaizen is a method that gives importance to the process and gives priority to production, since it is focused on improving processes. In this study, it is intented to find some better ways for problems occurred during production in package

manufacturing company in Turkey. Kaizen is implemented as "Before - After Kaizen" in this study in order to find solution of three problems of production. As a part of literature, Kaizen methods had been defined and after that necessary solutions were tried to be performed according to the methods.

MATERIAL AND METHODS

Lean Management

Lean philosophy can be considered as a structure that is based on value. All kinds of worthlessness undermines this structure. For this reason, the main purpose in lean thinking is to eliminate these worthlessness. All lean methods start on the basis of value origin and are converted into a flow of value to make it continuous. The flow of value ensures customer expectations **(**Nassereddine, A., & Wehbe,2018). Value and worthlessness are defined from the customer's point of view. Definitions such as Lean manufacturing, Lean management or Lean organization can be used for Lean philosophy. It is a management philosophy that is free from all the worthlessness in all structures and minimizes all kinds of factors that lead to error, stock, labor, cost, development process, production area, waste and customer dissatisfaction. The aim of this philosophy is to increase production speed and quality and reduce cost and delivery time. Lean philosophy constitutes an organization that aims to eliminate worthlessness, unnecessary workload and unnecessary energy and not to waste time. If production is not needed, it is definitely not done. Capacity utilization rate is reduced. This eliminates all unnecessary activities that use all capacity. Compared to mass production, faster cycles are used that require less. This results in a significant increase in product diversity, although inventory, faulty product and cost are less.

There are different types of lean methods:

- KAIZEN
- JIT
- VSM
- Kanban, Milk run
- HEIJUNKA
- SMED
- 5S
- HOSHIN KANRI
- YAMAZUMİ
- Poka-Yoke
- Jidoka

Kaizen

The word Kaizen is a Japanese concept derived from the words "kai" and "zen. "Kai" stands for change and "Zen" stands for better. The great inventions of Kaizen's basic philosophy are developed step by step, via making small improvements (Higuchi, Y., & Nam, H.V., & Sonobe, T, 2015). This is the main philosophy underlying Japan's current power after the Second World

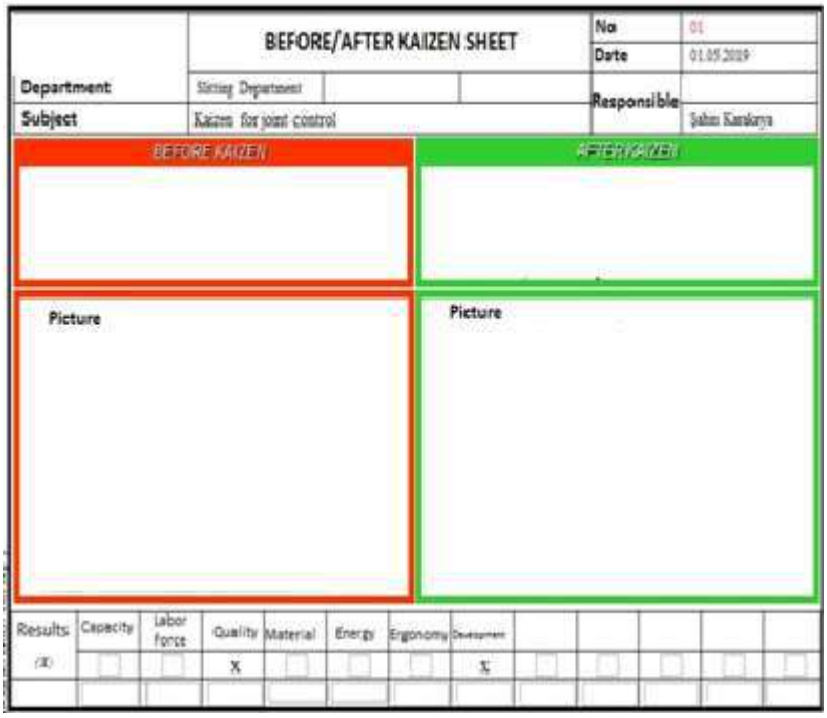
War. In the Second World War, Japan became very damaged, tired and wounded country. Japan entered a process where everything must be rebuilt. In this time, both employees and employers faced great difficulties, but in the face of these challenges, they took steps forward instead of giving up. At this time, Kaizen philosophy emerged as a way of life and management. After the war, experts such as W.E. Deming and J.M. Juran discovered various quality control tools that would contribute to the development of the Kaizen philosophy (Veres, C. & Marian, L., & Moica, S., 2017). Nowadays, Japan is a major economic power because it has adopted Kaizen philosophy very well. Japan has achieved a significant competition in the market in every sector, especially in automotive. Kaizen philosophy is so adopted in Japanese companies that employees and executives often do not even realize that they think of Kaizen.

There are three sorts of Kaizen;

1. Before / After Kaizen (Point Kaizen)

It is easy to explain with the photographs that show before and after as a single page form. This kind of improvements come out with suggestion of employees. It usually takes 8 days so as to finalize this Kaizen. The main aim of this type of Kaizen is to create motivation and awreness for improvements and lead employees to think of Kaizen. It is understood by everyone so it is very basic (Choomlucksana, J., & Ongsaranakorn, M., & Suksabai, P., 2015). The single page form is shown in figure 1.

Figure 1. Before/After Kaizen Form



2. Kobetsu Kaizen

A team consisting of employees from different departments works on this Kaizen. It is more complicated than Before/After Kaizen. Lean methods such as PDCA (Plan, Do, Check and Act), why-why analyze, brainstorm etc. are used in order to find the root cause of the problem (Tsao, L.,2015). It usually takes 40 days as average. The main aim of this Kaizen is to solve the problem permanently. Focusing on a topic with the team and improving current situation is the main objective.

3. Kaikaku Kaizen (System Kaizen)

Kaikaku is described as improvement activities in that more radical changes are made. There is a team that consists of experts. Production, sales, market situations and financial conditions that may change should be well analyzed at the beginning of the study. It takes 120 days as average. This is the longest time among other Kaizens. The radical changes are decided by top management (Kurdvea,M,& Sjögrenb,P., 2016).

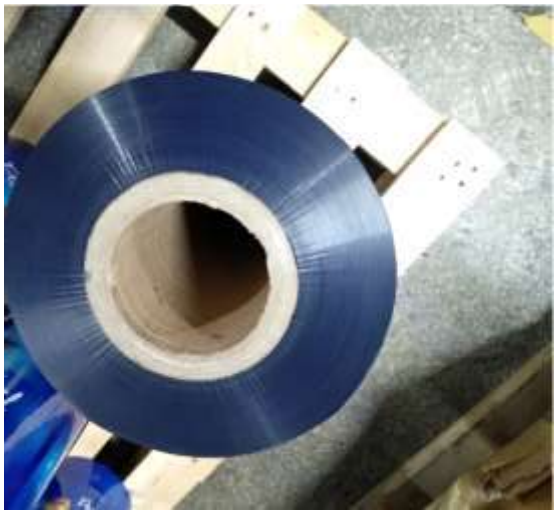
CASE STUDY

In this study, improvement works called as 1. Metrage verification, 2.Waste reduction and 3.Shipping inspections have been carried out in the production and shipping lines of the related company and the studies have been recorded with Kaizen forms. When problems in production and shipment were examined during the brainstorming by Kaizen team, these 3 problems were seen as the main reasons and solutions were developed for them.

Metrage verification

The Kaizen study was conducted on the metrage verification. Produced packages are wrapped in rolls in desired metrages and shipped to customers as shown in Figure 2.

Figure 2. Wrapped Roll



The products pass through extrusion, printing, lamination processes respectively and they are slitted to desired metrages in the slittiing process which is the final process. Then, they are shipped in pallets after labeling and packaging. In some cases, the rolls produced in the slitting machine are sent to the transfer machines. In the event of a defect in the packaging (printing error, lamination error, etc.), the rolls go to the transfer machine to clear the defects. After cleaning, they are shipped to customers. During production, wrinkle complaint was detected in the products produced on the slitting machine no 3. In order to remove the relevant wrinkles, the rolls were sent to the transfer process and cleaning was performed. Afterwards, it was noticed in the transfer machine that there was a deficiency-difference in metrage of rolls. As a result of measurements, it was seen that the average difference was 55 meters per 1000 meters. The difference was also detected by metrage verification encoder as shown in figure 3.

Figure 3. Metrage Verification Encoder

After that the machine was repaired by maintenance team and all of the electronic - mechanical corrections were done and verified . The study was recorded as before-after kaizen form as shown in figure 4.

Figure 4. Before-After Kaizen Form for Metrage Verification

Waste reduction

In the slitting process, which is the last process, the packages are slitted to requested metrages and then wrapped onto rolls and shipped to customers. During slitting, some materials are cut

as waste for proper winding and are not sent to the customer. Kaizen study was carried out to reduce these wastes. The wastes occur on both sides of the roll in equal amounts as shown in figure 5. and cannot be used afterwards.

Figure 5. Waste Material

The target tolerance of the materials set by the production planning department was 20mm. But it was found that the materials were produced wider than the tolerance. Due to the wide range of materials, the amount of waste increases. So as to solve this occasion, why-why analyse and PDCA were applied and it was seen that there was no Formula on computer system that blocks any kind of wider tolerance during production.

As a result of adequate studies, formulation was defined in the system and this formulation was provided to give error when there is production out of tolerance. After this formulation, Planning Department started to enter the production into the system within tolerances in accordance with the formulation. The material save has increased and thus the cost has been reduced. The study was recorded as before-after kaizen form as shown in figure 6.

Figure 6. Before-After Kaizen Form for Waste reduction

Shipping inspection

When feedbacks of customers were examined, it was found that there were problems caused by transportation and as a result there were damaged rolls and pallets as shown in the figure 7.

Figure 7. Damaged roll

By means of the examination, it was seen that the products were properly loaded into the transportation vehicles and the damages occurred later. As a precaution, shipment companies were warned regarding these problems but there was not any kind of real precaution for avoiding this in the future. The root cause was determined on fourth question in why - why analysis. The problem was defined as even though proper shipping was done as shown in the figure 8, damaged products were shipped to customers and this was caused by the shipping company.

Figure 8. Proper shipping

.

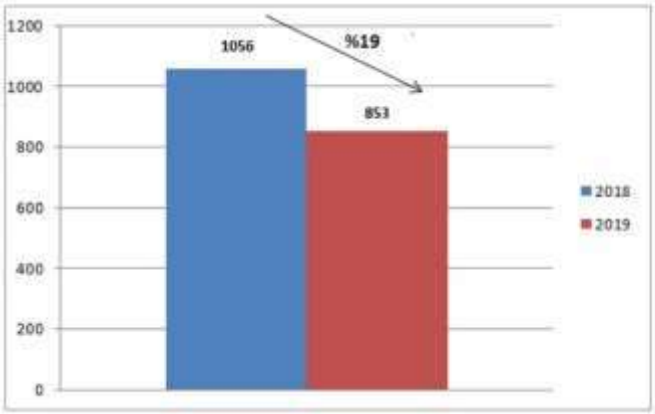
It was figured it out that there was not any kind of inspection report that shows capability of shipment company. After having done why-why analysis, it was decided to establish an audit team in order to determine the capability of the shipment companies and to supervise the shipment companies with audit form that was formed. Before Kaizen Study: There was no data for damaged products regarding whether the shipment companies were competent or not.

After Kaizen Study: Audit team was created with people from other departments such stock control, quality assurance and planning department. The audits have started for determining the appropriate shipment company by this team and audit form was formed as well. In this way, "quality and stock / transportation improvement" was achieved. The study was recorded as before-after kaizen form as shown in figure 9.

Figure 9. Before-After Kaizen Form for Shipping Inspection

Although there is no need for statistical data in before and after kaizen, 19% reduction has been achieved in the complaints received from customers compared to previous years, as shown in the figure 10.

Figure 10. Comparation of customer complaints



CONCLUSIONS

It is desirable to adopt lean thinking in enterprises in order to have knowledge about lean strategies and use competence techniques in appropriate areas. Applications and benefits can vary between businesses, and this is natural. The main aim is to have a lean culture and consider occasions with the philosophy of lean production. Lean production can also be defined as a common language used in enterprises. For example: when a German company opens a factory in China it is difficult to adopt employees to production of German style. So it is needed a common language so as to collaborate together. Lean philosopy is that common language (Altınbalık,T,&Karakaya,Ş.,2019). Because of this, it is very important for workers to adopt lean manufacturing methods in order to speak the common language with employees and when it is necessary, they look at the same point of view without any cultural difference in all companies. When applied Kaizens in the study are examined, it is seen that improvement is mainly aimed in three directions. Health and safety, quality and cost. In order to consider an improvement as Kaizen, at least one of these three options should be improved and should not have a negative effect on any of them. Before - after kaizen is done in this way, it affects employees positively and leads to continuous kaizen thinking (Hambach, J.,2017)**.**

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Hambach, J. (2017). Development of A Digital Continuous Improvement System for Production. Procedia CIRP 63 ('017) 330 – 335PROFILE OF FREE FATTY ACIDS IN THE ORGANIC YOGHURTS IN RELATION TO THE STORAGE TIME*

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ABSTRACT

Due to their high quality and proven health-promoting properties, fermented milk products from organic production have gained greater importance among consumers in the recent years. Organic milk yoghurts have a better fatty acid profile than conventional yoghurts. Lipid fraction also includes a small portion of fatty acids which are not esterified in triglycerides. Their content proves the quality of the product. Therefore, the aim of the study was to determine the profile of free fatty acids in yoghurts manufactured on the basis of organic cow milk with regard to storage time. It was showed that content of the certain free fatty acids (myrystic and palmitic acids) and groups of free fatty acids (short chain free fatty acids – SCFFA, long chain free fatty acids – LCFFA, polyunsaturated free fatty acids – PUFFA and all FFA) increased statistically ($P \leq 0.05$) significant during storage.

Keywords: *organic milk, yoghurt quality, free fatty acid, storage.*

INTRODUCTION

Nowadays, it is becoming more and more common to believe that modern methods of food production, which strive to constantly increase efficiency while reducing production costs, are not a good solution to produce food with exceptional nutritional values, including the desired, high health-promoting quality. The global increase in production of high-quality food, including organic food, which is perceived as such, is the direct result of the interest of consumers themselves, who wish to influence their own health in an informed manner. Interest in organic food as an alternative to mass-produced food is growing from year to year. The trend of acquisition of alternative food has persisted for at least 20 years (Brodziak et al. 2017, Zuba-Ciszewska et al. 2019). Global sales of organic food have rapidly increased between 2000 and 2018. In 2018, sales of organic food and drink amounted to 95 billion USD, up from nearly 18 billion in 2000 (Statista 2020). In 2017, organic agriculture was licensed in 181 countries. The global organic farming area increased from 11 million hectares in 1999 to 69.8 million hectares in 2017 (WOA 2019). The largest numbers of organic food producers were in India (835,000), Uganda (210,300), and Mexico (210,000) (Ostapenko et al. 2020). In Poland, there were 20,500 organic food producers in 2018, including about 900 processing companies and over 200 importers. The area covered by organic production was 500,000 ha (Raport 2019). The growing interest in organically produced food is unquestionably due to its specific characteristics. Organic food is produced without the use of artificial flavourings, additives, or synthetic

pigments. It comes from certified farms and processing plants, where it is produced without the use of GMO, artificial fertilizers, or toxic pesticides posing a risk to human health and life. It is recommended to produce food in the most natural conditions, and in the case of processing, use traditional methods of processing and preservation. Food placed on the market must be marked with a special logo for organic food (Regulation 834/2007, Regulation 889/2008). This type of food includes not only plant products, but animal products as well, including milk and dairy products. Organic cattle farming has a positive effect on the quality of raw milk, especially its chemical composition (Alothman et al. 2019). The milk does not contain antibiotics or other contaminants that could alter its quality or the organoleptic attributes of the product. Research has shown that milk from organic farms has much higher content of nutrients and a more favourable fatty acid profile (Bulter et al. 2020). Another advantage of organic milk is its higher antioxidant status, due in part to its higher content of lutein and zeaxanthin, which protect against ischaemic and diabetic retinopathy (Jia et al. 2017; Rock et al. 2017). Moreover, grazing of cattle on natural pastures in the spring and summer and the use of silage in the winter has been shown to increase the content of vitamins in milk, including vitamin E and beta-carotene, which are strong antioxidants protecting against free radicals (Puppel et al. 2017).

Milk fat is a natural composition of over 450 fatty acids (FA), of which 16 alone account for more than 1%. Saturated fatty acids (SFA) predominate in milk, with about 680 mg/g FA on average (Table 1).

Table 1. Comparison of the fat compositions (mg/g FA) of organic milk and non-organic milk obtained on the basis of meta-analysis (Średnicka-Tober et al. 2016)

Parameter	Organic milk	Non-organic milk
SFA	678	684
PUFA	41.4	36.31
MUFA	272	272
CLA (total)	9.72	6.98
CLA10 (t10c12 C18:2)	0.55	0.38
CLA9 (ci9t11 C18:2)	8.66	6.71
ALNA (18:3, n-3)	7.73	4.38
EPA (20:5, n-3)	0.87	0.53
Lauric acid (C12:0)	33.32	33.79
Myristic acid (C14:0)	113.08	111.23
n-3/n-6 ratio	0.42	0.26

FA – fatty acids
SFA – saturated fatty acids
PUFA – polyunsaturated fatty acids
MUFA – monounsaturated fatty acids
CLA – conjugated linoleic acid
ALNA – alpha-linolenic acid
EPA – eicosapentaenoic acid

Unsaturated fatty acids account for about 310 mg/g FA, of which monounsaturated fatty acids (MUFA) are dominant (about 270 mg/g FA), including oleic acid (C18:1). Polyunsaturated fatty acids (PUFA) are found in milk in the amount of about 40 mg/g FA, including α-linolenic acid (ALA; C18:3) and linoleic acid (LA; C18:2). Cow milk contains more oleic acid than goat or sheep milk (Barłowska et al. 2009; Manzi et al. 2017; Średnicka-Tober et al. 2016). Alternative (organic) milk contains more polyunsaturated and omega-3 acids and smaller amounts of omega-6 acids than milk from conventional farms (Benbrook et al. 2013). For this reason it is recommended for individuals with cardiovascular disorders, type-2 diabetes, or dementia (Lange, 2020; Lan et al. 2020). Organic milk and fermented dairy products are also a valuable source of conjugated linoleic acid (CLA), which exhibits anti-carcinogenic, immunostimulatory and weight-reducing properties (den Hartigh, 2019).
The fatty acid (FA) profile of milk is variable, depending mainly on the breed of cow, the stage of lactation, and the type of feeding (Stergiadis et al. 2018). This translates into the content and quality of fatty acids in milk products. The fat in fermented dairy products is the primary carrier of flavour and aroma. There have been very few reports on the effect of the amount of milk fat on the quality of fermented products. Shakerian et al. (2014) demonstrated that variation in the amount of fatty acids affects the production of organic acids. Processes taking place during fermentation (lipolysis) influence the formation of free fatty acids (FFA) and ketones (Bao et al. 2016). Free fatty acids make up a small part of the lipid fraction of milk and dairy products. These acids are not esterified in glycerides. They are freely dispersed in the lipid phase of milk, and to a minor extent in the aqueous phase. Their presence determines the quality, taste, texture, nutritional properties, and health-promoting properties of fermented dairy products. They may also influence the surface tension and foaming capacity of raw milk (Kilcawley et al. 2017). The usual FFA content in milk fat is 0.5-1.2 mmol/100 g (Hanuš et al. 2008). However, quality of raw milk, type of heat treatment or storage time and conditions determine FFA content in dairy products (Pereda et al. 2008; Mannion et al. 2016). Storage of dairy products may contribute to the release of FFA from triglycerides as a result of milk fat lipolysis, i.a., because of milk natural enzymes (lipases) – which can be thermoresistant or lactic acid bacteria's activity (Hanuš et al. 2008; Sumarmono et al. 2015).
The aim of the study was to determine the profile of free fatty acids in yoghurts manufactured on the basis of organic cow milk with regard to storage time.

MATERIALS AND METHODS

Raw milk

The milk for yoghurt production was obtained from three certified organic farms located in southern Poland (mountainous areas). All of the farms raised cows of the Simmental breed, which are predisposed to living in difficult environmental conditions. In the spring/summer season, their diet consisted primarily of pasture forage (*ad libitum*) supplemented with hay and cereal meal, while in the autumn/winter season they were fed haylage supplemented with hay and cereal meal. Bulk milk was collected three times during the spring/summer (pasture) season (May-July) and three times during the autumn/winter (indoor) season (December-February). A total of 18 (6 on each farm) bulk milk samples were collected for analysis. The milk was transported to the laboratory under refrigeration and immediately used for analysis and yoghurt production.

Yoghurt production

The yoghurts were produced six times by the water bath method (the thermostatic method). Milk was heat-treated at 85°C for 30 min. Next it was cooled to 40°C and inoculated with thermophilic yoghurt cultures, i.e. FD-DVS YC-380 Yo-Flex mixed strain culture, containing

Streptococcus thermophilus and *Lactobacillus delbrueckii ssp. bulgaricus* (Chr. Hansen, Denmark), in the amount of 0.15 g/l (Glibowski and Rybak 2016). The milk was incubated until pH=4.6 was attained. Then the products were cooled to 20°C to discontinue incubation. The yoghurts were stored at 4-6°C until the next day (about 14 h) for analysis. Testing of the yoghurts was continued every 7 days for 28 days of cold storage. The yoghurts were stored in closed containers under refrigerated conditions (4-6°C).

Analysis of free fatty acids

Fatty acid methyl esters (FAME) were prepared in order to determine the amount of free fatty acids in each yoghurt sample (three samples from one yoghurt). 2% (v/v) sulfuric acid in methanol was used as a derivatization reagent. Gas chromatograph (Shimadzu GC2010, Japan) interfaced with a quadrupole mass spectrometer (Shimadzu QP2010Plus, Japan) and autosampler (Shimadzu AOC-20i, Japan) was used. Chromatographic separation was performed on a FactorFour Type VF-5MS capillary column (CP8944, length – 30 m, internal diameter – 0.25 mm, film thickness – 0.25 μm) – Varian, USA. Dispenser heated to a temperature 250°C worked in the sample division mode (Split mode – 1:20). The mass spectrometer was operated in the SCAN mode (40-550 m/z), with the ion source heated to 250°C. The flow of carrier gas – helium was constant during the analysis (1 ml/min). Volume of dispensed sample was 0.001 mL. Mass spectra of all the analysed compounds were compared to reference mass spectra (NIST 5.0), which allowed to determinate a similarity coefficient of spectra. Qualitative analysis was performed on the basis of obtained retention times and mass spectra recorded for standards of analysed compounds. Quantitative analysis of FAMEs was performed by external standard method, using 1-bromotetradecane as an internal standard. The following groups of free fatty acids were determined: SCFFA – short chain free fatty acids (C4-C8), MCFFA – medium chain free fatty acids (C10-C14), LCFFA ¬ long chain free fatty acids (C15-C24), SFFA – saturated free fatty acids, UFFA – unsaturated free fatty acids, MUFFA – monounsaturated free fatty acids, PUFFA – polyunsaturated free fatty acids, and all FFA.

Statistical analysis

Statistical analysis of the results was performed using StatSoft Inc. Statistica ver. 13.1 (Dell 2016), by one-way analysis of variance (ANOVA). Significance of differences between means for groups was determined by the Mann-Whitney test at a level of p (alpha) = 0.05. The results are presented as mean ± SD.

RESULTS AND DISCUSSION

The changes in content of certain groups of FFA in yoghurts during storage are shown in Table 2. All FFA in the analyzed organic yoghurts accounted for 0.431 g/100 g fat in the first day of storage. It was stated that long chain free fatty acids (C15-C24) were the most dominant (0.310 g/100 g fat), and in particular palmitic – C16:0 (0.141 g/100 g fat) and oleic – C18:1n9c (0.102 g/100 g fat) acids – Table 3. These two acids were found to be the major FFA in all yoghurt samples. In the case of palmitic acid, the effect of the storage time turned out to be statistically significant ($P \leq 0.05$), as for the myristic acid. Content of unsaturated free fatty acids – UFFA (0.140 g/100 g fat) was two times smaller than saturated free fatty acids – SFFA (0.293 g/100 g fat). The levels of all groups of FFA were affected by the storage period – Table 2. It was noticed that at the end of storage their content increased, however, not statistically significant in all cases. The highest changes were obtained for short chain free fatty acids (SCFFA) which amount was two times higher on day 28 (0.007 vs 0.015 g/100 g fat). The changes during storage were statistically ($P \leq 0.05$) significant for short chain free fatty acids (SCFFA), long chain free fatty acids (LCFFA), polyunsaturated free fatty acids (PUFFA) and all FFA. With

the time of storage their content increased by 114%, 44%, 52% and 25%, respectively, during the whole period. Increase in SCFFA may account for the ability of C4-C8 release from triglycerides by the yoghurt cultures. These compounds are esterificated in triglycerides mainly at sn-3 position, which is easily hydrolysed by lipases of bacteria. However, bacterial lipases possess weak specificity to the linkage in sn-2 position in triglycerides, where more than 50% of C10-C14 acids are esterificated (Reguła, 2007; Güler and Gürsoy-Balcı, 2011). As reported Yadav et al. (2007), all FFA significantly ($P<0.05$) increased during fermentation and storage (after 10 days). In the research of Reguła (2007), fourteen-day-old fermented beverages were characterized by lower content of most of FFA than one-day-old. Intensity and direction of changes in FFA level depended on the starter culture.

Table 2. Content of the groups of free fatty acids in natural organic yoghurts during 28 days of storage at 4°C (mean±SD) (g/100 g fat)

Day of storage	Fat fraction							
	SCFFA	MCFFA	LCFFA	SFFA	UFFA	MUFFA	PUFFA	All FFA
0	0.007^{a} ±0.002	0.091± 0.005	0.310^{a} ±0.018	0.293± 0.021	0.140± 0.008	0.120± 0.010	0.021^{a} ±0.002	0.431^{a} ±0.025
7	0.007^{a} ±0.001	0.093± 0.009	0.376^{b} ±0.022	0.328± 0.027	0.160± 0.014	0.132± 0.007	0.023^{a} ±0.003	0.487^{b} ±0.033
14	0.009^{a} ±0.001	0.090± 0.007	0.394^{b} ±0.030	0.332± 0.024	0.162± 0.014	0.133± 0.011	0.027^{ab} ±0.004	0.496^{b} ±0.027
21	0.012^{b} ±0.002	0.099± 0.008	0.425^{c} ±0.026	0.345± 0.016	0.170± 0.018	0.138± 0.015	0.029^{b} ±0.003	0.522^{bc} ±0.030
28	0.015^{b} ±0.003	0.106± 0.008	0.446^{c} ±0.024	0.360± 0.029	0.168± 0.020	0.134± 0.012	0.032^{b} ±0.004	0.539^{c} ±0.022
Factor influence – day of storage (p value)	0.031	0.402	0.026	0.217	0.469	0.373	0.030	0.032

SCFFA – short chain free fatty acids (C4-C8)
MCFFA – medium chain free fatty acids (C10-C14)
LCFFA long chain free fatty acids (C15-C24)
SFFA – saturated free fatty acids
UFFA – unsaturated free fatty acids
MUFFA – monounsaturated free fatty acids
PUFFA – polyunsaturated free fatty acids
a, b, c – differences significant at $P\leq0.05$

According to Beshkova et al. (1998), the mixed cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) formed volatile free fatty acids more actively than the pure cultures. However, Güler and Gürsoy-Balcı (2011) obtained the significant differences in volatile free fatty acids (C2-C10) in goat and ewe milk yoghurts. In their research SCFFA reached a maximum level at the end of 21 days of storage while LCFFA (C16-C18:1) decreased during the storage.

Table 3. Content of selected free fatty acids in natural organic yoghurts during 28 days of storage at 4°C (mean±SD) (g/100 g fat)

Day of storage	Free fatty acids			
	C14:0 (Myristic)	C16:0 (Palmitic)	C18:1n9c (Oleic)	C18:0 (Stearic)
0	0.058^{a}±0.006	0.141^{a}±0.013	0.102±0.016	0.086±0.009
7	0.060^{a}±0.003	0.164^{b}±0.009	0.107±0.014	0.088±0.016
14	0.064^{a}±0.003	0.170^{b}±0.006	0.107±0.012	0.087±0.006
21	0.067^{ab}±0.004	0.174^{b}±0.010	0.112±0.008	0.093±0.007
28	0.071±^{b}0.007	0.184^{c}±0.013	0.115±0.011	0.100±0.011
Factor influence – day of storage (p value)	0.047	0.025	0.468	0.327

a, b, c – differences significant at P≤0.05

CONCLUSION

It has been showed that the storage of yoghurts contributes to the release of free fatty acids from triglycerides due to milk fat lipolysis. Content of the certain free fatty acids (myrystic and palmitic acids) and groups of free fatty acids (short chain free fatty acids – SCFFA, long chain free fatty acids – LCFFA, polyunsaturated free fatty acids – PUFFA and all FFA) increased statistically significant during storage. Intensity and direction of changes in FFA level probably depended on the starter culture. Therefore, further research is needed to gain detail information about the typical characteristic of yoghurts.

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CHANGES OF SATURATED HYDRAULIC CONDUCTIVITY AND SOME SOIL CHEMICAL PROPERTIES AT VARYING SOIL DEPTHS UNDER DIFFERENT SOIL MOISTURE REGIMES AND NITROGEN TREATMENTS IN A CLAY TEXTURED SOİL

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ABSTRACT

This study aimed to determine the changes of some soil physical and chemical properties at varying soil depths under different soil moisture regimes and nitrogen (N) treatments in a fine textured soil where pepper was grown in field conditions. Experiments included three different soil water regimes (100%FI (Full Irrigation), 75%FI and 50%FI) and 4 different N fertilizer doses (N_0: unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha). One-third of the nitrogen was applied at sowing in all treatments. The rest was applied through fertigation through growing period. Irrigation interval was set as four days based on evaporations from Class A pan. The results of the study showed that the effects of N treatments under different soil moisture regimes on the some soil physical properties (field capacity, permanent wilting point, available water capacity and bulk density) except saturated hydraulic conductivity (Ksat) for 0-30 cm depth did not differ significantly across treatments. However, the Ksat was not affected by treatments at the lower soil depths (30-60 and 60-90 cm). Soil moisture regime x N treatment interactions for 0-30 cm depth did have significant effects on the electrical conductivity (EC), cation exchange capacity (CEC) and Ksat. EC, available P_2O_5, available K_2O, organic matter and CEC values of the soils increased with increasing N treatments under different soil moisture regimes. In addition, the soil properties showed differences with soil depth. Significant positive correlations were observed between Ksat and EC (0.376*), between available P_2O_5 and CEC (0.554**), between available K_2O and CEC (0.479**).

Keywords: Electrical conductivity, Inorganic fertilizer, Saturated hydraulic conductivity, Soil nutrition, Soil water contents

INTRODUCTION

Soil nutrition are divided into mobile and immobile, which are absorbed by plants (Barber,1995). Water and N in form of nitrate required in greatest quantities by plants are most mobile. P required by crops is the highly immobile. In addition, K is relatively immobile (Marschner, 2012). Plant growth and yield are effected by concentrations of the macronutrients in the soils (Havlin et al., 2004). Thus, water and the contents of these macroelements (N-P-K) limit crop yield (Lynch, 2013). N treatments increases the growth and yield of crops and enhances soil quality by influencing microfauna and microflora of soils. Nitrogen deficiency in the soil reduces plant growth and productivity due to the reduce in the production potential and productivity of the soil. Thus, nitrogen which is absorbed in significant amounts by plants is the most essential plant nutrient (Sharma and Yadav, 1996).

Management practices such as crop cultivation and fertilization effects importantly soil hydraulic conductivity (Latif et al., 1992). The electrolyte concentration of soil solution causing the increase of soil electrical conductivity (EC) which is a significant factor for the sustenance of a good soil permeability modify fertilizer applications (Agassi et al., 1981). Most of the change of soil saturated hydraulic conductivity (Ksat) were explained by soil salinity measured

as EC. So, a larger flocculation of soil colloids due to saline impacts caused soil permeability improvements. Ksat is one of the most significant soil characteristics that determines the behavior of water flow systems (Klute and Dirksen, 1986). A detailed understanding of Ksat is critical in the assessment of infiltration rates, irrigation practices, groundwater recharge rates, runoff and drainage processes (Aimrun et al., 2004). Oosterbaan and Nijland (1994) found that soil salinity generally has a positive effect on the Ksat, especially in clay soils. This shows one way fertilizers can have a positive affect on the soil physical properties. Meena et al. (2020) found that soil Ksat did not differ importantly across applications (synthetic fertilizer and organic compost) at the 0-10 cm soil depth, while the Ksat for 10-20 cm soil depth was effected by applications. However, there are also studies negatively affected by fertilizer applications. For example, the destabilization of moist aggregates due to the dispersive action of ammonium fertilizer. In addition, Myers and Thien (1991) reported that N fertilizers that are characterized by high acidity index may decrease soil pH and dissolve soil organic matter thus effecting soil permeability. Besides, Intrawech et al. (1982) found that four different nitrogen sources had no effect on saturated hydraulic conductivity. Celik et al. (2004) stated that inorganic fertilizer applications do not affect the physical properties of the soil. However, fertilizer applications have generally produced contradictory influences on pore geometry or water retention: enhancement (Wang et al., 2010), suppression (Ghani et al., 2003), and no effects (Gong et al., 2009).

It is significant to determine the impact of inorganic and organic fertilizer applications on soil physical and chemical properties due to soil management systems effect soil chemical and physical productivity (Celik et al., 2004). This study aimed to investigate the variations of some soil physical and chemical properties at varying soil depth under different soil moisture regimes and nitrogen treatments in a fine textured soil where pepper (*Capsicum annuum* L.) was grown in field conditions.

Material and Methods

Experimental site description

Experiments were conducted in 2015 and 2016 growing seasons for two years over the experimental fields of Sarayköy Research and Application Station (35°27′ N and 28°69′ E, altitude 894 m) in Ankara, Turkey. Minimum and maximum monthly temperatures were 7.5–16.1 °C and 27.0–38.3 °C, respectively. Total precipitation during the growing seasons (June-September) was measured as 9.1 and 57.4 mm, respectively.

Experimental soils were clay (C) in texture, slightly alkaline (pH=7.83) with insufficient soil organic matter (0.27%), medium $CaCO_3$ (13.73%), EC (0.91 dS m^{-1}) (Soil Survey Staff 2014). Field capacity (FC) was measured as 40.02% and permanent wilting point (PWP) was measured as 20.29%. Available water capasity in the 0-30 cm soil depth is 19.73%.

Field study

Pepper *(Capsicum annuum* L.) seedlings were transplanted in plots on 2 June 2015 and 1 June 2016. Each plot had dimensions of 7 x 3.5 m. Treatments included three different soil water regimes (100%FI (Full Irrigation), 75%FI and 50%FI) and 4 different nitrogen (N) fertilizer doses (N_0: unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha). Four-day cumulative evaporation (Class-A pan) was used to determine the amount of irrigation water. So, irrigation interval was selected as 4 days. Seasonal irrigation water quantities varied between 354-624 mm in 2015 and varied between 318-568 mm in 2016 using drip irrigation. N (Amonium Nitrate, 33%) was applied through fertigation. One-third of the total N applied at

planting time and the rest applied through fertigation (2 times in the flowering period, 2 times fruit set and 1 times after the first harvest) through growing period (Sne, 2006). Experimental plots were supplemented with 100 kg ha^{-1} P_2O_5 at planting.

Soil sampling and analyses

Following the last harvest, soil samples (0-30, 30-60 and 60-90 cm) were taken from each plot and analyzed for some soil chemical and physical properties. Soil pH were measured with a pH meter and electrical conductivity (EC) values were measured with an EC-meter (Richard 1954). Scheibler calcimeter was used to determine soil lime contents (Soil Survey Staff 2014) and ammonia acetate extraction method was used to determine exchangeable cations (Kacar 1994). Soils were extracted with 0.5 M $NaHCO_3$ (pH =8.5) to determine soil available P contents (Olsen et al. 1954). Soil organic matter contents were determined with the use of Modified Walkley-Black method (Kacar 1994). Samples were extracted with NH_4OAc. (pH=7.0) to get soil available potassium contents (Jackson 1958). Cation Exchange Capacity (CEC) values were determined as described by Richard (1954).

Hydrometer method was used to get soil particle size distribution (Richard 1954). Field capacity (FC) and permanent wilting point (PWP) values were identified with the aid a pressure plate (Tüzüner 1990). Available water capacity (AWC) was found by calculating the difference between FC and PWP (Tüzüner 1990). Bulk density (BD) was identified as described by Tüzüner (1990). Saturated hydraulic conductivity (Ksat) was determined with the aid of constant head permeameter by using Equation 1 as described in US Salinity Lab. Staff (1954);

$$\text{Ksat} = \frac{\text{Q}}{\text{A t}}\left(\frac{\text{S}}{\text{S+H}}\right) \qquad (1)$$

where; Ksat = Saturated hydraulic conductivity (cm h^{-1}), Q = Outflow from the soil column (cm^3), A = Cross sectional area of soil column (cm^2), t = Time (hour), S = Length of soil column (cm), H: Water head over the soil column (cm).

Statistical analyses

Data were subjected to analysis of variance (ANOVA) with the aid of SPSS Statistical Package (SPSS 19.0, SPSS Inc., 2011). Significant means were compared with the aid of Duncan's multiple range test at $p< 0.01$ and $p< 0.05$ significance levels. Correlations between soil characteristics were assessed through Pearson's correlation coefficients.

Results

Changes of some soil chemical properties under different soil moisture regimes and nitrogen treatments in a fine textured soil at the 0-30 cm soil depth are given in Table 1. N applications under different soil moisture regimes resulted in changes in some soil chemical characteristics.

Table 2. Changes of some mean soil chemical properties under different soil moisture regimes and nitrogen (N) treatments in a fine textured soil at the 0-30 cm soil depth

Soil Moisture Regimes	N dose, kg N/ha	pH	EC, dS/m	$CaCO_3$, %	Av. P_2O_5, kg/ha	Av. K_2O, kg/ha	OM, %	Exc. Ca, me/100 g**	Exc. Mg, me/100 g	Exc. Na, me/100 g	Exc. K, me/100 g	CEC, me/100 g**	B, ppm
SMR_1	N_0	7.84	0.91 f	15.79	52.0	1704.8	0.59	20.08 a	7.33	1.90	1.75	31.06 cd	0.75
	N_{70}	7.87	1.05 def	16.65	48.9	1943.0	0.68	20.77 a	7.05	2.21	1.73	31.76 bcd	1.00
	N_{140}	7.85	1.21 c	14.95	70.7	1879.6	0.86	24.39 a	6.65	1.93	1.55	34.47 a	0.56
	N_{210}	7.76	1.34 b	14.05	71.8	2063.3	0.83	24.34 a	7.38	1.96	1.42	35.16 a	1.46
SMR_2	N_0	7.88	1.00 ef	18.28	48.9	1791.4	0.57	20.32 a	5.98	1.98	1.48	29.76 d	0.71
	N_{70}	7.72	1.10 cde	14.51	51.0	1893.0	0.61	20.85 a	7.10	1.75	1.63	31.34 cd	0.65
	N_{140}	7.85	1.40 b	16.09	61.0	1925.2	0.77	21.07 a	8.79	2.21	1.61	33.67 abc	0.60
	N_{210}	7.71	1.41 b	15.56	60.9	1907.5	0.81	21.02 a	9.60	2.15	1.54	34.31 ab	1.46
SMR_3	N_0	7.69	1.05 def	16.48	42.7	1682.4	0.61	12.93 b	9.55	1.92	1.64	26.04 e	1.12
	N_{70}	7.84	1.19 cd	16.58	47.1	1681.0	0.56	23.11 a	6.74	2.06	1.62	33.54 abc	0.46
	N_{140}	7.67	1.43 b	15.97	54.8	1702.8	0.69	23.74 a	6.44	1.70	1.48	33.36 abc	0.72
	N_{210}	7.64	1.72 a	16.55	57.7	1790.5	0.73	22.11 a	7.73	2.20	1.52	33.57 abc	1.85

**Significant at 1% level, *Significant at 5% level.

SMR_1: 100%FI (Full Irrigation), SMR_2: 75% of FI, SMR_3: 50% of FI, N_0: Unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha

Effects of N applications under different soil moisture regimes on available P_2O_5, plant available potassium (K_2O) and organic matter at the 0-30 cm soil depth were found to be significant (Table 3; Figure 1). There were significant ($p< 0.01$) effects of the soil moisture regimes on plant available K_2O (Table 3; Figure 2). Moreover, soil moisture regimes x N applications interactions did have significant affects on the EC, extractable Ca and CEC (Table 1 and 3). Soil pH, lime content, extractable Mg, Na, K and B did not differ significantly across treatments. EC, available P_2O_5, available K_2O, organic matter and CEC of the soils increased with increasing N treatments under soil moisture regimes. PH values of the soil under soil moisture regimes and nitrogen treatments varied between 7.64-7.88 with an average value of 7.78. The highest EC (1.72 dS/m) was observed in the N_{210} treatment (210 kg N/ha) of the lowest soil moisture regime (50%FI) and the lowest EC value (0.91 dS/m) was obtained from the N_0 treatment (without N fertilizer) of the highest soil moisture regime (100%FI). $CaCO_3$ values of the soil under different soil moisture regimes and N treatments varied between 14.0-18.3% with an average value of 16.0%. OM values of the soil under different soil moisture regimes and N treatments varied between 0.56-0.86% with an average value of 0.69%. CEC values of the soils varied between 26.0-35.2 me/100 g with an average value of 32.3 me/100 g.

Changes of some soil physical properties under different soil moisture regimes and N treatments in a fine textured soil at the 0-30 cm soil depth are given in Table 2. The results of the study obtained that the affects of N applications under different soil moisture regimes on the selected soil physical properties except Ksat for 0-30 cm soil profile depth did not differ significantly across applications (Table 4). However, the Ksat was not affected by treatments at the lower depths. The highest Ksat value (1.128 cm/h) was observed in the N_{210} treatment (210 kg N/ha) of the highest soil moisture regime (100%FI) and the lowest Ksat (0.040 cm/h) was obtained from the N_{70} application (70 kg N/ha) of the 75%FI treatment. AWC values of the soilS varied between 10.97-19.03% with an average value of 16.5%. BD values of the soil varied between 1.39-1.45 g/cm^3 with an average value of 1.42 g/cm^3.

Table 2. Changes of some mean soil physical properties under different soil moisture regimes and nitrogen (N) treatments in a fine textured soil at the 0-30 cm soil depth

Irrigation level	N dose (kg N ha^{-1})	FC, %	PWP, %	AWC, %	BD, g/cm^3	Ksat, cm/h
SMR_1	N_0	36.18	19.39	16.79	1.42	0.070 de
	N_{70}	40.32	21.30	19.03	1.41	0.050 e
	N_{140}	32.28	21.31	10.97	1.39	0.810 b
	N_{210}	37.16	20.85	16.31	1.45	1.128 a
SMR_2	N_0	38.96	21.30	17.67	1.42	0.050 e
	N_{70}	36.73	21.40	15.33	1.44	0.060 e
	N_{140}	40.27	21.70	18.57	1.42	0.350 cd
	N_{210}	39.68	21.29	18.40	1.45	0.478 c
SMR_3	N_0	35.80	19.55	16.25	1.39	0.060 e
	N_{70}	39.52	21.52	18.01	1.41	0.040 e
	N_{140}	33.65	21.29	12.36	1.41	0.095 de
	N_{210}	38.56	19.72	18.84	1.42	0.168 de

**Significant at 1% level, *Significant at 5% level. SMR1: 100%FI (Full Irrigation), SMR2: 75% of FI, SMR3: 50% of FI, N_0: Unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha

Table 3. Results of variance analysis for the some soil chemical properties at the 0-30 cm soil depth

Variation parameters	pH	EC	$CaCO_3$	Av. P_2O_5	Av. K_2O	OM	Exc. Ca	Exc. Mg	Exc. Na	Exc. K	CEC	B
N	ns	**	ns	*	*	**	**	ns	ns	ns	**	ns
SMR	ns	**	ns	ns	**	ns	ns	ns	ns	ns	**	ns
N x SMR	ns	*	ns	ns	ns	ns	**	ns	ns	ns	**	ns
Error	0.013	0.006	3.05	1.41	196.2	0.01	5.31	2.94	0.29	0.033	1.06	0.56
CV (%)	1.7	18.9	11.3	24.3	9.0	18.9	16.4	23.7	24.5	11.8	8.1	8.3

ns; not significant, ** significant at 1% level, * significant at 5% level. N: nitrogen applications, SMR: soil moisture regimes, CV: coefficient of variation.

Table 4. Results of variance analysis for the some soil physical properties at the 0-30 cm soil depth

Variation parameters	FC	PWP	AWC	BD	Ksat
N	ns	ns	ns	ns	*
SMR	*	ns	*	ns	**
N x SMR	ns	ns	ns	ns	**
Error	5.044	1.088	6.016	0.001	0.013
CV (%)	8.9	5.5	19.1	2.3	28.7

ns; not significant, ** significant at 1% level, * significant at 5% level. N: nitrogen applications, SMR: soil moisture regimes, CV: coefficient of variation.

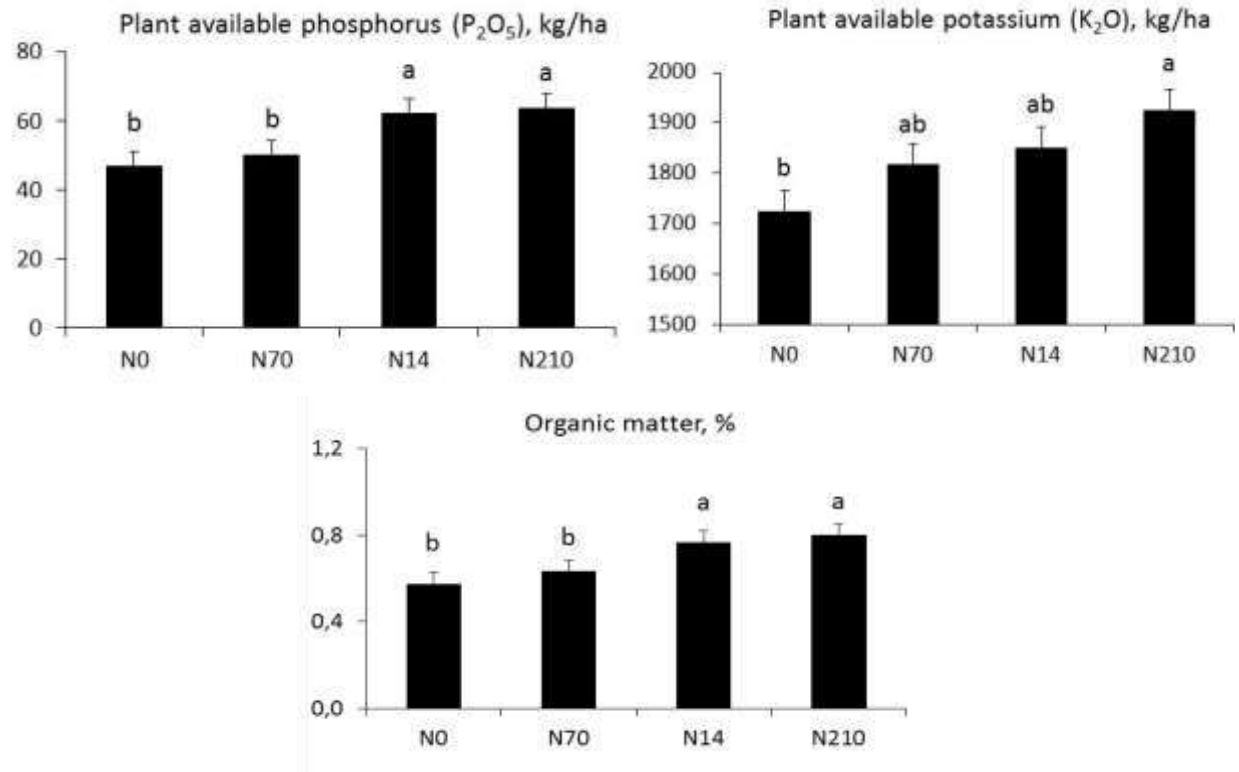


Figure 1. Influences of N applications under different soil moisture regimes on available phosphorus (P_2O_5), potassium (K_2O) and organic matter at the 0-30 cm soil depth (N_0: Unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha)

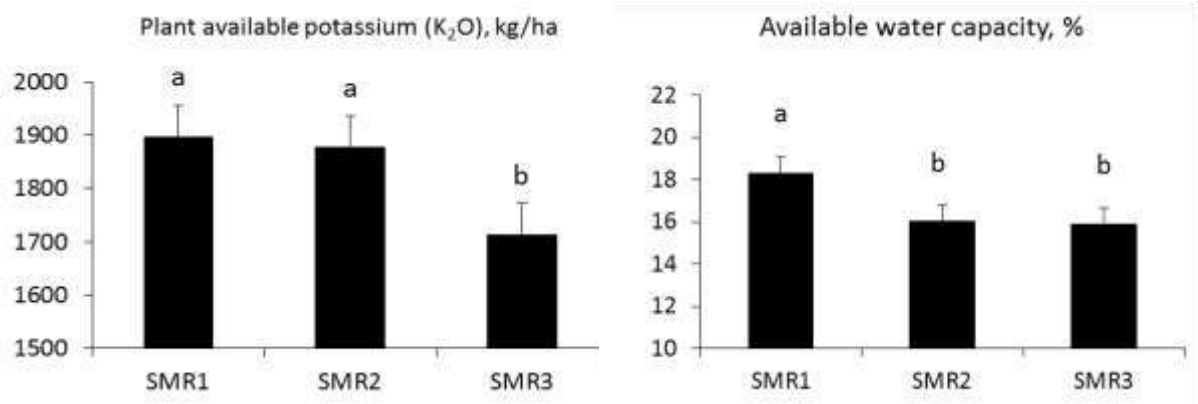
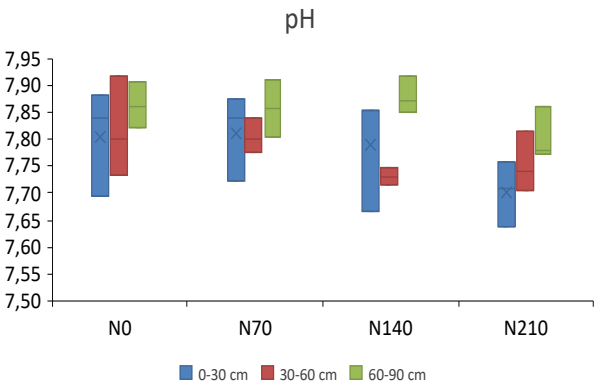
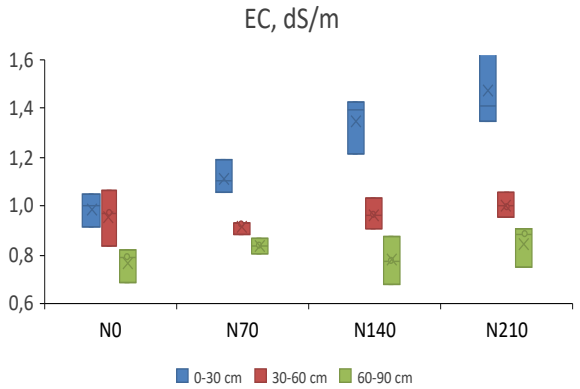
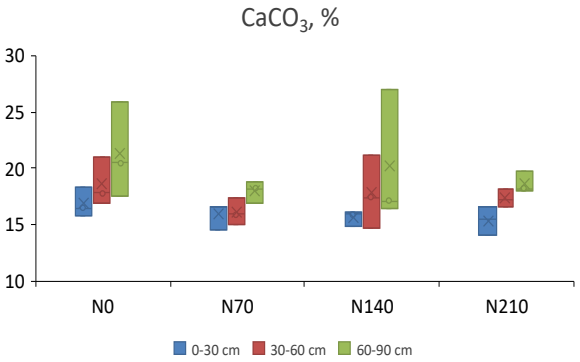


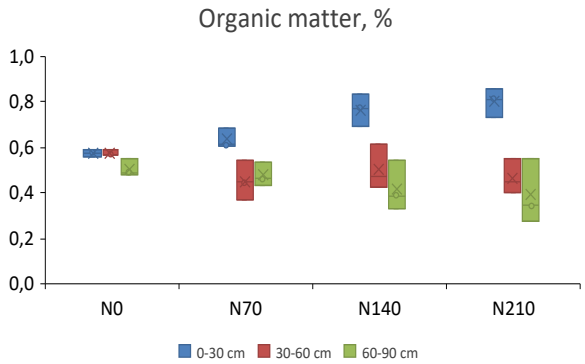
Figure 2. Effects of different soil moisture regimes on plant available potassium (K_2O) and available water capacity (AWC) at the 0-30 cm soil depth (SMR1: 100%FI (Full Irrigation), SMR2: 75% of FI, SMR3: 50% of FI).

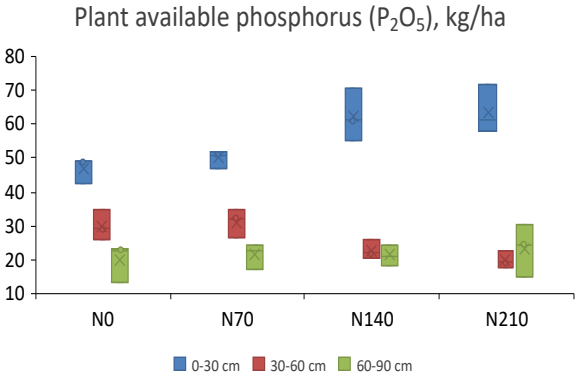
Changes on some soil chemical properties at the soil depths (0-30, 30-60 and 60-90 cm) in the experiment field are shown (Figure 3 and 4). Analyses showed that mean pH and lime content were higher at the subsurface (60-90 cm depth). However, EC, OM, available P_2O_5 and K_2O values of the soils was higher at the top (0-30 cm soil depth). Mean EC, OM, available P_2O_5 and K_2O values of the soils also decreased as soil depth increased. At the end of the research, pH values of soils varied in the range from 7.64 to 7.92 with average 7.80, EC values from 0.68 dS/m to 1.78 dS/m with average 1.00 dS/m, lime values from 14.0% to 26.9% with average 17.7%, OM values from 0.27% to 0.86% with average 0.55%, plant available P_2O_5 values from 13.6 kg/ha to 71.8 kg/ha with average 34.4 kg/ha, plant available K_2O values from 715 kg/ha to 2063 kg/ha with average 1366 kg/ha, B values from 0.33 ppm to 1.85 ppm with average 0.82 ppm.

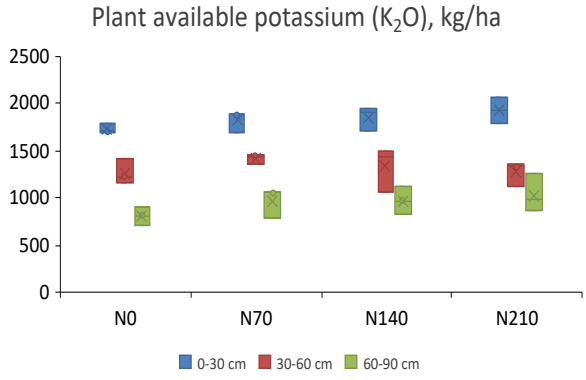

pH
7,95
7,90
7,85
7,80
7,75
7,70
7,65
7,60
7,55
7,50
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm


EC, dS/m
1,6
1,4
1,2
1,0
0,8
0,6
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm


CaCO3, %
30
25
20
15
10
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm


Organic matter, %
1,0
0,8
0,6
0,4
0,2
0,0
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm


Plant available phosphorus (P2O5), kg/ha
80
70
60
50
40
30
20
10
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm


Plant available potassium (K2O), kg/ha
2500
2000
1500
1000
500
0
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm

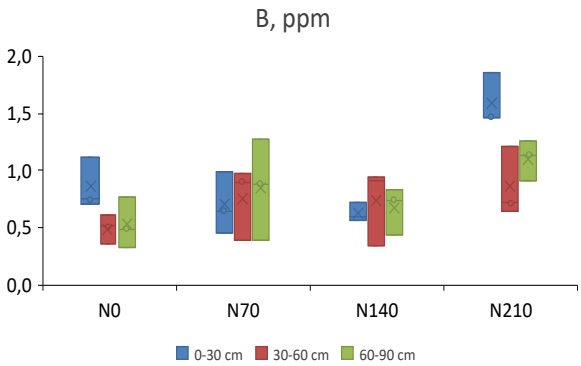

B, ppm
2,0
1,5
1,0
0,5
0,0
N0
N70
N140
N210
0-30 cm
30-60 cm
60-90 cm

Figure 3. Changes on some soil chemical properties at different soil depths (N_0: Unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha)

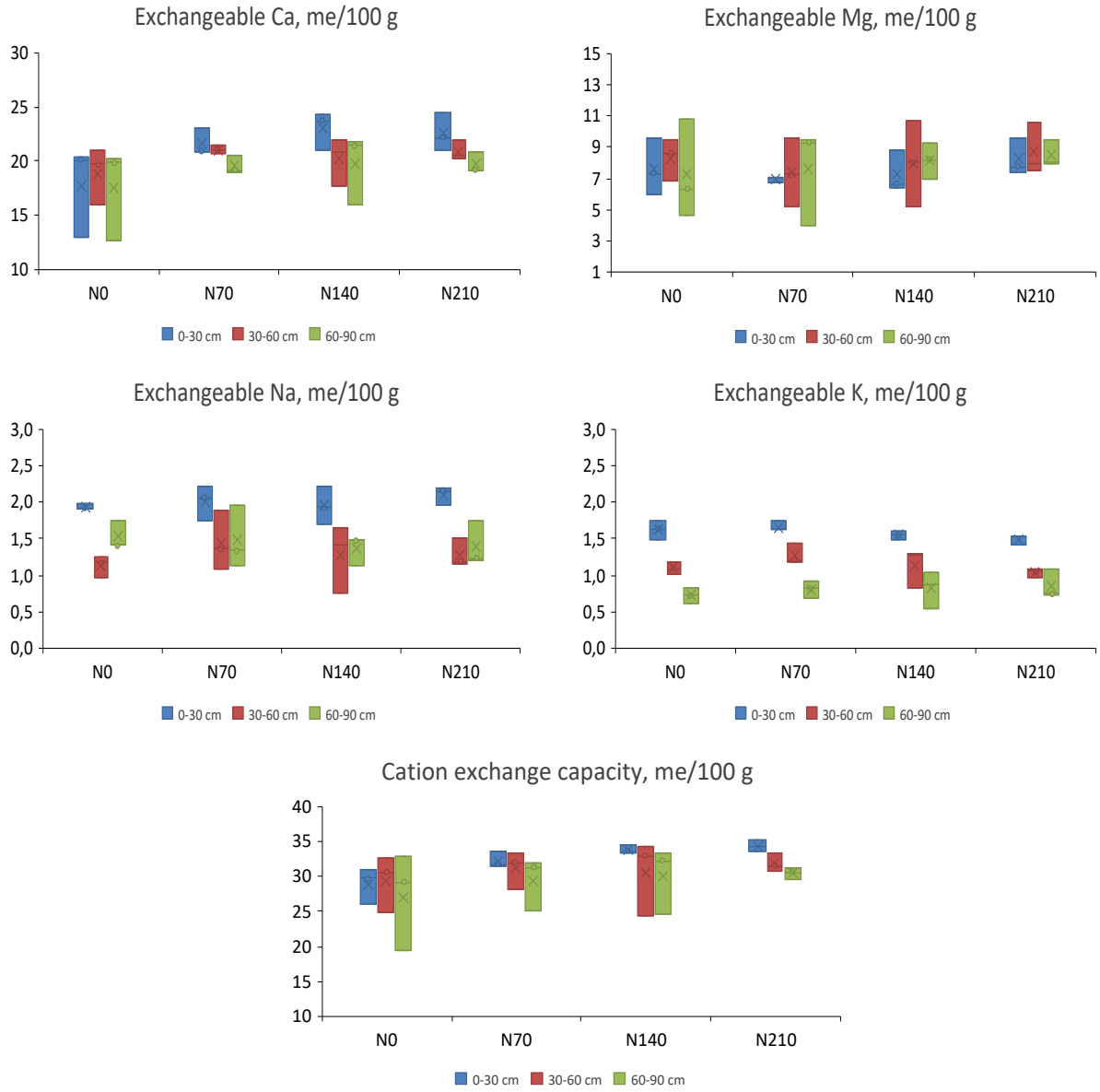


Figure 4. Changes on exchangeable Ca, Mg, Na, K and cation exchange capacity at different soil depths (N_0: Unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha)

Changes on some soil physical properties at the soil profile depths (0-30, 30-60 and 60-90 cm) in the experiment field are shown (Figure 5). FC values of soils varied in the range from 33.2 to-42.1% with average 36.6%, PWP values from 16.8 to 21.7% with average 20.0%, AWC values from 12.3% to 20.6% with average 16.7%, BD values from 1.34 g/cm^3 to 1.50 g/cm^3 with average 1.44 g/cm^3, Ksat values from 0.015 to 1.128 cm/h with average 0.244 cm/h.

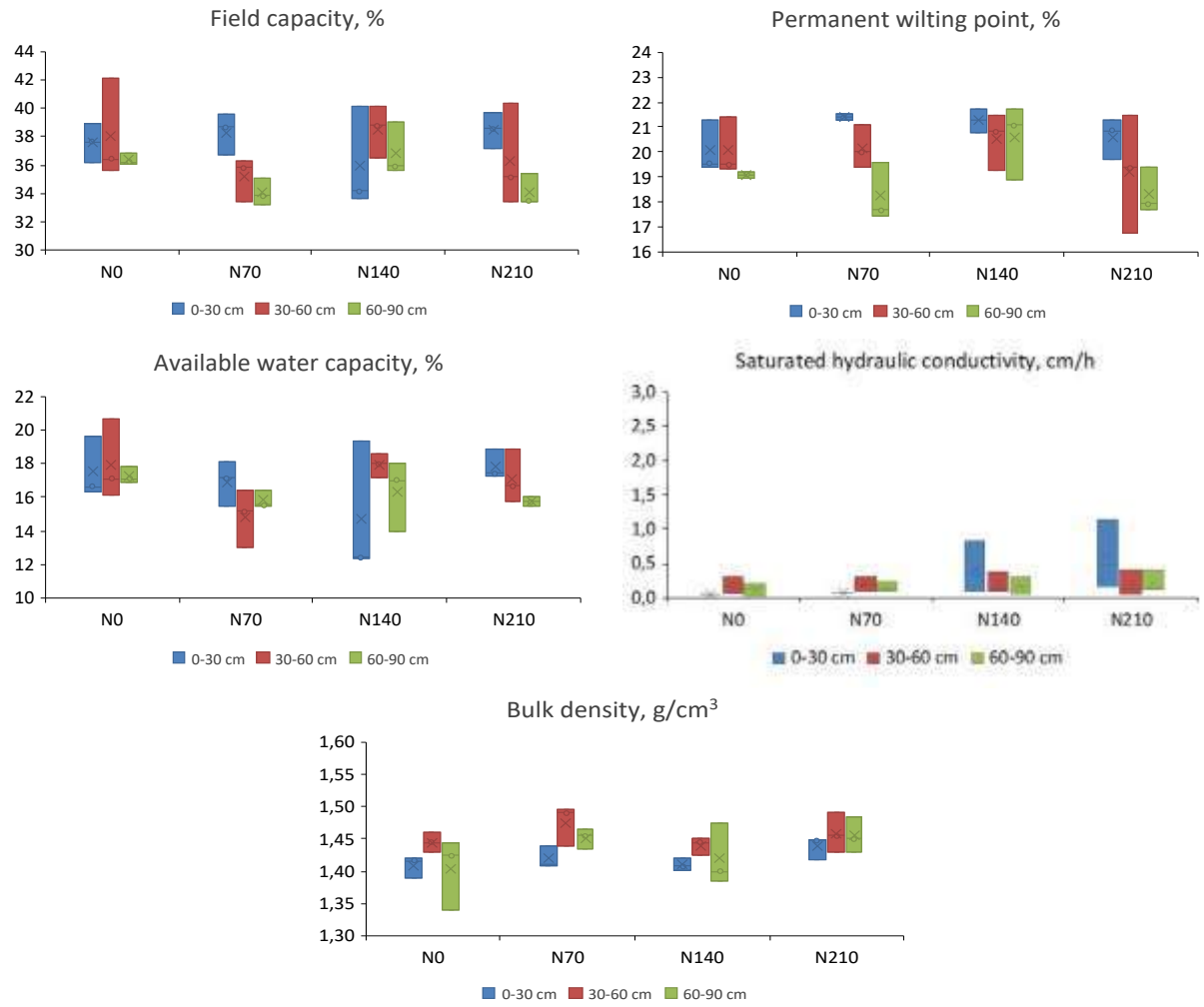


Figure 5. Changes on some soil physical properties at different soil depths (N_0: Unfertilized, N_{70}: 70 kg N/ha, N_{140}: 140 kg N/ha, N_{210}: 210 kg N/ha)

Discussion

The results of the study showed that the effects of N fertilizer applications under soil moisture regimes on the selected soil physical properties except Ksat for 0-30 cm depth were found to be insignificant across applications. Similarly, Roberson et al. (1995) and Rodríguez et al. (1999) found that Ksat were increased in response to N fertilizer treatments. Meena et al. (2020) found that soil Ksat for 0-10 cm soil depth did not differ significantly across applications (synthetic fertilizer and organic compost), while the Ksat was effected by applications at the 10–20 cm soil depth. Oosterbaan and Nijland (1994) found that soil salinity generally has a positive effect on the Ksat, specially in clay soils. The changes of soil Ksat for 0-30 cm depth may be explained by the increment of soil salinity. Soil permeability depends on the flocculation of colloids which is influenced by electrical conductivity (Abu-Sharar et al., 1986). So, a larger flocculation of soil colloids due to saline impacts caused soil permeability improvements. The electrolyte concentration of soil solution causing the increase of soil electrical conductivity which is an significant factor for the sustenance of a good soil permeability modify fertilizer applications (Agassi et al., 1981). Therefore, fertilizers can positively affect the physical properties of the soil

(Rodríguez et al., 1999). Abu-Sharar et al. (1986) reported that the increment in electrical conductivity decreases the stabilizes aggregates and double-diffusive layer of the colloids, bring about more permeability and less slaking. Ammonium concentration was increased in relation to the original level in the soil. It can be thought that the electrolytes narrowed soil the double-diffusive layer, hence enhancing the flocculation of soil colloids (Rodríguez et al., 1999). Rodríguez et al. (1999) found that electrical conductivity showed the high correlation with saturated hydraulic conductivity among the examined soil characteristics. In this study, significant positive correlation was observed between Ksat and EC (0.376*) (Table 5).

Table 5. Pearson correlation coefficient between soil parameters at the 0-30 cm soil depth

	EC	pH	$CaCO_3$	P_2O_5	K_2O	OM	Ca	Mg	Na	K	CEC	B	FC	PWP	AWC	BD
pH	-0.198															
$CaCO_3$	-0.064	0.151														
Av.P_2O_5	0.371*	-0.010	-0.180													
Av.K_2O	0.297*	0.152	-0.321	0.392*												
OM	0.492**	-0.077	-0.152	0.711**	0.494**											
Exc.Ca	0.356*	0.322	-0.175	0.460**	0.430**	0.445**										
Exc.Mg	0.168	-0.325	-0.030	-0.084	-0.108	0.002	-0.658**									
Exc.Na	0.120	-0.170	0.128	0.055	-0.096	0.166	-0.143	0.078								
Exc. K	-0.347*	-0.090	-0.271	-0.181	-0.046	-0.392*	-0.167	-0.060	-0.019							
CEC	0.590**	0.169	-0.249	0.554**	0.479**	0.599**	0.846**	-0.184	0.048	-0.196						
B	0.309	-0.146	0.085	0.205	0.306	0.030	0.129	-0.080	-0.073	0.167	0.115					
FC	-0.185	0.056	-0.245	-0.129	0.189	0.052	0.135	-0.035	-0.070	0.164	0.156	0.049				
PWP	0.103	0.308	0.001	0.139	0.145	0.039	0.225	0.059	-0.052	-0.068	0.326	-0.032	0.011			
AWC	-0.210	-0.064	-0.228	0.067	0.121	0.034	0.041	-0.055	-0.045	0.178	0.021	0.057	0.926**	-0.176		
BD	0.081	-0.066	0.119	0.142	0.070	0.120	0.105	0.017	0.150	-0.016	0.179	-0.021	-0.122	0.054	-0.133	
Ksat	0.376*	0.135	-0.336*	0.580**	0.492**	0.662**	0.363*	0.089	0.046	-0.310	0.533**	-0.058	0.280	0.063	0.236	0.265

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level.
pH: soil reaction, EC: electrical conductivity, Av. P_2O_5: available phosphorus, Av. K_2O: available potassium, OM: organic matter, Exc.Ca: extractable calcium, Exc.Mg: extractable magnesium, Exc.Na: extractable sodium, Exc. K: extractable potassium, CEC: cation exchange capacity, B: boron, FC: field capacity, PWP: permanent wilting point, AWC: available water capacity, BD: bulk density, Ksat: saturated hydraulic conductivity.

Results showed that pH values reduced with N treatments and water deficit but the differences were not significant (Table 1). Myers and Thien (1991) reported that N fertilizers that are characterized by acidity index may decrease soil pH and dissolve soil OM thus effecting soil permeability. Guo et al. (2010) reported that the mean soil pH reduced 0.5 units because of the utilization of N fertilizer in the past two decades in China. Li et al. (2013) found that the soil pH decreased from 8.76 to 8.56 in North region, China. On the other hand, mean pH values of the soils increased as soil depth increased (Figure 3). The reason could be an increase in basic cation along soil depth which increases soil pH from top to down the soil profile. The result concurs with reports by Eyayu Molla et al. (2009), Amare Haileslassie et al., (2005) and Kumar et al. (2012).

N fertilizer treatments under different soil moisture regimes increased the available P_2O_5, available K_2O and organic matter of the soils. Similarly, Zhang et al. (2019) found that available K_2O, available P_2O_5, OM and available N in the soil were higher than the control. Halvorson et al. (1999) found that the increase in soil organic carbon with N fertilization contributes to increased

soil fertility, and increased efficiency of C sequestration into the soil. Therefore, the treatment of N fertilizer can improve soil fertility (Zhang et al., 2019).

Cation exchange capacity (CEC) of soil increased with the increase of N fertilizer dose. This may be explained by retaining the ammonium ion by adsorptive complex or by clay minerals in the soil (Radulov et al., 2011). In addition, Radulov et al. (2011) found that the increase of the CEC values were significantly influenced by available K_2O, available P_2O_5 values of soil. In this study, available phosphorus and potassium contents of the soils increased as the N fertilizer dose rise (Figure 1). Significant correlations were obtained between available P_2O_5 and CEC (0.554**), between available K_2O and CEC (0.479**), between organic matter and CEC (0.599**) (Table 5). On the other hand, mean CEC values were affected by increasing soil depth where it decreased by 4.90% at the 30-60 cm and 9.51% at the 60-90 cm soil plofile depth. The findings were in agreement with the results by Bewket and Stroosnijder (2003).

Conclusions

In the present study, N fertilizer treatments under different soil moisture regimes were carried out to determine the changes of some soil physical and chemical properties in a clay textured soil where pepper was grown in field conditions. The findings of the study showed that the affects of N applications under soil moisture regimes on the selected soil physical properties except Ksat for 0-30 cm depth did not differ significantly across applications. However, the Ksat was not effected by applications at the lower depths. Soil moisture regime x N treatment interactions for 0-30 cm soil depth did have important influences on the EC, CEC and Ksat. EC, available P_2O_5, available K_2O, organic matter and CEC of the soils increased with increasing N applications under soil moisture regimes. In addition, the soil attributes showed differences with soil depth. Saturated hydraulic conductivity and some soil chemical properties were effected by the topsoil depth and the soil management practices.

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COMPARISON OF TOTAL CHLOROPHYLL CONTENT, CHLOROPHYLL A, B AND CAROTENOIDS IN PRUNUS ROOTSTOCKS; *CAB 6 P* IN VITRO (*PRUNUS CERASUS*) AND IN VIVO OF *P. MAHALEB* (*PRUNUS CERASUS*)

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ABSTRACT

Rootstocks of Prunus, *CAB 6 P*, after being subjected to micropropagation, in In Vitro cultures, have been compared with rootstock plants of *P. mahaleb* (In Vivo), for the number of total chlorophylls, chlorophylls a, b and carotenoids. Thus, has been evaluated and compared the spectral reflection potential extracted from FISS (Field Imaging Spectrometer System) data and the derivative spectrum in evaluating the chlorophyll content of plant leaves; in vivo and in vitro. Determination of chlorophylls in two prunus subspecies acquired in various technologies they also verify the quality of the plants. Displacement of "in vitro" plants in the greenhouse of acclimatization, verified increased chlorophyll content (chlorophyll) a and chlorophyll b, in these plants compared to *Mahaleb* (In Vivo). The study was conducted at UBT at the Research and Nutrition Center, Faculty of Biotechnology .The chlorophyll content was calculated by comparing the absorption in a UV spectrophotometer. The results and verification of FISS performance were achieved after a quantitative analysis through the assessment of chlorophyll content and comparison with a traditional non-imaging spectrometer. A relatively high correlation coefficient with four absorbances was verified: 490-550, 600-670, 680-710 and 725-775 nm, for the fuel spectrum, for the chlorophyll content that was determined using the rapid extraction method with one ethanol-acetone mixture. Total chlorophyll levels are rated at higher levels in plants obtained from in vitro and turned out to be 121.6 mg / g. Spectro photometer absorbance the total chlorophyll of *P. Mahaleb* in vivo was 14. 8 mg / g. Chlorophyll a, b and carotenoids were respectively in “in vitro”: 170.6; 307.6; 114.3. Chlorophyll a, b and carotenoids were respectively in in vivo (*P. Mahaleb):* 11; 3.5; 6.1.

Keyword: *CAB 6P, P. Mahaleb*, Chlorophyll a, Chlorophyll b, carotenoids

INTRODUCTION

Plant pigments

The life on Earth primarily relies on sunlight energy captured by plant pigments to drive the process of photosynthesis. The main photosynthetic pigments in plant leaves are chlorophylls a

and b. The main carotenoids in green leaves of terrestrial plants are α- and β-carotene, lutein, zeaxanthin, violaxanthin, antheraxanthin, and neoxanthin (Esteban et al. 2015a, Nisar et al. 2015).[1] The spectral properties of individual pigments are well studied in solution where interactions with other pigments and pigment-protein complexes are absent (Porra 2002)[2], but less is known of spectral properties of pigment-binding complexes as they occur and function in intact leaves (Marin et al. 2011)[3].

Changes in chlorophyll a to b ratio can also reflect multiple adjustments in response to light quality and quantity. Ratio of two photosystems (PSI and PSII) as well as the size of light-harvesting antenna complexes modify chlorophyll a to b ratio (Hansen et al. 2002;[4]

A photocopying law that is evalueble for all the biological effects of light, is that this pigment in the greenhouse, hlorophyll, absorbs most of the light used in plants, green pigment chlorophyll absorbs most of the light used for photosynthesis.. The absorption of light is clearly the first step in photosintheses. (Holt, A. S., and E. E. Jacobs 1954-1955) **[5]**

Cab 6 P Selection of P.Cerasifera, acquired by the University of Bologna. Good suitability to different types of soil. Moderate high viscosity, with moderate fit. It fits into heavy and smoky soils. Water-sensitive and Armillaria, tolerant to Phytophthora and Verticillium. Maturity period comparable to or close to Colt and F12. Suitable for medium-density fertile soils. De Salvador et al 2005**[6]**

The main problem in sweet cherry (Prunus avium L.) production is large tree size, which increases the costs of management activities, especially harvesting. A lot of research has been done over the years to find a solution by introducing spur-type scion cultivars [7] Ducci F., et al (2013)

Nowadays the knowledge on genotype requirements and behaviour in relation to different micro-propagation factors, and on the interactions they have with the different environments, is well developed and it is nowadays possible to transfer this knowledge to private/ public laboratories for vegetative mass production[8] (Barzanti et al. 2004 ; De Rogatis et al. 2006, 2009)[9]

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MATERIAL AND METHODS

Propagation patterns within cherry rootstocks (macro-micro propagations) as well as within seed propagation of cherry trees (*P. Mahaleb*) also gave a view of how rootstocks cherry interacts in In Vitro propagation.
The pattern of distribution of diversity throughout Mediterranean Europe has been partially examined, referred the propagations technology.
Most technologies of micro-macro propagation issues have been answered: most cherry rootstocks stand cannot produce enough diversity, looking at their size and observed patterns of intra-population diversity.[10] Wilkins D, et al (1994). So, *CAB 6 P* was finding interesting
However, in order to make public demonstration plots in rootstocks cherry forests in our country, analyses within Mahaleb and CAB 6P cherry populations (especially incoming from in vitro stands) could be useful. The introgression with propagations forms is an important aspect to be observed.

Experimental designer

The study conducted at the Agricultural University of Tirana, in the laboratory of the Research and Food Center, Faculty of Biotechnology, AUT.
An investigational scheme was applied in order to study the quality of plantlets formed from micropropagation by estimating the content of total chlorophylls, chlorophyll a, b and carotenoids Some leaf of *CAB 6 P* and *P. Mahaleb*, obtained, one-to-one by in vitro and in vivo propagation,
Chlorophyll extraction: The content of chlorophyll is determined using the quick extraction method with an ethanol-acetone mixture (1: 1 in volume).
Fresh leaves were cut into pieces and 0.2 g of pieces were weighed and placed in a 25-mL cap for 18-24 hours in the dark. The tube was shaken once per hour; absorption was measured with an UV spectrophotometer to calculate the content of chlorophyll.
Spectrophotometry is a shared technique used to quantity the absorbance of light by the chlorophyll molecules. The Nano Drop One/One C UV-Vis Spectrophotometer can be used to portion of the optical density of chlorophyll. Chlorophyll a and chlorophyll b absorb light at slightly diverse wavelengths
The computer system includes hardware and software: the hardware is a computer, and the software includes the FISS operating software, data acquisition software and data processing software. The instrument operating software and data acquisition software are used for setting the instrument parameters (integration time, aperture, field of view, cooling temperature, etc.) and can display images and spectra in real time. The primary functions of the data processing software are data format conversion, geometric correction, radiometric correction, image segmentation, interception and image stitching.

RESULTS AND DISCUSSIONS

The techique of macro- micro propagation of cherry trees rootstocks, applied across Mediterranian nurseries and laboratories is not well known. Continuing the exploration and inventory of new in situ resources is planned in all these countries.

[10] Wilkins D, Van Oosten JJ, Besford RT (1994) Effects of elevated CO (2) on growth and chlorophylloroplast proteins in Prunus avium. Tree Physiol 14(7–9):769–779

In the same way, new and possibly useful variations can be researched throughout extended experiment of micro propagations of rootstocks of cherry and by selecting genotypes within existing progeny tests. In view of this, the disccussions can help all specialist of products development to understand new entry of sedlings of cherry rootstocks, like CAB 6P.

Chlorophyll is one of the most important biochemical parameters of plants and is usually an indicator of plants' nutritional stress, photosynthetic capacity and the health status of plants; therefore, it is an important information parameter in this research on in vitro propagation quality monitoring.

To estimate the use of different spectral characteristics as indicators of pigment content, we have calculated the linear coefficients of correlation between these characteristics and the pigment content (shown in Figures 1 and 2).(Aronoff, S. 1950)[11]
Spectral variables as indicators of the content of pigments

International Standards according to the literature for SPECORD 40 - 232E160:
Parameter- Design SPECORD 40 - 232E160
Subject Definition of chlorophyll
Operator Date / Time 07/06/2018 09:23
Screen Display - Absorbance
Reference Correction
Lamp change at 320 nm
Meas. scanning mode
Range [nm] 350 - 880
Lambda Delta [nm] 10
Speed [nm / s] 10
Cycle Mode Manual

The results obtained on the PC screen of the spectrophotometer were as follows:

Chlorophyll a (mg/g) = (12.7 * A663) − (2.59 * A645) (1)
Chlorophyll b (mg/g) = (22.9 * A645) − (4.7 * A663) (2)
Chlorophyll total (mg/g) = (8.2 * A663) + (20.2 * A645)
Carotenoids (mg/g) = (7.6*A480) -(1.49*A510)

For the spectrum of derivatives, there were four relatively high coefficient of correlation absorption: 490-550, 600-670, 680-710 and 725-775 nm.
As shown in the summary table and Figures 5 and 6, the maximum coefficient of correlation between the spectral reflections of the average data derivatives and the chlorophyll content was -0.90 (corresponding to a wavelength of 650 nm).

[11] Aronoff, S. 1950. Chlorophyll. Bot. Review 16, 52-8

Chlorophyll *In vivo*

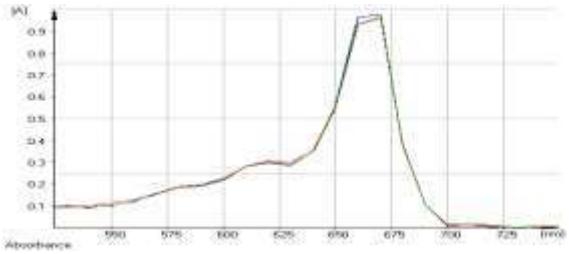


Figure 1. Absorbance of chlorophylls that in vivo plants of CAB 6 P

Chlorophyll *in Vitro*

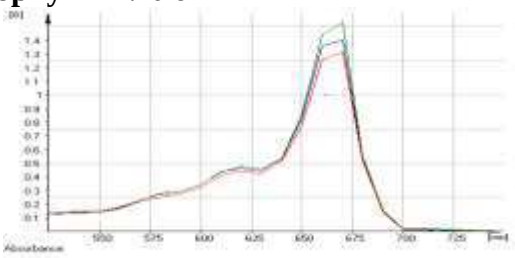
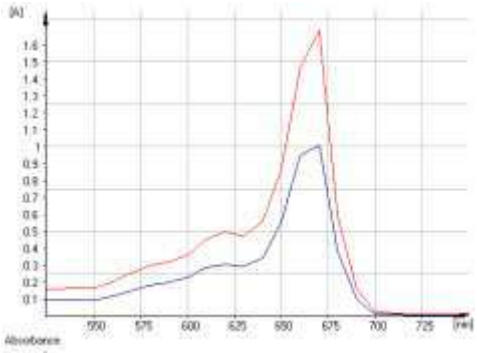


Figure 2. Absorbance of chlorophyllorophiles that in vitro plants of CAB 6 P



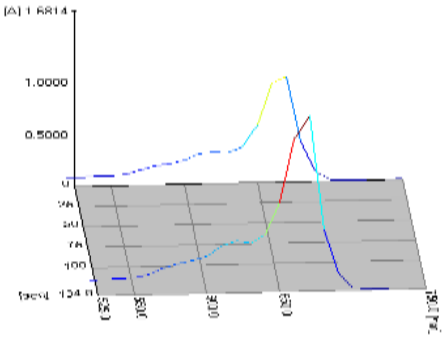
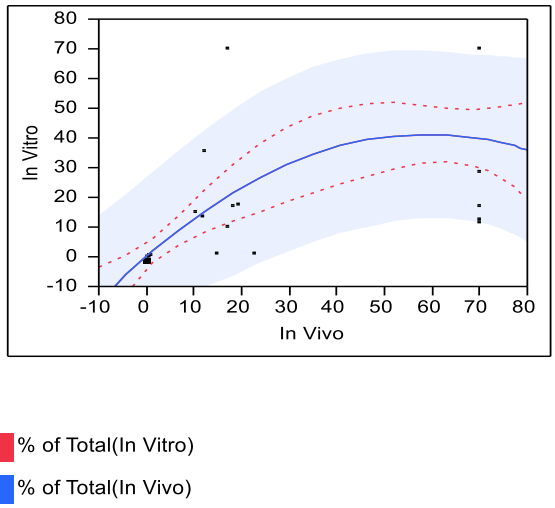


Figure 3 & 4. In vivo vs. in vitro comparison (in vivo blue and red in vitro)

Correlation quantity of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in two forms of prunus CAB and mahaleb (in vitro and in vivo) were the same in form, but had different wavelength verses with high correlation coefficients, which will be illustrated below (table 1)



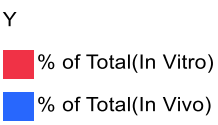


Figure 5. Correlation of total chlorophyllorophiles in vivo and in vitro

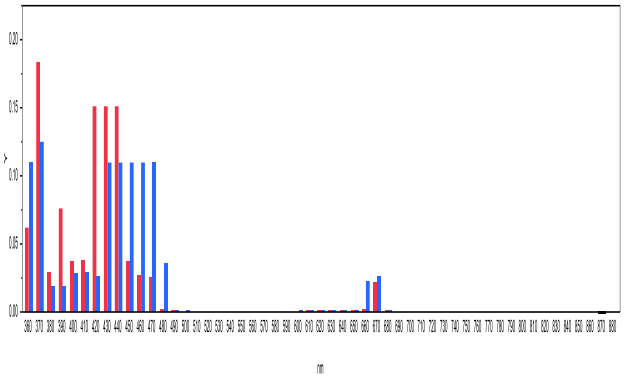


Figure 6. The maximum and maximum values of total chlorophyllorofiles

	Chlorophyll A				**Chlorophyll A**		
	A660	A640			A660	A640	
	14.418	0.5314	181.732		0.9674	0.3466	11.388
In vitro	13.604	0.5102	171.449	In vivo	0.9301	0.3571	10.887
	12.609	0.5386	158.739		0.9360	0.3464	10.990
	Chlorophyll B				**Chlorophyll B**		
	A640	A660			A640	A660	
	0.5314	14.418	327.6746		0.3466	0.9674	3.39036
In vitro	0.5102	13.604	309.1337	In vivo	0.3571	0.9301	3.80612
	0.5386	12.609	286.2147		0.3464	0.9360	3.53336
	Total Chlorophyll				**Total Chlorophyll**		
	A660	A640			A660	A640	
	14.418	0.5314	128.9619		0.9674	0.3466	14.934
In vitro	13.604	0.5102	121.8588	In vivo	0.9301	0.3571	14.84024
	12.609	0.5386	114.2735		0.9360	0.3464	14.67248
	Carotenoids				**Carotenoids**		
	A480	A510			A480	A510	
	14.368	0.1588	108.9602		0.7922	0.1100	5.85682
In vitro	11.702	0.174	88.67594	In vivo	0.8897	0.1119	6.594989

Table 1. In vivo Mahaleb and CAB 6P in vitro content of chlorophylls a, b and carotenoids

Analysis of chlorophyll content

The content of chlorophyll (Chlorophyll) a and Chlorophyll b increased after in vitro transition to the acclimatization greenhouse.
After conception, CO2 absorption of leaves was similar to that of plants grown in vitro or slightly higher, and a large increase in CO2 was observed only in newly formed leaves (J. Pospóšilova et al1999, Deng and Donnely 1993). [12]

[12] J. Pospóšilová, I. Tichá, P. Kadleček, D. Haisel & Š. Plzáková (1999) Acclimatization of Micropropagated Plants to Ex Vitro Conditions, Biologia Plantarum, volume 42, p 481–497
Ribo Deng and Danielle J. Donnelly, (1993) In Vitro Hardening of Red Raspberry by CO2 Enrichment and Reduced Medium Sucrose Concentration, in HortScience, DOI: https://doi.org/10.21273/HORTSCI.28.10.1048
P: 1048–1051, Volume 28: Issue 10

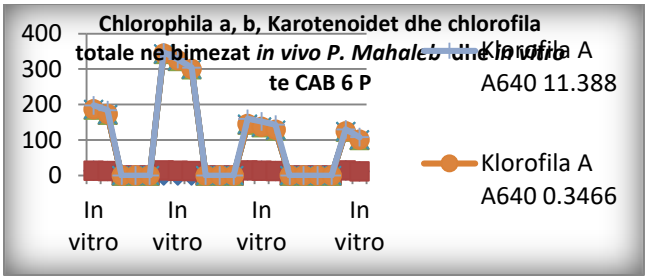


Figure 7. The frequencies of chlorophyll a, b and total for in vivo P. mahaleb and in vitro CAB 6

A comparison of two figures revealed that the correlation coefficient between the spectral derivatives and the pigment content was generally higher, indicating that the derivative spectral information was a better indicator of the pigment in the in vitro propagation

The mean a / b ratio of chlorophyll in vivo and in vitro CAB 6 P was 3.2 and 6.3, respectively (Table 1). The determination coefficient between the absolute concentration of chlorophyll and the a / b ratio was 0.68 for CAB 6 P

Studies of chlorophyll absorption spectrum in vivo and in vitro, its measurements, are the application of chemical kinetics to photochemical photosynthesis reactions and other photochemical processes involving chlorophyll - these are undoubtedly tools to get some information on our problems

CONCLUSIONS

This study has shown that these cv of rootstocks of cherry interact differentially to instantaneous (measuring) and long-term (acclimation) changes in PSII excitation pressure with regard to their photosynthetic behaviour

Spectrophotometric analysis showed high chlorophyll a / b ratio, which shows good photosynthetic competence and ability to protect photons in vitro plants. Thus, photosynthetic acclimation is essential for proper energy balancing during the acclimation process

The level of total chlorophyllorophiles is predominantly dominant in plants obtained from in vitro and has resulted to be 121.6 mg / g

Spectrophotometric absorbance of total CI6 C in vivo CAB 6 P was 14. 8 mg / g

Chlorophyll a, b and carotinoids were respectively in vitro: 170.6; 307.6; 114.3

Chlorophyll a, b and carotinoids were respectively in vivo: 11; 3.5; 6.1

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SOIL CLASSIFICATION IN NAGARI SUNGAI KAMUYANG, WEST SUMATRA, INDONESIA

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ABSTRACT

This study was aimed to examine the classification of soil in Nagari Sungai Kamuyang, West Sumatra, Indonesia by the USDA Soil Taxonomy up to the level of family. This research was conducted in Nagari Sungai Kamuyang, Luak District, Limapuluh Kota Regency, West Sumatra-Indonesia, Laboratory of Soil Science Andalas University, and Laboratory of Soil Chemistry and Laboratory of Soil Mineralogy of Soil Research Center, Bogor. This research used a surveiy method with several stages: Preparation, Pre-Survey, Main Survey, Soil Analysis, and Data Processing. Based on the results on the research of soil classification in Nagari Sungai Kamuyang, found differences in the results in classification of Soil in Nagari Sungai Kamuyang served Land Unit Map Sheet Solok by Soil and Agroclimate Research Center in 1990, which is only on the profile SL3 showing characteristics of Andisols while on the other profiles show Inceptisols chararacteristics. The results of soil classification based on the USDA Soil Taxonomy System, on SL1, SL2, SL4, SL5, SL6, and SL7 profiles were classified into Ordo: Inceptisols, Sub Ordo: Udepts, Greatgroup: Dystrudepts, Sub Group: Andic Dystrudepts, Family: Fine silt, Kaolinit, Isohypertemic, Andic Dystrudepts. SL3 profiles was classified into Ordo: Andisols, Sub Ordo: Udands, Great Group: Hapludands, Family: Medial, Amorphic, Isohypertermic, Typic Hapludands.

Keywords: *Soil classification, USDA soil taxonomy system, Sungai Kamuyang.*.

INTRODUCTION

Nagari Sungai Kamuyang, West Sumatra is located in the north of Mount Sago so that this area was classified as stratovolkan physiography. The area is located at an altitude of 506 – 2.225 meters above sea lecel. The geology in this region based on the Solok Sheet Geological Map (0815) has two types of parent material. Qamg parent material is the product of Mount Malintang Andesite rocks, Qpt2 is the result of Tuff pumice and andesite basalt. This parent material will give a big influence on the soil properties.

Based on the Land Unit Map Solok Sheet (1085) scale of 1 : 250.000 by Soil and Agroclimate Research Center in 1990, the soil type in Nagari Sungai Kamuyang was classified into greatfroup Hydrandepts and Dystrandepts. Based on the Soil Classification System in 1975, Dystrandepts and Hydrandepts in the 7th Approxination Classifisication Systems are a greatgroup of Andept as a subordo of Inceptisols. By Smith, this Andept Subordo was used in the Classification of Soil

Taxonomy A Basic System of Soil Classification for Making and Interpreting Soil Surveys in 1978 and officially used in the Soil Taxonomy in 1990 and now as a new Ordo, namely Andisols.
Andisols are soils formed from volcanic ash, pumice, and/or other volcanic eruptions, which have ≥60% of Andic soil charactersitics to a depth of 60 cm. This soil has an exchange complex dominated by amorphous compound of Al, Si, and/or Fe, and humus. This soil was composed of sand fraction's minerals in the form of quartz, plagioclase, hornblende augite, hypersteine, olivine, and volcanic glass. The clay minerals are dominated by non-crystalline clay minerals consistine of allophane, imogolite, and/or ferritic acid in addition to halloysite, gibsite, and cristobalite, has bulk density ≤ 0,90 g/cm^3, phosphate retention ≥85%. Epipedon identified of this soil, among others melanic, umbric, or ochric and have cambic horizon on the bottom layer (Smith, 1978; Parfitt and Childs, 1988).
However, based on Sari (2018), there is no specific characteristics of Andisols on the soil layer to a depth of 60 cm. In addition, from the results of soil properties analysis, which found the available phosphorus content was very high with a value of 39 ppm. This is inversely proportional to the statement of Tan (1984) that Andisols is a soil that has a low level of available phosphorus due to high phosphate sorption up to 90% by Al and allophane clay minerals.
The incompatibility of soil types information on the Nagari Sungai Kamuyang based on Solok Sheet Land Unit Map issued by the Soil and Agroclimatology Research Center in 1990 with the results of research conducted by Sari (2018) allegedly caused by maps issued as source data was small scale maps 1 : 250.000. According to Burrough (1986), map scale is a measure of the detail of data in conventional mapping system. Soil data presented in the form of maps will be related to scale. In addition, maps with a scale of 1 : 250.000 are known as derivative maps, which are maps that were not made directly from stereoplatting or surveys but were made using maps that already existed as a source on a larger scale. This affects the appearance of points, lines, and areas that result in changes in the amount of length and area (Hisanah *et al*., 2015). Therefore, to get more accurate and detailed information, a soil classification study in Nagari Sungai Kamuyang is needed. Based on the above, a study was conducted on "Soil Classification in Nagari Sungai Kamuyang, West Sumatra, Indonesia.

MATERIAL AND METHODS

The research was conducted in Nagari Sungai Kamuyang, Luak District, Limapuluh Kota Regency, West Sumatra, Indonesia. The soil analysis conducted in Soil Science Laboratory of Agriculture Faculty of Andalas University and Soil Chemistry Laboratory of Soil Research Center in Bogor. The main tools used in this study are maps, climate data research area (rainfall, temperature, and soil temperature), GPS, Abney level, drill of soil mineral, ring samples, Munsell Soil Colour Charts, knive, soil profile description cards, stationery, and laboratory equipment. The materials used are labels, rubber bands, plastic bags, and chemicals needed for soil analysis in the laboratory.
The method used in this study is a semi-detailed survey method with a map scale of 1 : 50.000. Sampling was done by Purpossive Random Sampling based on land units with an area of more than 25 hectares. The stages of this research consisted of: preparation, pre-survey, main survey, laboratory analysis, and data processing. Data obtained in the field and in the laboratory are used as a basis for determining soil classification according to the USDA Soil Taxonomy System by Soil Survei Staff (2014) from Ordo to Family level. Observation of soil characteristics in the field is done through observing the soil profile. Soil profile is done on each land units with a size of 1,5

m (length) x 1.5 m (width) x 1.5 m (height). Soil sampling is done in two ways, namely satellite soil sampling and undisturbed soil sampling. Satellite soil samples are taken from each horizon A and B in the soil profile. Undisturbed soil samples is carried out using the ring samples on horizon A and B. The physichal characteristics of land observed in this study are slope, erosion, drainage, and surface rocks. Soil properties analyzed in this study were soil texture, base saturation, pH (H_2O, KCl, and NaF), C-Organic and organic matter, base cations (Ca, Mg, K, and Na), melanic index, and Al, Fe, and Si Oxalate extract.

RESULTS AND DISCUSSIONS

Climate research area

Based on the Schmidth Fergusson Climate Classification Systems, during the 10 year observation period, Nagari Sungai Kamuyang was classified as type B climate (wet; 14,3% < Q < 33,3%) with a Q value of 24,8%. The pattern of rainfall from the average rainfall for 10 years (observation period 2008 – 2017) shows that in Nagari Sungai Kamuyang have two peaks of rainfall in April with an average rainfall of 242,6 mm/month and November with an average rainfall of 350,7 mm/month. There were no dry month because the lowest rainfall was 91,6 mm. The humid month occur in June and July with rainfall ranging between 91,6 to 92,9 mm/month. The wet month occur in January, February, March, April, May, August, September, October, November, and December with rainfall ranging from 131,2 to 350,7 mm.month.

Soil moisture regime can be determined by looking at the pattern of the rainfall in the research area. Based on existing rainfall data, note that the rainfall in research area is distributed troughout the year so that the soil does not dry for 90 days cumulative throughout the year. Based on this, the soil moisture regime at the research area is Udic.

Temperatures in Nagari Sungai Kamuyang are in the range of values from 12,95 ^oC to 23,26 ^oC. The soil temperature at the research area was obtained from the formula of Van Wambeke (1982) by adding an air temperature of 2,5 ^oC. It is based on reserach Van Wambeke (1981) which is the average in the United States by an additional 1 ^oC to 2 ^oC temperature of the soil and elsewhere. Whereas in the tropics there was an increase in the soil temperature of 2,5 ^oC from the air temperature, so that the soil temperature obtained at the research area was 22,6 – 25,57 ^oC. From the soil temperature value, the soil temperature regime at the research area is isohypertermic with a soil temperature > 22 ^oC with an average temperature difference in the summer and winter less than 6 ^oC. The difference in soil temperature less than 6 ^oC is supported by the statement of Hadi (2010) that changes in soil surface temperature in Indonesia in the period 1979 – 2009 in the rainy and dry season ranged from 0,5 to 1,5 ^oC. In addition, Fajrin and Driptufany (2017) stated that changes in the soil surface temperature in West Sumatra during the period 2007 – 2013 were recorded at a maximum of 4 ^oC.

Soil Morphology Characteristics

Of the seven soil profiles observed, each has a different morphology. In 5th profile, it was found horizons that were already dominated by rocks called C or B/C horizons at a depth of 30 cm. However, on 1st, 2nd, 3rd, 4th, 6th, and 7th profiles, horizon B was found in deeper solum with an average depth more than 100 cm.

The soil color on each profile tends to be darker (brown to black) with Hue 7,5 YR and 10 YR on 1st, 2nd, 3rd, 4th, and 7th profiles with varying values and chroma of 2/2 to 5/6, while the 5th and 6th soil profiles has a color with a 7,5 Y Hue with a value of values and chroma 2/2 to 6/1.

Picture 1. 1st Soil Profiles

Picture 2. 2nd Soil Profiles

Picture 3. 3rd Soil Profiles

Picture 4. 4th Soil Profiles

Picture 5. 5th Soil Profiles

Picture 6. 6th Soil Profiles

Picture 7. 7th Soil Profiles

Soil Physical Characteristics

Table 1. Soil Bulk density and Texture in Nagari Sungai Kamuyang

Profile	Horizon	Depth (cm)	Texture				Bulk density (g/cm^3)
			% Sand	% Silt	% Clay	Class	
1	A	0 – 14	21,95	59,33	18,72	Silty loam	0,82
	B1	14 – 70	8,75	12,15	79,1	Clay	0,77
	B2	70 – 130	20,21	16,48	63,31	Clay	
2	A0	0 – 10	20,81	16,46	62,73	Clay	0,88
	A1	10 – 30	17,35	50,2	32,45	Clay	
	A2	30 – 62	41,12	8.81	50,07	Clay	
	B1	62 – 89	26,08	17,71	56,21	Clay	0,82
	B2	89 – 100	43,27	10,61	46,12	Clay	
3	A0	0 – 2	17,07	3,18	79,75	Clay	0,69
	A	2 – 18	20,18	6,64	73,18	Clay	
	B1	18 – 48	13,62	12,31	74,07	Clay	0,59
	B2	48 – 79	17,78	10,25	71,97	Clay	
	B3	79 – 100	15,55	10,88	73,57	Clay	
4	A	0 – 39	20,42	30,24	49,34	Clay	0,72
	B	39 – 110	23,86	35,07	41,07	Clay	0,88
5	A0	0 – 9,5	28,91	7,88	63,21	Clay	0,83
	A1	9,5 – 30	9,86	46,65	43,49	Silty clay	
6	A	0 – 10	35,37	15,35	49,28	Clay	0,87
	A1	10 – 26	37,09	17,11	45,8	Clay	
	B1	26 – 40	40,73	2,57	56,7	Clay	0,74
	B2	40 – 67	18,8	19,44	61,76	Clay	
	B3	67 – 100	75,03	3,1	21,87	Sandy clay loam	
7	A	0 – 30	9,84	9	81,6	Clay	0,84
	B1	30 – 50	9,86	18	72,14	Clay	0,79
	B2	50 – 100	9,88	31,46	58,66	Clay	

The color of the soil will affect other soil properties through radiation from sunlight energy. The heat energy contained in the darker soil will result in higher evaporation rates so that the darker soil will dry out faster than lighter colored soils (Hillel, 1982). The color of the soil can indicate organic matter in the soil because organic matter gives a dark or blackish color. According to Hakim (1986), the higher content of soil organic matter the darker color of the soil. In addition, the color of the soil can be used to determine the level of weathering. The reddish soil color indicates the level of weathering is getting further.

Soil structure was also observed in observing soil profiles. The soil structure in each soil is dominated by granular structure and angular with a moderate level of development. Soil structure affects the movement of water in the soil. Crumb structure will have more porous space than angular structure so that water will enter the soil more quickly (Foth, 1999).

Bulk Density

Based on the results, that the average of the soil bulk density is < 1 g/cm^3 is equal to 0,59 to 0,88 g/cm^3. The low bulk density can be caused due to an amorphous mineral content in the soil so that number of micropores is quite a lot, especially intra and inter particle pore space. Higher bulk density will affect the pore spaces of the soil, the lower bulk density will be higher pore space (Juarti, 2016). Low soil bulk density due to allophane contributes to the development of pores in the soil structure. The pore arrangement of allophane structure unit causes allophane porosity to be high (Wada, 1985). Shoji *et al* (1993) stated that allophane has a perforated structure and with a diameter of 3 – 4 mm. The heavy of soil bulk density will be lower along with the increase in allophane content because of 25 – 45% of the pore volume of Andisol is occupied by allophane.

Soil Texture

All soil profiles have a very high clay fraction with a range of 18,72 – 81,6 %, with the highest clay fractions found in the 7th profile and the lowest clay fraction found in the 1st profile. The high clay fraction in each profile shows that the soil has undergone further weathering. Most of the soil has more clay content in horizon B compared to horizon A. The older the soil, the difference in clay content between horizon A and B will be even greater. The content of each fraction (sand, silt, and clay) in each soil horizon is influenced by three things, the movement of clay from horizon A to horizon B duet o percolation water, chemical weathering from clay in horizon A and leaching duet o drainage water, and weathering silt and sand so as to form clay on the horizon B (Thompson, 1957).

Soil Chemical Characteristics

Soil Reaction (pH)

Based on the results, pH values ranged from 4,56 to 6,75. The diversity of soil pH values is caused by weathering that occurs. In the weathering process, when base cations (Ca, Mg, K, and Na) are detached from spcace between the micelles then the pH will increase, whereas if the loose cations of Al, Fe, and Si then the pH will tend to decrease (Fiantis, 2006). In soils with moderate acidity, aluminium and hydrogen compunds cause hydrogen ions to dissolve in the soil. Al-hydroxide ions adsorbed and transformed into exchangable cations. In the soil solution, Al-hydroxide will produce H ions due to Al hydrolysis reaction (Buckman and Brady, 1982). The pH delta values obtained to negative value, this indicates that many colloidal soils have negative charges so that it has ability to bind positively charged base cations (Fiantis, 2006). The pH values will determine the amount of positive and negative charges on the surface of the colloid. If the delta pH value of ± 0,5 indicates that the soil is variable, that is the charge changes according to the increase and decrease of soil pH (Arifin, 1994). Increase and decrease of soil pH is influenced by the input given to the soil, either in the form of inorganic and organic fertilizers or various types of ameliorant material such as phosphate rock or volcanic ash. The value of NaF pH obtained in each soil ranged from 8,7 to 11,49. The high pH of NaF in this soil indicates that the soil is contains high amorphous material which dominates the exchange complex. This is based on the ligand exchange between F- and OH- which is on the edge of allophane so that OH- is free and will quickly replace the pH of solution (Simamore *et al*., 2015).

Table 2. Soil Reaction in Nagari Sungai Kamuyang

Profile	Horizon	Depth (cm)	pH		Delta pH	pH NaF	
			H_2O	KCl		1 minute	60 minute
1	A	0 – 14	4,65m	4,16	-0,49	9,61	10,29
	B1	14 – 70	4,84m	3,92	-0,92	9,9	10,58
	B2	70 – 130	4,79m	4	-0,79	10,09	10,76
2	A0	0 – 10	5,67am	5,02	-0,65	10	11,2
	A1	10 – 30	5,23m	4,48	-0,75	10,43	10,76
	A2	30 – 62	5,09m	4,32	-0,77	10,08	10,81
	B1	62 – 89	5,19m	4,4	-0,79	10,37	11,08
	B2	89 – 100	5,16m	4,4	-0,76	10,38	11,1
3	A0	0 – 2	4,56m	4,18	-0,38	10,17	10,85
	A1	2 – 18	4,62m	4,16	-0,46	10,73	11,34
	B1	18 – 48	4,98m	4,53	-0,45	11,35	11,71
	B2	48 – 79	5,07m	5,09	-0,02	11,49	11,76
	B3	79 – 100	4,98m	4,2	-0,78	10,79	11,38
4	A	0 – 39	6,75n	6,02	-0,73	9,7	10,14
	B	39 – 110	6,37am	5,07	-1,3	10,68	11,21
5	A0	0 – 9,5	6,09am	4,87	-1,22	8,7	8,97
	A1	9,5 – 30	6,49am	5,52	-0,97	8,91	9,25
6	A	0 – 10	5,33m	4,36	-0,97	8,69	9,05
	A1	10 – 26	6,01am	4,96	-1,05	9,27	9,73
	B1	26 – 40	5,75am	4,85	-0,9	9,46	9,96
	B2	40 – 67	6,05am	5,15	-0,9	8,81	9,18
	B3	67 – 100	6,02am	5,05	-0,97	8,99	9,33
7	A	0 – 30	4,75m	4,17	-0,58	9,57	10,06
	B1	30 – 50	4,96m	4,16	-0,8	10,27	10,81
	B2	50 – 100	5,15m	4,17	-0,98	10,22	10,9

Soil Organic Matter

C-organic content in each soil ranges from 2,72 to 3,15% with moderate to high criteria. The content of soil organic matter in the soil ranges from 4,67 to 5,41%. According to Tan (1998), the high content of organic matter in the soil may be due to the presence of amorphous clay. Organic matter will produce humus that will bind to the Al and Fe into the Al-humus and Fe-humus or would happen polycondensation with amorphous minerals. Amorphous mineral compounds stabilized organic matter and protect them against biodegradation of microorganisms and stimulate the accumulation of organic compounds in the soil. These compound will be stable and remain in the soil and will not have movement (Resman, 2010). In addition, high clay content in the soil also effects the content of organic matter in the soil. Clay fraction has an influence on soil organic matter because the largest specific surface area is 800 m^2/g. The large surface area has a high activity in water adsoprtion, so that the soil which is dominated by clay fraction will have a high water holding capacity with low pore aerase. The low pore aerase causes a semi-anaerobic state so that the air exchange is not smooth. This affects the decomposition of organic matter that will

undergo humification process to produce organic compounds that are resistant to weathering (Stevenson, 1982; Darmawijaya, 1992).

Table 3. C-organic content and Soil Organic Matter in Nagari Sungai Kamuyang

Profile	Horizon	Depth (cm)	% C-organik	% Organic Matter
1	A	0 – 14	3,23	5,55
	B1	14 – 70	2,87	4,93
	B2	70 – 130	2,83	4,86
2	A0	0 – 10	3,1	5,33
	A1	10 – 30	3,05	5,24
	A2	30 – 62	3,05	5,24
	B1	62 – 89	2,93	5,03
	B2	89 – 100	3	5,16
3	A0	0 – 2	3,03	5,21
	A1	2 – 18	3,03	5,21
	B1	18 – 48	3,12	5,36
	B2	48 – 79	3,1	5,33
	B3	79 – 100	2,9	4,98
4	A	0 – 39	3,25	5,59
	B	39 – 110	2,9	4,98
5	A0	0 – 9,5	3,05	5,24
	A1	9,5 – 30	2,85	4,990
6	A	0 – 10	2,87	4,93
	A1	10 – 26	2,82	4,85
	B1	26 – 40	2,72	4,67
	B2	40 – 67	3	5,16
	B3	67 – 100	2,82	4,85
7	A	0 – 30	3,15	5,41
	B1	30 – 50	3,12	5,36
	B2	50 – 100	3,05	5,24

Based on the results, the value of base cations in the soil varies from very low to high, base saturation is very low to high with a value of 16,8 – 70,98%. Ca^{2+} content in the soil ranges from 1,96 – 4,51 me/100g of soil (very low to high), Mg^{2+} content in the soil ranges from 1,25 – 1,95 me/100 g of soil (moderate), K^{+} content ranges from 0,59 – 0,92 me/100 g of soil (high), and Na^{+} content ranges from 0,46 – 0,9 me/100 g of soil (high). The low Ca and Mg values in the soil caused by leaching due to high rainfall. Ca and Mg cations are the most occupying the surface of colloids, so that when rainfall was high, leaching will occur and base cations will be lost (Yulius *et al*., 1985). Bases that can be exchanged have a relationship with base saturation, cation exchange capacity, and pH. If the soil pH is high, the base saturation will tend to be high because many base cations are absorbed in the soil colloid, conversely if the pH is low, the base saturation will tend to be low because the cation ansorbed in the ground colloid is dominated by Al and Fei ons (Nyakpa *et al*., 1988).

Base Cations and Base Saturation

The phosphate retention in each soil show values ranging from 35,5 to 97,5%. The high phosphate retention in soil is due to the amorphous iron and aluminium content derived from aluminium hydroxides and allophane. Al-OH and Al-OH_2 functional groups contained in amorphous mineral are very important in absorbing phosphate ions. Aluminol group is a hydroxyl anion which has a single bond to the aluminium metal. This single bond causes OH to be easily exchanged by phosphate ions through the ligand exchange mechanism. The more aluminol groups in amorphous materials such as allophane, the more phosphate is absorbed in the soil (Bohn *et al*., 1979). According to Tan (1984), a high phosphate retention resulted in phosphate becomes insoluble and unavailable to plants. Phosphate retention in one of the requirements of andic soil properties if phosphate retention more than 85%. Fiantis (2002) states that high phosphate retention in soils is directly proportional to allophane present in volcanic soil and the P-bond by allophane cannot be released by the help of organic matter.

Table 4. Base Cations and Base Saturation in Nagari Sungai Kamuyang

Profile	Horizon	Depth (cm)	Base Cations (me/100 g)				Base Saturation (%)
			Ca	Mg	K	Na	
1	A	0 – 14	1,99	1,95	0,68	0,53	21,69
	B1	14 – 70	3,32	1,65	0,64	0,71	35,68
	B2	70 – 130	1,96	1,88	0,7	0,5	23,35
2	A0	0 – 10	2,38	1,68	0,77	0,58	28,14
	A1	10 – 30	2	1,55	0,68	0,62	23,11
	A2	30 – 62	2,46	1,45	0,72	0,67	28,02
	B1	62 – 89	2,47	1,58	0,59	0,67	26,85
	B2	89 – 100	2,87	1,25	0,71	0,69	26,90
3	A0	0 – 2	2,66	1,44	0,86	0,84	29,95
	A1	2 – 18	2,32	1,83	0,68	0,74	26,31
	B1	18 – 48	2,99	1,79	0,66	0,82	34,07
	B2	48 – 79	2,27	1,58	0,73	0,84	33,89
	B3	79 – 100	2,51	1,48	0,61	0,85	18,42
4	A	0 – 39	3,36	1,81	0,6	0,9	22,70
	B	39 – 110	2,01	1,85	0,68	0,76	20,76
5	A0	0 – 9,5	2,36	1,85	0,79	0,7	22,68
	A1	9,5 – 30	4,51	1,9	0,92	0,84	70,98
6	A	0 – 10	3,08	1,74	0,86	0,89	20,69
	A1	10 – 26	3,9	1,62	0,63	0,65	38,09
	B1	26 – 40	3,14	1,74	0,67	0,58	28,93
	B2	40 – 67	3,24	1,62	0,84	0,59	16,80
	B3	67 – 100	3,15	1,67	0,83	0,46	23,71
7	A	0 – 30	3,62	1,8	0,82	0,77	20,91
	B1	30 – 50	3,9	1,89	0,73	0,71	27,28
	B2	50 – 100	4,44	1,94	0,76	0,85	27,06

Phosphate Retention

Table 5. Phosphate retention and Al, Fe, Si Oxalate Extract Content in Nagari Sungai Kamuyang

Profile	Horizon	Depth (cm)	Oxalate Extract (%)			Al_o+ ½ Fe_o	Phosphate retention (%)
			Al	Fe	Si		
1	A	0 – 14	0,73	1,02	0,14	1,24	61,14
	B1	14 – 70	0,58	0,88	0,24	1,02	66,1
	B2	70 – 130	0,5	0,41	0,14	0,705	57,8
2	A0	0 – 10	1,03	1,76	0,21	1,91	78,5
	A1	10 – 30	1,15	1,91	0,28	2,105	82,1
	A2	30 – 62	0,79	1,42	0,29	1,5	77,9
	B1	62 – 89	0,73	1,98	0,26	1,72	77,7
	B2	89 – 100	0,73	2,02	0,24	1,74	84,6
3	A0	0 – 2	1,38	2,67	0,26	2,715	87,6
	A1	2 – 18	1,48	2,96	0,3	2,96	82,1
	B1	18 – 48	1,91	3,54	0,38	3,68	94,3
	B2	48 – 79	2,38	3,89	0,52	4,325	97,5
	B3	79 – 100	0,9	1,79	0,29	1,795	83,1
4	A	0 – 39	0,89	2,61	0,32	2,195	58,9
	B	39 – 110	1,1	2,18	0,34	2,19	77
5	A0	0 – 9,5	0,26	2,59	0,27	1,555	35,5
	A1	9,5 – 30	0,26	3,26	0,26	1,89	35,5
6	A	0 – 10	0,34	3,98	0,26	2,33	53,8
	A1	10 – 26	0,4	3,49	0,22	2,145	51,3
	B1	26 – 40	0,45	4,02	0,25	2,46	52,6
	B2	40 – 67	0,29	5,8	0,32	3,19	58,3
	B3	67 – 100	0,46	9,1	0,26	5,01	60,6
7	A	0 – 30	0,63	1,45	0,21	1,355	63,3
	B1	30 – 50	0,65	0,86	0,24	1,08	69
	B2	50 – 100	0,59	0,75	0,3	0,965	68

Al, Fe, and Si Oxalate Extract Content

The metal content of Fe oxalate extract (0,41 – 9,2%) was higher compared to Al (0,26 – 2,38%) and Si (0,14 – 0,52%) for all soil profiles. The content of Si is lower than Al and Fe because Si ions are more mobile and easier to leaching than Al and Fe so Si leached in the top soil will be lossed and accumulated in the sub soil, whereas Al and Fe more easily make humus complex (Ajidirman, 2010). This is supported by the agreement of Van Ranst (1993) that materials which are rich in weathered minerals such as volcanics substnaces which produce top layers containing high Al and Fe. This situation increases the composition of complexes with relativiely high metals to that only slighlty soluble in air. Soils that have Andic soil properties must meet the requirements set forth in Soil Taxonomy. One of the requirements that must be met is the value of Al_o + ½ Fe_o contents ≥ 2%. The value of Al_o + ½ Fe_o eligible andic soil is on 3rd, 4th, and 6th soil profiles with values ranging from 2,145 to 5,01%. The high content of Al, Fe, and Si Oxalate extract in these three soils indicates the presence of amorphous material. Amorphous clay minerals such as allophane, imogolith, Al/Fe-Oxide, Al/Fe-hydroxides, and ferrihydrite has a main constituent mineral soil colloids (Wada, 1985).

Table 6. Soil melanic index in Nagari Sungai Kamuyang

Profile	Horizon	Depth (cm)	Melanic index
1	A	0 – 14	1,76
	B1	14 – 70	1,5
	B2	70 – 130	0,75
2	A0	0 – 10	1,92
	A1	10 – 30	1,65
	A2	30 – 62	1,89
	B1	62 – 89	1
	B2	89 – 100	2
3	A0	0 – 2	1,88
	A1	2 – 18	1,86
	B1	18 – 48	1,87
	B2	48 – 79	1,77
	B3	79 – 100	1,6
4	A	0 – 39	1,76
	B	39 – 110	1
5	A0	0 – 9,5	2
	A1	9,5 – 30	1,33
6	A	0 – 10	2
	A1	10 – 26	1,56
	B1	26 – 40	1,76
	B2	40 – 67	1,39
	B3	67 – 100	1,15
7	A	0 – 30	1,68
	B1	30 – 50	1,5
	B2	50 – 100	1,5

Melanic Index

The deeper the depth of the soil, the value of the melanic index also decreases. This is duet o the low fulvic and humic substances as indicators of the melanic index (Hulu, 2019). Melanic index is used for the melanic epipedon characteristics requirements which the melanic indeks value must be ≤ 1,7 in cumulative soil thickness of 30 cm. In this research, there was no soil profiles that qualified as a melanic epipedon. This index is associated with the rasio of humic and fulvic acids in the organic fraction of the soil (Honna *et al*., 1988). The melanic index is used to differentiate accumulate organic matter which is though to be produced from large amounts of graminae vegetation from humidified organic matter formed from forest vegetation. Melanic index is calculated as the absorbance of the extract solution at a wavelength of 450 nm divided by the absorbance at a wavelength of 520 nm (Soil Survey Staff, 2014).

Soil Classification

In this research, soil parent material derived from pumice tuff formation and rocks andesite Mount Malintang and produce volcanic soil with Andic soil properties. Based on Soil Survey Staff (2014), Andic soil is a soil that has a bulk density ≤ 0,90 g/cm^3, phosphate retention ≥ 85%, and $Al_o + ½ Fe_o$ content ≥ 2%. From the results, it is obtained in 3rd soil profiles meets the characeristics of the Andic soil. In classifying the soil, it is necessary to determine the identifier epipedon and horizon based on data obtained from observing and analyzing soil properties in the laboratory. Based on the data, all soil profiles meet the requirements of umbic epipedon which the soil has a thickness of more than 18 cm, the soil structure is sufficiently developed and soft if it dry has colors with values and chroma ≤ 3 (wet) and ≥ 5 (dry), base saturation (NH4OAc) ≤ 50%, and average C-organic content ≥ 0,6% and moist for 90 cumulative days throughout the year.

After identifying the epipedon, it is followed by identification identifier diagnostic horizon, which both soils meet the requirements as a cambic horizon. Soil has a subsurface horizon thickness of ≥ 15 cm, with a fine soil texture and not hard soil, soil color does not change when opened in the air, and genetic soil development without extreme clay accumulation.

After determining the identifier epipedon and horizon on the soil profiles, it is followed by determining soil ordo based on the Soil Taxonomy by Soil Survey Staff (2014). The 1st, 2nd, 4th, 5th, 6th, and 7th soil profiles when viewed from morphology, identifier epipedon as well as subsurface horizons belong to the Ordo Inceptisols. According to Hardjowigeno (2015), Inceptisols are soils with a cambic horizon with the upper limit at a depth 10 cm from the surface and its lower limit at a depth of more than 25 cm. The 3rd soil profile meet the requirements of the andic soils so that belong to Ordo Andisols. Andisols are soils that have 60% of the thickness of the andic soil properties with 36 cm thick or more at depths of less than 60 cm (Soil Survey Staff, 2014).

Soil classification at the Sub Ordo level was determined based on the soil moisture regime, which in the research site has a Udic regime so that the Sub Ordo at 1st, 2nd, 4th, 5th, 6th, and 7th soil profiles are Udepts and the 3rd soil profiles is Udands. The Greatgroups category in 1st, 2nd, 4th, 5th, 6th, and 7th soil profiles is Dystrudepts because the soil has a base saturation (NH4OAc) less than 60% and 3rd soil profile is Hapludands because the soil properties does not qualify for the other greatgroups. At the Sub Group level, the 1st, 2nd, 4th, 5th, 6th, and 7th soil profiles were classified into Andic Dystrudepts because all horizons has a thickness more than 16 cm with fine soil fractions with bulk density less than 1 g/cm3 and have a total Al_o and ½ Fe_o percentage more than 1%. The 3rd soil profile was classified as Typic Hapludands because the soil properties does not meet requirements of other subgroups.

At the Family level, soil classification based on three types were grain structure, mineralogy class, and soil temperature regime. In the 1st, 2nd, 4th, 5th, 6th, and 7th soil profiles have fine silt grain size (having less than 15% weight of particles with 0,1 – 7,5 mm in diameter, fine soil fractions having clay content of 18 – 35%). In the 3rd soil profile, it has a medial grain size because it only has fractonal of stones less than 35%. The mineral classes in 3rd soil profile belong to the Amorphic class with values of $8 \times Si_o + 2 \times Fe_o > 5\%$ and $8 \times Si_o > 2 \times Fe_o$. The parent material derived from volcanic material and high rainfall causes the formatio of amorphic clay minerals at a further development will be followed by the kaolinite mineral. This can indicate that the mineral class in the 1st, 2nd, 4th, 5th, 6th, and 7th soil profile is kaolinite. The soil temperature regime class in all soil profiles were classified as isohypertermic.

Based on the results of the soil classification, it found differences in results of soil classification in Nagari Sungai Kamuyang presented by Solok Sheet Land Unit Map by the Soil

and Agroclimate Research Center in 1990, of which only the 3rd soil profile shows the characteristics of the Andisol while the other profiles show the Inceptisol characteristics. This difference was due to the map issued as a data source was a small scale map of 1 : 250.000 while the map used in research sampling was a large scale map of 1 : 50.000. The difference in the map scale can causes differences in the detail of information obtained. According to Burrough (1986), map scale is a measure of the detail of data in conventional system mapping. The soil data presented in map form will be related to the scale. Large scale maps wills show detailed typology and information of soil series, while medium to small scale maps will presents associations of several soil series and family. In addition, maps with a scale of 1 : 250.000 are known as derivative maps, which are maps that were not made directly from stereoplatting or surveys but were made using maps that already existed as a source on a larger scale. This affects the appearance of points, lines, and areas that results changes in the amount of length and area (Hisanah *et al*., 2015). This is also supported by Hariyono's research (2018) found in the analysis of maps with two different scale of 1 : 50.000 and 1 : 250.000, there has been a change in the geometrical shape of objects on the scale change from medium to small scale. There are classes that changes in a sizem decrease in size, or are eliminated.

CONCLUSIONS

Based on the results of research on Soil Classification in Nagari Sungai Kamuyang, Luak District, Limapuluh Kota Regency, West Sumatra, it was found that differences in the results of soil calssificaton were presesented by the Solok Sheet Land Unit Map by Soil and Agroclimate Research Center in 1990, which is only on the 3rd profile shows the Andisol characteristics while in other soil profiles it shows the characteristics of Inceptisols. The results of soil classification based on the Soil Taxonomy System on the 1st, 2nd, 4th, 5th, 6th, and 7th soil profiles in Jorong Madang Kadok, Jorong Batang Tabik, Jorong Rageh, Jorong Tabing, and Jorong Subaladung were classified as Ordo: Inceptisols, Sub Ordo: Udepts, Greatgroups: Dystrudepts, Subgroups: Andic Dystrudepts, Family: Fine silt, Kaolinite, Isohypertermic, Andic Dystrudepts. In the 3rd soil profile which is in Jorong Madang Kadok area, was classified as Ordo: Andisols, Subordo: Udands, Greatgroups: Hapludands, Subgroup: Typic Hapludands, Family: Medial, Amorphic, Isohypertermic, Typic Haplundands.

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EFFECTS OF ULTRASONICATION ON ANTHOCYNANIN CONTENTS OF FRUIT JUICES

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ABSTRACT

Fruit juices are processed against microbial contamination, most commonly via thermal preservation techniques, of which reduce nutritional composition and organoleptic properties. Rising public interest in safer and more nutritional foods have increased the popularity of non-thermal methods, including ultrasonication. Ultrasound, when applied to liquid mediums like fruit juices, creates acoustic cavitation that generates bubble formation, which then will be collapsed causing thermal, mechanical and chemical impacts. This impacts of ultrasonication, make it useful in the fruit juice processing. Anthocyanins are colored pigments that are found in plants including fruits, especially berries, cherries and grapes. As a functional ingredient, anthocyanins show anti-diabetic, anti-inflammatory and antimicrobial effects, also helps preventing cancer and cardiovascular diseases. This review summarizes the effects of ultrasonication on anthocyanin contents of fruit juices.

Keywords: Ultrasound, Ultrasonication, Anthocyani

INTRODUCTION

Unprocessed fruit juices are vulnerable to microbial contamination which adversely effects sensory and nutritional properties [9], hence preservation methods are applied. Besides being the most common and the most effective preservation techniques, thermal methods reduce organoleptic properties and freshness of juices [9]. It has been reported that anthocyanins degrade during thermal processing, because of their instability [16]. Rising public interest in safer and more nutritious foods on the other hand, have increased the popularity of non-thermal preservation techniques [2]. It has been documented that nonthermal methods have much less effect on anthocyanin degradation when compared to the thermal techniques [16]. Moreover, considering the reduction in water consumption, wastewater production, energy requirement and chemical consumption [3]; non-thermal preservation methods including ultrasound, have gained acceptance as green and innovative technologies. Ultrasound also meets the US. Food and Drug Administration's (FDA) requirement of 5-log reduction in microorganisms in fruit juices [17].

Ultrasound is the term given to the sound waves which have higher frequencies than human hearing, ranges from 20 kHz to some gigahertz level and ultrasonication [4] is the name given to the process that utilizes ultrasound. Ultrasound is categorized into two [4] as low power and high power. While low power ultrasound has intensities below 1 W/cm2 and frequencies above 100 kHz, high power ultrasound has intensities higher than 1 W/cm2 and has frequencies in between 20 kHz and 500 kHz [4]. Although high power ultrasound is used to have insight on physicochemical properties of foods, low power ultrasound is used to affect these properties instead [4]. When ultrasound is applied to a liquid medium like fruit juices, acoustic cavitation occurs, creating bubbles [4] which will be collapsed ending up thermal, mechanical and chemical impacts in the environment. Such impacts of ultrasound are convenient to be used in the field of food preservation.

Anthocyanins are colored pigments, a member of flavonoid [11] class, found in plants, being the source of red, orange [11], purple and blue colors [10]. Cyanidin-3-glucoside (Cy-3-glu) is the most common anthocyanin type that found in fruits [14].

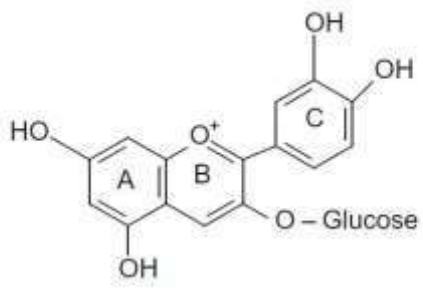

Figure-1: Molecular structure of cyanidin-3-glucoside.

As a functional ingredients [9] they show anti-diabetic, anti-inflammatory and antimicrobial effects, help preventing cancer and cardiovascular diseases, all mainly because of their anti-oxidative properties [10]. Most common anthocyanin containing fruits are berries, cherries, strawberries and grapes [11].

In this paper, the effects of ultrasonication on fruit juices in terms of their anthocyanin content was examined.

BACKGROUND

There are vast amount of studies in the literature on ultrasonication of fruit juices, such as on bayberry juice (Xiamin et. al., 2019) pomegranate juice (Alighourchi et. al., 2013), orange juice (Valtramidis et. al., 2010), strawberry juice (Tiwari et. al., 2008), blackberry juice (Tiwari et. al., 2009) and the like.

Besides generally being at a lesser degree than thermal methods [13], studies proved that anthocyanin content of fruit juices treated with ultrasound, decreases [6][8][9][13][14]. These factors affecting anthocyanin degradation are reported as; duration of ultrasonication time [13], temperature [13] and amplitude level [13]. Most studies was run at a stable temperature of 25 °C [9][16], whereas different temperatures such as 40°C [13] and 55°C [13] was also examined. Installations like heat exchangers using water, preferred to stabilize the temperatures of the samples [9][13][16]. Amplitudes tested in the studies varies between %20 [8] and %100, concentrated mostly between %40 and %100 [9][13][16][17]. Sonication durations studied in most works were between 0 and 10 minutes [9][13][16][17].

Dubrovic et. al. (2011) reported an inverse proportion between the anthocyanin content and duration of ultrasonication or temperature [13]. An example of the effects of amplitude and treatment time on total anthocyanin content are shown in Figure-2.

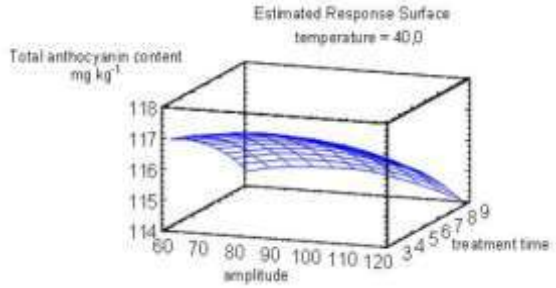

Figure-2: Response surface plots for total anthocyanin content in sonicated strawberry juice [15].

Xiamin et. al. reported no considerable differences in anthocyanin concentration at low amplitude values (90, 181, 271 W/cm^2) with treatment times under 8 minutes and also high amplitude values (362, 452 W/cm^2) with treatment times under 6 minutes [8]. Similarly, Tiwari et. al reported a high level of stability of anthocyanins against ultrasound treatment [16]. Nevertheless, while Xiamin et. al. observed increased durations cause anthocyanin degradation, up to %9.95 as shown in Figure-3 [8], Alighourchi et. al. recorded %8.41 degradation level as maximum in their research with pomegranate juice [9] and Tiwari et. al. reported a maximum decrease of %5 in blackberry juice [16].

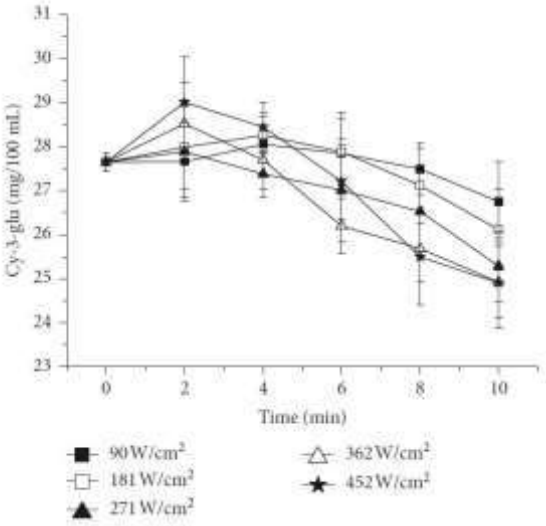

Figure-3: Effects of application time of ultrasound treatment on Cy-3-glu [8].

Anthocyanin decomposition that occurs during ultrasound treatment is caused mainly by 3 factors; (a)high temperature and pressure caused by cavitation, (b)forming of free OH^- radicals and (c)mechanical shear forces [8]. With a more precise suggestion, Yao et. al. (2016) reported that the main factor affecting anthocyanin degradation is cavitation, which makes water molecules create OH^- free radicals that deteriorate anthocyanins [14]. Kamal et. al. (2014) reported that free radicals produced by high intensity ultrasound may have undesired effects on fruit based foods [2] and similarly Jianxia et. al. (2016) observed formation of OH^- free radicals during high intensity ultrasonication of berry juices, causes degradation of anthocyanin, up to a ratio of %3.2 and lower their antioxidant activities up to %74.77 [6]. Yao et. al. reported a direct relationship between OH^- radicals and the degradation rate of anthocyanin [14]. Figure-4 shows the reduction in the clearance rate of OH^- free radicals by Cy-3-glu, depending on treatment time and ultrasonic power [14].

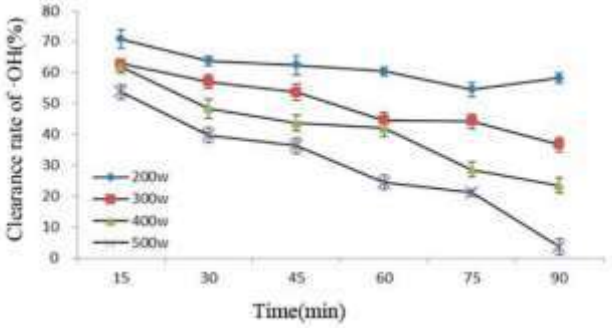

Figure-4: Correlation between clearance rate of OH and ultrasonic power and treatment time (n=3) [14].

Xiamin et. al [8], Alighourchi et. al. [9] and Tiwari et. al. separately recorded increases in anthocyanin contents, up to a level of %6.84, at especially low amplitudes and durations of ultrasonication process, and both researchers explained this happening with extraction of bound anthocyanins in fruit pulp [8]. However, Dubrovic et. al. [13] studied ultrasound on strawberry juice and did not report such an increase in anthocyanin level [13].

Tiwari et. at. (2009), Dubrovic et. al. (2011) and Espinosa et. al. (2019) used response surface methodology to design their experiments and optimize the conditions of ultrasonication. All researches noted that RSM is applicable and useful for the stated purpose.

CONCLUSION

However ultrasound causes degradation of anthocyanins in fruit juices, depending on treatment time, temperature and amplitude, recent studies showed that the degradation ratio varies between %5 and %10, a lesser degree than thermal techniques. Hence ultrasound is considered an effective preservation technique to be utilized in fruit juices.

Many studies make use of response surface methodology (RSM) to determine optimum conditions of the laboratory-scale tests to specify minimal anthocyanin degradation level. This method can also be applied in full-scale designing of ultrasonication processes to be used for fruit juice pasteurization.

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ULTRASOUND-ASSISTED EXTRACTION OF TANNINS FROM PLANT SOURCES

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ABSTRACT

Growing industrial demand for phytochemicals in regard to their rising commercial values, draws increasing attention to more effective extraction methods. Ultrasound-assisted extraction is a powerful alternative to conventional methods and offers several advantages such as effective applicability in varying matrices, higher extraction yields, reduced process times, lower water and energy demands and higher purity of extracts. Tannins are the second most abounding natural phenolic compounds that can be found in almost all plant sources and are considered to be a precious renewable aromatic resource as bio-based polymers. In this study, ultrasound-assisted extraction of tannins from plant sources and the parameters affecting extraction yield were examined.

Keywords: ultrasound, extraction, phytochemical, tannin

INTRODUCTION

Industrial notice and need for naturally occurring bio-compounds are increasing continuously [1]. Natural sources of phenols that can be extracted from plants are of specific interest, in regard to their arising commercial benefits in pharmaceuticals, food and cosmetics industries [1] [4].

Tannin is the second most abounding naturally occurring phenolic compound, after lignin [4], which is essentially can be found in all plants [3]. However, only a limited number of plant species have adequate amounts of tannin to be feasibly commercialized [3]. In spite of the fact that most tannin-rich plants like wattle, myrobalan and banana originate in tropical and subtropical climates, hot climate zones also have significant sources of tannins [3].

Tannin is a valued source that is used in tanning process while producing leather goods [4] and almost %90 of the plant-based tannin that is extracted today, is used in the leather industry [2]. But being a green chemical as a bio-based polymer, tannin is potent to be a renewable aromatic resource [4].

Tannins are water soluble phenolic compounds [2] and thus, they are conventionally extracted from tannin-containing plants via hot water extraction method [3]. Although water is a green solvent that is used in the extraction process successfully, it is not good enough in every case, in terms of maximizing the extraction yield [4]. Other conventional extraction techniques including maceration, infusion and soxhlet extraction needs longer process durations and utilize toxic solvents for extraction, creating a risk of intoxication of final extracts [1]. Moreover, high temperature operating conditions of these conventional methods may damage tannins [1] that are heat sensitive chemical compounds [2].

Since the accessibility of plant-based tannins are limited [2], there is an increasing demand to accomplish more efficient extraction of these valuable biomaterials from plants [3].

Sound waves with frequencies over human hearing are called ultrasound and frequency ranges that are in between 16-100 kHz, are named as power ultrasound or low frequency ultrasound [3]. Recent developments in ultrasound have created new paths to power ultrasound practices on a wide range of application areas including homogenization, emulsification, crystallization, filtration, as well as extraction [2].

Ultrasound-assisted extraction is an encouraging alternative to traditional methods, in regard to applicability in various matrices, better extraction yields, lesser process times, lower water and energy requirements and better extract purities [1][3]. Moreover, ultrasound-assisted extraction can be successfully utilized at lower process temperatures when compared to the conventional methods, which makes it a potent alternative for extraction of heat-sensitive bio-compounds [1].

In this paper, the application of low frequency ultrasound-assisted extraction of tannins from plant sources and the parameters affecting the extraction yields, were examined.

BACKGROUND

Several researchers studied different plant sources for ultrasound-assisted extraction of tannins. Myrobalan nut [2], acorn cupule [3], banana [4], mangosteen [5], walnut green husk [6], galla

chinensis [7] and oak [8] are some examples of which were subjected to the tannin extraction studies.
Efficiency of ultrasound-assisted extraction is dependent on some factors such as the reactor type, extraction time, ultrasound frequency, power density, solvent characteristics and process temperature [1].

Ultrasound baths and probe type devices are the two most common reactor designs. Figure 1. illustrates both the bath and probe type ultrasonic devices. Between the two, probe type reactors have various supremacies over the bath type designs, as they focus the energy on a local zone, hence provide higher efficiencies in terms of creating cavitation [3]. The ultrasound intensity that can be provided by probe type reactor can be up to 100 times higher than the ultrasonic bath design. In this wise, the extraction of the biomolecules of interest, from the solid samples of plants, can be accelerated drastically [3]. The disadvantage of probe type reactors is the risk of decomposition of heat sensitive biomolecules via the intense cavitation effect [1]. However, the bath type reactors are more widely seized [1].

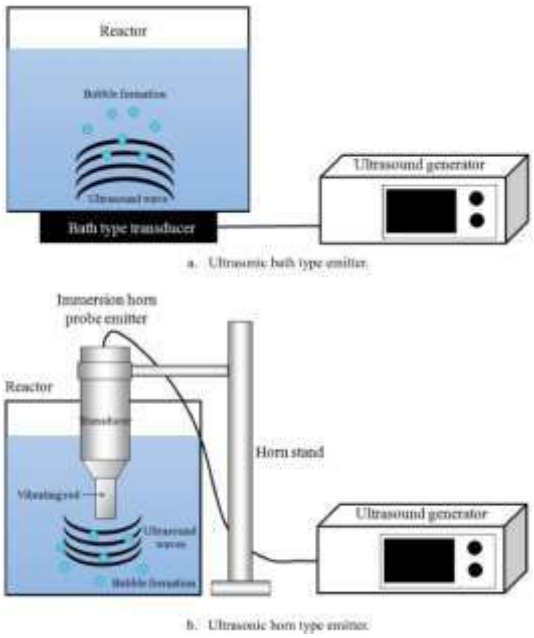

Figure 1. Ultrasonic reactor types [9]

Figure 2. shows the effects of ultrasonication time on extraction quantity at various power outputs in between 20W and 80W. It can be seen that increasing time leads to increasing amounts of tannin extracted, however this increase in extract quantity stabilizes at the fourth hours. Kilicarslan and Ozgunay (2012) similarly reported that extending process time from 6 hours to 8 hours, resulted an increase in overall extraction yield but they recorded no improvements neither the tannin ratio nor the amount of tannin in the extract. This was explained by the increased extraction times had led the passage of non-tannin molecules into the extract [3].

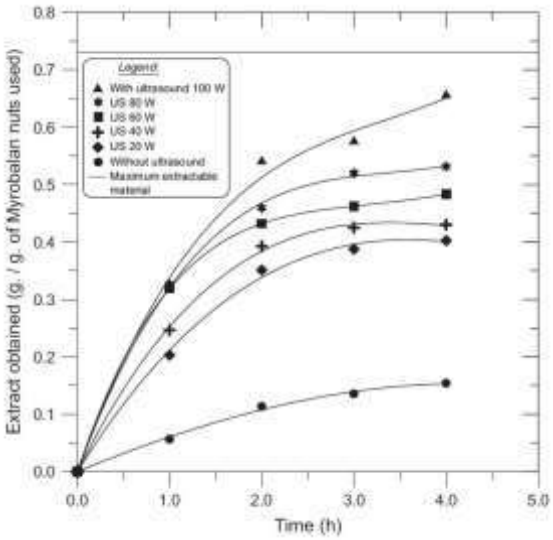

Figure 2. Effect of proses time on extraction quantity at different power outputs [2].

Studies indicated that the temperature has a major effect on extraction yield [3]. Cavitation effect of ultrasonication raises the temperature during the process, even up to the boiling point of the extraction liquor in some instances [3], which can be controlled externally via a cooling system such as a chiller. Panda and Manickam (2019) recorded a decrease in the extraction yield of phenolic compounds when the temperature was raised to 70°C from 60°C and explained this with less intense bubble collapses when the temperature increases. Unlikely, Figure 3. indicates the effect of temperature on extract quantity. From Figure 3. it can be clearly seen that increasing temperatures result in higher amount of tannin extraction. It is a vital parameter for the extraction process to determine the optimum operation temperature, according to the matrice. Literature shows that extraction temperatures may differ between 10 and 80 °C, also depending on ultrasound intensity and solvent type that is used in the extraction [1].

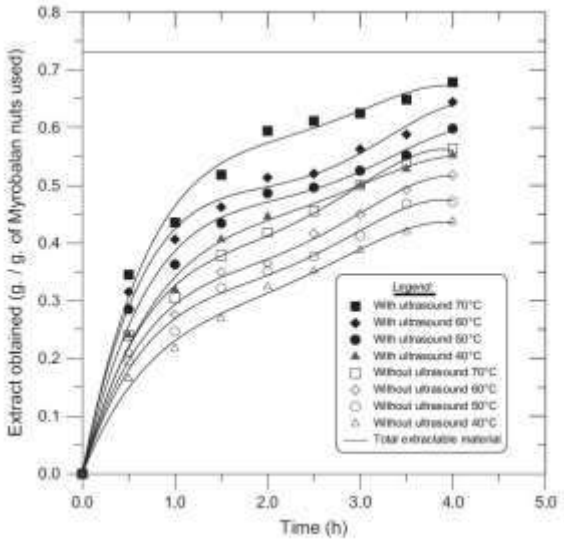

Figure 3. Effect of temperature varying in between 40°C and 70°C on extraction amount [2].

When applied power increases, the generation of bubbles and also their sizes rise. The bigger the bubbles causes the higher temperatures after collapsing. This can explained by the

conversion of potential energy into heat energy. Also bigger bubbles causes better mixing in the reactor. Therefore, it is generally accepted that the higher power density the higher extraction yield [1]. However, according to the type of target biomolecules, this fact may change [1]. Figure 4. shows the effect of power output on extraction yield of tannin from myrobalan nuts. It can be clearly seen that extraction yield increases with increasing power. And according to the Figure 4., experiments with higher power outputs than 100W can be studied for investigation of even reaching higher yields.

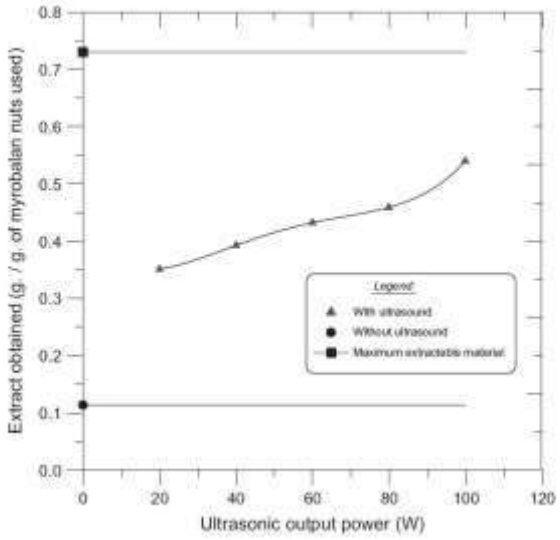

Figure 4. Effect of ultrasonic power between 20W and 100W, on tannin extraction from myrobalan, for 2h extraction time [2].

With increasing frequencies, cavitation becomes less brutal, eventually lose all effectiveness when MHz range is reached [3]. It was observed that the highest cell surface damage was reached via frequency ranges below 100 kHz [1] and for flexible matrices including plant sources, frequencies in between 20 and 40 kHz are most effective [1].

Solvent takes over a requisite role on transmitting the cavitation energy to the matrices as the cavitation incident relies on viscosity, surface tension and the polarity of the environment [1]. Although water is the most preferred among other organic and inorganic solvents, it is not the most effective in every case [1]. Eventually the determination of the solvent to be used is matrix dependent [1].

Sivakumar et. al. (2007) reported that the extraction efficiency is also affected with the size of the matrix that is used in the process, hence they cut their matrices into smaller pieces during their experiments.

It is assumed that the benefit of ultrasound extraction comes from its effect on vegetable cell wall break down and the release of cell materials thereafter into the solvent [3]. The variance in the yield for various plant sources may be due to the differences in cell wall structure, its sensitivity to outer impacts as ultrasonic bubble collapsing and possibly the composition of inner cell materials [3].

CONCLUSION

Studies showed that ultrasound assisted extraction of tannins has higher extraction yields up to %17, when compared to conventional hot water extraction technique that is widely used in industry today.

To design feasibly operating industrial scale reactors; multi-frequency applications, configurations that use multiple transducers and continuous flow reactor designs should be studied.

Weakening of ultrasonic waves in concentrated dispersed phases and the inequality of dispersed extract materials away from the ultrasound generator are major obstacles standing in front of developing feasible industrial-scale reactors.

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DISTRIBUTION OF DTPA-EXTRACTABLE MICRONUTRIENTS AND THEIR RELATIONSHIP WITH SOME SOIL PROPERTIES IN RICE GROWING SOILS OF BIRBHUM DISTRICT, WEST BENGAL, INDIA

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ABSTRACT

A study was under taken to assess the DTPA-extractable micronutrients in the soils under rice based cropping system in Birbhum district, West Bengal, India. The aim of this study was to examine the micronutrient status of selected soils to investigate the soil fertility and to determine correlation between physico-chemical properties (pH, EC, OC) and available zinc (Zn), iron (Fe), manganese (Mn)and copper (Cu). A total of hundred surface soil samples (0-15 cm), twenty soil samples from each block were collected from five selected blocks *viz*. Suri II, Rampurhat II, Md Bazar, Nalhati I, Sainthia of Birbhum district. The pH of the collected soil samples varied from 4.93-7.35, indicating that the soils were strongly acidic to neutral in reaction. The electrical conductivity (EC) values were low (0.04-0.19 dS/m), indicated low salt concentration in all five blocks. All the soil samples under investigation were found to contain low (<0.5%) to medium (0.5-1.0%) soil organic carbon content. About 4% of the collected soil samples were deficient, 76% marginal and 20% sufficient in available Zn. All the collected soil samples were sufficient in available Fe, Mn and Cu. Soil pH correlated significantly and negatively with DTPA-extractable micronutrients. DTPA-extractable micronutrients significantly and positively correlated with soil organic carbon. Significant and negative correlation was found between EC and available micronutrient cations (Zn, Fe, Mn and Cu). Results obtained from multiple regression analysis revealed that soil pH and organic carbon content are of immense importance in the determination of micronutrient availability.

Keywords: *Micronutrients, Rice Field, Physico-chemical properties of soils, Correlation Studies, Birbhum district, West Bengal, India.*

INTRODUCTION

Micronutrient deficiency is a well documented public health problem in the developing world. It can cause severe health problems. Currently, 2 billion people are facing micronutrient deficiency globally (Velu *et al*., 2014). In the last two decades, the concept of hidden hunger (deficiency of certain vitamins and micronutrient nutrients despite eating enough calories) has been well established (Nilson and Piza, 1998). Micronutrient deficiency appears to be a major problem for the rice consumers. Rice (*Oryza sativa* L.) is the staple food for more than 60% of world population (Parthipan and Ravi, 2016) and it contributes 45% to the total food grain production in India (Ram *et al*., 2013). Thus, even a small increase in the nutritive value of rice can highly contribute to human nutrition (Graham *et al*., 1999). India ranks first in the world in area of rice cultivation with 43.97 million ha and second in production with 104.32 million tons (Anonymous, 2013). Rice is the major food crop in West Bengal and it is being cultivated in

eighteen districts of the state. Micronutrients are very important for maintaining soil health and also in increasing productivity of crops (Rattan *et al*., 2009, Warjri *et al*., 2017; Warjri *et al*., 2019; Nisab *et al*., 2019). Soils which have higher finer fractions (*viz*., silt and clay) and higher organic matter are found to provide a greater reserve of these elements whereas, coarse textured soils (having sand fraction in large amount) have fewer reserves and tend to get depleted rather quickly. Three major reasons of micronutrient deficiency have been identified: some soils can be naturally deficient, some can be deficient due to crop removal and some can be due to erosion together with destruction of soil organic matter. The deficiency of micronutrients has become a major constraint to productivity and sustainability in many Indian soils (Athokpam *et al*., 2016). Due to negative or positive interactions of major nutrients, uptake of micronutrient is affected. High phosphate content of soils or high fertilization with phosphate may reduce the uptake of Zn and other nutrients (Kizilgoz and Sakin, 2010). The micronutrients which are essential for plant growth are Zn, Fe, Mn, Cu and boron (B). Through their involvement in various enzymes and other physiologically active molecules, these micronutrients are important for gene expression, biosynthesis of proteins, nucleic acids, growth substances, chlorophyll and secondary metabolites, metabolism of carbohydrates and lipids, stress tolerance, etc. (Rengel, 2007; Gao *et al*., 2008). As micronutrients are associated with enzymatic system of plants, hence their deficiency and toxicity can reduce plant yield (Tisdale *et al*., 1995). Although these micronutrients are required in small quantities for plant development and crop production, they appear to be equally vital as the macronutrients. By determining the uptake of micronutrient, its availability to plants can be known. Availability of micronutrient can be determined by correlating it with the quantities of micronutrients extracted chemically from it. Currently, 48.1% of Indian soils are lacking diethylene-triamine pentaacetate (DTPA) extractable Zn, and are deficient to about 11.2% in Fe, 7% in Cu and 5.1% in Mn (Singh *et al*., 2017). Crop yield is controlled by soil fertility. Determination of soil fertility status gives an idea of available nutrients present in the soil under study. The information obtained by this assessment is used for recommending fertilizer in order to maximize crop yield. Hence an attempt was made to assess the available status of micronutrients in the paddy growing areas and their relationship with different properties of soils of selected blocks of Birbhum district, West Bengal, India.

MATERIALS AND METHODS

The geographical area of the district is 4545 sq. Km with 5.12% of the total area of the state. It is the 9th biggest district by area in the state. The district is situated between 23°32'30" (right above the tropic of cancer) and 24°35'0" north latitude and 87°5'25" and 88°1'40" east longitudes. During summer, the temperature can shoot well above 40°C (104°F) and in winters it can drop to around 10°C (50°F) (Mondal, 2006). Red and lateritic soils occupy an area of about 28,000 sq. km in West Bengal which is about 28% of the total geographical area of the state (Anonymous, 1989). Of these, red soil occur in the districts of Birbhum, Bankura, Burdwan, Midnapore and some other parts of Malda and West Dinajpur and gravelly soils are found in the districts of Purulia, part of Midnapore, Bankura and Birbhum. Some parts of Birbhum, Burdwan, Bankura and Midnapore have also laterites and lateritic soils (Panda *et al*., 1991). Only 54.7% of the geographical area under red and lateritic soils in West Bengal have been brought under cultivation and *kharif* rice is the major crop occupying about 75% of the gross cropped area (Chattopaddhyay and Ghosh, 2006). In fact, the red, lateritic and associated soils of eastern India are acidic in soil reaction, light textured, low in organic matter and phosphorus and are often deficient in sulphur (Panda *et al*., 1991; Sakal and Singh, 1997; Ghosh, 2001; Chakraborty *et al*., 2002; Ghosh *et al*., 2005; Patra *et al*., 2012). The main crop of the district is rice. 100 soil samples were collected from five selected blocks of Birbhum district (20 samples from each block) at a depth of 0-15 cm. The soil samples were collected from paddy growing areas of the district. These soils were stored and labelled properly in polythene bags,

then taken to the laboratory for physico-chemical analyses. The soils were air dried, grinded using porcelain mortar and pestle, sieved through 2 mm sieve. Particle size distribution (relative distribution of sand, silt and clay in soils) and textural class was determined by the method proposed by Bouyoucos (1927). pH, EC of the collected soil samples were determined using standard procedure given by Jackson (1973). Soil organic carbon content was determined following the wet digestion method as suggested by Walkley and Black (1934). The DTPA-extractable Zn, Cu, Fe and Mn in the soil samples were extracted with a solution of 0.005M DTPA, 0.01M calcium chloride ($CaCl_2$) and 0.1M triethanolamine, adjusted to pH 7.3 as outlined by Lindsay and Norvell (1978). 1:2 soils to extractant ratio were taken, shaken for two hours and filtered. The filtrate was then used for determining Zn, Fe, Mn and Cu using Atomic Absorption Spectrophotometer (AAS). The relationship between various soil properties and micronutrient distribution were established by using simple correlation coefficient. For statistical analysis of data, Microsoft Office Excel 2007 (Microsoft Corporation, USA) and a statistical software IBM SPSS 25.0 (windows version 8.0) packages were used. The level of significance in the results is $P<0.05$. Classification of the soil samples depending on soil pH values and EC values are given in Table-1 and 2 respectively. The rating chart of the micronutrient status and organic carbon content of soil samples are represented in Table-3 and 4 respectively.

Table 1. Classification of soil pH values

Strongly Acid	Moderately acid	Slightly Acid	Neutral	Moderately Alkali	Strongly Alkali	Reference
<5.5	5.5-6.0	6.0-6.5	6.5-7.5	7.5-8.5	>8	Muhr *et al*., (1965)

Table 2. Classification of total soluble salts (EC dS/m)

No deleterious effect on crop	Critical for germination	Critical for Salt Sensitive Crop	Injurious to most crops	Reference
<1.0	1.0-2.0	2.0-3.0	>3.0	Muhr *et al*., (1965)

Table 3. Rating Chart of Micronutrients in soil required for Plant growth

Content	Zn	Fe	Mn	Cu	Reference
Deficit	< 0.6	< 4.5	< 2.5	< 0.2	Lindsay and Norvell (1978)
Marginal	0.6-1.2	4.5-9.0	2.5-3.5	0.2-0.4	Lindsay and Norvell (1978)
Sufficient	1.2-2.4	9.0-18.0	3.5-7.0	0.4-0.8	Lindsay and Norvell (1978)
High	>2.4	18.0-27.0	> 7.0	0.8-1.6	Lindsay and Norvell (1978)
Very high	-	> 27.0	-	1.6-3.2	Lindsay and Norvell(1978)

Table 4. Rating chart of organic carbon content

Parameter	Low	Medium	High	Reference
Organic Carbon (%)	<0.50	0.5-1.0	>1.0	Muhr *et al*., (1965)

RESULTS AND DISCUSSION

Physico-chemical properties and Micronutrient status of the collected Soil samples

The different physico-chemical properties and available micronutrient status of the collected soil samples from paddy growing areas are represented in Table5.

Table 5. Physico-chemical properties and available micronutrient status of the collected soil samples from selected Blocks of Birbhum district

Block		pH	EC (dS/m)	% OC	Available Zn (mg/kg)	Available Fe (mg/kg)	Available Mn (mg/kg)	Available Cu (mg/kg)	Textural Class
Suri II	Range	4.92-5.71	0.04-0.11	0.50-0.74	0.62-2.08	37.56-42.00	19.16-37.94	1.48-2.75	Silt loam
	Average	5.24	0.06	0.61	1.30	39.76	29.11	2.11	
	Standard Deviation	0.23	0.02	0.09	0.57	1.32	7.31	0.50	
Rampurhat II	Range	5.95-6.46	0.04-0.09	0.50-0.76	0.56-0.87	22.12-29.04	18.10-39.12	1.02-1.71	Silt
	Average	6.24	0.06	0.66	0.67	26.40	34.44	1.46	
	Standard Deviation	0.13	0.01	0.06	0.09	1.83	4.88	0.20	
Md Bazar	Range	5.51-6.17	0.04-0.12	0.37-0.71	0.99-1.40	25.60-33.80	13.36-37.92	1.85-2.50	Silt
	Average	5.85	0.08	0.61	1.16	30.86	31.24	2.28	
	Standard Deviation	0.18	0.02	0.08	0.10	2.18	6.43	0.20	
Nalhati I	Range	6.81-7.37	0.04-0.20	0.56-0.79	0.95-1.26	24.68-34.89	15.64-36.44	1.81-2.41	Silt loam
	Average	7.04	0.11	0.71	1.09	31.76	27.30	2.07	
	Standard Deviation	0.19	0.04	0.07	0.09	2.81	6.39	0.21	
Sainthia	Range	4.66-7.06	0.07-0.19	0.38-0.98	0.62-1.49	21.48-43.16	28.64-40.60	1.48-2.58	Silt
	Average	5.30	0.12	0.61	0.96	34.87	38.00	1.90	
	Standard Deviation	0.84	0.04	0.13	0.29	6.26	2.97	0.39	

Textural class of the collected soil samples at 0-15 cm depth were found to be silt loam, silt, silt, silt loam and silt for Suri II, Rampurhat II, Md Bazar, Nalhati I and Sainthia respectively (Table5).

pH of the collected soil samples varied from 4.92-5.71 in Suri II with an average of 5.24, 5.95-6.46 in Rampurhat II with an average of 6.24, 5.51-6.17 with an average of 5.85 in Md Bazar, 6.81-7.37 with an average of 7.04 in Nalhati I, 4.66-7.06 with an average of 5.30 in Sainthia. Based on the ratings of pH value as suggested by Muhr *et al*., (1965), the pH values were strongly acidic to moderately acidic in Suri II, Moderately acidic to slightly acidic in Rampurhat II and Md Bazar. Neutral soil pH was observed in Nalhati I, strongly acidic to neutral soil pH was found in Sainthia. Low soil pH value might be attributed to the impact of parent material

(granite and sandstone) and leaching down of basic cations in soils by heavy rainfall during monsoon (Mini *et al*., 2007). Athokpam *et al*., (2013) reported that acidic in reaction of the district might be due to the high rainfall leading to the leaching losses of bases from the surface soils in Senapati district, Manipur. They further reported that application of nitrogenous fertilizers and decomposition of organic residues hastened the soil acidity. Application of liming materials, organic manures and in situ rice residues incorporation was required to bring the pH to neutral levels (Kumar *et al*., 2017a).

EC values ranged from 0.04-0.11 dS/m in Suri II with an average of 0.06 dS/m, 0.04-0.09 dS/m in Rampurhat II with an average of 0.06 dS/m, 0.04-0.12 dS/m with an average of 0.08 dS/m in Md Bazar, 0.04-0.20 dS/m with an average of 0.11 dS/m in Nalhati I, 0.07-0.19 dS/m with an average of 0.12 dS/m in Sainthia. According to the ratings given by Muhr *et al*., (1965), EC values were low and soils are non-saline in nature i.e. low salt concentration was observed in all five blocks. Similar results were given by Chakravarti andChakravarti (1957); Ray and Mukhopadhyay (2012). Low EC in these soils might be due to proper management of soil and by the leaching of salts from surface to subsurface and also due to the application of acidulating fertilizers in salt affected areas (Vijayakumar *et al*., 2011a). Kumar *et al*., (2017) reported that, in all the Talukas, 100% area was under non-saline condition in rice-chickpea Chhattisgarh plain region. Similar result was also found in soils of Akaltara block of Janjgir district of Chhattisgarh as described by Chandrakar *et al*., (2013).

The OC content varied from 0.50-0.74% with an average of 0.61% in Suri II, 0.50-0.76% with an average of 0.66% in Rampurhat II, 0.37-0.71% with an average of 0.61% in Md Bazar, 0.56-0.79% with an average of 0.71% in Nalhati I, 0.38-0.98% with an average of 0.61% in Sainthia. On the basis of limits suggested by Muhr *et al*., (1965) all the soil samples under investigation was found to contain low (<0.5%) to medium (0.5-1.0) soil OC content. Medium to higher organic carbon status of the soils could be attributed to the regular addition of organics in the form of FYM, compost and green manures. Similar observations were noticed by Chidanandappa (2003) indicating that application of organic manures enhances the organic carbon content in the soils. Poor vegetation is one of the major causes of low organic carbon content of soil. Low organic carbon content of the soils is characteristics of the rapid decomposition and mineralization of organic matter and to poor management, sometimes by burning of crop residues by farmers (Lawal *et al*., 2012). Mahendra Kumar *et al*., (2017) found that the organic carbon content ranged between 0.12-1.05%, indicating medium to high organic carbon content in the rice growing soils of Chamarajanagar District, Karnataka, India. Other than that, organic matter gets decomposed under hyperthermic temperature leading to extremely high oxidizing conditions. Poor vegetation is another cause of low organic carbon content of soil.

Available Zn content varied from 0.62-2.08 mg/kg with an average of 1.30 mg/kg in Suri II, 0.56-0.87 mg/kg with an average of 0.67 mg/kg in Rampurhat II, 0.99-1.40 mg/kg with an average of 1.16 mg/kg in Md Bazar, 0.95-1.26 mg/kg with an average of 1.09 mg/kg in Nalhati I, 0.62-1.49 mg/kg with an average of 0.96 mg/kg in Sainthia. According to the rating chart given by Lindsay and Norvell (1978), 4% of the collected soil samples were found deficit in available Zn, 76% were having marginal available Zn and 20% were found sufficient in available Zn. Singh *et al*., (1988); Sharma and Lal (1992); Nisab *et al*., (2020) reported that the higher amount at the surface layer was related to higher organic carbon content of the soils due to regular addition of plant residues. Similar results were observed by Tiwari and Mishra (1990); Krishnamurthy and Srinivasamurthy (2005); Chidanandappa *et al*., (2008). Gupta (2005) reported that 48.1% of Indian soils were deficient in DTPA-extractable Zn, 11.2% in Fe, 7% in Cu and 5.1% in Mn. Available Zn was found in the range from 0.60 to 0.97 mg/kg with mean value of 0.79 mg/kg in the alluvial soils West Bengal, India (Mahata *et al*., 2012). DTPA-

Zn content in the soils varied from 0.07 to 1.29 mg/kg with mean value of 0.43 mg/kg, 0.11 to 3.16 mg/kg with mean value of 0.41 mg/kg and 0.04 to 1.72 mg/kg with mean value of 12.16 mg/kg, respectively indicating Zn deficient soil is 97% in Jintur, 99% in Selu and 97% in Pathri tahsil, Parbhani District, Maharastra (Mandavgade *et al*., 2015). The low content of DTPA-Zn in these soils might be due to fact that under alkaline conditions, Zn cations are changed largely to their oxides or hydroxides and thereby lowering the availability of Zn. Similar results were reported by Meena *et al*., (2006). Zn was found to be deficient in the entire study area of Jhotwara Panchayat Samiti of Jaipur District, India (Choudhary *et al*., 2017). Mahendra Kumar *et al*., (2017) reported that available Zn content of the soils under paddy land use in Chamarajanagar district was ranged from 0.69 to 2.96 mg/kg. DTPA-Zn ranged between 0.42-1.95 mg/kg with mean value of 0.84 mg/kg (Meena and Mathur, 2017). The higher content of DTPA-extractable Zn in Kollegal Taluk may be due to higher organic carbon content of the soils. Available Zn in the studied surface soils varied from 0.02 to 0.56 mg/kg with mean value of 0.23 mg/kg in the soils of Chandel district, Manipur (Singh *et al*., 2017). DTPA-Zn content in soils of mustard cultivation area of Gharsana Tehsil of Rajasthan ranged between 0.20 to 0.59 mg/kg with mean value of 0.36 mg/kg (Kumar *et al*., 2018).

As per the critical limits suggested by Lindsay and Norvell (1978), all the collected soil samples were found sufficient in available Fe. Its value ranged from 37.56-42.00 mg/kg with an average of 39.76 mg/kg in Suri II, 22.12-29.04 mg/kg with an average of 26.40 mg/kg in Rampurhat II, 25.60-33.80 mg/kg with an average of 30.86 mg/kg in Md Bazar, 24.68-34.89 mg/kg with an average of 31.76 mg/kg in Nalhati I, 21.48-43.16 mg/kg with an average of 34.87 mg/kg in Sainthia. Prasad and Sakal (1991) were in the opinion that the higher amount of available Fe might be due to the presence of organic matter indicating that organic matter influenced the solubility and availability of Fe which might be due to the chelation of Fe which protects it from oxidation and precipitation of available Fe into unavailable form with a consequence of increasing its availability in the soil. Therefore the distribution pattern of DTPA-Fe followed the pattern of distribution of organic carbon which might be attributed to their regular addition through crop residues on the surface (Tiwari and Mishra, 1990). Rattan *et al*., (2008) found that available Fe in Indian soils varied from 3.40 to 68.10 mg/kg. Higher amount of DTPA-Fe content in soil may be due to the presence of minerals like Feldspar, Magnetite, Hematite and Limonite which together constitute the bulk of trap rock in these soils (Vijaya kumar *et al*., 2013). Srinivasan and Poongothai (2013) reported that available Fe content ranged from 2.52-34.66 with mean value of 18.59 mg/kg in the soils of Tamilnadu, India. DTPA-Fe content varied from 1.00 to 76.68 mg/kg with mean value of 13.43 mg/kg in the soils of Chhattisgarh plain region of India (Kumar *et al*., 2017). Mahendra Kumar *et al*., (2017) reported that the available Fe content of the soils under paddy land use in Chamarajanagar district varied from 6.09 to 32.14 mg/kg. DTPA-Fe ranged from 2.26 to 28.40 mg/kg with mean value of 10.12 mg/kg in the soils of Ghatol Tehsil, Banswara District of Rajasthan, India (Meena and Mathur, 2017). Available Fe content in the surface soils ranged from 3.75 to 56.65 mg/kg with mean value of 22.93 mg/kg in the soils of Chandel district, Manipur, India (Singh *et al*., 2017). Rai *et al*., (2018) reported that available Fe content of the soil under five different cropping system varied from 9.45-23.54 mg/kg with mean values of 18.41, 15.34, 16.34, 21.35 and 16.54 mg/kg respectively in the soils of Kishtwar District (J&K), India. Available Fe was found sufficient in the soils assuming 4.5 mg/kg as a critical limit as suggested by Lindsay and Norvell (1978). These findings were in conformity with that of Nazif *et al*., (2006); Kirmani *et al*., (2011).

All the collected soil samples were found sufficient in available Mn according to the limits suggested by Lindsay and Norvell (1978). Available Mn value varied in the range 19.16-37.94 mg/kg with an average of 29.11 mg/kg in Suri II, 18.10-39.12 mg/kg with an average of 34.44 mg/kg in Rampurhat II, 13.36-37.92 mg/kg with an average of 31.24 mg/kg in Md Bazar, 15.64-

36.44 mg/kg with an average of 27.30 mg/kg in Nalhati I, 28.64-40.60 mg/kg with an average of 38.00 mg/kg in Sainthia. Srinivasan and Poongothai (2013) observed that available Mn in the studied soils varied from 4.05-21.44 mg/kg with mean value of 12.75 mg/kg. Considering 2.5 mg/kg as a critical limit for Mn deficiency (Lindsay and Norvell, 1978), zero % was found in deficient and marginal range, and rest of the soils had sufficient amount of available Mn. The relative high content of Mn in the soils of Parbhani District, Maharastra could be due to the soils derived from basaltic parent material which contained higher ferromagnessium minerals (Mandavgade *et al.,* 2015). Aich *et al*., (2017) reported that, all the soil samples collected from organic farm in Pune were sufficient in available Mn, as the critical limit of available Mn is 2.5 mg/kg (Lindsay and Norvell, 1978). Available Mn ranged from 0.08 to 31.60 mg/kg with a mean value of 8.87 mg/kg in the surface soils of Chandel district, Manipur, India (Singh *et al*., 2017). Rai *et al*., (2018) reported that DTPA-Mn varied from 11.32-31.23 mg/kg in five different cropping system with mean values of 26.51, 29.41, 19.41, 20.54 and 18.64 mg/kg respectively. Mn content of the soils varied widely and was higher under rice-mustard cropping than other cropping system. Due to different pedogenic processes during soil development, variations in available micronutrient content is observed. Higher micronutrient availability is observed with soils having higher organic carbon content. This is due to the fact that during decomposition of organic matter, chelating ligands are released which prevent fixation, oxidation, leaching and precipitation of the micronutrient cations, hence micronutrient cations remain available for the take up by plants.

According to the critical limits suggested by Lindsay and Norvell, (1978) all the collected soil samples were found sufficient in available Cu. Its value ranged from1.48-2.75 mg/kg with an average of 2.11 mg/kg in Suri II, 1.02-1.71 mg/kg with an average of 1.46 mg/kg in Rampurhat II, 1.85-2.50 mg/kg with an average of 2.28 mg/kg in Md Bazar, 1.81-2.41 mg/kg with an average of 2.07 mg/kg in Nalhati I, 1.48-2.58 mg/kg with an average of 1.90 mg/kg in Sainthia. All the soil samples were found sufficient in available Cu as per the critical limits suggested by Lindsay and Norvell (1978). The higher amount of DTPA-Cu in surface layer might be due to higher biological activities and chelating effects (Kadao *et al*., 2002; Jibhakate *et al*., 2009). Rattan *et al*., (2008) found that the contents of Cu in Indian soils varied from 0.2 to 5.0 mg/kg. Available Cu content of the soil samples varied from 0.32-1.52 mg/kg with mean value of 0.86 mg/kg (Vijayakumar *et al*., 2011b). Athokpam *et al*., (2013) reported that available Cu content in the surface soils ranged from 0.65 to 2.60 mg/kg with mean value of 1.52 mg/kg in the soils of Senapati district, Manipur (India). Considering 0.2 mg/kg as critical limit for Cu deficiency (Lindsay and Norvell, 1978), all the soils were found to be in adequate range. Choudhary *et al*., (2017) reported that the available Cu content of surface soils was ranged from 1.10 to 4.75 mg/kg with mean value of 2.96 mg/kg. Meena and Mathur (2017) reported that DTPA-Cu varied from 0.37 to 4.15 mg/kg with mean value of 1.07 mg/kg in the soils of Ghatol Tehsil, Banswara District of Rajasthan, India. Rai *et al*., (2018) reported, DTPA-extractable Cu ranged from 1.20-4.89 mg/kg with average values of 1.67, 2.22, 1.98, 2.44 and 2.46 mg/kg under different cropping system indicating no deficiency as the values are above the critical limit of 0.2 mg/kg (Lindsay and Norvell, 1978). This was in agreement with the study of Chattopadhyay *et al*., (1996). Satish *et al*., (2018) noted that accumulation of Cu in surface horizons of all the soils may be due to its turnover by plant residues.

Correlation between physico-chemical properties and Micronutrient Status of collected Soil samples

In order to determine the relationship between available micronutrients with soil properties and the inter-relationship amongst different micronutrients, correlation statistics was performed. The correlation between soil physico-chemical properties and micronutrient status of the collected soil samples are represented in Table6.

Table 6. Correlation Coefficients between soil physico-chemical properties and micronutrients of the collected soil samples from selected Blocks of Birbhum district

Parameters	pH	EC	OC	Available Zn	Available Fe	Available Mn	Available Cu
pH	1						
EC	0.486**	1					
OC	0.007	-0.480**	1				
Available Zn	-0.382**	-0.368**	0.486**	1			
Available Fe	-0.700**	-0.417**	0.296**	0.653**	1		
Available Mn	-0.466**	-0.416**	0.503**	0.256**	0.230*	1	
Available Cu	-0.324**	-0.325**	0.546**	0.923**	0.625**	0.317**	1

*. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.

Relation between available Zn and soil physico-chemical properties

Soil pH correlated significantly and negatively with available Zn (r= -0.382**). Inverse relationship existed between soil pH and DTPA-extractable micronutrients. Increased soil pH caused decreased availability of DTPA-extractable micronutrients. Lindsay (1991) reported that at higher pH metal cations remain as hydroxides, hence decrease in their availability is observed. Chandrakar *et al*., (2013) reported that available Zn showed significant and negative relationship (r= -0.063*) with pH, thereby indicating that availability of Zn decreased with increase in soil pH. Thakur and Bhandari (1986) also reported same relationship in soils of Saproon valley of Himanchal Pradesh. It may be due to the increased solubility of oxides and hydroxides of Zn at lower pH condition (Talukdar *et al*., 2009). Srinivasan and Poongothai (2013) reported non-significant and negative correlation with soil pH and available Zn (r= -0.084). This finding is in conformity with the result obtained by Mandavgade *et al*., (2015). They further reported negative correlation with soil pH which may be attributed to their precipitation as hydroxides, consequently making them immobile and unavailable to the plants. At higher pH, insoluble Zn hydroxide $Zn(OH)_2$ is formed, which does not come into the solution. Tundup and Akbar (2014) reported negative correlation between available Zn and soil pH (r = -0.083) which is in line with the findings of Vadivelu and Bandyopadhyay (1995); Mahendra Kumar *et al*., (2017); Rai *et al*., (2018). Ray and Banik (2016) reported that DTPA-Zn negatively and significantly correlated with pH (r= -0.72**) of the new alluvial and old alluvial soils of West Bengal, indicating the increased availability of the metallic cation Zn at lower pH. Reshma *et al*., (2016) observed significant negative correlation (r= -0.175**) between pH and available Zn. Significant and negative correlation was found between available Zn and pH (r= -0.448**) (Choudhary *et al*., 2017). Meena and Mathur (2017) reported inverse relationship between available Zn and soil pH (r= -0.265**). The findings of the present investigation are confirmed by the results of Singh (2006); Mehra and Jat (2007); Karar *et al*., (2018); Nisab *et al*., (2020). Regression line was fitted between available Zn and soil pH (Fig 1).

Significant and positive correlation was observed between available Zn and organic carbon (r= 0.486**). Chandrakar *et al*., (2013) reported significant and positive correlation between available Zn and organic carbon (r = 0.1**). Tundup and Akbar (2014) noted that Zn positively and significantly correlated with organic matter (r= 0.417**). The positive correlation may be due to the formation of organic complexes between organic matter and Zn that protect it from leaching. Choudhary *et al*., (2017) reported significant and positive correlation (r= 0.965**) between available Zn and organic carbon. Available Zn content of the soil increases with increase in the organic carbon content of the soil. Singh *et al*., (2017) reported significant and positive correlation between available Zn and organic carbon (r= 0.576**) in the soils of

Chandel district, Manipur. Kumar *et al*., (2018) reported highly significant and positive correlation between available Zn and organic carbon content (r= 0.381**) under mustard cultivation area. Similar result was obtained by Minakshi *et al*., (2005) in soils of Patiala district of Punjab; Athokpam *et al*., (2013); Rai *et al*., (2018). Similar relationship between available Zn and soil organic carbon content (r= 0.744**) were confirmed by Mandal *et al*., (2019) in the paddy growing soils of Birbhum district, West Bangal, India. Regression line was fitted between available Zn and soil organic carbon content (Fig 2).

Available Zn correlated significantly and negatively with EC (r= -0.368**). Maqbool *et al*., (2016) reported significant and negative correlation between EC and available Zn (r= -0.615**). Padhan *et al*., (2016) reported significant negative correlation between EC and available Zn (r= -0.294**) in the rice soils of three villages (*viz*. Saharapali, Nuagarh and Adgaon) of Bargarh district under the Hirakud Command Area of Odisha. Ray and Banik (2016) reported significant and negative correlation between EC and DTPA-Zn (r= -0.88**) in the soils of different districts (Uttar Dinajpur, Dakshin Dinajpur, Malda and Murshidabad) of West Bengal.

Relation between available Fe and soil physico-chemical properties

Available Fe of all the soil samples collected showed significant and negative correlation with pH (r= -0.700**). Available Fe found to decrease with increase in soil pH. High pH is responsible for the oxidation of Fe^{2+} (ferrous ion) to Fe^{3+} (ferric ion). The most readily form of Fe is Fe^{2+} which is converted to Fe^{3+} after its oxidation. At higher pH, Fe^{3+} is precipitated as insoluble $Fe(OH)_3$ which is not readily available to plants. Chandrakar *et al*., (2013) reported significant negative correlation (r = -0.079*) between Fe and pH. Similar results were reported by Minakshi *et al*., (2005) in soils of Patiala district of Punjab. Researchers (Verma *et al*., 2005; Pati and Mukhopadhyay, 2011) reported the influence of soil pH on the solubility and availability of micronutrients. DTPA-Fe bears negative and significant relationship with pH (r = -0.367**) (Tundup and Akbar, 2014). It can be observed that Fe like the other micronutrients like Cu and Mn, decreases with the increase in soil pH. These results are supported by Rajakumar *et al*., (1996); Chatterji *et al*., (1999); Chinchmalatpure *et al*., (2000); Choudhary *et al*., (2017); Rai *et al*., (2018). Mandavgade *et al*., (2015) reported non-significant negative correlation between available Fe and pH (r= -0.119) in the soils of Northern Tahsils (Jintur, Selu and Pathri) of Parbhani District, Maharastra. Mahendra Kumar *et al*., (2017) reported significant and negative correlation between soil pH and available Fe (r= -0.50**). This negative correlation indicated the precipitation of soluble Fe into insoluble products. Mandal *et al*., (2019) reported similar result in their studies. Regression line was fitted between available Fe and soil pH (Fig 3).

Available Fe correlated significantly and positively with organic carbon (r= 0.296**).The micronutrient cations react with certain organic molecules to form organometallic complexes as chelates and these soluble chelates can increase the availability of the micronutrient and protect it from precipitation reactions. These chelates may be synthesized by the plant roots and released to the surrounding soil. The chelate may also be present in the soil humus or may be synthetic compound added to the soil to enhance micronutrient availability (Brady and Weil, 2002). Meena *et al*., (2006) reported that the availability of metal ions (Fe, Cu) increases with increase in organic matter content because organic matter supplies chelating agents. Organic matter and manure applications affect the immediate and potential availability of micronutrient cations (Rengel, 2007). Yadav and Meena (2009) reported similar relationship in Degana soil series of Rajasthan. Athokpam *et al*., (2013) reported significant and positive correlation between available Fe and soil organic carbon (r= 0.835**) in the soils of Senapati district, Manipur. Chandrakar *et al*., (2013) reported that availability of metal ion Fe increases with

increase in organic matter which might be due to the fact that organic matter supplies chelating agents. Mandavgade *et al*., (2015) reported positive relationship between available Fe and organic carbon (r = 0.063). Choudhary *et al*., (2017) reported significant and positive correlation between available Fe and organic carbon content (r= 0.864**) in the soils of Jhotwara Panchayat Samiti of Jaipur District, India. Meena and Mathur (2017) reported that available Fe significantly increased with increase in OC (r = 0.607**) in the soils of Ghatol Tehsil, Banswara District of Rajasthan, India. Singh *et al*., (2017) reported significant and positive correlation between available Fe and organic carbon (r= 0.540**). Kumar *et al*., (2018) reported that availability of Fe enhanced significantly with increase in organic matter, which might be due to the fact that organic carbon content of the soil is helpful in improving soil structure and aeration. Organic carbon protects Fe from the oxidation and precipitation of Fe into unavailable forms and supply soluble chelating agents which increase the solubility of Fe compounds. Similar result was reported by Mandal *et al*., (2019) in the rice growing soils of Birbhum district, West Bengal, India. Regression line was fitted between available Fe and soil organic carbon content (Fig 4).

Significant and negative correlation was observed between EC and available Fe (r= -0.417**). Chandrakar*et al*., (2013) reported significant negative correlation between EC and DTPA-Fe (r= -0.067*) in the inceptisols of Akaltara block of Janjgir district, Chhattisgarh. Similar relationship was obtained by Sharma *et al*., (2006) in soils of cold arid region of Leh district, Ladakh. Maqbool *et al*., (2016) reported significant and negative correlation between EC and available Fe (r= -0.723**). Significant negative correlation between EC and available Fe (r= -0.72*) was recorded by Ghode*et al*., (2020) in the soils of Nanded district of Maharashtra.

Relation between available Mn and soil physico-chemical properties

Available Mn of the collected soil samples showed significant and negative correlation with soil pH (r= -0.466**). Mn may get complexed by organic matter and hence Mn oxides are formed. Dissociation of these complexes is dependent on pH; lower the pH, more is the available Mn (Smiley *et al*., 1986). Kumar *et al*., (2009) showed negative significant correlation of available Mn with soil pH in Dumka series in soils of Santhal Paraganas region of Jharkhand. Chandrakar *et al*., (2013) reported negative and significant correlation between available Mn and soil pH (r = -0.122**). This is due to the formation of insoluble higher valent oxides of Mn at higher pH. At higher pH, less soluble compound $Mn(OH)_2$ is formed. The higher pH favours the formation of less soluble complexes of Mn, which reduces the availability of Mn and the activity of soil micro-organism which oxidizes soluble Mn^{2+} (Singh *et al*., 2013). Tundup and Ali (2014) reported that Mn had negative highly significant correlation with soil pH. The correlation value (r) of Mn with pH was -0.455**, which showed that with the increase of pH, availability of Mn decreases. Reshma *et al*., (2016) reported that Mn (r= -0.084**) showed significant negative correlation with soil pH. Choudhary *et al*., (2017) reported similar relationship (r= -0.477**) in the soils of Jhotwara Panchayat Samiti of Jaipur District, India. Rai *et al*., (2018) reported significant and negative correlation between pH and available Mn (r= -0.397**). Regression line was fitted between available Mn and soil pH (Fig 5).

Significant and positive correlation was found between available Mn and organic carbon content of the collected soil samples (r= 0.503**). Increased availability of micronutrients with increasing organic carbon content might be due to complexation of micronutrients with organic acids (Lindsay, 1991; Venkatesh *et al.,* 2003) or due to the release of H^+ ions during the decomposition of organic matter (Sharma and Chaudhary, 2007). Sharma *et al*., (2006) reported that available Mn positively correlated (r=0.029) with organic carbon. Organic colloids can retain Mn by complex formation (Bassirani *et al*., 2011). Chandrakar *et al*., (2013) reported positive and significant correlation (r = 0.071*) between available Mn and organic carbon.

Similar relationship was given by Athokpam *et al*., (2013) in the soils of Senapati district, Manipur (r= 0.7882**); Singh *et al*., (2017) in the soils of Chandel district, Manipur. Organic matter indirectly brings about Mn transformation due to the release of an array of organic compounds during the decomposition (Reshma *et al*., 2016). Choudhary *et al*., (2017) reported significant and positive correlation between available Mn and organic carbon (r= 0.950**) in the soils of Jhotwara Panchayat Samiti of Jaipur District, India. Increase in the availability of Mn with increase in organic carbon content might be due to the influence of organic carbon on the solubility and availability of Mn which protect itself from oxidation and precipitation of available Mn into unavailable forms (Kumar *et al*., 2018). They reported significant and positive correlation between soil organic carbon and available Mn (r= 0.352**). Regression line was fitted between available Mn and soil organic carbon content (Fig 6).

Available Mn correlated significantly and negatively with EC (r= -0.416**). Maqbool *et al*., (2016) reported significant and negative correlation between EC and available Mn (r= -0.729**). Ray and Banik (2016) reported that significant and negative correlation between EC and DTPA-Mn (r= -0.80**) in the soils of different districts (Uttar Dinajpur, Dakshin Dinajpur, Malda and Murshidabad) of West Bengal. Aich *et al*., (2017) reported significant and negative correlation between available Mn and EC (r= -0.328*). Kondvilkar *et al*., (2017) reported that soil EC was negatively and significantly correlated with DTPA-Mn (r=-150*) in the soils of Sakri Tehsil of Dhule District, Maharashtra.

Relation between available Cu and soil physico-chemical properties

Available Cu content of all the collected soil samples showed significant and negative correlation with soil pH (r= -0.324**). This emplies that with increase in soil pH, availability of Cu decreases. This is due to the precipitation of Cu^{2+} as relatively insoluble hydroxide $Cu(OH)_2$. Such inverse relationship between DTPA extractable micronutrients with soil pH are confirmed by Somasundaram *et al.*, (2009); Vasuki (2010). Alkaline condition leads to the reduction in the availability of micronutrients. Chandrakar *et al*., (2013) observed negative significant correlation (r = 0.174**) with soil pH and available Cu. Tundup and Akbar (2014) obtained significant and negative correlation between available Cu and soil pH (r= -0.281*). Mandavgade *et al*., (2015) reported non-significant and negative correlation between available Cu and pH (r= -0.065) in the soils of of Northern Tahsils (Jintur, Selu and Pathri) of Parbhani District, Maharashtra. Reshma *et al*., (2016) obtained non-significant and negative correlation between available Cu and soil pH (r= -0.030). Non-significant negative correlation between soil pH and available Cu (r= -0.049) was reported by Kumar *et al*., (2018). Rai *et al*., (2018) obtained significant and negative correlation between available Cu and soil pH (r= -0.295*) indicating that decrease in soil pH leads to significant increase in Cu availability. Similar results were reported by Jalali *et al*., (1989) in some benchmark soils of Kashmir; Meena *et al*., (2006) in soils of Tonk district of Rajasthan. Regression line was fitted between available Cu and soil pH (Fig 7).

Available Cu of the collected soil samples correlated significantly and positively with soil organic carbon (r= 0.546**). With increase in organic content of the soils, micronutrient availability increases. This is due to the formation of soluble Cu complexes which decreases the chance of fixation of Cu in soils. Cu has high affinity for complexation with organic matter which increases its solubility. Sharma *et al*., (2005) reported non-significant and positive correlation between soil organic carbon and available Cu (r= 0.90) in soils of Rajasthan. Significant positive relationship (r= 0.116**) was observed between organic carbon and available Cu (Chandrakar *et al*., 2013). Tundup and Akbar (2014) indicated that Cu positively and significantly correlated with soil organic carbon (r = 0.256*). Ray and Banik (2016)

obtained significant and positive correlation between soil organic carbon and available Cu (r= 0.47**) in some soils of West Bengal. The organic acid molecules present in organic matter solubilise Cu^{2+} ions by chelation and complexion and as a result of this organic binding, there is more dissolved Cu in the soil solution than normally occurs in the absence of organic matter (Meena and Mathur, 2017). Singh *et al*., (2017) obtained similar relationship (r= 0.749**) between available Cu and organic carbon. Rai *et al*., (2018) obtained similar relationship (r= 0.266*) in the soils under different cropping system of Kishtwar district (J&K), India. Certain complexing agents are supplied by organic matter which enhances the availability of that particular element. Similar results were reported by Khalifa *et al*. (1996); Rajakumar *et al*. (1996); Meena *et al*., (2006); Athokpam *et al*., (2013); Choudhary *et al*., (2017) who found positive and significant correlation between Cu and organic matter. Regression line was fitted between available Cu and soil organic carbon content (Fig 8).

Significant and negative correlation was found between available Cu and EC (r= -0.325**). Negative significant correlation was observed between available Cu and EC (r = -0.093**) in Inceptisol of Akaltara block of Janjgir district, Chhattisgarh (Chandrakar *et al*., 2013). Maqbool *et al*., (2016) reported significant and negative correlation between EC and available Cu (r= -0.761**). Ray and Banik (2016) reported similar relation in some soils of different districts (Uttar Dinajpur, Dakshin Dinajpur, Malda and Murshidabad) of West Bengal (r= -0.34*). Available Cu showed significant negative correlation with EC (r= -0.053*) in soils of Salem district of Tamil Nadu (Reshma *et al*., 2016). Annepu *et al*., (2017) reported that there existed significant negative correlation between EC and available Cu (r= -0.180**).

Inter-Relationships between DTPA-extractable micronutrients

Avaialble Zn of the collected soil samples showed significant and positive correlation with other micronutrients. Similar result was given by Bassirani *et al*., (2011).

Available Fe showed positive significant correlation with other micronutrient cations. Similar results were obtained by Verma *et al*., (2005); Jiang *et al*., (2009); Bassirani *et al*., (2011).

Available Mn also had positive significant correlations with other micronutrient cation contents in the soils. Soil micronutrient cations Zn, Fe, Cu have significant correlation with available Mn, suggesting variation in their distribution dependent upon common soil factors (Follect and Lindsay, 1970).

Athokpam *et al*., (2013) obtained significant and positive correlation between DTPA-extractable micronutrients (r= 0.8491** between available Fe and Mn; r= 0.7675** between available Fe and Cu; r= 0.7201** between available Fe and Zn; r= 0.8260** between available Mn and Cu; r= 0.7594** between available Mn and Zn; r= 0.7798** between available Cu and Zn). Choudhary *et al*., (2017) reported that available Mn correlated significantly and positively with available Zn (r= 0.927**), available Fe (r= 0.828**), available Cu (r= 0.920**). They further reported significant and positive correlation between available Cu and Zn (r= 0.934**), between available Cu and Fe (r= 0.825**). Available Fe correlated significantly and positively with available Zn (r= 0.848**). Singh *et al*., (2017) reported positive and significant correlation between DTPA extractable micronutrients.

Kumar *et al*., (2018) reported significant and positive correlation between available Zn and Mn (r= 0.385**); positive non-significant correlation between available Zn and Fe (r= 0.029); significant and positive correlation between available Fe and Cu (r= 0.239**).

Multiple Regression equations of DTPA-extractable micronutrients (Zn, Fe, Mn, Cu) with soil properties

Linear stepwise multiple regression equations were carried out to check the relative contributions coming from different soil properties (pH, EC, OC) towards DTPA-extractable micronutrients (Zn, Fe, Mn, Cu). Availability of micronutrients (Zn, Fe, Mn, Cu) in the surface soils was found markedly affected or influenced by soil physico-chemical properties. Researchers (Lindsay, 1991; Brady and Weil, 2008) reported that the variation in DTPA extractable micronutrients in case of different cropping systems may be attributed to the effects of various soil properties such as soil organic carbon content, pH. Multiple regression equations relating to available micronutrients (Zn, Fe, Mn, Cu) with different soil properties (soil pH, EC, soil organic carbon content) were computed and represented in Table 7. 39.10%, 58.90%, 48.00% and 42.30% variabilities recorded in available Zn, Fe, Mn, Cu respectively can be related to the combined effects of soil pH, organic carbon content and electrical conductivity. Soil pH and organic carbon content together accounted for 38.50%, 58.00%, 47.40% and 40.50% variations in available Zn, Fe, Mn, Cu respectively. Soil organic carbon content had greater effect on the predictability of available Zn, Mn, Cu in comparison to soil pH as 23.60%, 25.30% and 29.80% variabilities in the changes of available Zn, Mn, Cu respectively can be related to soil organic carbon content whereas 14.60%, 21.80% and 10.50% variabilities in the changes of available Zn, Mn, Cu respectively are related to soil pH. On the other hand, in case of the predictability of available Fe, more contribution is coming from soil pH (48.90%) in comparison to soil organic carbon content (8.80%). Results of the stepwise multiple regression analysis showed that pH and organic carbon content are the main variables which influence the predictability of the amount of DTPA-extractable micronutrients in soil.

Table 7. Multiple regression equations relating DTPA extractable micronutrients (Zn, Fe, Mn, Cu) with soil properties

Step-down regression equations	($R^2 \times 100$)
DTPA-Zn	
$Y_1 = 0.877 – 0.202\ X_1 + 1.003\ X_2 + 1.986\ X_3$	39.10
$Y_1 = 0.944 – 0.178\ X_1 + 1.802\ X_3$	38.50
$Y_1 = 2.085 – 0.177\ X_1$	14.60
$Y_1 = – 0.108 + 1.792\ X_3$	23.60
DTPA-Fe	
$Y_2 = 50.161 – 5.456\ X_1 + 19.622\ X_2 + 20.747\ X_3$	58.90
$Y_2 = 51.466 – 5.000\ X_1 + 17.148\ X_3$	58.00
$Y_2 = 62.322 – 4.984\ X_1$	48.90
$Y_2 = 21.968 + 16.856\ X_3$	8.80
DTPA-Mn	
$Y_3 = 32.539 – 4.593\ X_1 + 19.673\ X_2 + 39.194\ X_3$	48.00
$Y_3 = 33.847 – 4.135\ X_1 + 35.587\ X_3$	47.40
$Y_3 = 56.375 – 4.103\ X_1$	21.80
$Y_3 = 9.450 + 35.344\ X_3$	25.30
DTPA-Cu	
$Y_4 = 1.365 – 0.223\ X_1 + 2.065\ X_2 + 2.711\ X_3$	42.30
$Y_4 = 1.502 – 0.175\ X_1 + 2.332\ X_3$	40.50
$Y_4 = 2.979 – 0.173\ X_1$	10.50
$Y_4 = 0.471 + 2.322\ X_3$	29.80

Y_1 = DTPA-Zn; Y_2 = DTPA-Fe; Y_3 = DTPA-Mn; Y_4 = DTPA-Cu; X_1 = pH; X_2 = EC; X_3 = OC.

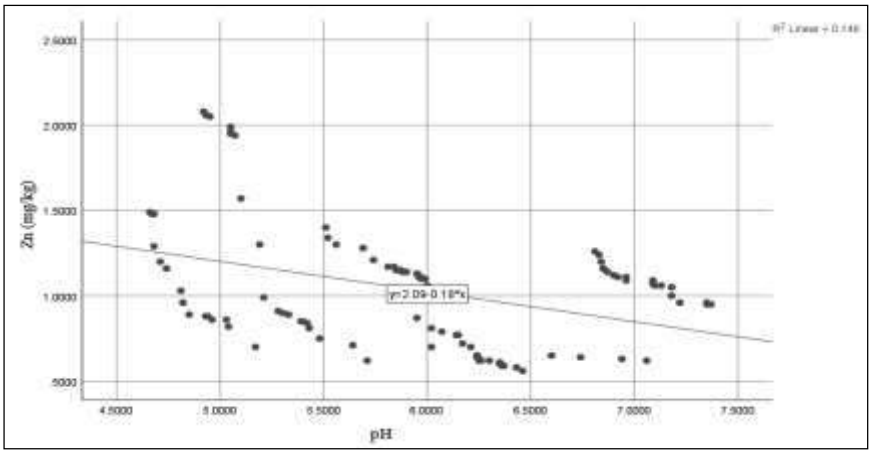

Figure 1. Relationship between soil pH and DTPA-Zn (mg/kg)

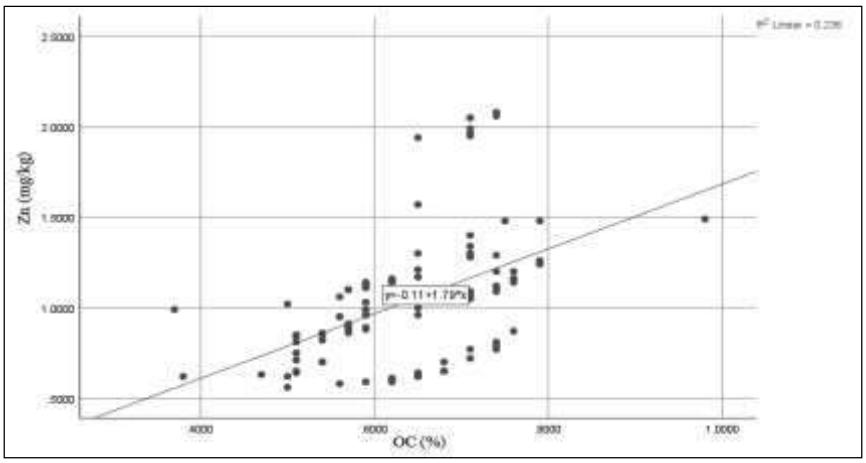

Figure 2. Relationship between soil organic carbon (%) and DTPA-Zn (mg/kg)

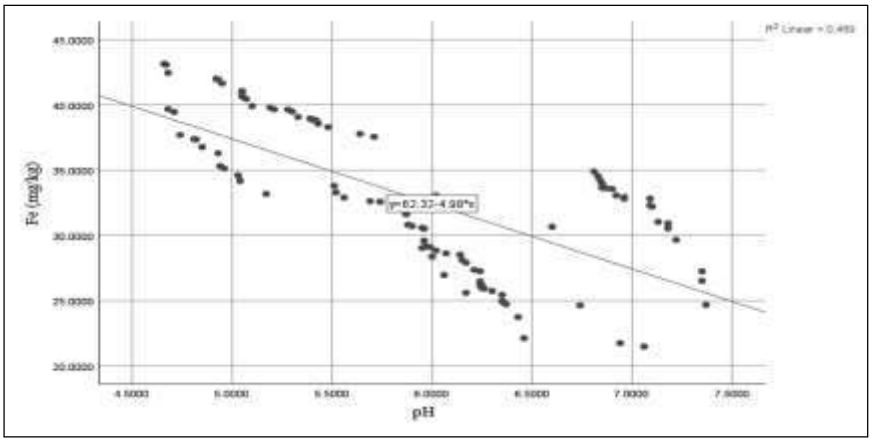

Figure 3. Relationship between soil pH and DTPA-Fe (mg/kg)

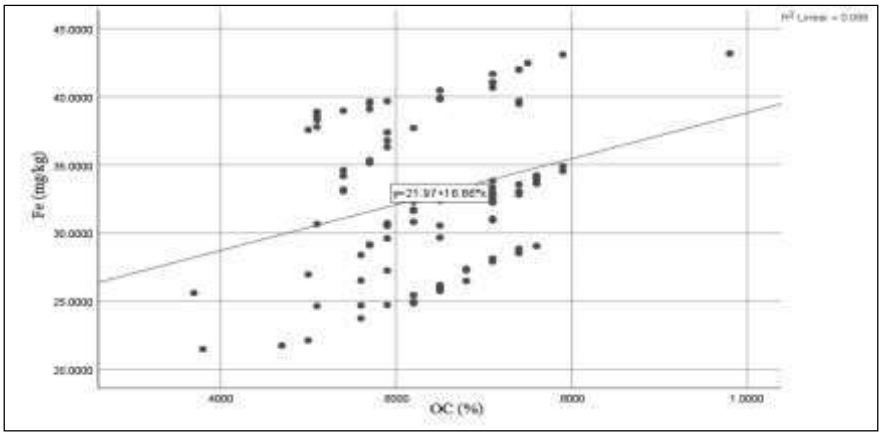

Figure 4. Relationship between soil organic carbon (%) and DTPA-Fe (mg/kg)

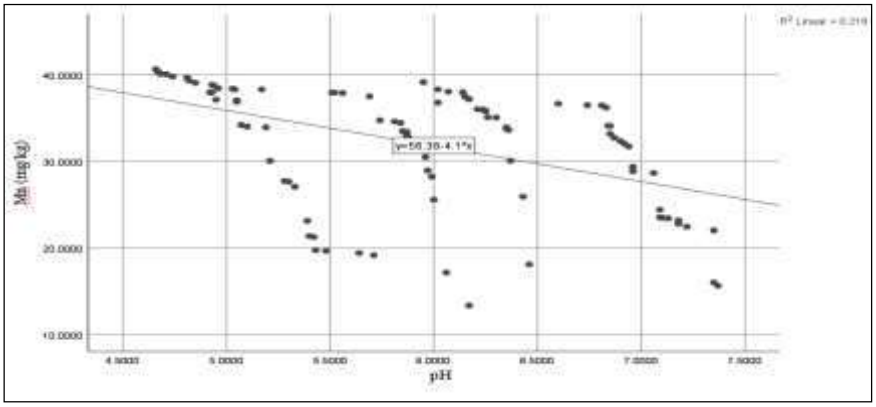

Figure 5. Relationship between soil pH and DTPA-Mn (mg/kg)

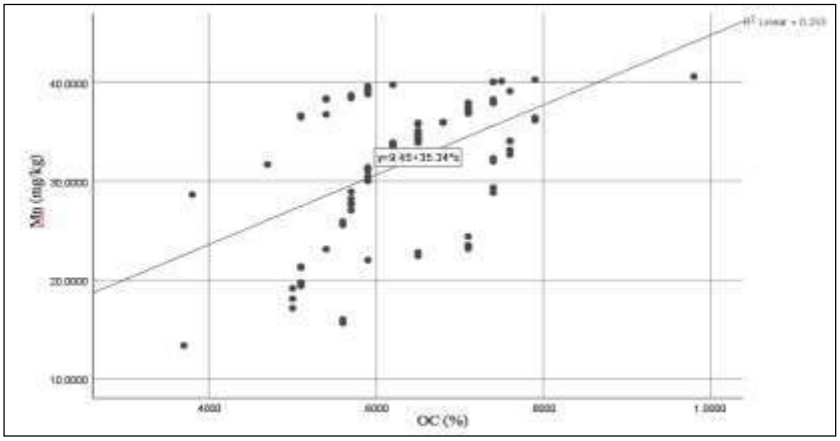

Figure 6. Relationship between soil organic carbon (%) and DTPA-Zn (mg/kg)

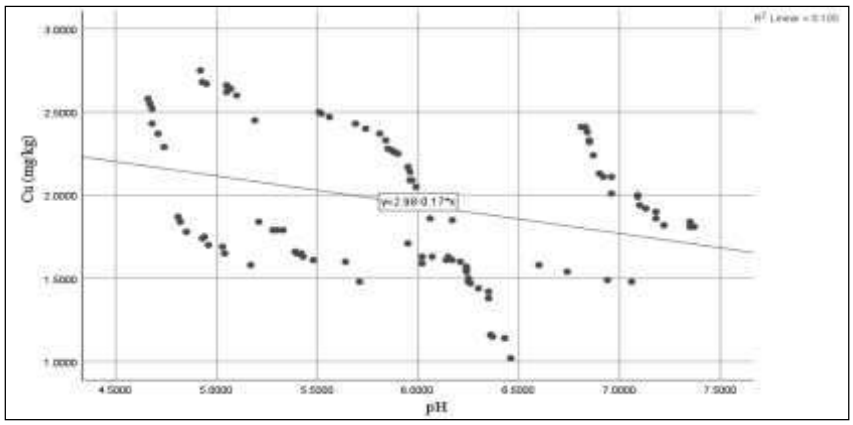

Figure 7. Relationship between soil pH and DTPA-Cu (mg/kg)

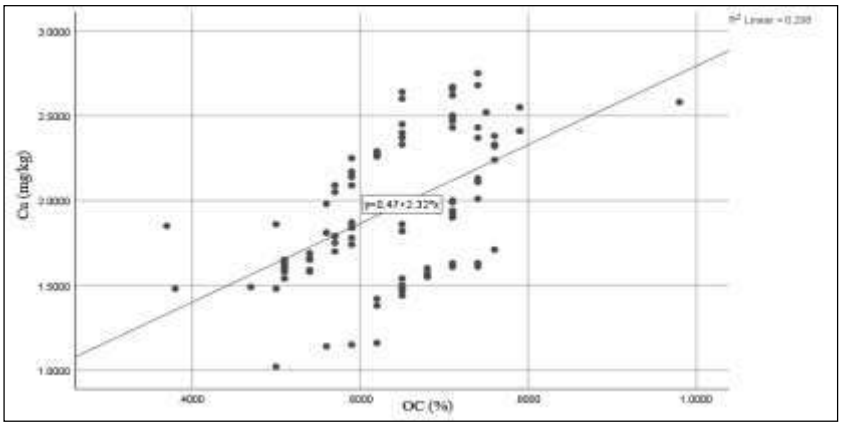

Figure 8. Relationship between soil organic carbon (%) and DTPA-Cu (mg/kg)

CONCLUSIONS

Results revealed that the soils of Birbhum district, West Bengal were strongly acidic to neutral in soil reaction, with low salt concentration and low to medium in soil organic carbon content. 4% of the soil samples were deficient in available Zn, 76% were marginal and 20% were sufficient in available Zn. All the soil samples were sufficient in available Fe, Mn and Cu. The studied soils contained adequate amounts of available micronutrients such as Fe, Mn and Cu whereas deficiencies recorded in case of available Zn. As for maintaining soil health and increasing productivity of crops micronutrients play important role, hence their deficiency appears to be major constraint when it comes to productivity of crops and soils require attention for nutrient management practices. From the correlation study it is found that soil properties such as pH, EC, organic carbon influence greatly on availability of micronutrients in soil. Stepwise multiple regression analysis showed that the major contributions towards available Zn, Fe, Mn, Cu are due to soil pH and organic carbon content.

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THE RACIAL BELONGING OF *PUCCINIA HELIANTHI* SCHWEIN – THE PATHOGEN OF SUNFLOWER RUST IN SOME REGIONS OF THE RUSSIAN FEDERATION

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ABSTRACT

Sunflower rust, caused by a highly specialized fungus *Puccinia helianthi* Schwein, was observed in Russia as early as 1866. There were descriptions of systematically recurring epiphytotics of the disease until 1983. The presence of race 1(100) of rust pathogen isolates, collected in the south of the USSR, was determined in 1962. The dominance of this race in the Northern Caucasus was confirmed in 1981-1983 and the presence of a less virulent race 3(300) was also noted. At the same time, a native sunflower variety Kremniy, resistant to rust, was developed in VNIIMK. Due to the introduction of this variety and its analogues into production, the rust problem in the Russian Federation was solved for a long time. Since then, the determination of *Puccinia helianthi* races has not been carried out in Russia. However, over the past two decades, in different years, depending on weather conditions, a strong prevalence of rust has been observed in the Tambov, Lipetsk, Saratov, Krasnodar regions, and in others places. The objectives of our research was to determine the races of rust pathogen from the Krasnodar, Saratov and Lipetsk regions, using an international standard set of sunflower differentiating lines. Eight lines were used: SM-90, SM 29, P-386, HAR-1, HAR-2. HAR-3, HAR-4, HAR-5. The sunflower variety VNIIMK 8883 was used as susceptible to all races. Six races of *P. helianthi* were identified. The samples from the Krasnodar region were races 100 and 300. These races, including the other two (700 and 772), were found in the Lipetsk region. The isolates from the Saratov region were races 300, 700, 710 and 772. Thus, in three regions of the Russian Federation six races of *Puccinia helianthi* were found on sunflower: 100, 300, 700, 710, 722, 772. It is possible that other races are present in the country; therefore, it is necessary to continue the study by surveying a larger number of fields in different regions.

Keywords: sunflower, rust, obligate parasite, fungus, races

INTRODUCTION

The pathogen of sunflower rust *Puccinia helianthi* Schwein is a basidial fungus (Basidiomycetes), an obligate parasite that is widespread in the world. Rust affects sunflower in almost every country that cultivates this crop: Argentine, USA, Canada, Australia, the European and Asian countries, as well as the Russian Federation (Sackston, 1962; Yang et al., 1986; Gulya, 1990; Gulya, 1997; Sendall et al., 2006; Friskop et al., 2012; Qi et al.,2011; Jing et al., 2015). The occurrence of rust and damage degree of plants vary by year, depend on the time of disease beginning and on the climate conditions during the growth period of sunflower. All development stages of this monoecious fungus take place on sunflower. *P. helianthi* is an autoecious macrocyclic pathogen, thus allowing frequent sexual recombination events and earlier disease onset (Friskop and Markell, 2016).

Since the rust pathogen is an obligate parasite, its variability is determined by the appearance of new races. Many authors have reported on the different racial belonging of *P. helianthi* in

different countries. Bailey (1923) described two "forms" of the pathogen. Further characterization of sunflower rust races was accomplished using three Canadian derived differentials and races were named North American race 1, 2, 3, and 4 (Sackston, 1962). Sackston (1962) has also identified *P. helianthi* isolates from Russia as belonging to race 1. Then, in 1981, race 3 was also identified in Russia (Slyusar, 1981). At that time, the Kremniy variety, resistant to both races of the rust pathogen, was developed in VNIIMK (Slyusar, 1983). Since then, the racial belonging of *P. helianthi* in the Russian Federation has not been determined.

Since 1989, sunflower rust surveys have been completed in the U.S. (Gulya, 1990; Gulya and Viranyi, 1994;; Gulya T.J. 1997; Gulya et al., 1997) During that time, the four differentials were expanded to nine internationally accepted sunflower differentials. Race nomenclature was established using a triplet coding system. Virulence phenotypes were assessed on the nine differentials and virulence formulas could be converted to a three digit race designation (Gulya and Masirevic, 1996). The survey conducted in 2008 determined that the predominant race detected from bulk isolates were race 334 and 336 (Gulya and Markell, 2009). During that time a total of 25 and 31 bulk races were detected in 2007 and 2008 respectively (Gulya and Markell, 2009). Sunflower disease surveys have shown that sunflower rust increased in incidence and severity from 2007 and 2011 (Jing et all, 2015).

The rust infection of sunflower plants at the early stages of development can lead to the growth stop and consecutive death (Lekomtseva, 1976). As Markell et al. (2009) noted, severe yield losses in amounts of 80 % can occur in severely infected fields (Markell et al., 2009). Over the past two decades, in some regions of the Russian Federation (Tambovsky, Saratovsky, Lipetsky, Krasnodarsky), an increase in the prevalence and degree of sunflower damage by rust has been observed (Fig.1).

Figure 1. The sunflower affection by rust in the Saratovsky (left) and Lipetsky (right) regions in 2017 and 2018 years accordingly (orig).

Regularly, an epiphytotic situation occurs. In 2015, in the Tambovsky region, at the end of the sunflower growth period the prevalence of rust at individual fields reached 100 % with a damage rate of 80 % (Vypritskaya, 2015). In the Krasnodar region, sunflower varieties that were previously resistant to rust began to be severely affected. It was noted that the damage degree depends not only on the climatic conditions prevailing during the growth period of sunflower, but also on the ripeness group of the variety (Detsyna et al., 2018). This indicates the possible emergence of new, more virulent races of the pathogen.

The purpose of this work is to determine the racial belonging of *P. helianthi* isolates from some regions of the Russian Federation using internationally accepted differentiating lines of sunflower resistance to rust.

MATERIALS AND METHODS

The leaves affected by rust were collected in 2017-2019 in sunflower sowings in the Saratovsky, Lipetsky, Krasnodarsky regions and were stored in a refrigerator (+4…+6 ºC). Eight differentiating lines of sunflower resistance to rust from the generally accepted standard set were used (Table 1) (Gulya and Masirevic, 1996). Instead of a line susceptible to all races of the pathogen, a sunflower variety VNIIMK 8883 was used, which has never been bred for resistance to rust.

Table 1. Differential-lines for rust pathogen identification (Gulya and Masirevic, 1996)

Set	Line-differentials	Resistance gene	Scoring value
1	7350*		1
	CM90	R_1	2
	CM29	R_2 +R_{10}	4
2	P386	R_{4e}	1
	HA-R1	R_{4a}	2
	HA-R2	R_5	4
3	HA-R3	R_{4b}	1
	HA-R4	R_{4c}	2
	HA-R5	R_{4d}	4
Note: * - instead of line 7350 sunflower variety VNIIMK 8883 was used that is susceptible to all races of *P. helianthi*			

The seeds of differentiating lines of sunflower resistance were sown in rows in flower boxes with a capacity of 6 kg of soil. Then, they were placed on a stack in a growth chamber, where plants were grown at a temperature of 23-25 ºC at day and 20 ºC at night, and with 16-hour photoperiod under artificial light until the emergence of a second pair of true leaves (Fig. 2). Watering was carried out on a daily basis.

Figure 2. The plants of differentiating lines of sunflower resistance in a growth chamber before inoculation with rust pathogen *P. helianthi* (orig.).

The samples of rust-affected leaves with uredopustules were placed in a humid chamber for 24 hours to resume sporulation. Then, the spores were washed off with a brush in distilled water. By using the Goryaev camera, the amount of uredospores in the suspension was calculated. Their optimal concentration for affection the susceptible sunflower plants for 100 % should be 100… 110 thousand pieces in 1 ml of water (Slyusar, 1981). The plants that formed the second pair of true leaves were sprayed with the prepared suspension and the boxes with them were placed in a humid chamber for 24 hours. After inoculation, the plants were grown under the same conditions until the appearance of affection signs on the leaves. The incubation period at 25 ºC took 9 -14 days. When pustules appeared on the leaves, the number of affected plants of each differential-line was taken into account. Infection type was evaluated 13-15 days post-inoculation according to a modified 0-5 scale from Yang et al., (1986). Infection types 3 or greater were considered to be a susceptible reaction (Yang et al., 1986).

RESULTS AND DISCUSSION

Each tested isolate of *P. helianthi* affected the differential-lines susceptible to it with a degree exceeding 3-4 points of the known scale described by Yang et al. (1986) (Fig. 3, 4).

Figure 3. The affection of differentiating line CM 90 of sunflower resistance to rust with *P. helianthi* isolate from the Krasnodarsky region with virulence code 300 (orig.)

Figure 4. Uredopustules of *P. helianthi* isolate from the Saratovsky region with virulence code 700 on a leaves of differentiating line of sunflower resistance CM 29 (orig.)

Among the entire sample of *P. helianthi* isolates collected in the sunflower sowings of the Saratovsky, Lipetsky and Krasnodarsky regions, a total of six races were differentiated with virulence codes 100, 300, 700, 710, 722 and 772 (Table 2). Moreover, the races 700, 710, 722 and 772 were identified in the Russian Federation for the first time.

The isolates of rust pathogen, collected over the period of three years in the Saratovsky region, were divided into races with code numbers 300, 700, 710 and 722. Race 700 was present in isolate samples of all three years of collection. Race 300 has been identified among isolates collected in 2018 and 2019.

Among the 14 isolates of *P. helianthi*, collected in the Lipetsky region in 2018, race 100, 300, 700 and 772 were differentiated. Nine isolates collected the same year in the Krasnodar region were represented by races 100 and 300 (Table 3). According to this preliminary data, it can be assumed that races 300, and 700 are quite widespread in the studied regions.

Table 2. The response of differential-lines of sunflower resistance to rust during their artificial infection with the *Puccinia helianthi* pathogen from some regions of the Russian Federation

Line-differential	Scoring value	Reaction on inoculation: S –susceptible; R -resistant					
Triplet 1							
VNIIMK 8883	***1***	S	S	S	S	S	S
CM 90	***2***	R	S	S	S	S	S
CM 29	***4***	R	R	S	S	S	S
Triplet 2							
P386	***1***	R	R	R	S	R	S
HA-R1	***2***	R	R	R	R	S	S
HA-R2	***4***	R	R	R	R	R	S
Triplet 3							
HA-R3	***1***	R	R	R	R	R	R
HA-R4	***2***	R	R	R	R	S	S
HA-R5	***4***	R	R	R	R	R	R
Virulence code		100	300	700	710	722	772

Such a long existence of races with code numbers 100 and 300 in the Russian Federation can be explained by the fact that here, in addition to hybrids, both oil and confectionery sunflower varieties are still widely cultivated. Besides, the selection for rust resistance has not been carried out since 1983. It is known that *P. helianthi* is capable of affecting both oil and confectionery sunflower, as well as wild-growing species (Gulya, 2006). Cross-pollination among plants of sunflower varieties contributes to the preservation of genotypes on which these races can reproduce themselves. It is known that the races of obligate parasites, to which the rust pathogen belongs, disappear as the cultivation of susceptible to them range of varieties of agricultural crops, on which they could sustain their existence, ceases.

Sunflower varieties that were not selected for rust resistance can be called reserves for the preservation of old races of rust pathogen in the Russian Federation. At the same time, the presence of other races of *P. helianthi* is not excluded, both in the studied regions and in other areas of the Russian Federation where sunflower is cultivated. On large areas of the country, various sunflower hybrids of foreign breeding are cultivated. Moreover, the periods for crop returning to the former field are often reduced to 1-3 years due to its high profitability. This situation contributes to the acceleration of race formation in obligate parasites of sunflower and to their rapid spread in agrocenoses. A large-scale expedition is necessary to collect *P. helianthi* isolates, to differentiate them, and to determine the dominant races in different regions.

Table 3. Race codes of *Puccinia helianthi* isolates, collected in Saratovsky, Lipetsky, Krasnodarsky regions of Russian Federation, 2017-2019 years

The region of isolate collection	The year	The quantity of isolates	Race code
Saratovsky	2017	6	700
		5	722
	2018	7	300
		8	700
	2019	4	710
		2	700
		6	300
Lipetsky	2018	2	100
		3	300
		4	700
		5	772
Krasnodarsky	2018	5	300
		3	100

CONCLUSIONS

Thus, in three regions of the Russian Federation (Saratovsky, Lipetsky, and Krasnodarsky) 6 races of sunflower rust pathogen were discovered. Four races of them (700, 710, 722, and 772) are highly virulent and were identified in Russia for the first time. Old races 100 and 300 were also discovered. It is assumed that their long-term preservation is facilitated by the wide cultivation of sunflower varieties that have not been selected for rust resistance.

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EVALUATION OF MULTIELEMENT EXTRACTANTS FOR PREDICTION OF AVAILABLE PLANT NUTRITIENTS IN SLIGHTLY ALKALINE SOILS IN KAHRAMANMARAS-TURKEY

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ABSTRACT

Multi-element soil tests such as ammonium bicarbonate-DTPA (AB-DTPA) and Mehlich 3 have been employed by soil testing laboratories in some countries, since they reduce labor, time, and chemical losses and allow simultaneous measurement of many elements by using the Inductively Coupled Plasma (ICP). However, information on their efficiencies for assessing the availability of nutrients in Turkey's soils is still inadequate. The objective of this study was to evaluate AB-DTPA and Mehlich 3 in comparison to routine soil tests (Olsen for P; NH4OAc for K, Ca, Mg, and Na; DTPA for Fe, Mn, Cu, and Zn; Hot water for B) for simultaneous measurements of 10 elements (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), and boron (B)) in slightly alkaline Kahramanmaras soils. Pearson correlation analysis were applied to investigate the relationship between AB-DTPA or Mehlich 3 and the respective soil test. The results can be summarized as follows: 1) the best correlations were found for K and Cu with both extractants and routine soils tests; 2) The AB-DTPA method showed significant correlations with routine test for all the elements studied but Mehlich 3 did not have any correlations with routine tests for Na and B; 3) the highest correlation coefficients were achieved in Zn (r= 0.998**) with AB-DTPA and in K (r= 0.876**) with Mehlich 3 compared with routine soil tests; 4) while a low negative relationship at the level of 1% was found with AB-DTPA for Ca, a moderately positive relationship at the level of 1% was determined with Mehlich 3. The findings of the study strongly encourages new research on multielement methods and plant nutrient uptake studies in the region.

Keywords: *Multielement extractants, AB-DTPA, Mehlich 3, available plant nutrition, slightly alkaline soils.*

INTRODUCTION

Diagnosis of plant nutrients in the soil by chemical analysis is essential in determining the amount of fertilizer to be applied (Yanai et al., 2000). The suitability and usability of the soil analysis method in terms of the territory of the region reveals the quality of that analysis process. Therefore, a diagnostic method is required both to save labor and time and to quickly identify nutrients in the soil, because many soil samples are taken at once in a season (Uwasawa, 1994).

The United States has a history trying to implement and develop soil analyzes since the early 1940s (Jones, 1998). Due to improvements in soil testing techniques, Quebec and Canada's Prince Edward Islands (Liu and Bates, 1990), the Netherlands (Houba, 1998), Malawi (in East Africa) (Chilimba et al., 1999), Spain (Monterroso et al. ., 1999), Turkey (Yıldız et al., 1999), Czech Republic (Zbiral and Nemec, 2000), Thailand (Aramrak, 2007), India (Joshi et al., 2014) and many other countries are trying to adapt these methods to their own soils. These developed techniques allowed the plant nutrient to be determined by reading it with a single extraction solution by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Yıldız et al. (1999), engaged in research related to the first multi-extraction methods in Turkey. In this study, Mehlich 3 extraction method was tried in 20 Erzurum plain soils and 14 acid soils, but no relation was found between Mehlich 3 method and extraction of available nutrients to the plant. Later, Gürbüz and Günay (2013) measured the amount of nutrients P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B in the acid, neutral and alkaline soils with conventional and multiple extraction methods and determined the correlations between them. According to this study, it was reported that Mehlich3 and acid ammonium acetate-EDTA (AAAc-EDTA) methods in acid soils and Mehlich3 and AB-DTPA methods in neutral and alkaline soils gave high correlations. However, it still remains uncertain since there is not enough work done on this subject in our country. The purpose of this study; using multiple elemental analysis methods such as AB-DTPA and Mehlich 3, to determine the amount of available plant nutrients in the soil and to obtain the relationship between the methods and the routine soil tests.

MATERIAL AND METHODS

Soils

The soil samples were collected from Afsin and Elbistan districts of Kahramanmaras province (Figure 1). A total of 239 surface (0-20 cm) soil samples were taken from Afsin (99 samples) districts and from Elbistan districts (140 samples). Soil samples were collected from agricultural lands by determining their coordinates with GPS device. The soil samples brought to the laboratory were laid in clean polyethylene tubs, and stone, all kinds of garbage and plant particles were removed and dried in the open air. The dried soils were beaten with wooden mallets, passed through a 2 mm steel sieve and made suitable for analysis.

Laboratory analysis

Some properties of the soil samples were determined like soil texture determined according to the hydrometer method (Bouyoucos, 1951), pH in saturated soil (saturated mud) was measured by pH meter with glass electrode (Thomas, 1996), electrical conductivity (EC) measured from saturated mud with electrical conductivity device (Richards, 1954), total lime it was determined volumetrically in Scheibler calcimeter (Klute, 1986), organic matter (OM) it was determined by the modified Walkley-Black method (Nelson ve Sommers, 1996), available P determined by spectrophotometer device according to Olsen method (Olsen et al., 1954), available K, Ca and Mg and extractable Na determined by measuring with ICP-OES according to ammonium acetate method (Helmke ve Sparks,1996), available Fe, Mn, Cu and Zn filtered soil solutions obtained from soils extracted with DTPA solution were determined by measuring with ICP-OES (Lindsay and Norvell, 1978) and available B determined according to the hot water method (Klute, 1986), AB-DTPA (Soltanpour and Schwab, 1977) and Mehlich 3 (Mehlich, 1984).

Ammonium bicarbonate-DTPA method

On the basis of this method developed by Soltanpour and Schwap (1977), 20 ml of AB-DTPA solution (0.005 M DTPA + 1 M NH_4HCO_3, pH: 7.6) was added to 10 g of the soil sample ready for analysis, shaken in shaker for 15 minutes and then filtered. Amounts of P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn and B passing from soil samples to the solution were determined by the ICP-OES device.

Mehlich 3 method

According to the method determined by Mehlich (1984), 15 ml of Mehlich 3 solution (0.2 N glasial acetic acid (CH_3COOH), 0.25 N ammonium nitrate (NH_4NO_3), 0.015 N ammonium fluoride (NH_4F), 0.13 N nitric acid (HNO_3) and 0.001 M EDTA (Ethylene diamine tetra acetic acid), pH: 2) was added to 2 g of the soil sample ready for analysis, shaken in shaker for 5 minutes and then filtered. Amounts of P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn and B passing from soil samples to the solution were determined by the ICP-OES device.

Statistical evaluation

Relationships between routine analysis results and multiple elemental analysis results such as AB-DTPA and Mehlich 3 were determined according to Pearson correlation analysis in SPSS (2018) statistical software program.

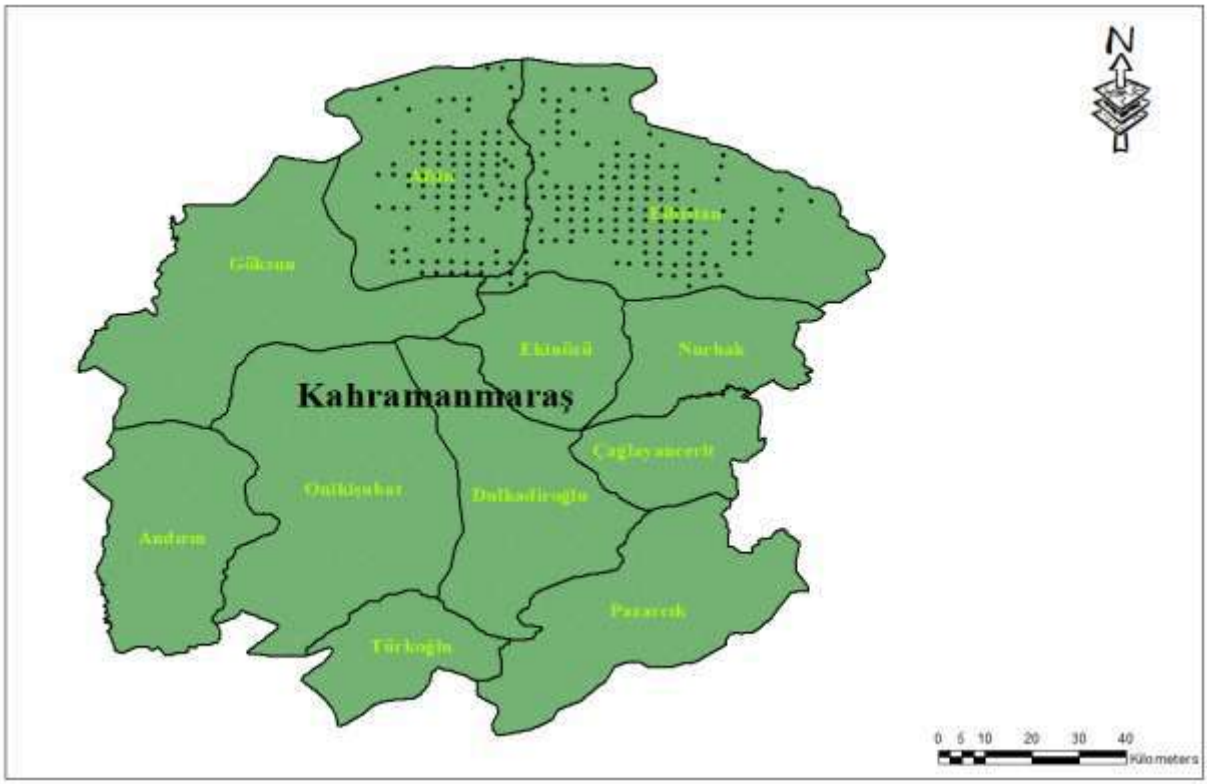

Figure 1. Map showing the points where soil samples were collected

The significance level of the correlation coefficients between routine and multiple extraction methods were evaluated according to the data of Atan (Gürbüz and Günay, 2014) in Table 1.

Table 1. Correlation coefficient significance level (Gürbüz and Günay, 2014)

Correlation coefficient	Significance level
0 - 0.25	Very low relationship
0.26 - 0.49	Low relationship
0.50 - 0.69	Moderate relationship
0.70 - 0.89	Highly relationship
0.9 – 1.0	Very highly relationship

RESULTS

Soil Properties

Determined soil parameters are given in Table 2. According to this; the amount of sand was between 10.4% and 81.2% and the average was 40.4%, the amount of silt was between 1.0% and 54.1%, the average was 16.9%, the amount of clay was 15.2% and 81.2%, and the average was 42.7%. The average texture class of the soils was found as clay. Soil pH changed between 6.47-8.18, and average was 7.65 (slightly alkaline), soil EC ranged from 0.38 dS m^{-1}-2.66 dS m^{-1}, and mean was 0.95 dS m^{-1} and slightly saline, total lime of soil changed between 0.88-39.56% and the average was 20.53% too chalky and, the organic matter of the soils ranged from 0.42% to 4.81%, and the average was determined as 2.14% and medium class.

Table 2. Minimum, maximum and average values of the determined soil properties

Determined soil parameters	Sand (%)	Silt (%)	Clay (%)	Texture	pH	EC (dS m^{-1})	$CaCO_3$ (%)	OM (%)
Range	10.4-81.2	1.0-54.1	15.2-81.2	C	6.47-8.18	0.38-2.66	0.88-39.56	0.42-4.81
Mean	40.4	16.9	42.7		7.65	0.95	20.53	2.14

The minimum, maximum and average values of the soils determined as results of these analyzes are given in Table 3. According to the mean values for routine, AB-DTPA and Mehlich 3 methods, macronutrients were found as 14.92, 8.91 and 52.73 mg kg^{-1} for P, 381, 255 and 386 mg kg^{-1} for K, 8089, 247 and 15767 mg kg^{-1} for Ca, 373, 130 and 343 mg kg^{-1} for Mg, and 50.97, 9.91 and 345 mg kg^{-1} for extractable Na respectively. Microelements were determined as 4.48, 7.05 and 40.64 mg kg^{-1} for Fe, 10.19, 8.83 and 110 mg kg^{-1} for Mn, 1.46, 2.87 and 3.53 mg kg^{-1} for Cu, 1.06, 1.37 and 2.51 mg kg^{-1} for Zn, and 0.71, 0.03 and 6.46 mg kg^{-1} for B respectively.

Table 3. Minimum, maximum and average values (mg kg^{-1}) of some parameters of soils obtained by routine and multiple elemental analysis methods

Tests	Range-Mean	P	K	Ca	Mg	Na	Fe	Mn	Cu	Zn	B
Routine tests	**Range**	2.25-55.50	69.49-1070	1138-13977	91.94-2524	4.46-198	0.80-20.66	2.35-69.43	0.30-6.21	0.09-68.79	0.01-3.07
	Mean	14.92	381	8089	373	50.97	4.48	10.19	1.46	1.06	0.71
AB-DTPA	**Range**	0.58-711	34.60-823	123-352	9.67-607	1.41-107	1.16-46.81	0.88-73.11	0.38-12.26	0.10-76.35	0,00-0,25
	Mean	8.91	255	246.80	130	9.91	7.05	8.83	2.87	1.37	0.03
Mehlich 3	**Range**	2.21-596	48.42-1289	1492-30031	90.03-2608	214-697	5.48-261	26.30-426	0.63-16.46	0.30-26.40	0.24-52.74
	Mean	52.73	386	15767	343	345	40.64	110	3.53	2.51	6.46

After the properties of each soil were determined by the routine, AB-DTPA and Mehlich 3 extraction methods, the relationships between these methods were also examined (Table 4).

According to this; all the relationships among the methods are at 1% level and very important. Among all the correlations, it is seen that there is only in Ca element a negative relation. The negative correlation was found between ammonium acetate and AB-DTPA methods. However, this negative relationship is significant at the level of 1% again. The highest correlations were determined between the routine and AB-DTPA methods for Zn (r= 0.998**) and K (r= 0.878**). With the Mehlich 3 method, the highest correlation was obtained in the K element (r= 0.876**). The highest relationship between AB-DTPA and Mehlich 3 multielemental analysis methods was determined in K (r= 0.804**). No significant relationship was found between Routine and Mehlich 3 for sodium and boron (NS). Similarly, no significant relationship was detected for calcium and boron (NS) also between AB-DTPA and Mehlich 3.

Table 4. Relationships between extraction methods

Methods and correlations	P	K	Ca	Mg	Na	Fe	Mn	Cu	Zn	B
Routine x AB-DTPA	0.283**	0.878**	-0.256**	0.675**	0.509**	0.869**	0.850**	0.783**	0.998**	0.468**
Routine x Mehlich3	0.426**	0.876**	0.596**	0.637**	NS	0.622**	0.454**	0.740**	0.310**	NS
AB-DTPA x Mehlich3	0.636**	0.804**	NS	0.276**	0.381**	0.559**	0.207**	0.747**	0.329**	NS

****:** Significant at p< 0.01; **NS:** Not significant

DISCUSSION

Mean values

It is seen that the same nutrient elements are extracted in different amounts by all three extraction methods. It is thought that this is due to the fact that each extraction method has different pH values and different shaking times. Looking at the averages, it is understood that the order of the methods that extract the most nutrients is Mehlich 3 > Routine > AB-DTPA in macro elements. However, in the ammonium acetate method, it is seen that the mean of Mg is higher than the Mehlich 3 method. This can be explained by the fact that the shaking time of 60 minutes in the routine analysis method caused more of the magnesium to pass to the soil solution than the clay surfaces according to the Mehlich 3 method. The order of methods that extract the most nutrients in microelements is Mehlich 3> AB-DTPA> Routine, for iron, copper and zinc. It is understood from Table 3 that this ranking altered to Mehlich 3> Routine> AB-DTPA for manganese and boron. This is because power of AB-DTPA to extract Mn and B is less than the DTPA solution of which pH is 7.3 and the shaking time is 2 hours.

Correlations

It is seen that all correlations obtained between extraction methods are significant at the level of 1% (p< 0.01).

P correlations

In this study, for the obtained P values, between the Olsen method (routine method) and the AB-DTPA method was found a significant at the level of 1% (p< 0.01) but a low relationship (r= 0.283**). While there was determined a significant at the level of 1%, but low relationship between Olsen and Mehlich 3 extraction methods, there was found a moderate relationship between AB-DTPA and Mehlich 3 extraction methods (r= 0.426** and r= 0.636**, respectively). Gürbüz and Günay (2014) stated that both the AB-DTPA-P method (r= 0.426**) and Mehlich 3-P method (r= 0.413**) showed significant at the level of 1% but low relationships with the Olsen-P method on 123 neutral and alkaline soils collected from the

agricultural lands. Elrashidi et al. (2003) reported that in alkaline soils both AB-DTPA and Mehlich 3 showed high correlations with Olsen method, but Mehlich 3-P gave a stronger relationship with Olsen method (r= 0.926**) than AB-DTPA-P (r= 0.689**). The amount of P obtained by AB-DTPA and Mehlich 3 multi-nutrient extraction methods were strongly correlated (r= 0.636**).

K, Ca, Mg and Na correlations

Correlations between ammonium acetate (AA) (routine method) x AB-DTPA and ammonium acetate (AA) x Mehlich 3 for potassium (r= 0.878** and r= 0.876**, respectively) and magnesium (r= 0.675** and r=0.637**, respectively) were found very close to each other. Both AB-DTPA-K and Mehlich 3-K methods gave a high relationship for potassium, while both methods showed a moderate relationship for magnesium. Some researchers also reported that they obtained high correlations for K and Mg between both AA x AB-DTPA and AA x Mehlich 3 methods in alkaline soils (Elrashidi et al., 2003; Gürbüz and Günay, 2014). Relationships between AB-DTPA and Mehlich 3 methods were also found to be high in K and low in Mg. It showed that these two methods are more reliable for K than Mg. For calcium, the correlation between AA-Ca and AB-DTPA-Ca methods was significant at the level of ($p< 0.01$), but a negative and low relationship was found (r= -0.276**). In other words, while the amount of Ca in AA increased, the amount of Ca in AB-DTPA decreased. Or the opposite is the case. A similar relationship (r= -0.273**) was also reported by Elrashidi et al. (2003), Gürbüz and Günay (2014). Precipitation of lime explains the failure of the AB-DTPA multiple extraction method in alkaline soils, where the amount of soluble Ca is too high (Soltanpour, 1991). A moderately significant relationship at the level of 1% level was obtained between AA-Ca and Mehlich 3-Ca (r= 0.596**), but no significant relationship was found between AB-DTPA-Ca and Mehlich 3-Ca (NS). For extractable Na, a moderately significant relationship at 1% level between AA-Na and AB-DTPA-Na (r= 0.509**), and a weak relationship at 1% level between AB-DTPA-Na and Mehlich 3-Na (r= 0.381**) were determined. On the other hand, no significant relationship was found between AA-Na and Mehlich 3-Na (NS). Elrashidi et al. (2003) reported that they obtained significant correlations at the level of 1% between routine analysis and AB-DTPA (r= 0.946**) and Mehlich 3 (r= 0.996**) methods in alkaline soils. Madurapperuma and Kumaragamage (2008) stated in their study that they found a significant relationship at the level of 0.1% ($p <0.001$) between AA and AB-DTPA methods (r= 0.78***) for Na.

Fe, Mn, Cu, Zn and B correlations

For Fe, Mn, Cu and Zn elements, significant relationships were determined at the level of 1% among all methods. For B, only between hot water-B (routine-B) and AB-DTPA-B extraction methods were obtained a significant relationship. Gürbüz and Günay (2014) found a similar result for the B nutrient element between routine and AB-DTPA (r= 0.300**) in a study they conducted in alkaline soils. No significant relationship was found between routine-B x Mehlich-3-B and AB-DTPA-B x Mehlich 3-B methods (NS). While between DTPA (routine) and AB-DTPA was determined a significant and high relationships at 1% level for Fe, Mn and Cu (r= 0.869**, r= 0.850** and r= 0.783**, respectively), a very high correlation was found for Zn (r= 0.998**). While all of the relationships between DTPA and AB-DTPA were high for the Fe, Mn, Cu and Zn elements, the correlation of only Cu element was high in the other two (r= 0.740** and r= 0.747**). Some researchers had also reported that the relationships between routine and AB-DTPA for Fe, Mn, Cu and Zn in alkaline soils were high (Elrashidi et al., 2003; Gürbüz and Günay, 2014).

CONCLUSIONS

In the light of the data, it was concluded that AB-DTPA extraction method could be used for macroelements (P, K and Mg), and extractable Na and microelements (Fe, Mn, Cu, Zn and B) in alkaline soils. However, Mehlich 3 multielement extraction method could also be recommended for simultaneous measurement of all these elements except for Na and B. Yet, upon careful examination, it can be considered that AB-DTPA is a better method for these soils than Mehlich 3. Further research is necessary in order to test the extractants in several soil types in Turkey and to determine other nutrients particularly when ICP is used for measurement of the quantity.

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THE EFFECTS OF DEFICIT IRRIGATION ON NET PHOTOSYNTHESIS RATE, STOMATAL CONDUCTANCE, INTRACELLULAR CO2 CONCENTRATION AND TRANSPIRATION RATE IN SUGAR BEET

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ABSTRACT

In this study, the effects of deficit irrigation applications on net photosynthesis rate (A), stomatal conductance (gs), intercellular CO2 concentration (Ci) and transpiration rate (E) physiological parameters in sugar beet were examined in Bursa Uludağ University Agricultural Application and Research Center in 2019. Plants were irrigated with drip irrigation method and physiological measurements were made on 12 August, 29 August and 9 September. Irrigations were scheduled based on the replenishment of 100% (S1), 67% (S2), 33% (S3), and 0% (S4) of soil water depletion from a soil depth of 90 cm using a 7-day irrigation interval. According to the research results; It was determined that all physiological parameters were significantly affected by different water stress conditions ($p < 0.01$) on all of the measurement days. The A, gs, Ci and E values measured in the irrigation treatments have changed in that intervals respectively; 6.03-19.00 µmol CO2 m-2 s-1, 0.0747-0.4742 mol H2O m-2 s-1, 237.83-294.00 µmol CO2 mol-1, 1.89-5.31 mmol H2O m-2 s-1.

Keywords: Deficit Irrigation, Sugar Beet, Net Photosynthesis Rate, Drip Irrigation

INTRODUCTION

In the world, sugar was produced from sugar cane until the end of the 1800s. The existence of sugar in sugar beet was first determined in studies conducted in Germany in the 18th century, and sugar production from sugar beet began at the beginning of the 19th century. The climatic zones located our country are not suitable for sugar cane production. Since our country is located between 36-42° north latitudes in the northern hemisphere and in the subtropical climate zone, all of the sugar produced is obtained from sugar beet due to the suitability of climate and soil conditions (Anonymous, 2018). In 2018, the total sugar beet production area in the world was 4 809 490 hectares. In the same year, the world sugar beet production amounted to 274 886 306 tons (FAOSTAT, 2018). In 2019, 18 085 528 tons of sugar beet were obtained from 310 000 hectares of production area in Turkey (TUIK, 2019). Between the years 1961-2018, according to the average yield for Turkey and the world, it is observed that the two values are close to each other all the time. (FAOSTAT, 2020).

Today, the increasing population due to global warming and world conditions has increased the use of agricultural land and water and reduced the sustainability rate of these resources. In irrigated agriculture areas in the world, especially in arid and semi-arid climates; insufficient precipitation and high evaporation increase soil dryness. Due to the decrease in the quantity and

quality of water resources worldwide, deficit irrigation practices are becoming more important day by day. As a result of the planning of the irrigation system according to limited conditions, there is a significant decrease in water, energy and capital needs, and consequently, the income of the enterprise can be increased (English et al., 1990). For this reason, it has become necessary to consider approaches such as deficit irrigation management that provides water increase in order to be able to irrigate more effectively and irrigate more areas with our existing water resources allocated to agriculture (Ünlü et al., 2008). This approach, which aims to use less water in crop production with acceptable losses in yield parameters in plants with different irrigation levels throughout the growing period, is named with different names; Partial Irrigation, Regulated Deficit Irrigation, ET Deficit Irrigation and Limited Irrigation (English et al., 1990; Kanber et al., 2007). On the other hand, photosynthesis, which is an important parameter of plant physiology, is affected by different irrigation levels. For plants which irrigating by deficit irrigation, the decrease in photosynthesis rate can be attributed to the decrease in leaf width or the water stress. Along with these, it can be attributed to the decrease in the amount of C by physiological closure of the stomata or the decrease in the amount of light per unit area per leaf (Bloch et al., 2006). The level of different irrigations affects photosynthesis parameters independent of stomatal factors. In addition to closing and opening of stomata, inhibition of mesophyll activity (non-stomatal restriction of photosynthesis) occurs only under severe or prolonged drought stress. With photosynthesis values changing with different irrigation levels; changes in net photosynthesis rate, stomatal conductivity, intercellular CO2 concentration and transpiration values are also observed.

The researches of the effects on physiological parameters of deficit irrigation on sugar beet plants in Turkey and in the world are limited in number (Tognetti et al., 2003; Bloch et al., 2006; Köksal, 2006; Li et al., 2019). Studies on sugar beet irrigation in Turkey are usually about water-yield relationships (Süheri et al., 2007; Poçan, 2008; Tari et al., 2013;). This study aims to determine effects of deficit irrigation applied with drip irrigation on the net photosynthesis rate (A), stomatal conductivity (gs), intercellular CO2 concentration (Ci) and transpiration (E) in sugar beet in the sub-humid climate.

MATERİAL AND METHODS

The research was carried out on the experimental area of Bursa Uludağ University Faculty of Agriculture Agricultural Application and Research Center. The experimental area is located at an average altitude of 112 m and 40º 13 '33 "north (N) latitude, 28º 51' 34" east (E) longitude. The soils of the experimental area are in the clay structure. For 0-120 cm soil depth, considering 30 cm soil layers, the volumetric mass is 1.35-1.38 g cm-3; the field capacity ranged from 38.17%-43.01%and the wilting point ranged from 23.18%-27.07%. Considering the effective rooting depth of sugar beet (90 cm), the water-holding capacity for 0-90 cm soil depth was calculated as 163.3 mm. Bursa, located in the south-east of the Marmara Region, has a mild climate, with hot and dry summers and cool and rainy winters (Candoğan et al., 2013). The long-term climate data (1960-2019) (Anonymous, 2020a)and the 2019 growing season climate data (Anonymous, 2020b) of the experimental area are given in Table 1.

"KWS Akazia" sugar beet (Beta vulgaris L) variety was used as the plant material. Sugar beet seeds were hand-sown on May 1, 2019, approximately 3 cm deep in the soil, with a row spacing of 45 cm and a plant-plant spacing of 20 cm. After planting, 15-15-15 NPK compound fertilizer at an effective substance dose of 5 kg da-1 was perfused by hand on the plots. Irrigation water was applied to the plots by sprinkler irrigation method for the germination and emergence of seeds. The experiment was carried out with three replications according to the randomized

blocks design.The area of each trial plot was formed by 5 plant rows in planting; Plot area was designed to be 2.25 m x 6.0 m = 13.5 m2. In the research, 4 different experimental treatments were created with different irrigation levels. Irrigations were scheduled based on the replenishment of 100% (S1), 67% (S2), 33% (S3), and 0% (S4) of soil water depletion from a soil depth of 90 cm using a 7-day irrigation interval. Gravimetric method was used to determine the irrigation water to be applied.

Table 1. Long terms (1960-2019) monthly averages and experimental area 2019 climate data for growing period.

	Average temperature (ºC)		Relative Humidity (%)		Wind Speed (m/s)		Precipitation (mm)	
Months	2019	1960-2019	2019	1960-2019	2019	1960-2019	2019	1960-2019
May	19.6	17.6	67.3	68.1	2.3	2.0	40.4	46.0
June	23.7	22.0	68.6	62.3	2.9	2.0	51.2	36.7
July	23.6	24.4	64.6	59.6	2.8	2.3	37.9	15.8
August	24.5	24.2	64.3	61.5	3.2	2.3	39.1	18.9
September	21.3	20.3	63.5	66.8	2.9	1.9	11.3	42.7

According to the water analysis results; Irrigation water to be used in the experimental area is classified as C2S1. Irrigation water was applied by drip irrigation. A polyethylene lateral drip line was designed for each row using 16 mm-dia. lateral pipes with 2 L h-1 in-line drippers spaced at 20 cm. Net photosynthesis rate, stomatal conductivity, intercellular CO2 concentration and transpiration physiological measurements were measured with a portable photosynthesis system (Li-6400, LI-COR Inc., Lincoln, NE, USA) between 13:00 and 14:00. In the measurements, young and mature upper leaves were used without detaching them, and measurements were made on two leaves in two plants in each plot. Portable photosynthesis system uses equations derived by von Caemmerer and Farquhar (1981) as the basis for net photosynthesis rate, transpiration, and stomatal conductivity.

Transpiration

The mass balance of water vapor in an open system is given by 14th equation.

sE = uowo – uiwi (14)

where s is leaf area (m-2), E is transpiration rate (mol m-2 s-1), ui and uo are incoming and outgoing flow rates (mol s-1) from the chamber, and wi and wo are incoming and outgoing water mole fractions (mol H2O mol air-1). Since

"E = " ("u" _"i" "(" "w" _"o" -"w" _"i" ")")/("s(1" -"w" _"o" ")") (15)

The relationships between the terms are 14-15 and what the LI-6400 measures are

ui = F/106 (16)

wi = Wr/103 (17)

wo = Ws/103 (18)

s = S/104 (19)

where F is air flow rate (μmol s-1), Ws and Wr are sample and reference water mole fractions (mmol H2O (mol air)-1), and S is leaf area (cm-2). The equation that the LI-6400 uses for transpiration is:

"E = " ("F(" "W" _"s" -"W" _"r" ")")/("100S(1000" -"W" _"s" ")") (20)

Data for A, gs, Ci, and E were subjected to analysis of variance (ANOVA). The F-test was used to determine the effects of irrigation treatment and block at the 0.05 and 0.01 probability levels, with F-protected least significant difference (LSD) calculated at 0.05, according to Turan (1995).

Total Conductance to Water Vapor

The total (includes stomatal and boundary layer) conductance of the leaf gtw (mol H2O m-2 s-1) is given by

"g" _"tw" " = " "E" ("1000" -("W" _"l" " +" 〖" W" 〗_"s")/"2")/("W" _"l" -"W" _"s") (9)

where Wl is the molar concentration of water vapor within the leaf (mmol H2O (mol air)-1), which is computed from the leaf temperature Tl (C) and the total atmospheric pressure P (kPa)

"W" _"l" " = " ("e(" "T" _"l" ")")/"P" "×1000" (10)

The function e(T) in 11th equation is saturation vapor pressure (kPa) at temperature T (C) measured in 12th equation (Buck, 1981).

"e(T) = 0.61365" "e" ^("17.502T" /"240.97+T") (11)

Stomatal Conductance to Water Vapor

The stomatal conductance gsw to water vapor (mol H2O m-2 s-1) is obtained from the total conductance by removing the contribution from the boundary layer.

"g" _"sw" " = " "1" /("1" /"g" _"tw" -"k" _"f" /"g" _"bw") (12)

where kf is a factor based on the estimate K of the fraction of stomatal conductances of one side of the leaf to the other,

"k" _"f" " = " ("K" ^"2" " + 1")/("K + 1")^"2" (13)

and gbw is the boundary layer conductance to water vapor (mol H2O m-2s-1) from one side of the leaf. The boundary layer conductance correction thus depends on whether the leaf has stomata on one or both sides of the leaf.

Net Photosynthesis Rate (A)

The mass balance of CO2 in an open system is given by

sa = uici – uoco (1)

where a is assimilation rate (mol CO2 m-2 s-1), ci and co are incoming and outgoing mole fractions (mol CO2 mol air-1) of carbon dioxide. Using (2), we can write

"a = " ("u" _"i" "(" "c" _"i" "-" "c" _"o" ")")/"s" -"E" "c" _"o" (2)

Portable photosynthesis system makes measurements using the following relationships.

ci = Cr/106 (3)

co = Cs/106 (4)

a = A/106 (5)

where Cr and Cs are sample and reference CO2 concentrations (µmol CO2 (mol air)-1), and A is net assimilation rate of CO2 by the leaf (µmol CO2 m-2 s-1).

"A = " ("F(" "C" _"r" -"C" _"s" ")")/"100S" -"C" _"s" "E" (6)

Transpiration in the equation (E) serves as a dilution correction. As water vapor is added to the leaf system, transpiration dilutes all other gases containing CO2.

Intercellular CO2 Concentration

The intercellular CO2 concentration Ci (µmol CO2 mol air-1) is given by

"C" _"i" " = " (("g" _"tc" -"E" /"2") "C" _"s" -"A")/("g" _"tc" "+" "E" /"2") (7)

where gtc is the total conductance to CO2, and is given by

"g" _"tc" " = " "1" /("1.6" /"g" _"sw" "+" ("1.37" "k" _"f")/"g" _"bw") (8)

1.6 is the ratio of the diffusivities of CO2 and water in air, and 1.37 is the same ratio in the boundary layer.

"g" _"tw" " = " "E" ("1000" -("W" _"l" " +" 〖" W" 〗_"s")/"2")/("W" _"l" -"W" _"s")"W" _"l" " = " ("e(" "T" _"l" ")")/"P" "×1000" "e(T) = 0.61365" "e" ^("17.502T" /"240.97+T") "g" _"sw" " = " "1" /("1" /"g" _"tw" -"k" _"f" /"g" _"bw")"k" _"f" " = " ("K" ^"2" " + 1")/("K + 1")^"2" "" ""

RESULTS

For four different irrigation treatments applied, results of variance analysis and average values of A, Ci, gs and E values performed on 12.08.2019, 29.09.2019 and 09.09.2019 are given in Table 2. According to the results of variance analysis, all physiological parameters were significantly affected by different water stress conditions for each measutement date (P <0.01).

Table 2. Results of variance analysis and mean values of net photosynthesis rate (A), stomatal conductivity (gs), intercellular CO2 concentration (Ci) and transpiration (E) under deficit irrigation treatments

	A			gs			Ci			E		
Treatment												
	12.08.19	29.08.19	09.09.19	12.08.19	29.08.19	09.09.19	12.08.19	29.08.19	09.09.19	12.08.19	29.08.19	09.09.19
S1	15.63 a1	18.18 a	19.00 a	274.67 a	294.00 a	284.83 a	0.37 a	0.47 a	0.44 a	4.48 a	5.31 a	3.43 a
S2	12.77 b	15.15 b	16.73 b	260.33 b	283.17 a	270.83 a	0.26 b	0.36 b	0.36 b	3.95 b	4.32 b	2.68 b
S3	10.92 c	12.23 c	13.77 c	232.17 c	237.33 b	235.33 b	0.11 c	0.18 c	0.15 c	2.73 c	3.15 c	1.80 c
S4	6.03 d	8.27 d	9.72 d	200.33 d	222.83 b	221.83 b	0.07 d	0.09 d	0.08 d	1.48 d	1.89 d	0.97 d
LSD0.05	1.56	13.98	0.03	0.5	1.65	15.05	0.05	0.3	1.24	26.08	0.07	0.5
Blocks	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Treatments	**	**	**	**	**	**	**	**	**	**	**	**

1: means with different letters in the same column are significantly different

ns: non significant

**: significant at the 1% of probability level (P<0.01)

It was determined that the highest A values were measured in S1 and were between 15.63 and 19.00, and the lowest values were measured in S4 and varied between 6.03 and 9.72. The highest gs values were measured in S1 and were between 274.67 and 284.83 and the lowest values were measured in S4 and varied between 200.33 and 222.83. The highest Ci values between 0.37 and 0.47 were measured for S1, while the lowest Ci values were between 0.07 and 0.09 for S4. E values were measured in S1 with the highest values between 4.48 and 5.31, and the lowest in S4 between 1.48 and 0.97 (Table 2).

The changes of all physiological parameters during the measurement dates are also given in Figure 1. As seen in Figure 1, except A; gs, Ci, E values showed a tendency to decrease in the last measurement (09.09.2019). The reason for this situation can be attributed to the approaching of the sugar beet plant to the harvest period.

Figure 1. Changes of net photosynthesis rate (A), stomatal conductance (gs), intercellular CO2 concentration (Ci) and transpiration (E) parameters during the measurement dates

DİSCUSSİON

When Figure 1 is examined, it is observed that the A, gs, Ci and E values decrease with the decreasing of water application levels. Bloch et al. (2006), in their study on 3 different sugar beet varieties in Germany, observed physiological parameters for different irrigation levels, 100%, 50% and 20%, and determined a decrease in net photosynthesis rate, stomatal conductivity and perspiration values with increasing of water deficit. Photosynthesis values of 3 different genotypes for full irrigation subject varied between 13 and 15 μmol CO2 m-2 s-1, for 50% subject between 7 and 10 μmol CO2 m-2 s-1, and for 20% subject between 3 and 4 μmol CO2 m-2 s-1 values. For the same study, transpiration values ranged from 5 to 6 H2O m-2 s-1 for full irrigation, 3 to 4 H2O m-2 s-1 for 50%, and 1 to 2 H2O m-2 s-1 for 20%. At the last, stomatal conductivity values varied between 350-420 H2O m-2 s-1 for full irrigation, 160-200 H2O m-2 s-1 for 50%, and 20 to 70 H2O m-2 s-1 for 20%. Tognetti et al. (2003) in the study conducted in Southern Italy measured the responses of sprinkler and drip irrigation techniques to sugar beet physiological parameters and determined 3 different irrigation levels for the drip irrigation method: 100%, 75% and 25%. In the 2-year results of the experiment, it was observed that as the irrigation level decreased, the net photosynthesis rate (A) and stomatal conductivity (gs) values decreased. For the full irrigation treatment irrigated with drip irrigation, net photosynthesis rate values varied between 25 and 20 μmol CO2 m-2 s-1 during the season, between 22 and 17 μmol CO2 m-2 s-1 for 75%, and 17 and 14 μmol CO2 m-2 s-1 for 50%. Stomatal conductivity values for 100%, 75% and 50% irrigation levels varied respectively, between 450-200, 320-180, 220-140 mol H2O m-2 s-1 values. Li et al. (2019) created 3 different irrigation levels in their studies conducted in China, observed the physiological responses of deficit irrigation on sugar beet, and encountered decreases in A, gs, and Ci values as the water stress increased. Experimental subjects are designed at 3 different irrigation levels as 70%, 50% and 30%. At the end of the research average values were found as follows: 25.5, 18.5, and 12.5 μmol CO2 m-2 s-1 for the net rate of photosynthesis and for the stomatal conductance; 405, 190 and 110 mol H2O m-2 s-1, lastly for the intercellular CO2 concentration: 260, 220 and 200 μmol CO2 mol air-1. Many similarities were found between the results obtained in the study and the results determined in the above studies.

CONCLUSIONS

The effects of deficit irrigation applications on physiological characteristics of sugar beet A, gs, Ci, E in semi-humid climates was found to be significant at the level of P <0.01. As the water stress increased, a decrease was observed in A, gs, Ci and E values. It is thought that these conclusions will be useful in evaluating the results to be obtained in studies to be conducted in similar climatic conditions.

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EFFECTS OF DIFFERENT PRUNING PRACTICES ON LEAF DEVELOPMENT AND LIGHT OBSTRUCTION OF KIWI (*ACTINIDIA DELICIOSA*)

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ABSTRACT

In this study, the relationship between types of pruning and light obstruction was investigated for Hayward kiwi species under ecological conditions of the province Samsun. Prunings were performed at two different periods as of winter pruning (long, medium and short pruning) and summer pruning (heavy and light pruning). In was found that light-obstructions rates (%) of winter prunings were ordered from higher to lower as long, medium and short pruning. Additional summer prunings beside winter prunings didn't have statistically significant effects on light-obstruction. It was determined that percent light-obstruction got a constant state 4 weeks after the beginning of shoot development (end of May – first week of June). Light-obstruction rates were generally about 60-80% for all types of prunings. Increases were also observed in leaf areas based on pruning types in the order of short, medium and long pruning. Based on the results of this study, it can be stated that short pruning was a significant practice in kiwi culture for sufficient light intensity; however, a mixed pruning practice mostly short pruning with some medium pruning was recommended to prevent excessive exhaustion of the plant.

Keywords: kiwifruit, pruning, light, growth, light obstruction

INTRODUCTION

There are several researches conducted about the significance and impact mechanisms of light, temperature and relative humidity in plant production. These studies are mostly concerned about pruning, shading, and rarefaction. Almost all of the life on Earth is connected to plants that supply oxygen to the atmosphere by photosynthesis. Photosynthesis is the state of the appearance of organic components as a result of the change of chlorophyll with light energy. (Yakar and Bilge, 1987).

Light is one of the most important factors in the realization of photosynthesis event. Plants need light to be able to photosynthesize, grow and develop. As the light requirement may vary according to the plants, the requirement of a plant to light can also be at different levels at different times (Schmitt & Wulff, 1993).Photosynthesis is the most significant process in plant culture. Beside the components of photosynthesis such as temperature, water, CO_2 and chlorophyll, light-obstruction is another critical factor effecting photosynthesis. Dry matter accumulation of plant and distribution of dry matter over the various parts of plant and plant yield are directly related to plant canopy – light relationships (Uzun et al.1998). The main source in the realization of photosynthesis is the sun. A very high level of energy comes from the sun to the earth. Green plants make photosynthesis using sunlight and produce nutrients. In photosynthesis, 85-90% of active light is absorbed by plant leaves. The remaining light is

reflected or transmitted by the leaves. Chlorophyll in plant leaves absorbs light with wavelength in the blue and red region (Kaçar et al., 2010).

Plant ecology is controlled by two factors based on light intensity. The first one is the position of leaves within the canopy of the plant and the second one is utilization capability of leaves from the light obstructed by the leaves (Hay and Walker 1989). Optimum light distribution and utilization are the critical issues for quality products.

The leaf area index (LAI-The Leaf Area Index) is an important variable in evaluating the shading process of the plant's canopy. The relationship between the atmosphere and the plant canopy is effective in photosynthesis and the water consumption of the plant, that is, the conversion of energy into mass (Ahmad et al., 2015).

Due to the arrangement of the leaves in the plants and their overlapping, the rate of light utilization occurs gradually. In addition, plant height, plant density and leaf shape also affect the quality and amount of light utilized. Leaf structure and thickness are effective on light transmittance (Kapucu, 2016).Kiwi has a cuddling, climbing and strongly-developing shooting structure. Therefore, pruning is a significant practice in kiwi culture for sufficient light utilization of leaves, sufficient accumulation of carbohydrates and prevention of diseases stimulated by slow air movements within the canopy. In this study, it was aimed to determine the effects of different pruning practices on leaf development and light-obstruction of kiwi.

MATERIAL AND METHODS

Experiments were conducted under ecological conditions of the province Samsun during the years 2003 and 2004. Climate of the study area is hot and dry in summers and cool and rainy in winters (Anonim 2005). Although, average temperatures in both years have followed a similar course between the months of May-September in which light-intensities were measured, the temperature in the year 2003 was slightly lower than the year 2004. It was even below 5 ^{0}C during the months February and March. Again, although relative humidity in both years has followed the similar course during the same period, it was lower in the year 2003. Relative humidity decreased to 70% especially June and July of the year 2003.

7 years of kiwi orchard constructed by 4x4 planting spacing with Hayward species has constituted the material of the study. The orchard was constructed by T- pole system. Following practices and investigations were used as the methods of the study.

Pruning Practices Used in This Study

Winter pruning: Long pruning (15-18 shoots, U), medium pruning (10-12 shoots, O) and short pruning (5-7 shoots, K) (Samancı 1990) with 4 replications.

Summer pruning: Heavy pruning (6 leaves after the last fruit, shortening after 6 leaves over vegetative shoots, Ş) and light pruning (tip trimming from both fruits and vegetative shoots, H) (Galliano et al. 1990) with 4 replications. Control branches were also left for both subjects.

A single plant was used in winter prunings for each practice. Since kiwi-fruit exhibits a two-directional development as of North-south, each plant was used for two practices in summer prunings. Therefore, 12 plants were used in these experiments.

Each practice was also carried out by taking data according the directions as of ‘sea side – north’ and ‘road side – south’. Symbols used for practices in experiments were given in Table 1.

Performance and Evaluation of Leaf Measurements

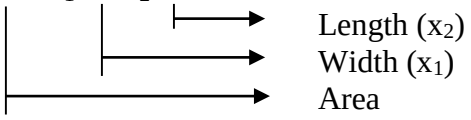
Number of leaf over the shoot: Leaves over each annual shoots of experimental plan or laterals were counted.

Leaf width and length: At the end of harvest, all the leaves over the previously selected annual shoots were counted, their lengths (cm) and widths (cm) were measured.

Leaf dry weight: All the leaves over which the measurement were carried out were put into paper bags and dried at 70 ^{0}C for 3-4 days until reaching a constant weight. Dried leaf samples were weighed at balances with 0.01 g sensitivity.

Determination of leaf areas and total leaf area (TSYA): A random 85 leaf samples were taken from the experimental plants to find out leaf area equation. Area, width and length of each leaf required for leaf area formula were measured. Based on these measurements, average leaf area (cm^2), average leaf width (cm) and average leaf length (cm) of mature leaves were determined. Leaf area measurements were performed by a digital planimeter. The widest section of the leaf was measured to find the leaf width. The length between the tip and the bottom of the leaf were measured as leaf length. Then, mature leaf area formula for kiwi was determined by using regression analysis carried out Excel software. Following formula was determined for mature kiwi leaves:

$y = a + bx_1 + cx_2$



Area = -200,52 + 16,01 . Width + 10,01 . Length

Standard Error	= 7,77***	0.73***	1,01***

$r^2 = 0,97^{***}$

By using the above formula, leaf area for each year was calculated and the total leaf area was determined by summing up the leaf areas of the shoots.

Performance and Evaluation of Light Measurements

Delta-T Devices Type SS1 Sun Scan Canopy Analyzer was used to measure the light absorbed by leaves above and below the plants used in experiments. Measurements were made once in a week from both sides of the plant, above and blow the canopy and once every two week after the fifth week since the vegetative development slows down. Light measurements were continued from the start of shoot development (May) to the end of shoot development (end of September).

Light data taken during the experiments were transformed into % light-obstruction values by using the following formula:

$$\% \text{ Light-obstruction} = \frac{(a - b) \times 100}{a}$$

a: Amount of light falling on upper section of plant

b: Amount of light falling on lower section of plant

Performance and Evaluation of Statistical Analysis

Number of leaves, leaf area indexes and leaf dry weights were statistically analyzed based on randomized block design by using "**MSTAT-C Software**". **"Duncan Multiple Range Test"** under the **RANGE** sub-program of the same software was used to test the differences among

the averages exhibiting differences based on the performed statistical analysis. During the statistical evaluations of the results, significance level (P) between the differences was selected as 5% (significant) and 1% (very significant). **"Slide Write 2.0"** software was used to create the graphs and error bars specified in graphs were given based on 5% probability limit.

RESULTS AND DISCUSSIONS

3.1. Light Intensities Measured over the Plants in Experimental Orchard

Variation of % light-obstruction of kiwi in both years based on short winter pruning and summer pruning was given in Figure 1.

Variation of % light-obstruction of kiwi in both years based on medium winter pruning and summer pruning was given in Figure 2.

Variation of % light-obstruction of kiwi in both years based on long winter pruning and summer pruning was given in Figure 3.

It was observed that summer prunings in all three summer prunings didn't have any significant impacts on light-obstruction. Generally, the % light-obstructions have reached to a constant state after the 4th week (end of May – the first week of June). When the % light obstructions of winter prunings were evaluated, it was seen that the general order from higher to lower was as long-medium-short. The average values for all prunings in both years were about 60-80%. Although the light-obstruction values are close together, rate of solar radiation utilization and fruit quality can be different. Palmer (2005) states that light-obstruction rates of apples could be increased by increasing leaf area index. It was also stated that light-obstruction has given only the yield potential of the orchard and if the yield was lower than expected and fruits were smaller than normal sizes, the trees could have leaved in shadow. As it was in this study, the researcher has stated that although total yield and dry matter accumulation increases by increasing light utilization, additional increases in leaf areas could cause smaller size fruits.

Impact of light over the leaf consequently the fruit quality was investigated by several researchers. Uzun et al. (1998) stated that amounts of light not obstructed by leaf, directly reached below the canopy, absorbed by leaf, passed through the lower sections of tree and reflected from the leaf were depend on types, distribution, thickness and number of leaves within canopy. Kumar et al. (2014) performed pruning of figs in 4, 6, 8 buds / shoots at each different planting intervals. They obtained the maximum leaf production from pruning in the form of 4 buds / shoots in the planting range of 5x2m or 5x2.5m. Maximum leaf production, shoot growth and internode length were witnessed under closer spacing of 5x2m or 5x2.5m, and, under 4 buds/shoot pruning regimen) Minchin et al. (2010) found that the rate of increase in both fresh fruit and dried fruit weight in kiwi decreases with increasing number of leaves. They found that a large part of the carbohydrate requirement that occurs with the increasing number of leaves is used as a dry matter content in the fruit with vegetative parts. In other words, they stated that the dry matter content of a fruit increases with increasing number of leaves. In their study, when a fruit is 2-3 leaves, there is no competition, but when a fruit is left with 4 leaves, they saw that vegetative growth and fruit growth compete and the fruit fresh weight decreases by 28% and dry weight decreases by 39%. Biasi et al. (2005) observed that light utilization and distribution within plant canopy significantly effected the physiology of the tree and consequently the fruit quality. Researchers also evaluated the impact of light on quality of kiwi fruits and observed the impacts of light on individual fruits and whole tree. Uzun (2000) found that although light intensities at some degree increased the yield based on light absorption of leaves and light utilization in photosynthesis, yield decreased after certain intensities. Öner and Sezer (2007) found that the effect of light and temperature on leaf area was statistically significant ($P<0,5$) in their study in Egypt. Morgan et al. (1985) investigated the impacts of light on Hayward and Matua kiwi species and stated that growth in some parts of plants under shadow light was due to lower rates of infrared lights within this shadow light.

3.2. Total Leaf Area and Leaf Characteristics

Effect of summer pruning over the variations in total leaf area of winter pruned kiwi plants was given in Figure 4. The reason to have higher total leaf areas for medium and long prunings of the first year than the second year was due to lower temperatures observed in June of the year 2003 than the next year. Althoug the relative humidity was low during the same period, the temperature was also low and that was the reason of having higher leaf areas. Statistically significant differences were observed between winter and summer prunings. Leaf area increased in the order of short, medium and long pruning. Althoug the leaf area is higher in long pruning practices due to higher number of leaf, leaf quality was higher in short pruning.
Number of leaf over a shoot varied between 6-8 in all practices. Taking summer prunings also into consideration in both years, leaf widths were 9-10 cm in short pruning, 8-13 cm in medium and 8-10 cm in long pruning; leaf lengths were 10-12 cm in short, 9-12 in medium and 11-12 in long pruning. Leaf dry weights of the year 2003 varied between 6-16 g in short pruning, 7-12 g in medium and 8-13 g in long pruning. Leaf dry weights of the year 2004 varied between 10-14 g in short, 7-11 g in medium and 10-16 g in long pruning. Higher leaf dry weights in long pruning were due to higher number of leaf over a shoot. When evaluated based on shoots, differences were observed in long pruning, however when considered based on plant, number of shoots increases in short pruning and dry leaf weights get closer to other practices.
Various researches were conducted in this topic for kiwi fruit and different species. In this study, statistically significant differences were observed between winter and summer pruning. Leaf area increased in an order of short, medium and long pruning. Snelgar and Thorp (1998) determined that when the leaf area per fruit exceeded 300-700 $cm^{2,}$ a 100 cm^2 in leaf area caused 5-6 g increase in fruit weights. Cangi and Atalay (2006) conducted researches on kiwi fruits and determined that total leaf area was 21.047 - 58.61 m^2/plant; average lead area was between 185.51 - 194.17 cm^2. Researchers have also stated a positive relation between yield and average and total leaf areas. Althoug Özkan and Koçyiğit (1995) has stated that kiwi have significantly large leaves generally with 20-30 cm diameters, average leaf diameters measured in this study for Hayward species was about 10 cm. That may be due to species and climate factors. Palmer (2005) states that light-obstruction could be increased by increasing lead area index.
Özkan and Koçyiğit (1995) stated that a mature kiwi tree has average 2000-3000 leaves. Findings of this study exhibits parallelism with the findings of other researchers.
Uzun et al. (1998) stated that dry matter accumulation, distribution in various parts and yield were effected from plant canopy-light relationships. Gullo et al. (2013) applied two different summer prunings (removal of 30% and 60% of the current leaf area of the canopy) in kiwi. They found that when 60% of the canopy leaf area was removed, the titratable acidity and kiwi fruit increased the flesh stiffness at harvest, reducing average fruit weight and yield. They also found that when compared to the control kiwi, which was not pruned in summer, the total antioxidant capacity (TAC) in the fruit and total polyphenols (TPH) increased. They also stated that removing the 30% leaf area did not decrease the average fruit weight and yield, but also increased TAC and TPH by 20% at harvest. Cangi and Atalay (2006) determined the total leaf weight per plant (PTLW) as 9.04-25.68 kg/plant. In this study, although leaf dry weight in long pruning was higher, when shoot-based weights were evaluated, almost equal values should be obtained in short pruning due to higher number of shoots in short prunings.

CONCLUSION

Although light-obstruction varies based on winter pruning practices, it generally reaches to a constant state at the end of forth week (end of May – the first week of June). When the % light-obstructions were evaluated based on winter prunings, the order from higher to lower was as long-medium-short pruning. It was seen that summer prunings didn't have any effect on % light-obstruction.

As a result, it can be stated that short pruning was a significant practice in kiwi culture for sufficient light intensity, however, a mixed pruning practice mostly short pruning with some medium pruning was recommended to prevent excessive exhaustion of the plant. Therefore, under experimental conditions of this study, winter pruning should be performed from minimum 5-7 sprouts and maximum 10-12 sprouts. Summer pruning can also be performed at necessary rates and mixed with winter pruning.

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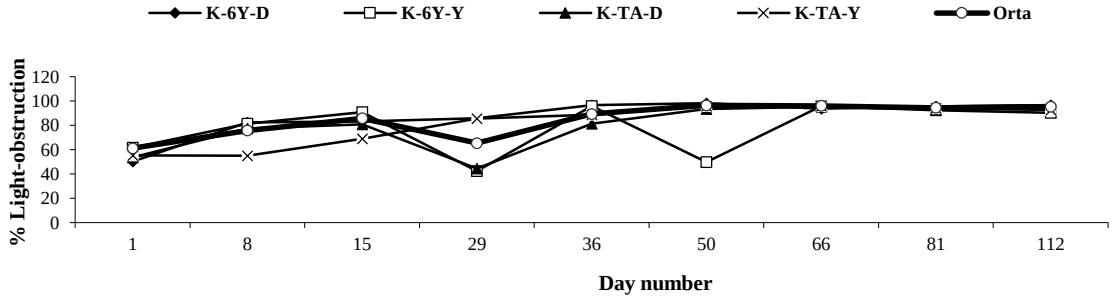
Table 1. Symbols used in practices in experiments

K-6Y-D...K(Short winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)	**KONT-O-6Y-D**...KONT(Control)-O(Medium winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)
K-6Y-Y... K(Short winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-Y(Road side)	**KONT-O-6Y-Y**...KONT(Control)-O(Medium winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-R(Road side)
K-TA-D...K(Short winter pruning)-TA(Sprout trimming = Light summer pruning)- D(Sea side)	**KONT-O-TA-D**...KONT(Control)-O(Medium winter pruning)-TA(Sprout trimming = Light summer pruning)- D(Sea side)
K-TA-Y...K(Short winter pruning)-TA(Sprout trimming = Light summer pruning)- Y(Road side)	**KONT-O-TA-Y**...KONT(Control)-O(Medium winter pruning)-TA(Sprout trimming = Light summer pruning)- R(Road side)
O-6Y-D...O(Medium winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)	**KONT-U-6Y-D**...KONT(Control)-U(Long winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)
O-6Y-Y...O(Medium winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-R(Road side)	**KONT-U-6Y-Y**...KONT(Control)-U(Long winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-R(Road side)
O-TA-D...O(Medium winter pruning)-TA(Sprout trimming = Light summer pruning)- D(Sea side)	**KONT-U-TA-D**...KONT(Control)-U(Long winter pruning)-TA(Sprout trimming = Light summer pruning)- D(Sea side)
O-TA-Y...O(Medium winter pruning)-TA(Sprout trimming = Light summer pruning)- R(Road side)	**KONT-U-TA-Y**...KONT(Control)-U(Long winter pruning)-TA(Sprout trimming = Light summer pruning)-R(Road side)
U-6Y-D...U(Long winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-D (Sea side)	**a**...(K,O,U-Short, Medium, Long winter prunings)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)

U-6Y-Y...U(Long winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-R (Road side)	**a***...KONT(Control)-(K,O,U-Short, Medium, Long winter prunings)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)
U-TA-D...U(Long winter pruning)-TA(Sprout trimming = Light summer pruning)- D(Sea side)	**b**...(K,O,U-Short, Medium, Long winter prunings)-6Y(from 6 leaves = Heavy summer pruning)-Y(Road side)
U-TA-Y...U(Long winter pruning)-TA(Sprout trimming = Light summer pruning)- R(Road side)	**b***...KONT(Control)-(K,O,U-Short, Medium, Long winter prunings)-6Y(from 6 leaves=Heavy summer pruning)-Y(Road side)
KONT-K-6Y-D...KONT (Control) K(Short winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-D(Sea side)	**c**...(K,O,U-Short, Medium, Long winter prunings)-TA(Sprout trimming = Light summer pruning)- D(Sea side)
KONT-K-6Y-Y...KONT(Control) K(Short winter pruning)-6Y(from 6 leaves = Heavy summer pruning)-Y(Road side)	**c***...KONT(Control)-(K,O,U-Short, Medium, Long winter prunings)-TA(Sprout trimming = Light summer pruning)- D(Sea side)
KONT-K-TA-D...KONT(Control) K(Short winter pruning)-TA(Sprout trimming = Light summer pruning)- D(Sea side)	**d**...(K,O,U-Short, Medium, Long winter prunings)-TA(Sprout trimming = Light summer pruning)- R(Road side)
KONT-K-TA-Y...KONT(Control) K(Short winter pruning)-TA(Sprout trimming = Light summer pruning)- R(Road side)	**d***...KONT(Control)-(K,O,U-Short, Medium, Long winter prunings)-TA(Sprout trimming = Light summer pruning)- R(Road side)



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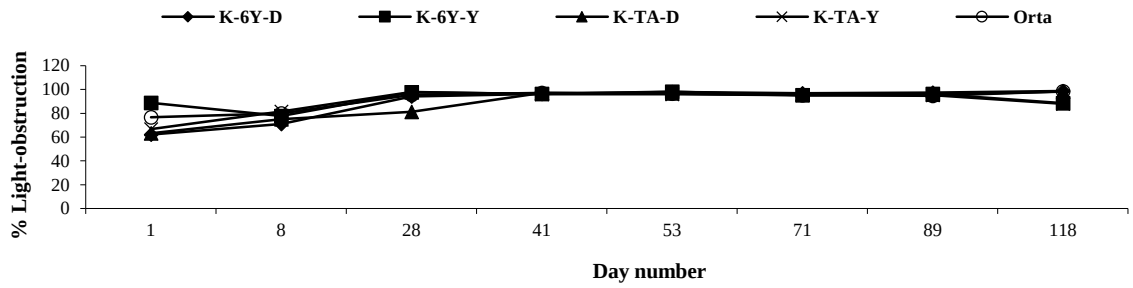

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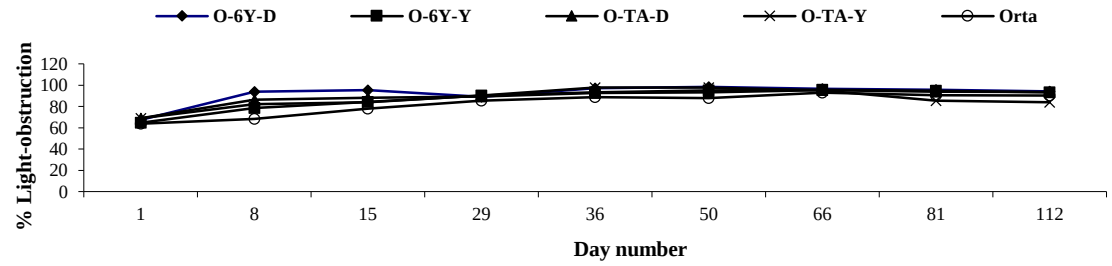
Figure 1. % light-obstruction values for plants with short pruning



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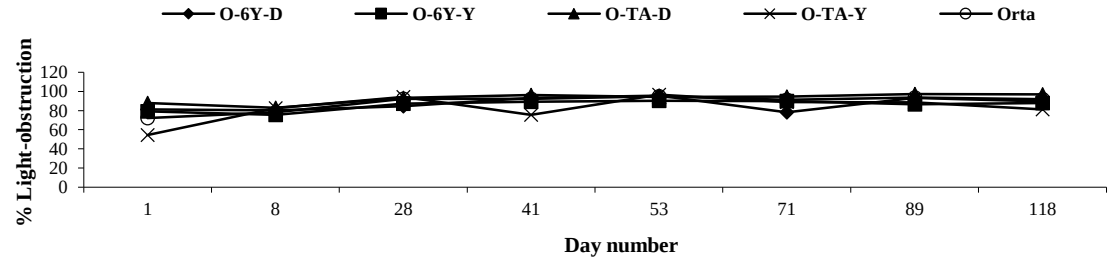

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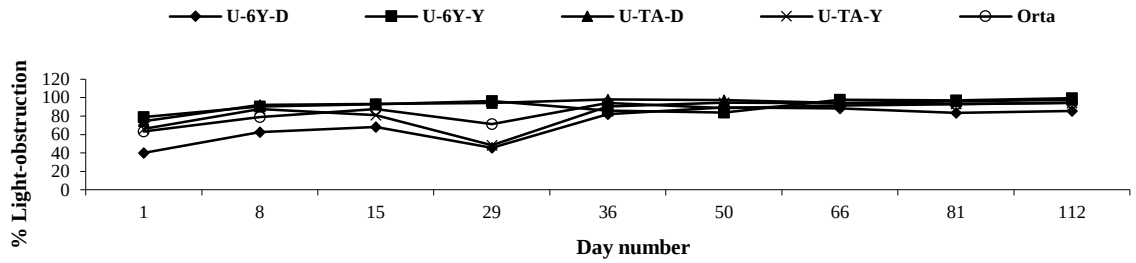
Figure 2. % light-obstruction values for plants with medium pruning



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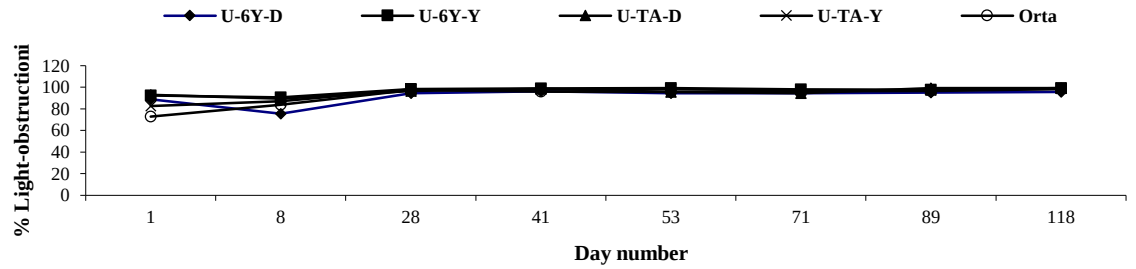

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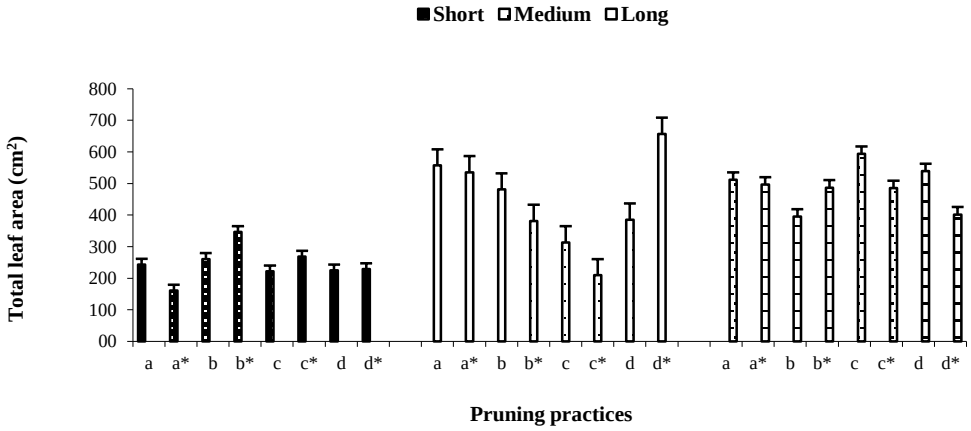
Figure 3. % light-obstruction values for plants with long pruning



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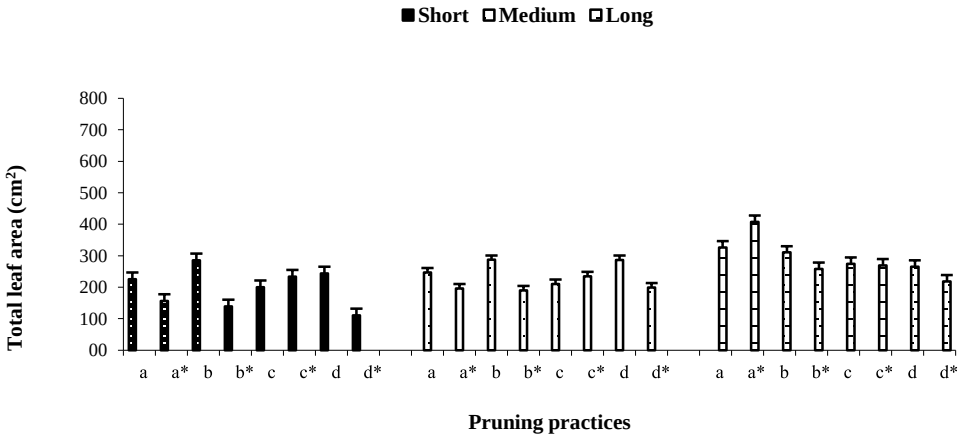

2004

Figure 4. Variation of total leaf area (cm^2) according to pruning practices

DEVELOPMENT AND CHARACTERIZATION OF FLOATING PECTIN HYDROGEL PELLET FORMULATIONS CONTAINING PREGABALIN

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ABSTRACT

Pregabalin (PG) is primarily prescribed for painful diabetic neuropathy, post-herpetic neuralgia, radicular pain, and fibromyalgia, also it is used in clinical practice to treat general anxiety disorder due to its anxiolytic properties. Pectin is a water-soluble ionic polysaccharide found naturally in the cell walls of many plants. As a result of the interaction of pectin with calcium ions, a hydrophilically coated insoluble carrier system is formed by complexing between surfaces and this provides sustained-release. In this direction, floating pectin pellets containing PG were developed in our study. It has been found that floating and pregabalin containing pectin pellets have not been studied in the literature. With this study, it was aimed that PG should make a sustained-release in the stomach content and reduce the daily dose intake of the patient. Within the scope of the study, validation of PG's quantification method, development of pellet formulations, and in-vitro characterization studies were performed. The formulations have been successfully developed without using any film coating agent to prolong drug release. Considering the results obtained, it was determined that the 6D code formulation continued PG release for 24 hours with an average encapsulation efficiency of ~82%. We anticipate that the formulation we developed will provide an alternative to the extended-release pregabalin preparations in the current pharmaceutical market.

Keywords: *Pregabalin, Pectin, Pellet, Sustained-Release, Neuropathic Pain.*

INTRODUCTION

Oral drug delivery is the most widely used of all the routes of administration investigated for the systemic delivery of drugs using different dosage forms for decades. Solid dosage forms are advantageous in terms of low cost, ease of administration, exact dosing, and most importantly patient compliance (Kantharao et al., 2019).

PG is a white and crystalline powder that dissolves in acidic, basic, and aqueous solutions and has anticonvulsant (antiepileptic) properties (Jadi et al., 2016; Velavan and Venkatesan, 2014). PG binds to calcium channels on the nerves and can change the release of neurotransmitters (the chemical that nerves use to communicate with each other) and act on pain and seizures, provided that the communication between nerves is reduced (Jadi et al., 2016). Although used in clinical practice to treat general anxiety disorder due to its anxiolytic properties, it is primarily prescribed for painful diabetic neuropathy, post-herpetic neuralgia, radicular pain, and fibromyalgia. Its doses range from 75 to 150 mg per day for neuropathic pain, and its bioavailability is quite high (% 90) (Azmi et al., 2019).

Pectin is a water-soluble ionic polysaccharide naturally found in the cell wall of many plants and contains α-D-galacturonic acid in straight chains (Alvarez-Lorenzo et al., 2013; Sriamornsak, 1998). Low methoxylated pectins need calcium or other multivalent cations to form gels (Sriamornsak, 2011). Pectinate hydrogels formed in the presence of calcium are stable

in low pH solutions and swell in weak basic solutions (Sriamornsak, 1999). Calcium pectinate is disintegrated by pectinolytic enzymes in the colon and delays drug release since it cannot dissolve in the upper gastrointestinal tract (cannot be broken down by gastric or intestinal enzymes). As a result of the interaction of pectin with calcium ions, an insoluble carrier system is formed, which is hydrophilically coated by interfacial complexation, and this ensures sustained-release (Sriamornsak,1998; Sriamornsak and Nunthanid, 1998).

In the preparation of delivery systems containing pectin, especially ionotropic gelation, and gel coating technique is frequently used. Both of these techniques are safe for the risk of toxicity (Sriamornsak, 2003). In the ionotropic gelation technique, polysaccharides (such as pectin) are dissolved in water or a weakly acidic medium. These solutions are then added dropwise into solutions containing oppositely charged ion, which is constantly stirred. During complexation with oppositely charged ions, polysaccharides undergo ionic gelation and precipitate as spherical particles. The pellets formed are filtered, washed with distilled water, and dried. Solvent toxicity and other undesirable effects are prevented by crosslinking by electrostatic interaction instead of chemical crosslinking (Racovita et al., 2009).

Pellets are multi-unit solid drug forms, with particle sizes generally ranging from 0.5-1.5 mm. Each particle is a matrix obtained by dispersing the active substance in the polymer (Choudhury et al., 2010). With pellets, drugs with different release properties and/or incompatible drugs can be combined, physically opposite materials can be combined and dissolution can be achieved in the desired rate by controlled release or extended-release (Kumari et al., 2013; Hossein et al., 2015).

Floating drug delivery systems are useful systems that increase the length of stay in the stomach. They are low-density systems that can remain floating in gastric fluid for a long time without being affected by gastric content and gastric emptying (Jain et al., 2006; Mayavanshi and Gajjar, 2008; Chowdary and Chaitanya, 2014; Patil et al., 2016). These systems are matrix type systems prepared with the help of swellable polymers and various effervescent compounds (such as sodium bicarbonate, tartaric acid, and citric acid). These hydrophilic systems are formulated to retain gas and release carbon dioxide (CO_2) when they encounter acidic gastric juice. They become floatable by trapping this gas through hydrogels that exhibit swelling (Shah et al., 2009; Khan and Bajpai, 2010).

Our aim in this study is to transform the PG active substance into floating pellets in the stomach content by using pectin. In this way, it is aimed to create a sustained-release and to decrease the daily dose intake of the patient. It is also possible to reduce the occurrence of side effects and/or toxic effects and to increase patient compliance with less dosing frequency.

Materials and Methods

Development of pregabalin quantification method

The PG quantification method developed by Mohan et al. was modified and re-developed. Standard solutions containing PG were prepared in ultrapure water at a concentration of 200 µg/mL and a calibration curve was drawn from 11 points by making the necessary dilutions (1, 5, 10, 25, 50, 75, 100, 125, 150, 175 and 200 µg/mL) (Mohan et al., 2014). HPLC conditions are given in Table 1.

Table 1. HPLC conditions

Mobile Phase	Phosphate Buffer-USP30/NF25 (pH adjusted to 6.5 using potassium hydroxide):MeCN:ACN, 75:10:15
Column	RP-C18, Silicyle® SiliaChrom, 150 x 4.6 mm
Detector	PDA
Wavelength	197 nm
Column Temperature	25 °C
Injection Volume	20 µL
Flow Rate and Duration	0.7 mL/sec.

Validation of the quantification method

Analytical method validation is the verification of the developed method. It ensures that the developed method achieves appropriate, repeatable, and reliable results for the desired targets. According to the rules of ICH Q2 (R1), the developed method has been validated for linearity, accuracy, precision, the limit detection (LOD), the limit of quantification (LOQ), and selectivity (specificity) (Udayalakshmi et al., 2014; Sreekanth et al., 2017; Mohan et al., 2014; Kavıtha et al. 2014).

Preparation of floating pellet formulations

The ionotropic gelation method was used in the preparation of floating hydrogel pellets (Muhamad et al., 2011; Lohani et al., 2016; Ren et al., 2016; Shirizadeh et al., 2017). First of all, the different amounts of pectin were swelled homogeneously on a multi-point magnetic stirrer at 750 rpm in pure water. Then, while mixing at the same speed, PG and $NaHCO_3$ (as a source of CO_2) were added in determined amounts and it was completely dissolved. These prepared dispersions were dropped with the help of a syringe into the mixing $CaCl_2$ solution at determined concentrations (0.1, 0.2, and 0.3 M) from a certain height. In order to form pellets in the gel structure and complete the complexation, dispersions were mixed for a certain period of more time. After the gel-shaped pellets were formed, filtration was applied and the pellets were washed several times with pure water and then frozen overnight at -20 °C. Then, lyophilization was applied for 24 hours, and dried pellets obtained for the later experiments were stored at room temperature in the desiccator (min. n=3). PG-free pellets (blank pellets) were also prepared without the PG addition only, as described above.

Determination of flotation degrees

The flotation degree determination of floating hydrogel pellets with and without PG was performed using a 50 mL pH 1.2 HCl buffer (USP30-NF25) in a 37 °C water bath. A certain number (such as 100-150 pieces) of lyophilized pellets were placed in this medium and those on the surface at certain time intervals (such as 5 min, 15 min, 30 min, 1 h) were counted. This study was continued for 24 hours. The flotation degree of the formulations was determined by proportioning the number of floating pellets over time and the number of non-floating pellets (min. n=3).

Determination of yield, drug loading capacity, and encapsulation efficiency

The floating pellets containing the known amount of PG were mixed in 5 mL mobile phase for 2 hours on a magnetic stirrer at 750 rpm and the pellets were broken and PG came into the open. Subsequently, the supernatant was separated, filtered through a 0.45 µm membrane filter, and quantified using the validated HPLC method (min. n=3). All dilutions were made with the mobile phase (Ahmed et al., 2014)

Determination of swelling degrees

For the swelling degree study, 50 mg of lyophilized samples from the floating pellet formulations with and without PG were weighed and carried out for 24 hours in 30 mL pH 1.2 HCl buffer (USP30-NF25) using a horizontal shaker water bath at 37 °C/50 rpm. Swelling pellets at the specified time intervals (such as 30 min, 1 h, 2 h) were filtered and weighed. The degree of swelling after the experiment was evaluated by calculating the difference between the dry weight before swelling and the subsequent wet weight in % and the increase in weight was determined (min. n=3) (Veronovski et al., 2014; Karki et al., 2016).

Morphological and organoleptic control

Properties such as shape, size, homogeneity, color, transparency, and surface of pellet formulations have been visually evaluated.

Determination of particle size and size distribution of wet-dry pellets

The sizes of freshly prepared and lyophilized PG containing and blank floating hydrogel pellets were measured from each series using a caliper (min. n=30).

In-vitro release study

Floating hydrogel pellets containing a certain amount of PG were placed in a horizontal shaker water bath set at 37 °C/50 rpm containing 50 mL pH 1.2 HCl buffer (USP30-NF25) and release was carried out for 24 hours. An appropriate amount of samples were taken at certain time intervals (such as 5 min, 30 min, 1 hr) and subsequently, the same amount of fresh buffer medium was added to the samples to maintain the sink conditions. Samples were filtered through 0.45 µm membrane filters and released PG amounts by a validated HPLC method were determined (Jelvehgari et al., 2015).

Determination of release kinetics

Release results in pH 1.2 HCl buffer were applied to the computer program in order to determine the kinetic model of the release from the selected hydrogel pellets containing PG. Whether formulations are compatible with Zero-Order, First-Order, Korsmeyer-Peppas, or Higuchi kinetic models were determined by mathematical operations and formulas (Kumar et al., 2014; Bhardwaj et al., 2010).

Surface and structural morphology

The presence of pores in the pellets, the roughness or smoothness of their surfaces were investigated using a scanning electron microscope (SEM). Since our formulations are insulating, they were examined after being covered with a thin layer (100 Å) with gold (Kathpalia et al., 2013).

Results

Development of pregabalin quantification method

A calibration curve was drawn from 11 points (1, 5, 10, 25, 50, 75, 100, 125, 150, 175, 200 µg/mL) with samples prepared from the stock solution so that the measurements were more precise. In the equation obtained, R^2 was determined to be 0.9999. The calibration curve and equation are given in Figure 1 below.

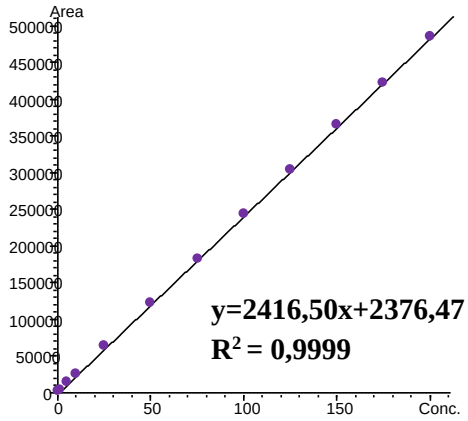

Figure 1. Calibration curve and equation

Validation of the quantification method

After the quantification method was developed, validation was initiated and it was determined that the method showed linearity in the PG dose range between 0.3 µg/mL and 200 µg/mL. It was determined that the blank solution containing all components used in formulations except PG was read and did not peak at the same place as PG and the developed method was specific for PG. The sensitivity of the method was determined experimentally that LOD was 0.3 µg/mL and LOQ was 1 µg/mL. As a result of the repeatability and reproducibility studies carried out for the accuracy and precision of the method, it has been determined that % relative standard deviation and% relative error do not exceed 2%.

Preparation of floating pellet formulations

As a result of the pre-formulation studies, different combinations were created by changing the formulation components. Used formulation components and quantities are given in Table 2.

Table 2. Formulation components and quantities (mg)

Formulation	PG	Pectin	$CaCl_2$	$NaHCO_3$	Drug phase:Aqueous phase
1D	10	40	0.1 M	20	1:5
2D	10	40	0.1 M	40	1:5
3D	10	40	0.2 M	20	1:5
4D	10	40	0.2 M	40	1:5
5D	10	40	0.3 M	20	1:5
6D	10	40	0.3 M	40	1:5
1B	-	40	0.1 M	20	1:5
2B	-	40	0.1 M	40	1:5
3B	-	40	0.2 M	20	1:5
4B	-	40	0.2 M	40	1:5
5B	-	40	0.3 M	20	1:5
6B	-	40	0.3 M	40	1:5

Determination of floating degrees

All prepared pellet formulations were left to float in pH 1.2 HCl buffer medium for 24 hours.

The results are given in Table 3. Considering the results, there have been 6B and 6D formulations that started floating as soon as possible and were completely flotation for 24 hours. Digital images of the flotation experiment of 6B and 6D are given in Figure 2.

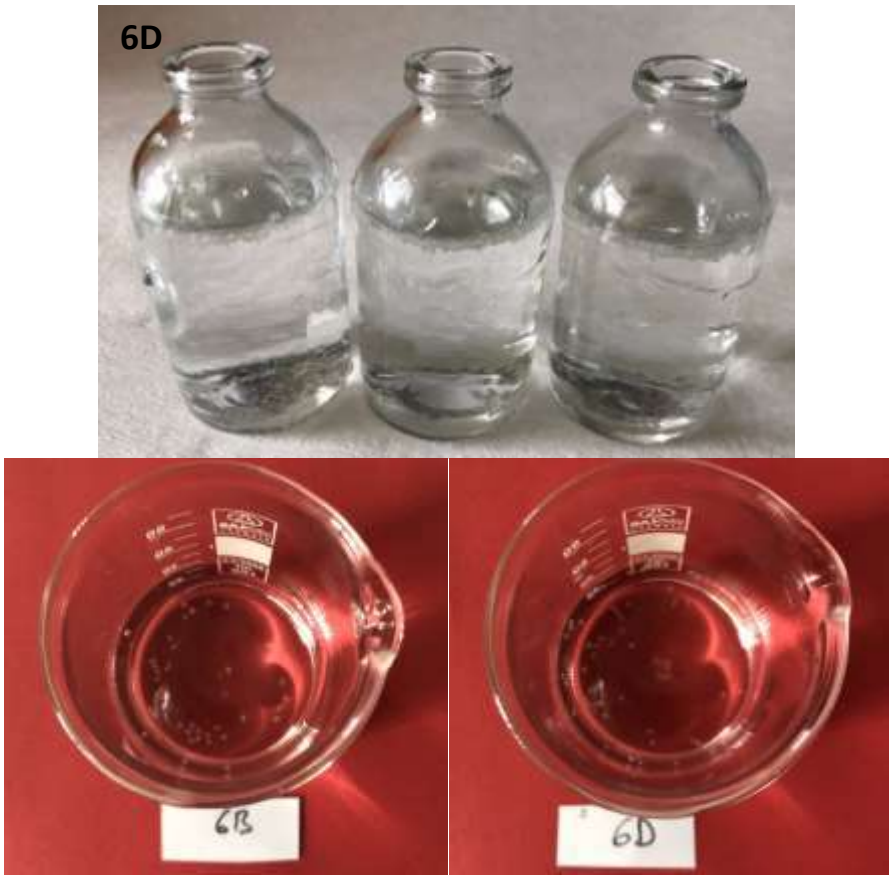

Figure 2. Formulations selected as a result of the flotation degree study

Table 3. Flotation degree data of formulations (%)

Formulation	5.sec	30.sec	1.h	2.h	4.h	8.h	24.h
1D	10	10	10	-	-	-	-
2D	60	60	60	-	-	-	-
3D	12.5	12.5	12.5	-	-	-	-
4D	100	100	90	-	-	-	-
5D	4.16	33.3	35	-	-	-	-
6D	100	100	100	100	100	100	100
1B	11.1	72.2	70.1	-	-	-	-
2B	76.47	76.47	75.09	-	-	-	-
3B	6.25	18.75	33	-	-	-	-
4B	80	90	90	-	-	-	-
5B	36	96	91	-	-	-	-
6B	100	100	100	100	100	100	100

Determination of yield, drug loading capacity, and encapsulation efficiency

The yield, drug loading capacity, and encapsulation efficiency data of the selected 6B and 6D

pellets are given in Table 4 (mean ± SD).

Table 4. Yield, encapsulation efficiency, and drug loading capacity of 6B and 6D pellets

Formulation	% Y	% EE	% DL
6B	10.74±1.29	-	-
6D	13.75±0.58	82.43±15.45	3.40±0.51

Determination of swelling degrees

The swelling degree study was carried out on the formulations of 6D and 6B, which had the best flotation degree from the prepared pellets. The swelling degrees were calculated with the formula given above and the results are given in Table 5 below (mean ± SD). The digital images of 6B and 6D as a result of the swelling degree study are given in Figure 3, and the swelling degree graph is given in Figure 4.

Table 5. Swelling degree data of 6B and 6D (%)

Formulation	30.sec	1.h	2.h	4.h	8.h	24.h
6B	150.00	150.60	161.80	195.07	204.40	247.73
6D	144.87	148.21	162.00	198.33	182.33	239.87

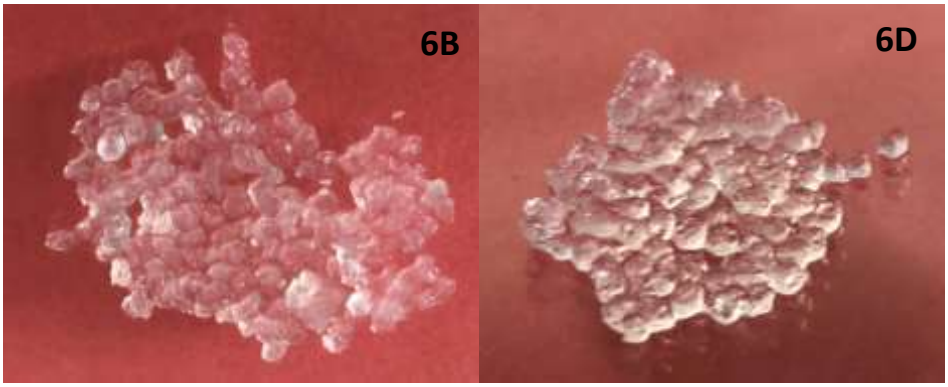

Figure 3. Swelling test results of 6B and 6D formulations after 24 hours

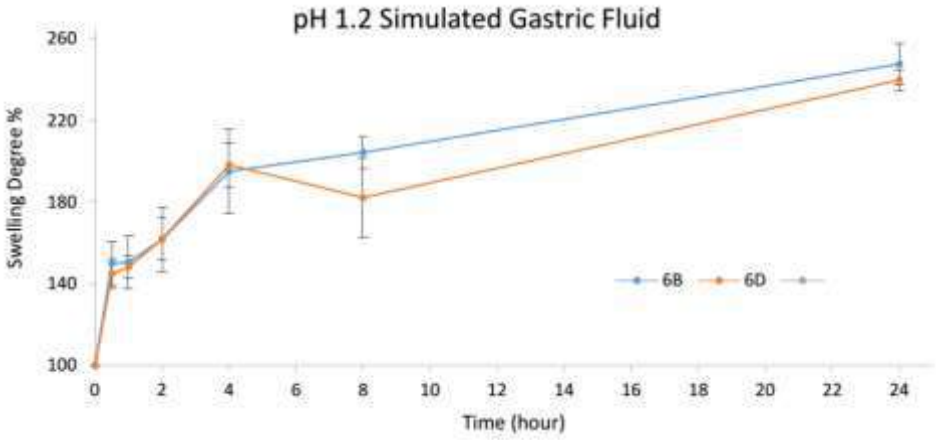

Figure 4. Graph of swelling degrees

Morphological and organoleptic control

After the pre-formulation development studies, all formulations were subjected to organoleptic control, and 6B and 6D formulations were selected both at this stage and after the quantification, yield, and swelling degree studies. Digital photos of 6B and 6D freshly prepared and after lyophilization are given in Figure 5.

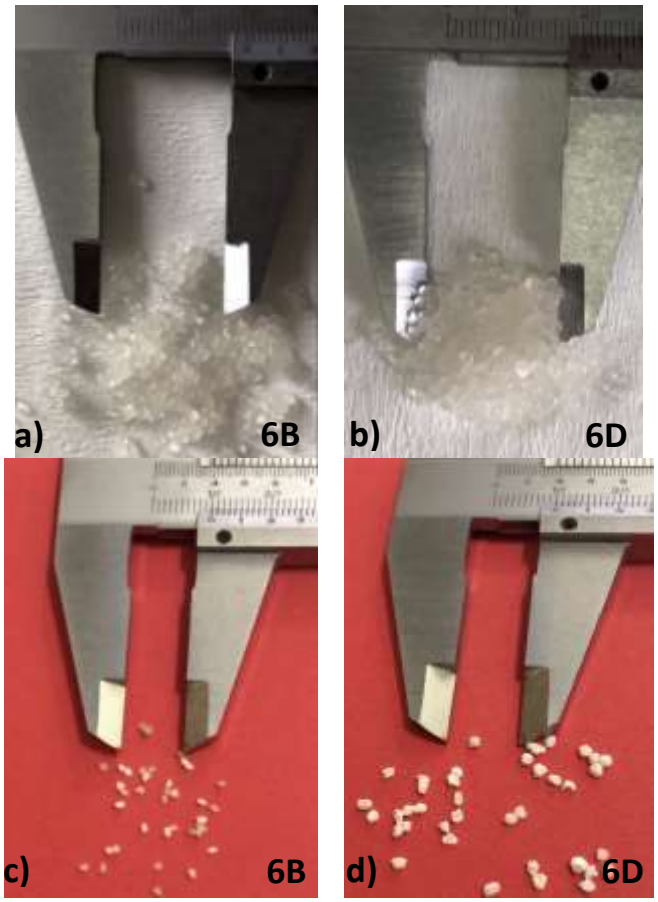

Figure 5. Images of 6B and 6D freshly prepared (a, b) and after lyophilization (c, d)

Determination of particle size and size distribution of wet-dry pellets

The sizes of the freshly prepared and lyophilized pellets of the selected 6D and 6B formulations and the pellets with and without PG are given in Table 6 as the mean and standard deviation.

Table 6. Sizes of formulations (min.=30, mean±SD)

Formulation	Freshly prepared (mm)	Lyophilized (mm)
6B	1.65±0.11	1.47±0.22
6D	1.85±0.12	1.77±0.14

In-vitro release study

Release results are given in Table 7 using pH 1.2 HCl buffer (USP30-NF25) (mean ± SD). It has been observed that pellets containing PG in the selected 6D formulation show a 'burst effect' in the first 5 minutes and then release for 24 hours. Approximately 99% of the PG loaded at the

end of 24 hours was released. The graph for release is given in Figure 6.

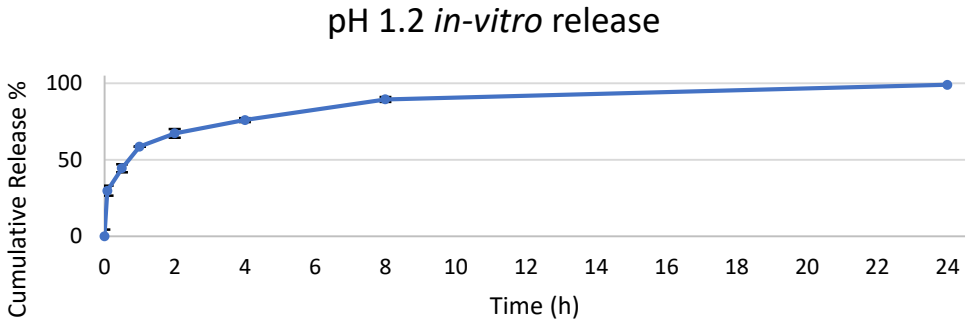

Figure 6. *In-vitro* release graph of 6D containing PG

Tablo 7. *In-vitro* release results % (n=3, mean±SD)

Formulation	5.sec	30.sec	1.h	2.h	4.h	8.h	24.h
6D	29.85± 4.41	14.75± 3.26	14.09± 2.61	8.74± 0.01	8.65± 2.82	13.48± 1.24	9.57± 1.54

Determination of release kinetics

The kinetic model of PG release from the selected 6D hydrogel pellet formulation was determined by a computer program. All release kinetics data of 6D formulation are given in Table 8 below. In the evaluation made on the value of R^2, it was observed that the 6D formulation showed a more suitable release for the Korsmeyer-Peppas release kinetic model.

Table 8. Release kinetics data of 6D formulation (n=3)

	R^2			
Formulation	Zero-Order	First-Order	Korsmeyer-Peppas	Higuchi
6D	0.6232	0.4802	0.9880	0.9428

Surface and structural morphology

SEM images of selected 6D and 6B formulations are given in Figure 7 below. It was observed that the pellets, especially the surfaces of the empty pellets were smoother and smaller, and the pellets containing PG were rougher and larger.

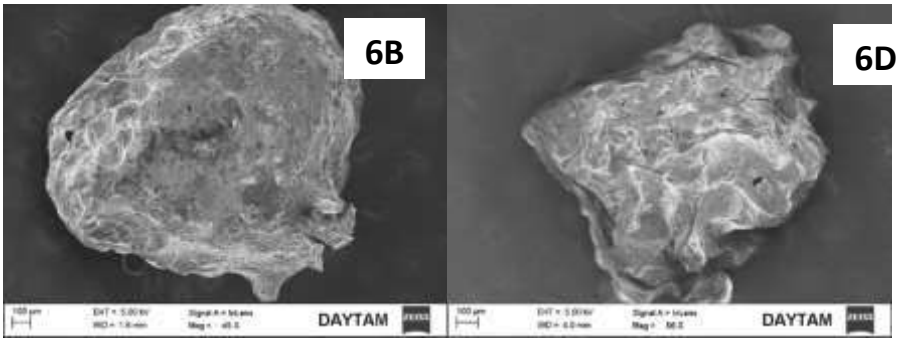

Figure 7. SEM images of 6B and 6D

Discussion

The method developed for PG quantification is sensitive, specific, reproducible, and validity has been proven with validation processes. The data obtained (quantification, EE%, and in-vitro release results) were evaluated precisely (R^2=0.9999). In the preformulation studies in which more than one parameter was evaluated, 100% floating pellets were preferred for our target and other studies were carried out on these formulations. The 6D formulation, which has an encapsulation efficiency of over 82%, has been found extremely satisfactory in terms of PG delivery and release. Given that gastric emptying varies between 1-3 hours in similar studies, it has been made possible for the formulation we developed to perform PG release on the gastric fluid surface for 24 hours (Uttam et al., 2016). When the degree of flotation of the formulations was examined, it was determined at the end of the first 1 hour that the fastest, longest, and the greatest amount of floating pellet formulations were 6B and 6D. In similar studies, it was observed that the flotation degree did not exceed 75% after 8 hours. In our study, this value was reached in the 5th minute and this situation continued until the end of 24 hours (Hemant and Patel, 2013).

The duration of staying drug formulations in the stomach generally increases as the size increases. However, for drug formulations larger than 9.9 mm, smaller sizes are known to prolong the stay, as the flotation potential will decrease (Uttam et al., 2016). Compared to similar carrier system formulations made with pectin, the pellets in our study have a larger size (~ 1.5-2.0 mm). Thus, the potential of pellets to swim in the stomach is increased and this theory is supported (Jiyoung et al., 2013).

The surfaces of the pellets were determined by SEM images that the 6D hydrogel pellets were rougher and larger than 6B hydrogel pellets. The growth of the size is also proof that PG is loaded. Also, the smoothness on the surface explains the “burst effect” observed in the in-vitro release. PGs adsorbed to the surface switched to the simulated gastric fluid in the first minutes of release and gave a high release percentage. This situation is also observed in similar studies (Sujata et al., 2016). This means that we can give the starting dose to the patient in treatment. In many of the sustained-release dosage forms available on the market, the starting dose is formulated separately and is placed in capsules or tablets. In our study, this has been realized spontaneously. Our study has been also gained value when viewed from this angle. In terms of release kinetics of the 6D formulation in which PG is released for 24 hours, it is very convenient to comply with the Korsmeyer-Peppas model, which explains the drug release behavior from polymeric systems. This model also shows that drug release is diffusion-controlled (Hemant and Patel, 2013). The 250% swelling of the developed formulations at the end of 24 hours compared to their first weight also supports this theory. Similar studies show formulations that exhibit swelling far below this value (Singireddy et al., 2016).

Conclusions

A new drug-bearing formulation has been developed for PG, which is prescribed for neuropathic pain, post-herpetic neuralgia, radicular pain, and fibromyalgia. With this study, a sustained-release has been achieved owing to pectin pellets, which can release for at least 24 hours in the contents of the stomach. Unlike extended-release preparations containing PG, available in the pharmaceutical market, this release was achieved without using any film coating agent. Formulations have been developed simple, reproducible, and easily adaptable to the industry, using fewer excipients. It is thought that this study will be an alternative dosage form that patients with neuropathic pain can benefit from, with fewer doses and fewer side effects.

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GREEN FERTILIZERS FROM MUSTARD AND RAPESEED PLANTS AS A WAY OF CONTROL OF BROOMRAPE (*OROBANCHE CUMANA* WALLR.) PARASITIZING ON SUNFLOWER

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ABSTRACT

O. cumana, an obligate parasite of sunflower, is widely spread in most countries cultivating this crop. It is one of the main factors limiting the cultivation of sunflower. Our laboratory experiments with broomrape seeds treated with extract from the crushed green mass of common mustard showed inhibition of both seed germination and growth of seedling, which penetrates the sunflower root. The aim of our research was to determine in greenhouse conditions the influence of application of oil crops (*Brassicaceae*) green fertilizers on the sunflower infection with broomrape. We used the green mass of white, black, common mustard and winter rapeseed as a green fertilizer. We added the crushed green mass of these crops (62.5-90.0 g) to vessels with a soil-sand mixture mixed with broomrape seeds. Such amount of green mass per vessel is comparable with the average green mass yield of these crops (15.5–22.5 t/ha) that is plowed into the soil as a green manure. After 15 days, we sowed the seeds of sunflower variety susceptible to all races of broomrape. We did not add the green fertilizers in the control variants. The growing of sunflower plants lasted for 45 days. We revealed the significant differences in the infection degree compared with the control at a 5 % significance level. The green fertilizer of white mustard (variety Ruslana) showed the highest percentage of decrease in the infection degree – 44.7 %. The common mustard (Yunona) and black mustard (Niagara) reduced the infection by 25.9 and 27.0 %, respectively, the rapeseed (Tavrion) – by 24 %. Thus, this experiment showed a significant decrease in sunflower plants infection with broomrape under presowing application of the crushed green mass of white, black, common mustard and winter rapeseed. We recommend using the green manures of these crops to reduce infection of sunflower crops with broomrape and as an environmentally safe method of clearing the field from its seeds.

Key words: sunflower, broomrape, green fertilizer, mustard, rapeseed.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a valuable oil crop that provides for the needs in vegetable oil not only in the Russian Federation but also in many other countries of the world. High profitability of sunflower cultivation makes it attractive for intensive cultivation. In the Russian Federation, the crop acreage occupied by sunflower is increasing each year. For instance, over the past 10 years the acreage has increased by 37.2 %. In 2019, according to the Russian Federal State Statistics Service, the crop acreage of sunflower in farms of all categories amounted to 8505.3 thousand ha. One of the main factors limiting sunflower production in the southern regions of the Russian Federation is the broomrape parasitic plant (*Orobanche cumana* Wallr.) (Antonova *et al*., 2012b). This weed parasitic plant also affects sunflower in many other countries of the world cultivating this crop. Broomrape is one of the main factors that significantly reduces the yield and worsens the seed quality (Kaya, 2014). *O. cumana* Wallr. is an obligate parasite that belongs to the higher flowering plants from the *Orobanchaceae* family.

Broomrape plants do not have their own roots and leaves; they are completely devoid of chlorophyll. The broomrape seedling grows into the sunflower root and feeds at its expense, developing the tubercle outside the root that is rich in nutrients. One or more apical points are formed in the tubercle, from which one or more stems grow. Under favorable conditions, the stems in *O. cumana* can reach a height of about 1 m and develop up to 100 flowers (Antonova *et al*., 2012a). The upper part of a stem with flowers of *O. cumana* Wallr. represents a loose spike-shaped inflorescence. In each flower, a fruit ripens that represented by a box containing hundreds of tiny seeds (360-500 x 160-250 μm (Plaza *et al*., 2004). When ripening, the leaves of boxes open, and the seeds spill out. A single broomrape plant can develop from 200 to 500 thousand tiny seeds that are easily spread by wind, water, agricultural machinery, tillage tools, and vehicles (Castejón-Muñoz *et al*., 1991). Laying in the soil, they can retain viability up to 20 years (Stoyanova *et al*., 1977; Molinero-Ruiz *et al*., 2008).

Such a high potential of the reproductive function of *O. cumana* and the frequent return of sunflower to its former field accelerate the emergence and rapid spread of new, more virulent parasite races that quickly overcome the resistance of new hybrids and varieties of sunflower. With frequent return of sunflower to its former field, the concentration of broomrape seeds in the arable layer of the soil increases disastrously. Under the current conditions, breeders do not have time to develop new sunflower genotypes that are resistant to new broomrape races. To solve the problem of broomrape control and to improve the phytosanitary condition of foul fields, an integrated approach to controlling this parasite is necessary.

It is necessary not only to cultivate sunflower genotypes, that are resistant to new virulent broomrape races, but also to comply with the crop cultivation technology, to apply the herbicides that kill the parasite without harming the sunflower plants, and to use the trap plants in crop rotations (corn, sorghum, millet, and Sudan grass) (Awad *et al.*, 2006; Yoneyama *et al.*, 2010; Goldwasser and Rodenburg, 2013; Antonova *et al*., 2015). Compared with the herbicides appliance, the use of trap crops is more preferable as an environmentally safe way to clear fields from broomrape seeds.

Another environmentally safe method is noteworthy. As our observations show, cruciferous oil crops (Fig.1) used as a green fertilizer can also help to suppress the germination of broomrape seeds. In 2016, we noticed a decrease in the sunflower broomrape infection at the farms in which white mustard (*Sinapis alba* L.) was grown on a field as a previous crop and its green mass (green manure) was crushed and plowed into the soil as a green fertilizer.

Figure 1. White mustard (*Sinapis alba* L.) before utilization as green manure.

It is known that cruciferous oil crops can have a fatal rhizospheric effect on a couch grass (*Elytrigia repens* L.), causing vessels occlusion of its rhizomes by metabolic products (Kukresh and Bysov, 1990). The application of green fertilizers into the soil and their plowing into the seed area of sown crops negatively affects their germination. The fresh plant residues almost always contain inhibitors of germination and growth; therefore, after their plowing into the soil,

it is necessary to wait some time for the decomposition by microorganisms (Novikov, 1993). According to Haramoto and Gallandt (2005), in spring, crushed and plowed into the soil green mass of cruciferous oil crops inhibits the growth of red-root amaranth (*Amaránthus retroflέxus* L.), shepherd's purse (*Capsella bursa-pastoris* L.), green foxtail (*Setaria viridis* L.), black nightshade (*Solanum nigrum* L.), and cockspur grass (*Echinóchloa crus-gálli* L.) (Haramoto and Gallandt, 2005). Apparently, the green mass of cruciferous oil crops can have a similar effect on broomrape (*O. cumana*).

The suppression of weeds during the cultivation of cruciferous oil crops occurs under the influence of thiocyanins, sulfur-containing compounds obtained during decomposition of glucosinolates (Zybalov, 2002). Glucosinolates are biologically active substances found in the leaves, stems, seeds, roots and root exudations of plants of the cruciferous family. Once in the soil, they inhibit the germination of seeds and the growth of the vegetative organs of weeds (biofumigation) (Sarwar *et al.*, 1998).

The application of green fertilizer of oil cruciferous crops in the seed fallows and intermediate sowings contributes to the soil improvement as well as to the increase of the crop yields. The low costs of cultivation and the subsequent plowing of green mass into the soil make green manuring practice economically advantageous and deserving more attention from agricultural producers. The application of oil cruciferous crops in crop rotation as a green fertilizer could also contribute to the improvement of the phytosanitary condition of the fields infested with broomrape seeds.

The aim of our research was to study the effect of green manure of oil cruciferous crops on the infestation of sunflower with broomrape in the controlled greenhouse conditions.

MATERIALS AND METHODS

To develop an infectious background, we used the seeds of broomrape population (*O. cumana*) collected in 2017 in the Morozovsky district of the Rostov region.

To obtain green mass, we grew mustard and rapeseed plants in vessels until the phase of budding – the beginning of flowering (Fig. 2.).

Figure 2.The plants of white mustard variety Ruslana grown in a greenhouse as a green fertilizer

The green mass of white mustard (*Sinapis alba* L.) – variety Ruslana, black mustard (*Brassica nigra* L.) – variety Niagara, common mustard (*Brassica juncea* L.) – variety Yunona and rapeseed (*Brassica napus* L.) – variety Tavrion was used as green manure. We grew the plants of these crops for 20 days and then crushed them to a size of 1 x 1 cm. We applied the

green mass of plants, crushed together with the roots (from 62.5 to 90 g), into the vessels with a mixture of sieved soil and sand in a ratio of 3:1 and the broomrape seeds at the rate of 200 mg per 1 kg of soil-sand mixture. The surface area of the growing medium in each vessel was 0.04 m^2. The green mass weight from 62.5 to 90 g, applied to such area, is comparable with the average green mass yield of these crops, respectively from 15.6 to 22.5 t/ha, which is plowed into the soil during green manuring. To create favorable conditions and accelerate microbiological processes, we moistened the growing medium with plowed green mass on a regular basis for 15 days.

After 15 days, during which the microorganisms decomposed the crushed parts of mustard and rapeseed, we sow the seeds of sunflower variety VNIIMK 8883 susceptible to all races of broomrape by 10 pieces in each vessel, in 2 replications in all variants of the experiment. In the control variant, we did not apply green manure. We grew the sunflower plants in a greenhouse for 45 days at a 16-hour photoperiod and at a temperature of 25-27 °C. Then, we dug the plants up and counted broomrape tubercles and shoots on the roots washed with water.

RESULTS AND DISCUSSION

The degree of infestation of sunflower plants of variety VNIIMK 8883 with broomrape from the population of Morozov district of the Rostov region in the control variant was very high.

The average number of tubercles and shoots of *O. cumana* was 99 pieces per affected plant (Table 1). Usually, such severe infection leads to the harvest failure of crop, especially in areas with insufficient moistening. We revealed significant differences in the degree of plant infection by broomrape in the variants with applied green manure, in comparison with the control at a 5 % significance level.

White mustard deserves special attention among the cruciferous family used for green manuring. The green manure of white mustard has prominent phytosanitary properties; it also helps to increase the microbiological activity of the soil and the accumulation of nutrients in it and to increase the yield of subsequent crop rotations.

Ruslana is a non-erucic variety of white mustard; it has a high seed yield potential, high initial growth rates, it is an excellent green fertilizer. The yield of green mass is 25 t/ha. The green manure of white mustard Ruslana showed the highest percentage of decrease in the infection degree of sunflower variety – 44.7 %, under application of 71.3 g per 6.7 kg of soil-sand mixture. Such amount of plant biomass, applied into one vessel is comparable with the average yield of green mass of white mustard – 17.7 t/ha, which is plowed into the soil during green manuring (Table 1).

Common mustard of variety Yunona is recommended for cultivation as a high-protein fodder additive and green manure. The harvest of its green mass can reach 33 t/ha. The green manure of common mustard reduced the infection degree of sunflower variety VNIIMK 8883 by 25.9 % (Table 1).

Niagara is the first black mustard variety in domestic production, it has the highest content of essential oil in the seeds – 1.0-1.1 %, and it is a source of allyl isothiocyanates (natural preservative). The green manure of black mustard reduced the sunflower infection in our experiment by 27.0 %, which is slightly higher than the indicator of common mustard.

Highly productive early-ripening variety of spring rapeseed Tavrion is stress-tolerant, and adapted for cultivation in various soil and climatic conditions. When we applied 90 g of spring rapeseed green mass per 6.4 kg of soil-sand mixture, the decrease in sunflower infection was only 24.2 % (Table 1).

Presumably, in comparison with common mustard, black mustard and rapeseed, the white mustard biomass exudes a greater amount of specific substances in the process of decomposition and mineralization that inhibit the germination of *O. cumana* seeds.

Table 1. The influence of green mass of oil crops from the family *Brassicaceae* on the degree of broomrape infestation of sunflower plants (variety VNIIMK 8883)

№ п/п	Variants of experiment	Quantity of soil per one vessel, kg	Broomrape seeds added per one vessel, g	Green mass added per one vessel, g	The degree of broomrape infestation*, pieces	The decrease of degree infestation, %
1	Control (without green manure)	6.3	1.7	0.0	98.8	0.0
2	White mustard, variety Ruslana	6.7	1.8	71.3	54.6	44.7
3	Common mustard, variety Yunona	7.2	1.9	62.5	73.2	25.9
4	Black mustard, variety Niagara	7.1	1.9	71.4	72.1	27.0
5	Rapeseed, variety Tavrion	6.4	1.7	90.0	74.9	24.2
5 % significance level					11.3	
* The degree of broomrape infestation – is the quantity of tubercles and shoots per one sunflower plant						

Earlier, we conducted laboratory experiments by treating broomrape seeds with different concentrations of extracts from the green mass of common mustard. The experiments showed inhibition of both seed germination and the growth of *O. cumana* seedling, which should penetrate the sunflower root (Fig. 3). The soaking of *O. cumana* seeds for 10 days in a 100 % extract of the cell sap of common mustard plants led to their loss of germination (Strelnikov and Trubina, 2019).

Economically speaking, the relatively low cost of resources for cultivating and subsequent plowing of green mass of oil cruciferous crops into the soil to reduce the infection degree of sunflower with broomrape makes the green manure method accessible and deserving consideration of agricultural producers.

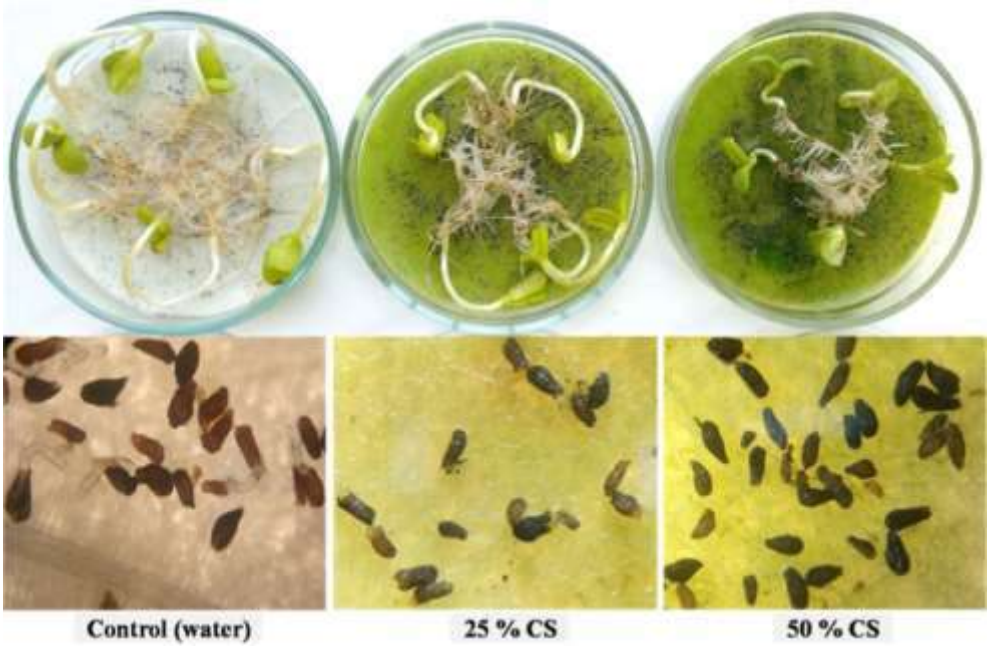

Figure 3. The effect of different concentrations of cell sap (CS) of plants of common mustard variety on the germination of *O. cumana* seeds in the presence of roots of sunflower variety VNIIMK 8883 (orig.).

CONCLUSION

Thus, the experiment conducted under controlled greenhouse conditions showed a significant decrease in the sunflower plants infection with broomrape under presowing application of the crushed green mass of white, black, common mustard and winter rapeseed. Varieties of mustard (white, black, and common) and rapeseed are approved for production in the regions of the Russian Federation. We recommend using green manures of these oil cruciferous crops to reduce the infection of sunflower crops with broomrape and as an environmentally safe way to clear fields from broomrape seeds in combination with already known control methods.

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COMPARISON OF THE STRUCTURE OF CHITIN EXTRACTED FROM PUPA, COCOON AND PUPA EXUVIAE OF THE TURKISH MULBERRY SILKWORM *BOMBYX MORI* L.

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ABSTRACT

Since the demand for valuable silk fabric is much higher than the amount of fabric produced, interest in silkworm cultivation is getting increase. With the increase in production, the low commercial value by-products of the silk industry such as pupa, pupa exuviae, and stained and hole cocoons go to waste. This study aimed 1) to identify the structure of the chitins isolated from pupa, waste cocoons, and pupa exuviae left in the cocoon, of Turkish mulberry silkworm, 2) to transform these biowastes from agricultural production into biotechnological product chitin, and to determine possible application areas of this chitin. The chitin samples were examined by Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), Scanning Electron Microscopy (SEM), and X-Ray Diffraction (XRD) analyses. Although the pupa and pupa exuviae chitin were determined to be in the α-form, we couldn't decide on that cocoon chitin is in which crystalline form. Considering the results of the analysis and previous studies, we believed that this material isolated from cocoon may be the residues of N-acetyl-D-glucosamine which is the degraded product of the chitin. The yield (17.2%), surface morphology, crystallinity (CrI: 63.1%) and thermal stability (DTGmax: 330 °C) of this cocoon chitin material were determined. Also, the crystallinity of the silkworm pupa chitin (CrI: 80.7%) and the thermal stability of the pupa exuviae chitin (DTGmax: 415 °C) were determined for the first time, and the thermal stability was quite high. Silkworm pupa exuviae can be used as the source of chitin because of its high chitin content (13% of dry weight). Also, porous pupa exuviae chitin can be used in tissue engineering and drug release and the chitins with high thermal stability from pupa and pupa exuviae can be used in heat treatment-resistant, fireproof garments, and thermal therapies.

Keywords: Turkish silkworm, chitin, thermal stability, crystallinity, biotechnological application

INTRODUCTION

Chitin is the second most abundant polysaccharides on earth. It consists of alternative N-acetylglucosamine units that are bound by β- (1-4) glycosidic bonds (Muzzarelli and Peter, 1997; Merzendorfer and Zimoch, 2003). Chitin is a component of exoskeletons of insects and crustaceans, the cell wall of fungi, yeasts and algae, and has three different structures: α, β, and γ (Yen et al., 2008). α-chitin is the most common form in nature and has a structure of antiparallel chains. The β-chitin has a structure of parallel chains, and it is less available than the α-chitin (Minke and Blackwell, 1978). The γ-chitin has a structure consisting of both parallel and anti-parallel chains and it is the least common form in nature (Rudall and Kenchington, 1973; Kramer and Koga, 1986). The polymorphic forms of the chitin differ according to the source, and the emergence of these polymorphic forms depends on the physiological role of the chitin in living organisms and the tissue characteristics (Purkayastha and Sarkar, 2020). The α-chitin was mostly extracted from marine crustaceans such as crab, lobster and shrimp (Gopal et al., 2019), β-chitin from the endoskeletons of cephalopods (Rinaudo, 2006), and γ-chitin from moth cocoon (Kaya et al., 2017). Since chitin is biodegradable, biodynamic, organic, natural, and ecological, it gains importance as a green product (Cervellon et al., 2011; Casadidio et al., 2019). Chitin and its deacetylated form chitosan have many biological properties such as biodegradable, biocompatible, anticancer, antiviral, antifungal, antimicrobial (Anitha et al., 2014). So, they are widely used in medicine, pharmaceutical industry, food industry, bioengineering, nanotechnology, textile industry, water treatment (Rinaudo, 2006). In previous studies, it has been stated that the inorganic portion in insects is generally less than 10% and therefore the chitin in insects can be isolated using less acid and base than aquatic crustaceans (Liu et al., 2012; Purkayastha and Sarkar, 2020). For this reason, in recent years, there has been a trend towards insect chitin (Zhang et al., 2000, Sajomsang and Gonil, 2010). In this study, the evaluation of waste products of the silk industry, which is in great demand in the world, as a source of chitin will be examined.

Silk is a woven raw material that is superior to other fabrics due to its lightness, natural luster, easy dyeability, high absorbency, softness, flexibility, and durability (Yılmaz et al., 2015; Gurjar et al., 2018, Ministry of Agriculture and Forest, 2020). According to the International Sericultural Commission data; global silk production was reported as 159648 tons in 2018. In terms of production, China ranks first with 120,000 tons, and India ranks second with 35,261 tons. In Turkey, 30 tons of silk production was carried out in 2018 (Inserco, 2020). Although silk is produced in a limited number of countries, it is consumed in many countries worldwide (Ministry of Agriculture and Forest, 2020). Silk demand in the world has been increasing especially in the last twenty years (Thiripura Sundari and Ramalakshmi, 2018). Due to the international demand for silk product consumption, many countries are closely interested in silk breeding.

Mullbery silkworm (*Bombyx mori* L.) is a holometabolous insect from the order Lepidoptera of the Arthropoda phylum (Klimenko, 1990). It is an economic species used in the production of silk, which is a product with high commercial value. It feeds only on mulberry (*Morus alba* L.) leaves (Gurjar et al., 2018). The silkworm undergoes a complete metamorphosis. During the metamorphosis, it passes through four stages including egg, larva, pupa, and adult stages (Paulino et al., 2006; Kumar et al., 2015; Supriya and Singh, 2020). The

pupa is the adult form of silkworm larvae (Paulino et al. 2006). Silkworm pupa is a by-product generated after the extraction procedure of silk thread and remain idle (Paulino et al., 2006; Kumar et al., 2015; Priyadharshini et al., 2017; Supriya and Singh, 2020). Silkworm pupae are high-quality protein sources containing the amino acids that the human body needs (Kumar et al., 2015; Supriya and Singh, 2020). In addition, pupa are very rich in fat, carbohydrates, and minerals (Priyadharshini et al., 2017). It is also consumed as food in some countries such as Assam, Korea, Japan, China, Vietnam, and Thailand (Kumar et al., 2015; Wikipedia, 2020). Pupa and pupa exuviae are generally considered to be waste products, and disposal of large amounts of pupa and pupa exuvia in silk production areas causes environmental pollution (Rosmiati et al., 2016; Priyadharshini et al., 2017). Silkworm designs a structure called cocoon during metamorphosis to protect itself against the attacks of other organisms such as birds, insects and bacteria, and harsh environmental conditions (Kumar et al., 2015; Gün Gök et al., 2019). Cocoon consists of a long silk thread ranging in size from 900 to 1500 meters (Gün Gök et al., 2019). Cocoon silk has been reported to consist of 45 different metabolites, containing amino acids, organic acids, carbohydrates, hydrocarbons, and fatty acids (Zhang et al., 2017). The quality of the cocoon and yarn produced depends on several factors, including health of the larvae and genetics (Wikipedia, 2020). Insects dying in cocoons stain silk cocoons. When the chrysalides inside the cocoons become butterflies and pierce the cocoon and go out, they decrease the commercial value of the cocoons. Thus, stained and perforated cocoons remain idle since they cannot be evaluated commercially.

The chitin is found in the silkworm's pupa, outer shell, and internal organs such as spiracle and trachea (Zhang et al., 2000; Kumar et al., 2015; Wikipedia, 2020). A few studies have been performed on the silkworm chitin before. Zhang et al. (2000) isolated chitin from silkworm pupa exuviae and characterized it by SEM, XRD CP/MAS, and NMR analyses. Paulino et al. (2006) obtained chitin and chitosan from silkworm chrysalides and characterized them by FTIR, SEM, TGA, DSC, and ^{13}C-NMR analysis and determined the molecular weight of chitosan. Suresh et al. (2012) investigated the chitin and chitosan yields of biovoltine and multivoltine silkworm pupa. Supriya and Singh (2020) extracted chitin from the silkworm cocoon and examined it by FTIR and XRD analyses. However, they did not provide sufficient and detailed information on the physicochemical properties of the cocoon chitin in these analyzes. The properties of the chitin differ depending on the source of the chitin and the geographical distribution (Gbenebor et al., 2017). Therefore, unlike other studies, we isolated chitin from Turkish mulberry silkworm's poor quality cocoons and the pupa exuviae remaining in them, we have made a detailed characterization of the chitin and examined the using potential of cocoon and pupa exuviae as a chitin source. The characteristics of the cocoon chitin were investigated in detail by XRD, SEM, TGA, and FTIR analyses, and compared with the pupa and pupa exuviae chitins. Thus, by determining the chitin content of the by-products of the Turkish silkworm and the structure of the chitin, it was aimed to transform biowastes originating from agricultural production into biotechnological products and to determine possible application areas.

MATERIALS AND METHODS

Silkworm cocoons were procured from a local silkworm breeder in Demirhanlı village of Edirne Province in 2018. Silkworm pupa were taken from the cocoons used for silk

extraction, and the chitin contents and the chitin structure of the pupa were analyzed (Figure 1a and 1c). In addition, chitin was obtained and analyzed from the idle cocoons left by the adult silk beetles, and the pupa exuviae remaining in the cocoon (Figure 1b and 1d).

Figure 1. By-products used in the chitin isolation from Turkish mulberry silkworm (*B. mori*). (a. Cocoon including silkworm pupa, b. Cocoon left by silkworms, c. pupa, d. pupa exuviae)

Isolation of the chitin from silk cocoons

The dry weight of the cocoons used for the chitin isolation was 15.399 g. For removing the minerals contained in the silk cocoon, the cocoons were first kept at room temperature in 2M 400 mL HCl for 1 day. Then, acid treatment was continued by stirring at 600 rpm in a magnetic stirrer for 6 hours at 60 ° C. After demineralization, silk cocoon samples were rinsed with pure water and filtered. Then, it was kept in 2M NaOH at 75 °C for 20 hours to get rid of protein residues in silk cocoon samples. After NaOH treatment, the sample was washed again with water until reaching a neutral pH and then kept in a mixture including chloroform, methanol, and distilled water (1: 2: 4) for 3 hours to provide decolorization. As a final step, the samples were washed with pure water and then stored in the oven at 50 °C until drying. The amount of isolated chitin material was 2.6611 g.

The chitin isolation from silkworm pupa

For the chitin isolation from silkworm pupa, 10.110 g of ground dry pupa were used. For removing the minerals in the silkworm pupa, the ground sample was refluxed in 2M 350 mL HCl at 70 °C for 5 hours. The sample was refluxed with 2M, 350 mL of NaOH solution for 22 hours to remove the protein residues in the structure. It was then washed with pure water

until the pH was neutral. After that the sample was exposed to decolorization in the same conditions with cocoon. The sample was then filtered by washing and dried in the oven at 50 °C. The dry weight of the isolated chitin was 0.319 g.

Isolation of chitin from silkworm pupa exuviae

For the isolation of the chitin from the silkworm pupa exuviae, 11.5 g of ground dry pupa exuviae sample was used. The sample was exposed to 2M, 300 mL HCl at 70 °C for 4 hours to remove the minerals. The sample was treated with 2M, 350 mL NaOH solution for 22 hours to remove the protein residues. Then, it was rinsed with pure water to obtain a neutral pH. Then, the sample decolorized in the same conditions with the cocoon and the pupa. After decolorization, the sample rinsed with water and stored in the oven at 50 °C to dry. The amount of the isolated chitin was 1.496 g.

FTIR (Fourier Transform Infrared Spectroscopy)

FTIR analysis was performed to confirm the presence of characteristic functional groups in the chitin obtained from silkworm cocoon, pupa, and pupa exuviae. In this study, FTIR spectra were taken with Bruker ATR FTIR from 400 to 4000 cm^{-1} wavelength.

SEM (Scanning Electron Microscopy)

SEM analysis was performed to determine the morphological characteristics of chitin from silkworm cocoon, pupa, and pupa exuviae. In this study, SEM images were taken at 500X, 2000X 4000X 10000X, and 20000X magnifications with Quanta FEI SEM device.

TGA (Thermogravimetric Analysis)

TGA thermograms were taken to determine the thermal stability of chitin obtained from silkworm cocoon, pupa, and pupa exuviae. In this study, measurements were made using a Perkin Elmer Pyris1 device. The thermograms were taken in a nitrogen atmosphere by heating the samples at a rate of 10 °C/min from 25 °C to 650 °C and weight changes in the samples were recorded.

XRD (X-Ray Diffraction)

XRD analysis was performed to examine the X-ray diffraction patterns of chitin obtained from silkworm cocoon, pupa, and pupa exuviae. The device used in this study is Rigaku Miniflex 600 Cu X-Ray tube. Samples were analyzed at 1.54 Angstrom wavelength (ʎ), 5°- 45° scanning range, and 40kV- 15mA 2θ scanning speed. The formula given below was used to calculate the crystalline index values (CrI) of the isolated chitin (Liu et al. 2012).

$$CrI_{110} = [(I_{110} - I_{am}) / I_{110}] \times 100 \quad (1)$$

In the formula, the intensity of amorphous diffraction at 2θ=16° was represent with I_{am}, while the maximum intensity at 2θ=20° was represented with $I_{110.}$

RESULTS AND DISCUSSION

FTIR

FTIR spectra of the pupa, pupa exuviae, and cocoon chitins of silkworm *B. mori* are given in Figure 2. Kaya et al. (2017) observed the characteristic absorption bands of the α-chitin isolated from freshwater crab (*Potamon ibericum*) at 1652.5, 1620.5, 1552.4, and 1307.5 cm^{-1}. In the same study, they observed the characteristic bands of the β-chitin obtained from the common squid (*Sepia sp.)* at 1640.2, 1552.9, and 1308.4 cm-1. The FTIR bands of the γ-chitin obtained from the moth (*Orgyia dubia*) cocoon was also recorded by them at 1654.2, 1621.4, 1549.8, and 1307.5 cm^{-1}. In this study, the characteristic absorption peaks were recorded at 1654, 1621, and 1539 cm^{-1} for the silkworm pupa chitin, and at 1653, 1620, and 1551 cm^{-1} for the pupa exuviae chitin. The pupa and pupa exuviae chitins were found to be in the α-form. The FTIR spectra observed in this study were similar to those of the α-chitin of other insects. In a previous study, Paulino et al. (2006) observed the presence of two bands at 1626 and 1656 cm^{-1} in the FTIR spectrum of the chitin isolated from silkworm chrysalides. The authors noted that these bands match up to the vibrations of the amide I band, and the band at 1656 cm^{-1} match up to the amide I stretch of CaO, while the band at 1626 cm^{-1} match up to the stretching of the C-N vibration of the superimposed CaO group connected to the OH group with H bond. The large peak at 3500 and 1650 cm^{-1} indicates the presence of free hydroxyl groups or fewer hydrogen interactions (Duarte et al., 2002).

Liu et al. (2012) identified the three important absorption bands at 1654, 1560 and 1310 cm^{-1} in the FTIR spectrum of the insect *Holotrichia parallela* which is attribute to the amide I stretching of C=O, the amide II of N-H and amide III of C-N, respectively. Purkayastha and Sarkar (2020) reported that the amide I band of the chitin extracted from black soldier fly *Hermetia illucens*, divided into two parts, at 1653 and 1622 cm-1 for pupa exuviae and at 1649 and 1620 cm^{-1} for imago. It has been reported that the split at 1660 cm^{-1} is caused by the formation of intermolecular hydrogen bond and that the split at 1620 cm^{-1} is caused by the formation of intramolecular hydrogen bond (Al Sagheer et al., 2009; Waśko et al., 2016). In this study, the FTIR peaks of chitins obtained from silkworm pupa and pupa exuviae were similar to those of the α-chitin of the studies given above.

Previous researches reported that the gamma-chitin consisting of parallel and antiparallel chains was recorded in insect cocoons, and the chitin obtained from the cocoon threads of spider beetle *Ptinus tectus* larvae was reported to be in the γ-form (Rudall, 1962, Rudal 1963). FTIR absorption bands of the γ-chitin from moth cocoon were recorded at 3430, 3261, 2918, 2850, 1652, 1621, 1549, 1423, 1376, 1307, 1154, 1113, 1068, 1013, 952, and 894 cm^{-1} wavelengths (Kaya et al, (2017). Also, the authors reported that the cocoon chitin showed an amide I band with a vertex divided into two sharp lower peaks at 1660 and 1620 cm^{-1}. Unlike the moth cocoon, Supriya and Singh (2020) observed absorption bands at around 3282, 2927, 1620, 1516, 1443, 1227, 1163, 1057, 685, 603, and 551 cm^{-1} in the FTIR spectrum of the silkworm cocoon chitin. In this study, the absorption bands of the chitin obtained from the silkworm cocoon are almost the same as those reported by Supriya and Singh (2020). However, based on the FTIR peaks (1697, 1620, and 1514 cm^{-1}) observed in this study, it is difficult to tell in which crystalline form the cocoon chitin is. Zhang et al. (2017) identified 45 metabolites in the silkworm cocoon, consist of carbohydrates, hydrocarbons, fatty acids, organic acids and

amino acids, and discovered that many carbohydrates in the cocoon can convert into each other. The authors reported that these included the degraded product N-acetyl-D-glucosamine of the chitin and that N-acetyl-D-glucosamine could be reused to produce chitin. Probably, this is the reason for the FTIR peaks of the cocoon chitin do not fully reflect any form of the chitin. Methods such as Nuclear Magnetic Resonance (NMR), thin-layer chromatography, and mass spectroscopy should be used to better identify the structure of the cocoon chitin.

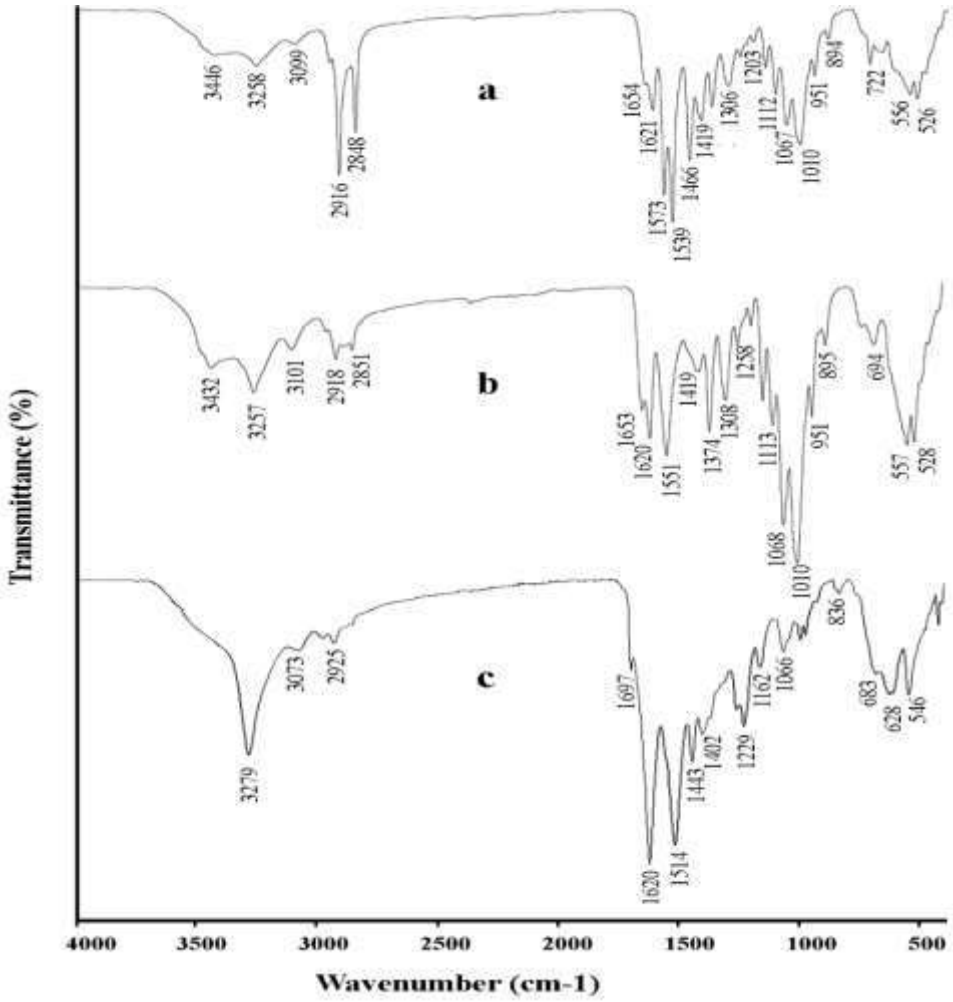

Figure 2. IR absorption spectra of chitin isolated from Turkish mulberry silkworm (a. pupa chitin, b. Pupa exuviae chitin, c. cocoon chitin)

Chitin contents of pupa, pupa exuviae and cocoon

The dry weight chitin contents of the by-products of silkworm *B. mori* were determined as 13% for silkworm pupa exuviae and 3.15% for pupa, respectively. The amount of the chitin material obtained from the silkworm cocoon was 17.2% of the dry weight of the cocoon. In previous studies on silkworm, the chitin content was found to be 15–20% for *B. mori* larva cuticle and pupa exuviae (Zhang et al. 2000), while it was reported as 2.59-4.23% for silkworm chrysalides (Paulino et al., 2006). Suresh et al. (2012) examined the chitin contents of different breeds of mulberry silkworm and the chitin contents of male and female silkworms were found

to be 3.225% and 3.078% in multivoltine pure breeds, respectively, and 3.078% and 2.945% in bivoltine pure breeds, respectively. We found that the chitin content of the pupa is quite low compared to that of the pupa exuviae, but the chitin contents of both pupa and pupa exuviae in this study are compatible with those found in previous studies. In a similar study, the dry weight chitin contents of adult and larvae of Colorado potato beetle (*Leptinotarsa decemlineata*) were found to be 20% and 7%, respectively (Kaya et al. 2014). The chitin content of *Tenebrio molitor* larva was reported as %18.01 and 4.92 for exuvium and whole body, respectively (Song et al. (2018). In other studies, the dry weight chitin contents were recorded as 9% for pupa exuviae of black soldier fly (*H. illucens*) (Purkayastha and Sarkar, 2020), 8.02% for pupa shells of house fly (*Musca domestica*) (Kim et al. 2016), 15% for the whole body of insect *H. parallela* (Liu et al. 2012), 36.6% for cicada slough (Sajomsang & Gonil, 2010), 5.43% for *Zophobas morio* larvae (Soon et al 2018) and 11.9% for *Vespula germanica,* 6.4% for *Vespa orientalis,* and 8.3% for *V. crabro* (Kaya et al. 2015a).

In a study, the chitin content of *Palomena prasina* cocoon was recorded as 10.8% (Kaya et al. 2015b). Apart from that, Kaya et al., (2017) obtained chitin from the cocoon of moth (*O. dubia*), and Supriya and Singh (2020) from silkworm cocoon, but they did not give the chitin contents. In this study, the yield of cocoon chitin material containing N-acetyl-D-glucosamine residues was determined to be 17% of the dry weight of the cocoon.

The studies presented above pointed out that insects can also be a new source of chitin. Song et al. (2018) suggest that larva exuviae (18.01%) and larvae (4.92%) of *T. molitor* can be an appropriate food additive as a source of chitin and chitosan in pet food. The chitin content of the silkworm in this study is high compared to other insect species. Therefore, it is envisaged that the silkworm pupa exuviae remaining idle in the cocoons can be considered as the alternative source for obtaining chitin. Thus, the wastes from agricultural production will be utilized in all aspects.

SEM

The morphology of the chitins from silkworm cocoon, pupa, and pupa exuviae were examined by SEM analysis at various magnifications (Figure 3). Chitin material obtained from the silkworm cocoon exhibited a smooth and loose structure (Figures 3a and b). While the chitin obtained from pupa exuviae displays a porous structure, the pupa chitin exhibits a more fibrous structure (Figures 3c and d). Kaya et al. (2017) observed long microfibers when they examined the surface morphology of the γ-chitin from the moth (*O. dubia*) cocoon. Liu et al. (2012) revealed that the chitin from insect *H. Parallela* exhibits a rough and thick surface morphology. Paulino et al. (2006) notified that the SEM image of the chitin prepared from silkworm chrysalides appears like a few thin loose combined leaves. They also observed a highly porous structure in this chitin. The surface of the chitin obtained from cicada slough showed a thick and rough morphology (Sajomsang and Gonil, 2010). The authors stated that this is caused by the removal of some inorganic materials and proteins from the structure.

Kaya et al. (2016) examined the surface of the chitins isolated from larva, pupa, and imago of *V. crabro* and remarked that all three chitin had fibrous and porous structure. Purkayastha and Sarkar (2020) described the properties of the chitins isolated from imago and pupa exuviae of *H. illucens,* a black soldier fly as follows. Pupa exuviae chitin exhibited a microfibrillar, non-porous, and hexagonal structure. The chitin obtained from the imago

showed a smooth structure with pores and parallel microfibrils. While the microfibrillar chitin has many applications in the textile industry (Ravi Kumar, 2000), the porous chitin has application in tissue engineering (Purkayastha and Sarkar, 2020). The surface morphology of the chitin from pupa exuviae in this study exhibited intense microporous structure and compatible with the results of Paulino et al. (2006) and Kaya et al. (2015a). We believe that the chitin of adult insects becomes more porous due to the accumulation of mineral substances carried in the cuticle.

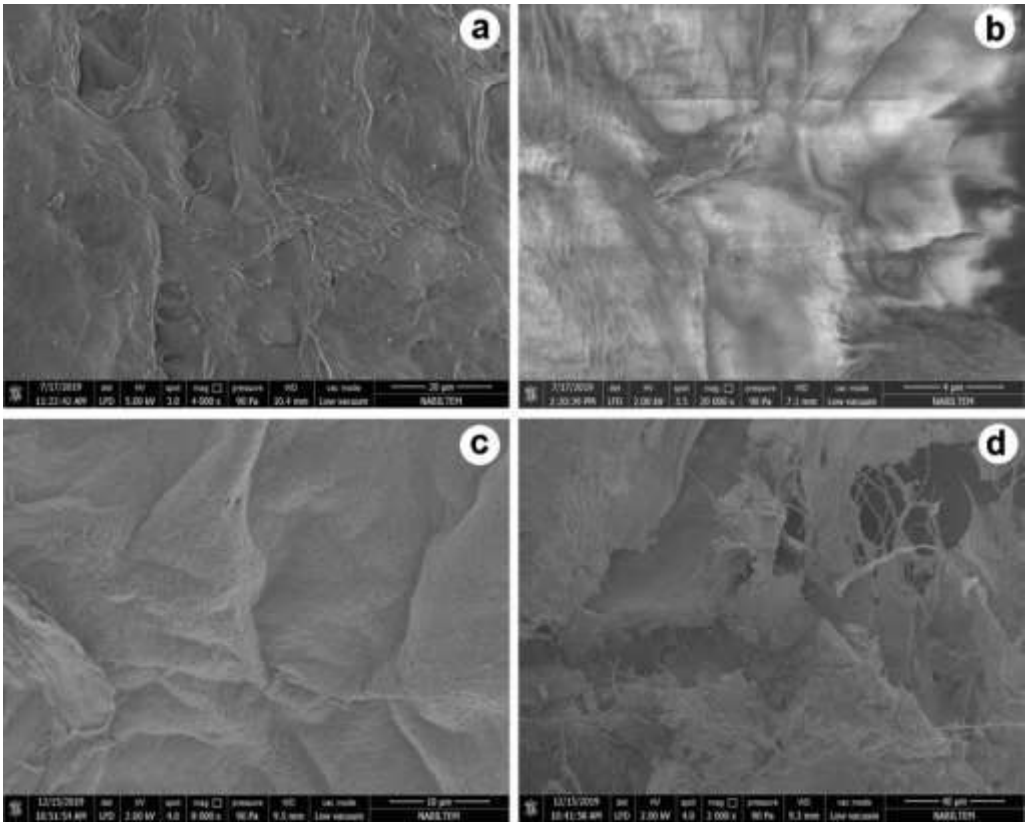

Figure 3. SEM images of Turkish mulberry silkworm chitin. a) chitin from cocoon (mag. 4000X), b) chitin from cocoon (mag. 20000X), c) chitin from pupa exuviae (mag. 8000X), d) chitin from pupa (mag. 2000X)

TGA

The TGA analysis showed that the chitins from the silkworm pupa, the pupa exuviae, and the chitin material isolated from the silkworm cocoon had a weigth loss in two stages (Figure 4). In the first stage, mass losses of 9.9% in the pupa chitin, 5.1% in the pupa exuviae chitin, and 7% in the cocoon chitin material result from the removal of water in the chitin structures. In the second stage, mass loss of 72% in the pupa chitin and mass loss of 85.7% in the pupa exuviae chitin were observed due to the degradation of the chitin structure. Mass loss in the cocoon chitin material was 57.1%. Similar to this study, Paulino et al. (2006) observed that the α-chitin obtained from silkworm chrysalides decomposed in two stages. The authors stated that the mass loss in the first stage (50 and 110 °C) was due to the evaporation of water, and the mass loss in the second stage (300 and 400 °C) was due to the removal of water from the saccharide rings, the decomposition and polymerization of the acetylated and deacetylated chitin units. They also suggested that the remaining residue of 36% after heating at 1000 °C

was due to the presence of non-extracted minerals at the acidic stage (Paulino et al. 2006). In this study, a residue of 35.761% was observed in the cocoon chitin material, after heating up to 650 °C. In the α-chitin isolated from the cicada slough, a mass loss of 7.3% was observed in the first stage (at 80 °C), while in the second stage (around 200 °C), a mass loss of 66.4% was observed (Sajomsang and Gonil, 2010). The chitins isolated from imago and pupa exuviae of black soldier fly (*H. illucens*) had a mass loss of 5-6% in the first stage (74-110 °C), while their mass losses in the second stage (250-1000 °C) were 70% for imago and 80% pupa exuviae (Purkayastha and Sarkar, 2020).

Another study reported that the chitin from moth (*O. dubia*) cocoon decomposed in two stages and the first weight loss (4.95%) occurred at ≈130 °C (Kaya et al. 2017). The authors stated that the cocoon chitin showed a low degradation (69.87%), and this may be due to the structural organization of the cocoon chitin consisting of thick rod-shaped microfibers. In this study, the decomposition rate of the chitin material obtained from the silkworm cocoon was 64.1%.

In this study, the maximum decomposition temperatures (DTGmax) were determined as 395 °C for the silkworm pupa chitin, 415 °C for the pupa exuviae chitin and 330 °C for the chitin material from the cocoon. The thermal stability of the silkworm pupa exuviae chitin is higher than others. The maximum decomposition temperatures of chitins obtained from various insect species range between 307 and 390 °C. The DTGmax values of Colorado potato beetle (*L. decemlineata*) chitins were measured as 379 °C for adult and 307 °C for larvae (Kaya et al. 2014). DTGMax values of the imago and pupa exuviae chitins of black soldier fly *H. illucens* were recorded as 363 °C and 371 °C, respectively (Purkayastha and Sarkar, 2020). In other studies, DTGmax values of chitins were reported as 362 °C for cicada slough (Sajomsang and Gonil 2010), 385-390 °C for female and male grasshoppers (Kaya et al. 2015c), 386 °C for the insect *P. prasina* (Kaya et al. 2015b) and 381.99 °C for the moth (*O. dubia*) cocoon (Kaya et al. 2017). In this study, DTGmax values of the pupa chitin and pupa exuviae chitin from Turkish silkworm were higher than other insect species. Gbenebor et al. (2017) reported that geographic location affects the physicochemical properties of the chitin.

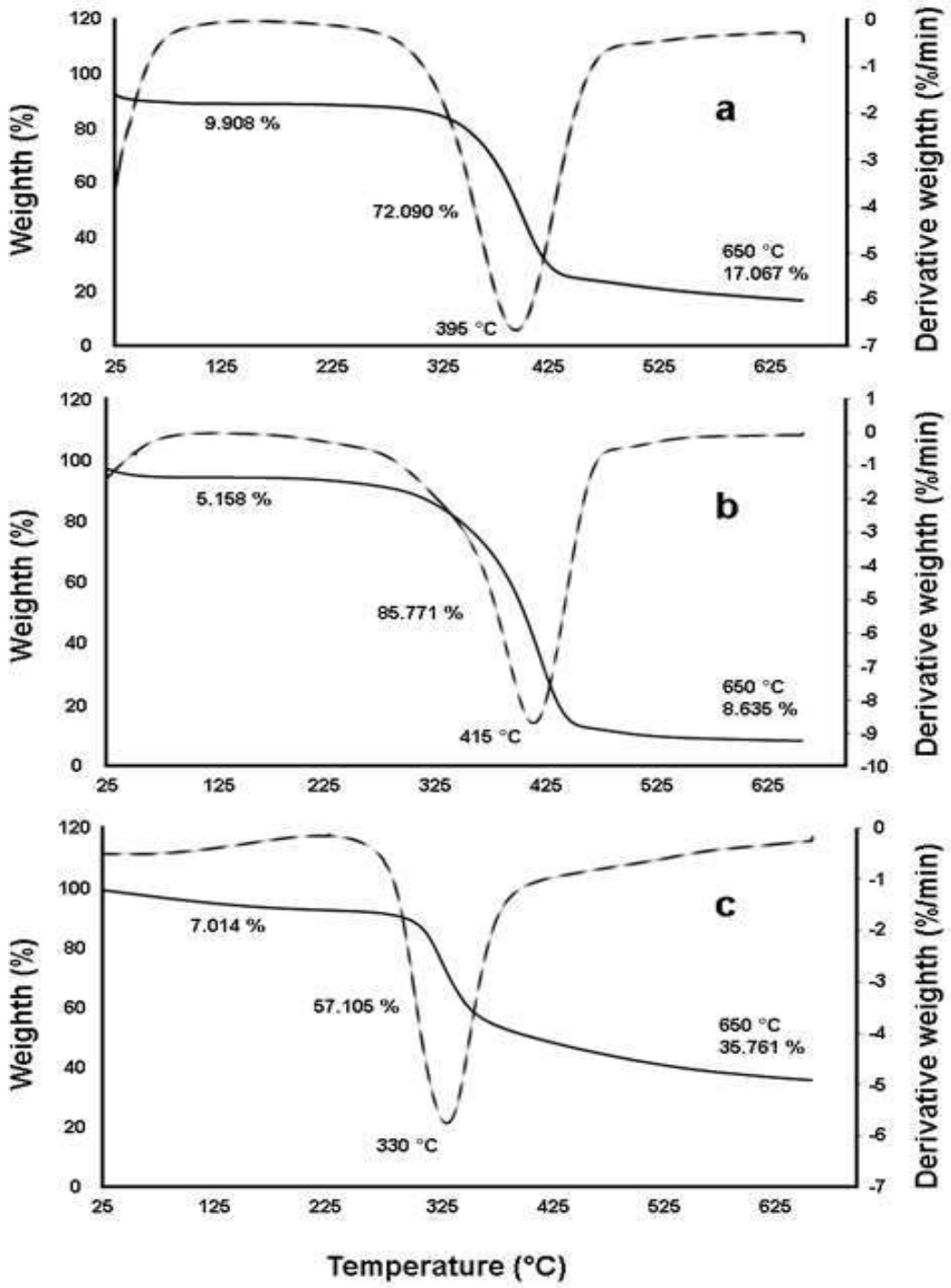

Figure 4. The thermal decomposition curves of Turkish mulberry silkworm chitin (a. Chitin from the pupa, b. Chitin from pupa exuviae, c. chitin from cocoon)

XRD

XRD diffractograms of the chitins obtained from silkworm pupa, pupa exuviae, and cocoons were presented in the Figure 5. As a result of XRD analysis, the crystalline reflections of silkworm pupa chitin were recorded at 3.82°, 5.56°, 9.42°, 13.14°, 19.12°, 26.14°, 35.1°, and 39.68° while the crystalline reflections of pupa exuviae chitin were recorded at 9.14°, 12.7°, 19.42°, 26.46°, 33.9°, and 38.68°. Also, the crystalline reflections of the chitin material isolated

from cocoon were recorded at 9.56°, 20.66°, 24.32°, 26.56°, 39.44°, and 44.44°. The peak of the cocoon chitin material at 9.42° is weaker compared to those of the pupa and pupa exuviae peaks. Jang et al. (2004) observed crystalline reflections for α-chitin at 9.6, 19.6, 21.1, and for β-chitin at 23.7°, 9.1° and 20.3°, and for γ-chitin at 9.6 and 19.8°.

Kaya et al. (2017) observed the I_{020} crystalline reflections of α-, β- and γ-chitin (from moth cocoon) at 9.46, 8.59, and 9.35°, respectively. The authors was observed the sharper second reflection peak at 12.74° for alpha and gamma chitin while the second reflection peak of β-chitin recorded at 12.29° was slightly weaker. The crystalline reflection peaks of α-chitin from cicada slough were observed at 9.20, 12.60, and 19.18° (Sajomsang and Gonil, 2010). The crystalline reflection peaks of the chitin from *H. parallela* were observed at 9.2, 12.6, and 19.1° (Liu et al. 2012) while they were observed at 9.3° and 19.8°, respectively for the pupa exuviae and imago chitins from the black soldier fly (*H. illucens*) (Purkayastha and Sarkar, 2020). Considering the previous researches, we concluded that the chitins isolated from silkworm pupa and pupa exuviae in this study are in the α-form.

Supriya and Singh (2020) observed the crystalline peaks of the silkworm cocoon chitin at 14.844°, 18.843°, 20.659°, 24.395°, 28.228°, 29.019°, and 30.114°. For the moth *(O. dubia*) cocoon chitin, the reflection value of the I_{020} was observed at 9.35°, while the second sharper reflection peak occured at 12.74° (Kaya et al. 2017). In this study, the crystalline reflections of the chitin material obtained from the silkworm cocoon yielded two sharp peaks at 9.56° and 20.66°, while there was no reflection peak at around 12°. Therefore, it differs from the XRD peaks of chitins obtained from cocoons in other studies. This may be due to the N-acetyl glucosamine residues in the structure. It is difficult to understand in which crystalline form the silkworm cocoon chitin material is, by considering these peaks. It should be checked by other analyzes.

Crystalline index values (CrI) were calculated as 63.1% for cocoon chitin material, 80.7% for pupa chitin, and 79.5% for pupa exuviae chitin, respectively. In another study, the crystallinity of chitins extracted from pupa exuviae and larva cuticles of *B. mori* were found to be very low (58% and 54%, respectively) (Zhang et al. 2000). The authors concluded that the low molecular weight catechol remaining in the chitin may be responsible for the low crystallinity. The CrI values of chitins from different insects are as follows. CrI values of chitins were calculated as 76% and 72%, respectively for the adult and larvae of potato beetle (Kaya et al. 2014), 49.4% and 25.2%, respectively for the imago and pupa exuviae of black soldier fly (Purkayastha and Sarkar, 2020), 89.05% for the insect *H. parallela* (Liu et al. 2012), 89.7% for the cicada slough (Sajomsang and Gonil, 2010), 75-80% for the male and female grasshoppers (Kaya et al. 2015c), and 50%, 69.88%, 53.92%, and respectively for *V. germanica, V. crabro,* and *V. orientalis* (Kaya et al. 2015a). In this study, the CrI values of the pupa and pupa exuviae are compatible with those of other insect species. Sajomsang and Gonil (2010) stated that a wide scattering and less dense peaks in the XRD graph were indicative of high crystallinity chitin. When considering the peak distribution and compared to other insect species, it can be said that the crystallinities of the pupa and pupa exuviae chitins are high. The CrI value (63.1%) of the cocoon chitin material is lower and is similar to the CrI value (68.6%) of the moth (*O. dubia*) cocoon chitin reported by Kaya et al. (2017). Gbenebor et al. (2017) reported that the decrease in the acetylation degree (DA) of the chitin promotes the creation of the hydrogen bond cleavage, and this division in the hydrogen bond causes changes in the crystal structure

of the chitin. Ioelovich (2014) reported that a decrease in the crystallinity of the samples was observed after a short time of milling, while the samples became completely amorphous in the long term milling. Since the increase of amorphous areas in the chitin samples increases the absorption ability of the chitin (Ioelovich, 2014), the amorphous chitin is effective in adsorbing dissolved pollutants such as heavy metal cations and it can be used in water treatment (Purkayastha and Sarkar, 2020).

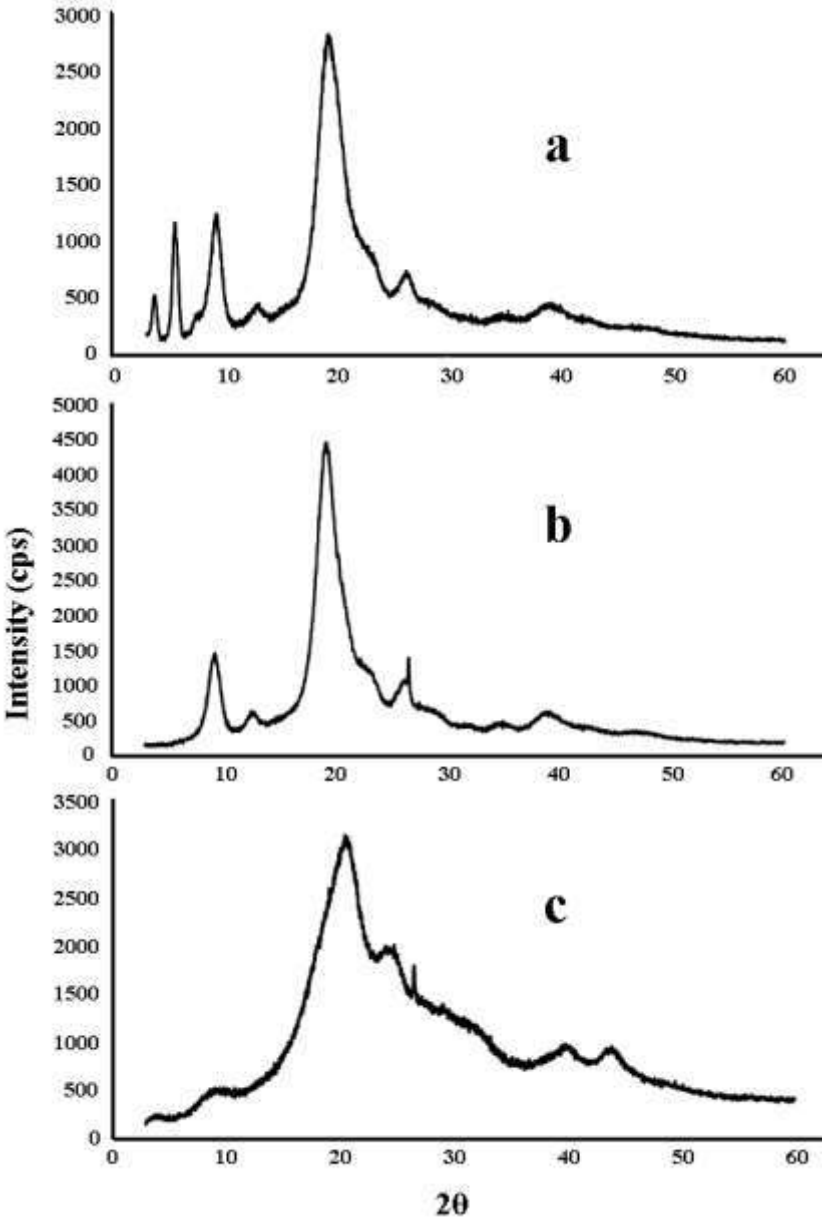

Figure 5. X-ray diffraction pattern of Turkish mulberry silkworm chitins (a. Pupa, b. Pupa exuviae, c. Cocoon)

Conclusion

The new information about the surface morphology, crystallinity, and thermal stability of the chitin material isolated from silkworm cocoon was revealed by SEM, TGA, and XRD analyzes. FTIR and XRD analyzes confirmed that the chitins isolated from silkworm pupa and pupa exuviae were in the α-form. However, as mentioned in an earlier study (Zhang et al. 2017), we believe that the cocoon chitin contains N-acetyl-D-glucosamine residues which is the degraded product of chitin. Methods such as Nuclear Magnetic Resonance (NMR), thin-layer chromatography, and mass spectroscopy should be used to identify the structure of cocoon chitin and to understand in which crystalline form it is. The chitin contents of the Turkish silkworm were determined as 13% for silkworm pupa exuviae, 17.2% for cocoon chitin material, and 3.15% for the pupa. Pupa exuviae can be used as a source of the chitin especially due to its high chitin content. While the pupa exuviae chitin has a porous structure, the pupa chitin has a more fibrous and cocoon chitin has a smooth loose structure. Porous pupa exuviae chitin has the potential to be used in tissue engineering and drug release. The maximum decomposition temperatures of the chitin from the pupa, pupa exuviae, and cocoon were determined as 395 °C, 415 °C and 330 °C, respectively, by TGA analysis. The pupa exuviae chitin was found to have very high thermal stability. Pupa and pupa exuviae chitin with high thermal stability can be used in thermal therapies, biosensor technology, and the production of fireproof garments. The crystalline index values were calculated as 63.1% for the cocoon chitin material, 80.7% for the pupa chitin, and 79.5% for the pupa exuviae chitin, respectively. The cocoon chitin material with low-crystallinity can be used in water treatment to absorb heavy metal cations and dissolved contaminants. The results of this study revealed the physicochemical structures of chitins obtained from pupa, cocoon, and pupa exuviae of the silkworm, and showed that these waste products of the silkworm industry can be evaluated in biotechnological applications.

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LENGTH-WEIGHT RELATIONSHIPS OF 16 COASTAL FISH SPECIES FROM THE SHALLOW WATERS OF ÇANAKKALE STRAIT, TURKEY

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Abstract
Length–weight relationships (LWRs) for 16 young of the year (YOY) fish species from the coastal areas of Çanakkale Strait, Turkey, were estimated. The YOY fish samples were collected by beach seine during the period of April 2019, and January 2020. The values of parameter b for the YOY 16 species ranged from 2.36 to 3.99, while the parameter a ranged from 0.0009 to 0.0542.

Keywords: Length-weight relations, young of the year, coastal, Çanakkale

INTRODUCTION

Length–weight relationships (LWRs) of fishes are used to get important information for many studies in each geographic region, such as comparing the condition, estimating the weight corresponding to a given length (Tesch 1968; Altin et al. 2015). However, life stages, sex, seasons and the environmental conditions could possibly affect the LWRs parameters (Froese 2006).

Coastal areas are important for the fish development. Fish need specific coastal areas to feed, protect, breed, and grow in the different life stages. Appropriate condition for fish development in these areas is directly related to physical and biological factors (Beck et al. 2003; Crec'hriou et al. 2008). Close coastal ecosystems support many important ecological organizations, such as the nutrient cycle in the coastal area. In this respect, these important coastal areas have been named as “basic fish habitats” and “habitats in this area; required areas for fish spawning, feeding or breeding” (Beck et al. 2003). Therefore, these species which lives in the coastal areas, are very important for fishery research and management.

In this study, we presented the length weight relationships for 16 young of the year (YOY) fish species from the coastal areas of Çanakkale Strait, providing some basic information for further fishery management.

MATERIALS AND METHODS

The current study was conducted in the coastal areas of the Çanakkale Strait, Turkey between April 2019, and January 2020. YOY fish samples were caught with a beach seine of 32 m in total length, with 15 m wing lengths. The net was constructed of 13 mm stretch mesh. The dimensions of the bag were 2 × 2 × 0.6 m, and the bag was constructed with 5 mm mesh.

In the laboratory, the total length of fish was measured to the nearest 0.1 cm and weight to the nearest 0.1 g. The LWRs of these species were estimated using log-transformed equation:

$$\log W = \log a + b \log L$$

where W is the total body weight (g), L is the total length (cm), 'a' is intercept and 'b' is slope. The parameters 'a' and 'b', 95% confidential limits (Cl) of 'a', 'b' and coefficient of determination (r^2) were estimated using the linear regression (Froese, 2006; Le Cren, 1951). Extreme outliers were removed from the analysis.

RESULTS

The LWRs were calculated for a total of 1476 individuals of 16 species belonging to 7 different families. The detailed information on the number of specimens measured (n), total length range, weight range, length-weight relationship parameters with 95% Cl of 'a' and 'b', r^2 is given in Table 1 for each species. Correlation of determination (r^2) was above 0.80 for all species. The values of parameter b for the YOY 16 species ranged from 2.36 to 3.99, while the parameter a ranged from 0.0009 to 0.0542.

Table 1. Estimated parameters of length-weight relationships for 16 YOY fish species from the coastal areas of Çanakkale Strait, Turkey. N, sample size; Min., minimum; Max., maximum; a and b, intercept, and slope of LWR; 95% CI of a, confidence intervals of a; 95% CI of b, confidence intervals of b; r^2, coefficient of determination.

Family	Species	N	TL (cm)		W (g)		Length–weight relationship parameters and statistics						
			Min	Max	Min	Max	a	95% CI a		b	95% CI b		r²
Belonidae	*Belone belone*	29	4.	30.	0.1	24.2	0.001	0.001	0.001	2.932	2.833	3.030	0.993
Bothidae	*Arnoglossus kessleri*	35	3.	7.	0.1	3.0	0.003	0.001	0.005	3.435	3.038	3.832	0.904
Moronidae	*Dicentrarchus labrax*	19	5.	9.	1.2	5.9	0.029	0.018	0.047	2.364	2.097	2.630	0.954
Mullidae	*Mullus surmuletus*	637	4.	9.	0.5	8.6	0.009	0.007	0.010	2.939	2.858	3.020	0.889
Scorpaenidae	*Scorpaena porcus*	64	5.	9.	2.6	18.7	0.010	0.005	0.019	3.268	2.962	3.574	0.880
Sparidae	*Dentex dentex*	22	6.	15.	3.0	37.1	0.011	0.004	0.031	2.962	2.538	3.387	0.914
	Diplodus annularis	132	2.	8.	0.2	10.6	0.009	0.008	0.010	3.269	3.182	3.355	0.977
	Diplodus puntazzo	79	2.	7.	0.0	6.8	0.005	0.004	0.007	3.567	3.401	3.733	0.960
	Diplodus vulgaris	64	2.	8.	0.1	12.3	0.010	0.008	0.012	3.185	3.062	3.308	0.977
	Lithognathus mormyrus	55	2.	7.	0.0	4.3	0.006	0.005	0.007	3.274	3.145	3.404	0.980
	Pagellus acarne	95	1.	4.	0.0	1.1	0.002	0.002	0.003	3.991	3.780	4.202	0.938
	Pagrus pagrus	13		11.	3.5	18.6	0.054	0.030	0.097	2.377	2.105	2.648	0.971
	Sarpa salpa	111		7.	0.8	5.7	0.003	0.001	0.005	3.770	3.414	4.125	0.802
	Sparus aurata	72	3.	10.	0.3	15.3	0.008	0.006	0.009	3.214	3.100	3.328	0.980
	Spondyliosoma cantharus	32	6.	9.		13.6	0.004	0.002	0.009	3.591	3.241	3.942	0.936
Triglidae	*Chelidonichthys lucerna*	17	2.	11.	0.	10.	0.010	0.007	0.016	2.846	2.613	3.079	0.978

DISCUSSION

In the current study, most values of parameter b were within the expected 2.5–3.5 range, except for 4 species, according to the estimates of the Bayesian LWR predictions (Froese 2006). This might be due to different factors such as stomach fullness, life stage, food supply, capture time, and perhaps narrow length intervals. In some species strong allometric growth is apparent

due to an over-proportional increase in length relative to growth in weight (Froese 2006). In our study, these differences can be explained due to the young of the year stages of fish.

In conclusion, the results of this study provide new information to better understand the ecological parameters of these coastal YOY fish.

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EFFECT OF TILLAGE PRACTICES ON YIELD AND PHOSPHORUS UPTAKE OF A DURUM WHEAT CROP UNDER SEMI-ARID CONDITIONS IN NORTHERN ALGERIA

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ABSTRACT

The aim of this study is to compare the effect of three tillage practices: conventional tillage (CT), reduced tillage (RT), and no-tillage (NT), on yield and phosphorus uptake of a durum wheat crop (*Triticum durum* var Simeto) in semi-arid conditions of northern Algeria. The study was carried out in 2017-2018 growing season in a trial established since the year 2015; the experiment is conducted in a randomized complete block design with three replicates. Grain, straw and biomass yields were assessed, phosphorus concentration in grain and straw was analyzed, and the total phosphorus uptake was determined by multiplying the grain and straw dry matters by their respective P concentrations. There were no effects of tillage on durum wheat production in this particularly rainy growing season. The favorable climatic conditions had a positive impact on durum wheat grain and biomass production, grain yields of 4.06 Mg ha^{-1}, 4.03 Mg ha^{-1} and 3.65 Mg ha^{-1} were obtained by RT, CT and NT systems respectively, which is superior to the average yields obtained usually in the region. A biomass production of 14.9 Mg ha^{-1}, 14.6 Mg ha^{-1} was recorded by RT and CT respectively and 10.9 Mg ha^{-1} by NT system. Phosphorus concentration in both grain and straw and the total P uptake by plants were not significantly affected by tillage systems, statistically similar P concentrations were obtained by the three treatments. P content values of: 5.1 g kg^{-1}, 5.2 g kg^{-1} and 4.8 g kg^{-1} were found in grain harvested from NT, CT and RT plots respectively, these values are above those reported by several authors, which indicate a fairly good phosphorus nutrition.

Key words: *No-tillage, conservation tillage, phosphorus, durum wheat, semi-arid regions.*

INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the most important cereal crop in Algeria and in the Mediterranean basin, it occupies more than 2Mha of the Algerian agricultural land with an annual production of 2Mt which is far from covering the demand of the growing population. The cereal production in the country is concentrated in the interior high plains characterized by a Mediterranean climate, with variable rainfall and frequent droughts. The severe natural conditions associated with the use of cropping systems based on monoculture of cereals, fallow

and deep and intensive tillage have exposed the soils of the region to degradation and loss of production potential. The adoption of the conservation agriculture techniques, especially, conservation tillage has proved its advantages in the Mediterranean region. Conservation tillage has considerable potential for stabilizing production in the semi-arid zones. Many authors have reported a greater yield production under no-tillage compared to the conventional tillage in conditions of water shortage (López-Bellido *et al*., 2000; De vita *et al*., 2007; Mazzoncini *et al*., 2008). Devita *et al*., (2007) estimated that under 300 mm rainfall a superior wheat yield is occurred under no-tillage system.

Phosphorus is one of the major nutrients that plants need for their growth, it is considered as a limiting factor for biomass production in many types of areas. The phosphorus availability can be improved by the conservation tillage techniques. Many researchers noticed a stratification of available P in the soil surface layers under reduced tillage and no-tillage systems (Piegholdt *et al*., 2013 and Peigné *et al.*, 2018), this may be attributed to a greater microbial activity under undisturbed soil. A higher P concentration in durum wheat grain under no-tillage system has also been reported by Mozafar *et al*. (2000), Mazzoncini *et al.* (2008) and Woźniak *et al.* (2014).

The aim of this study is to compare the effect of three tillage practices: conventional tillage, reduced tillage and no-tillage on yield and phosphorus uptake of a durum wheat crop under rain-fed conditions in a semi-arid region of northern Algeria.

MATERIAL AND METHODS

Experiment site

The experiment is conducted on a heavy soil (54% of clay) in a farm located in the region of El-Hachimia in northern Algeria (36° 14′ 21″ N, 3° 50′ 23″ E in an altitude of 713 m above the sea level), the region is characterized by a Mediterranean climate with a great variation of the rainfall in the year and between years, the mean annual rainfall in the region in the last 30 years is 479 mm.

The experimental design and crop management

This study is a part of an experiment started in the year 2015. The trial consists on a comparison between three tillage practices in a continuous durum wheat crop: (i) conventional tillage (CT), consisting of moldboard ploughing to 30 cm depth followed by a disk harrowing for bed seed preparation, (ii) reduced tillage (RT) using a cultivator to 7 cm depth, and (iii) no-tillage (NT) with crop residue retained in surface. The different tillage practices were arranged in plots of 60 m long and 10 m wide in a randomized complete block design with three replications.

No-tillage plots were sawn using a no-tillage seed drill, the seeding rate of all treatments was 180 kg ha^{-1}, Semito cultivar being the most cultivated in the region is chosen for the study. NT weed control was accomplished by applying glyphosate (2.5 l ha^{-1}), a fertilization as mono-ammonium phosphate (12% N and 52% P) in the rate of 150 kg ha^{-1} was applied before sowing, and 100 kg ha^{-1} urea fertilizer (46% N) was applied in the tillering stage of the plant, fungicides, pesticides and herbicides were also applied at the time of appearance of fungal diseases, pests or weeds.

Measurements and analysis

The harvest was mechanically done and grain yield was directly determined in each plot. In order to obtain the biomass yield, three samples of two linear meters have been harvested and weighted after they had been oven-dried at 75°C for 48 hours, the average of the dry matter weight of the three samples is reported to square meter and then per hectare; the straw yield was then obtained by subtraction. Grain and straw sub-samples were taken from each of the three samples, and P was determined by the colorimetric method and expressed in grams per dry matter kilogram (g kg^{-1}). The plant total P uptake was determined by multiplying the grain and straw dry matters by their respective P concentrations.

Statistical analysis

Data were evaluated by two-way ANOVA. The F test was applied at 5% probability level to check the significant differences between the three tillage practices.

RESULTS

Climatic conditions

The climatic conditions of the growing season 2017-2018 (table 1) are particular compared to the long term data in the region. The quantity of rainfall observed during the vegetative cycle of the crop (December to June) is 22 % higher than the cumulative rainfall obtained in the same period in the last 30 years. This significant amount of rainfall was characterized by a good distribution in the year which was beneficial for the vegetation development. The important quantity of precipitations obtained in the months of April and May which coincides with flowering and grain filling stages induced a good average yields in all the experiment treatments as well as in the region.

Temperatures of this season have followed the long term data, except for the month of May (grain filling stage) which was less hot than the long term average.

Table 1. Monthly rainfall and mean maximum and minimum temperature in the vegetative cycle of the durum wheat crop for the growing season 2017-2018.

Month	Rainfall (mm)	T min (c°)	T max (c°)
November	87	7.3	18.6
December	55	5.2	13.3
January	23	5.0	13.9
February	33	2.8	11.6
March	99	6.8	14.8
April	131	9.0	19.2
may	60	10.5	21.2
June	4	14.6	29.1
Total	492	8.0	17.6

Yields and Harvest Index

The grain, biomass and straw yields, as well as the harvest index were not significantly affected by tillage systems (table 2). Grain yields of all treatments (4.06 Mg ha^{-1}, 4.03 Mg ha^{-1} and 3.49 Mg ha^{-1} obtained by RT, CT and NT systems respectively) were about 50 % higher than the average obtained in the country in the last decade which is equivalent to 2 Mg ha^{-1} (statistics of agriculture ministry), this is due to the exceptional climatic conditions, in particular, the great amount of rainfall observed in the grain filling period. The means of grain, biomass and straw yields and the harvest index obtained by the different treatments are summarized in the table 3.

Phosphorus uptake

There were no significant effects of soil tillage on P concentration in grain and straw as well as on the total P uptake by durum wheat plants (table 2). The P content in the grain harvested from the different plots was ranged from 4.31 g kg^{-1} to 6.62 g kg^{-1}, which indicates fairly good phosphorus nutrition. Means are summarized in Table 3.

Table 2. Mean square and significance of traits studied

Traits	Mean square			
	Treatments (df = 2)	Blocks (df = 2)	Error (df = 4)	CV %
Grain yield (Mg ha^{-1})	16.29 ns	118.81*	12.23	8.9
Biomass yield (Mg ha^{-1})	1482.2 ns	242.4 ns	476.8	16.19
Straw yield (Mg ha^{-1})	1101.61 ns	643.96 ns	356.94	21
Harvest Index	0.003 ns	0.014*	0.002	13.45
Grain [P] g kg^{-1}	0.147 ns	1.461 ns	0.429	13
Straw [P] g kg^{-1}	0.308 ns	0.071 ns	0.429	58.5
Total P uptake (kg ha^{-1})	6.66 ns	11.82 ns	30.65	19.53

NB: ns = not significant at $p<0.05$, * significant at $p<0.05$.

Table 3. Means of all traits studied during the growing season 2017-2018

Traits	Means		
	NT	RT	CT
Grain yield Mg ha^{-1}	36.49	40.64	40.39
Biomass yield Mg ha^{-1}	104.30	142.60	140.50
Straw yield Mg ha^{-1}	67.86	101.93	100.08
Harvest Index	0.35	0.29	0.30
Grain [P] g kg^{-1}	5.12	4.77	5.18
Straw [P] g kg^{-1}	1.49	0. 92	0.95
Total P uptake (kg ha^{-1})	29.35	26.64	29.07

NB: NT: no-tillage, RT: reduced tillage, CT: conventional tillage

DISCUSSION

The grain yields obtained by all the tillage systems were statistically similar. These results are in disagreement with those obtained by several authors in similar environment. López-Bellido *et al*. (2000), De vita *et al*. (2007), Mazzoncini *et al*. (2008), Amato *et al.* (2013) and others affirm that durum wheat yield obtained by CT system is significantly higher than NT in conditions of a good water supply. De vita *et al.* (2007) estimate that in the Mediterranean conditions, a superiority of CT is occurred when rainfall recorded during the vegetative cycle is more than 300 mm.

Even the rainfall recorded in this season was about 492 mm, the CT grain and biomass production was similar to the conservation tillage systems. The reason why the CT could not express its superiority in this favorable climatic conditions may be attributed to its poorly made seed bed. Because of the wet conditions which characterized the period of soil preparation (87 mm rainfall in November) and the clay texture of the soil, a non-homogeneous seed bed has been performed in CT plots which had negatively affected the seed germination and plant density.

Our results show that no reduction of grain and biomass production results from the adoption of the conservation tillage systems (RT and NT), with their proven advantages of time, fuel and labor savings, added to their superiority to CT in the driest seasons. The conservation tillage techniques may be the perfect alternative for farmers in the region who, in addition to grain

production, are also envisaging the straw since the agricultural system is based on association of cereal production with livestock.

Phosphorus concentration in the grain was not significantly affected by tillage, similar P content was found in both conservation tillage systems and CT, this is in contradiction with the findings of Mozafar *et al*. (2000), Mazzoncini *et al.* (2008) and Woźniak *et al.* (2014) which have reported a significantly higher P content in durum wheat grain under NT. In the same manner, P content in straw did not significantly vary with tillage, which is in disagreement with the results of Mazzoncini *et al*. (2008) who find a higher P content in the straw under NT in nine out of ten years of experimentation. The total P uptake has not been affected by tillage systems; this can be attributed to the insufficient duration of the experiment to see the stratification of available P in the upper soil layers as reported in literature.

The grain P concentrations in the different treatments are above those reported by Mazzoncini *et al.* (2008) who find average values of 3.5 g kg^{-1} and 3.84 g kg^{-1} in CT and NT respectively, in a ten-year experiment conducted in southern Italy, similarly Woźniak *et al.* (2014) obtain values of 3.11 g kg^{-1} in CT and 3.47 g kg^{-1} and 3.68 g kg^{-1} in RT and NT respectively in more wet conditions. The grain obtained from all the plots in our experiment seems to be sufficiently rich in P, according to Bilal *et al*. (2019), a high P content in wheat grain can be a good strategy to overcome a P deficiency in the plant in its first growing stages.

CONCLUSION

Our results show a similar grain and biomass production under the three tillage techniques in this particularly wet season. A reduction or even a total removal of soil tillage did not affect the durum wheat production; several studies in similar environment have found an advantage of the NT and RT in the driest years. Since the NT adoption in the region still needs time to obtain the necessary equipment, the reduced tillage without soil returning seems to be the best alternative in the region, added to its time and labor saving compared to the CT, it allows a good seed bed preparation, a good drainage and a better seed germination.

The P content in grain and straw as well as its total uptake by the plant seems to be not affected by tillage, at least, after three years of the adoption of no-tillage and reduced tillage.

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ADSORPTION PROPERTIES OF XAD-16 RESIN FOR THE REMOVAL OF DIFFERENT CLASSES OF DYES

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ABSTRACT

In this study, Amberlite XAD-16 resin was used for the adsorption of brilliant green (BG), aniline blue (AB), malachite green (MG) and brilliant cresyl blue (BCB) dyes. Adsorption from aqueous media on the resin has been investigated in a batch system. In order to determine the capacity of the adsorbent, Langmuir and Freundlich isotherms were examined. In addition, initial concentration of the dye, amount of resin, contact time, temperature and pH values were adjusted experimentally in order to illuminate the adsorption mechanism. It was observed that the most effective adsorption occurred at the natural pH values of the dye solutions and the experiments were continued in that range. It was determined that the increase in temperature contributed positively to adsorption. The pseudo-first order and pseudo-second order kinetic mechanisms were examined and it was found that the adsorption processes of almost all dyes followed the pseudo-second order kinetics. Thermodynamic data of the reactions were also calculated and ΔG^o values were found to be negative when ΔH^o and ΔS^o values were positive for all dyes. This finding indicated that the adsorption of the dyes onto the resin was spontaneous and endothermic for all investigated dyes.

Keywords: *Adsorption, XAD-16, dyes, adsorption isotherms, adsorption kinetics.*

INTRODUCTION

It is common knowledge that one of the biggest problems that needs addressing today is environmental pollution. Many studies are carried out in different fields in order to eliminate pollution, that is perhaps the leading bad outcome of the progress of society, science, industry and technology. The increasing demand and research abundance in "green" applications in the field of chemistry also highlights the importance of this problem. The reduction of clean water resources, which is a current problem in our country as well as the world; has accelerated and diversified the researches on the treatment and preparation of wastewater for reuse.

Pollutants released into water are substances with quite different structures. These pollutants need to be removed from wastewaters because of their toxicity, preventing reuse of water, and being harmful to living organisms and ecological balance. For this purpose, one of two options can be preferred: degradation or separation of the pollutant from the environment. In addition to biological methods, organic pollutants can be broken down and wastewaters can be cleaned with chemical oxidative techniques such as ozonation (Van Aken et al., 2015), Fenton Reaction (Barndõk et al., 2016), photocatalysis (Kaya and San, 2017), or hybrid methods (Hou et al., 2015) where different techniques are applied together.

Dyes are one of the pollutant classes released into wastewater as a result of their widespread use in various industries. The excess in the variety of synthetic dyes causes different effects on aqueous systems. Dyes can affect the photosynthetic activity of underwater life by reducing the

light transmittance of water and also show toxic effects due to the metallic, aromatic etc. groups they contain (Fu and Viraraghavan, 2001). Dyes, which can cause intense colors even at very low concentrations, should be removed from wastewater as they make it difficult to reuse water and prevent the passage of light into water. Different methods have been developed for the treatment of dyes from wastewater due to their toxic effects (Shi et al., 2007, Akkaya et al., 2009, Mozumder and Islam, 2010). Especially, synthetic dyes containing aromatic groups are known to be carcinogenic (Bulut et al., 2008). Adsorption, which is one of the leading separation methods, is carried out by the attachment of the pollutant substance on the adsorbent, and there are many studies using this technique (Gupta and Suhas, 2009, Afkhami and Moosavi, 2010, Acar et al., 2012, San et al., 2016).

Adsorption can be defined as the process of condensing liquid or gas molecules in a phase on the surface of a solid phase interacting with it. Adsorption process takes place in the active areas of the adsorbents. The choice of adsorbent depends on its adsorption capacitiy, the type of process to be performed, the properties of the media, applicability, modification, reusability and cost.

In this study, adsorption of dyestuffs with different structures used in industry and cause pollution in wastewater onto the commercially available resin XAD-16 was investigated in a batch system. Brilliant green (BG), aniline blue (AB), malachite green (MG) and brilliant cresyl blue (BCB) were chosen as different classes of dyes such as anionic triphenyl, cationic triphenyl and cationic oxazine. Langmuir and Freundlich isotherms were plotted and the coefficients of the isotherms were calculated to determine the adsorption capacities of the adsorbent for the dyes. In order to investigate the adsorption kinetics, pseudo-first order and pseudo-second order models were applied and the obtained kinetic parameters were evaluated. It was also aimed to find the most effective conditions by changing the pollutant's initial concentration, amount of resin, temperature, contact time and pH values experimentally. Using the results of the experiments conducted at different temperatures, thermodynamic data of the reactions were calculated. Results showed that all the calculated ΔG^o values were negative for all dyes where ΔH^o and ΔS^o were found to be positive. This finding indicated that the adsorption of the dyes onto the resins were spontaneous and endothermic in nature.

MATERIAL AND METHODS

Amberlite XAD-16 resin (Fig. 1) which is a polystyrene divinylbenzene was purchased from Sigma Aldrich. The resin was thoroughly washed prior to use with distilled water and then with ethanol in order to wash away the possible salt residues. Afterwards, the resin was kept in a 100^{o}C oven for 24 hours until use. The chosen pollutants, BG, AB, MG and BCB were obtained from Merck and used without further purification. The solutions were prepared with purified water which was obtained from Human Corporation New Human Power 1 water purifier. All pH measurements were conducted using an inoLab pH 7110 pH meter. All batch adsorption experiments were carried out in a GFL 1083 horizontal shaker water bath at 120 rpm with temperature control. The concentration measurements were conducted with a Shimadzu UV-Mini-1240 UV-Vis Spectrophotometer at the corresponding λ_{max} values of the dyes. Dye solutions at desired concentrations were prepared by diluting from the stock solution of 1000 mg.L^{-1}. 25 mL of these solutions were introduced in 150 mL flasks and desired amount of adsorbent was added. The flasks were put in a constant temperature horizontal shaker water bath and samples were collected at regular intervals to determine the concentration of the remaining dye in the solution.

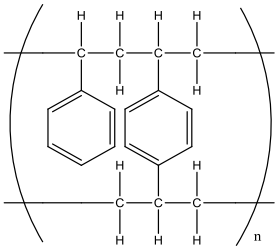

Figure 1. XAD-16 Resin

Analysis and Calculations

The adsorption performance is quantified in terms of adsorption capacity, q_t ($mg.g^{-1}$), which represents the amount of adsorbate adsorbed per gram of adsorbent at moment t and is calculated using the following Eqn. (1)

$$q_t = \frac{(C_0 - C_t)}{m} xV \quad (1)$$

where q is the amount of dye uptaken by the adsorbent ($mg.g^{-1}$); C_o is the initial dye concentration in contact with the adsorbent ($mg.L^{-1}$) and C_t is the concentration ($mg.L^{-1}$) after the batch adsorption procedure, m is the mass of the adsorbent (g) and V is the volume of adsorbate put in contact with the adsorbent (L).

Another parameter of interest and indicator of adsorption efficiency is removal percentage, Ads (%), is calculated using Eqn. (2)

$$Ads(\%) = \frac{(C_0 - C_t)}{C_0} x100 \quad (2)$$

where C_o and C_t have the same meaning as above.

Adsorption Isotherms

The basic assumption of the Langmuir adsorption process is the formation of monolayer of adsorbate on the outer surface of adsorbent and after that no further adsorption takes place (Purkait et al., 2007). Linear form of Langmuir isotherm equation is as follows,

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (3)$$

where C_e is the equilibrium adsorbent concentration ($mg.L^{-1}$), q_e is the adsorption capacity ($mg.g^{-1}$), q_m is the maximum amount of adsorbate per adsorbent unit mass that is a measure of monolayer adsorption capacity ($mg.g^{-1}$) and K_L is the Langmuir constant related to the free energy of adsorption ($L.mg^{-1}$). C_e can be yielded from the slope of the plot of C_e/q_e versus C_e and K_L can be calculated from the intercept.

Freundlich equation is actually a modification of Langmuir isotherm by following the assumption that the adsorbent surface is heterogeneous and multi-site adsorption takes place. Freundlich model is an indicative of the extent of heterogeneity of the surface of adsorbent. Linear Freundlich adsorption isotherm equation can be represented as,

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_F \quad (4)$$

where K_F is the relative adsorption capacity of the adsorbent ($mg.g^{-1}$) and n is the intensity of adsorption (dimensionless).

Adsorption Kinetics

Pseudo first order equation is,

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (5)$$

where q_e is the adsorption capacity in equilibrium ($mg.g^{-1}$),t is time (min), q_t is the amount of adsorbate adsorbed at time *t* ($mg.g^{-1}$), and k_1 is the pseudo first-order rate constant (min^{-1}).

Pseudo second order equation is as follows;

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \quad (6)$$

where k_2 is the pseudo second-order rate constant ($g.mg^{-1}.min^{-1}$) and q_e, q_t and t have the same meaning as above.

Thermodynamic Calculations

The equilibrium constant, K_C, is defined as the ratio of adsorbed equilibrium dye amount and equilibrium dye concentration in the solution and is calculated according to Eqn. (7).

$$K_C = \frac{q_e}{C_e} \quad (7)$$

Eq. (8) is used for the calculation of ΔH^o and ΔS^o of the adsorption: the slope and intercept of the plot of $\ln K_C$ and 1/T yields ΔH^o and ΔS^o, respectively. R is the ideal gas constant.

$$\ln K_C = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \quad (8)$$

Another thermodynamical property, Gibbs free energy of the reaction can be calculated from Eq. (9)

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (9)$$

RESULTS AND DISCUSSION

Effect of Reaction Time

In order to determine the reaction time, the reaction was continued until the adsorption equilibrium was reached and samples were taken at regular intervals. For this purpose, 25 mL of the dye solution with a concentration of 20 mg L^{-1} was taken and placed in a 150 mL conical flask and 0.2 g of XAD-16 resin was added. Flask was then taken into a shaking water bath, the temperature of which was set to 30°C, and adsorption was achieved. In Fig. 2, the adsorption

of four dyes on XAD-16 is plotted against time. The inset of Fig. 2 has been added for better interpretation of the initial adsorption and shows the first 30 minutes of the adsorption. Considering the time to reach equilibrium, adsorption on XAD-16 was listed as AB> BG> MG> BCB), with AB taking 10 mins to reack equilibrium, while BCB takes 240 mins, respectively.

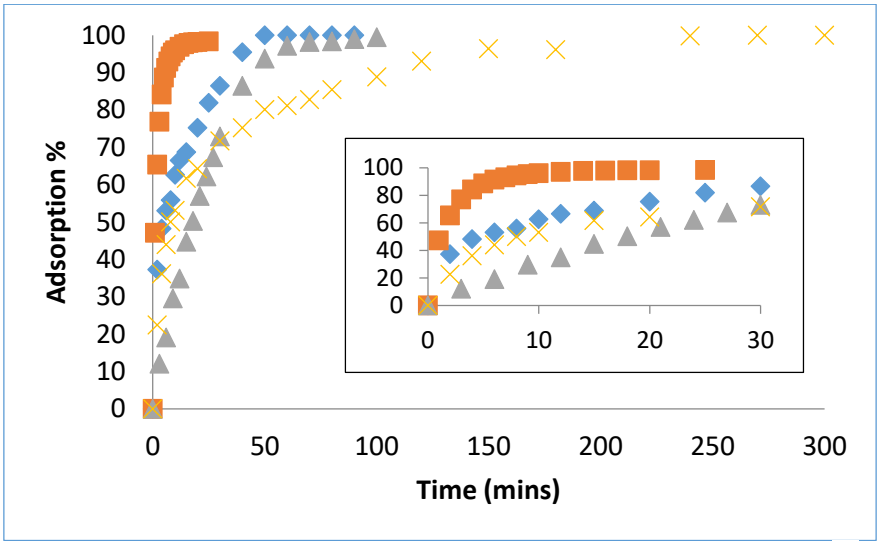

Figure 2. Effect of contact time on adsorption (◆ BG, ■ AB, ▲ MG, × BCB)

Effect of Adsorbent Amount

For determining the initial adsorbent amount, the adsorption of 25 mL, 20 mg L^{-1} dyes from solution onto XAD-16 were investigated for four different adsorbent amounts: 0.05, 0.1, 0.15 and 0.2 g / 25 mL. The temperature of the water bath was kept constant at 30°C and 120 rpm. In order to make comparisons, adsorption percentages are given for different time periods in Table 1.

Table 1. Adsorption percentages of dyes on different amounts of XAD-16

XAD-16 (g)		***BG***	***AB***	***MG***	***BCB***
0.05	10 mins	2,34	55,24	9,75	17,81
	30 mins	11,94	61,20	37,73	32,98
	60 mins	23,98	63,29	63,42	40,79
	120 mins	52,46	69,54	91,76	46,58
0.1	10 mins	3,56	68,75	25,98	32,45
	30 mins	16,02	76,01	68,28	44,30
	60 mins	33,88	80,08	98,33	51,58
	120 mins	73,33	82,86	99,46	57,81
0.15	10 mins	18,36	75,81	31,75	44,74
	30 mins	42,43	83,26	70,63	55,09
	60 mins	73,36	85,64	98,11	63,16
	120 mins	99,20	92,59	99,12	70,88

0.2	10 mins	62,66	96,03	34,85	53,12
	30 mins	86,46	98,18	71,36	71,70
	60 mins	99,96	98,41	97,23	81,18
	120 mins	99,68	98,44	99,61	93,07

It was observed that for all dyes as the amount of resin increases, the adsorption rate increases as well. The increase in adsorption efficiency is much more evident for BG and BCB. For example, for BG, after 0.05 minutes, 52% adsorption with 0.05 g XAD-16, while the amount of resin increased to 0.2 grams, the adsorption rate reached almost 100%. For AB, which is affected less by the initial adsorbent amount, when the amount of XAD-16 was quadrupled, only a 30% change in adsorption efficiency was observed.

Effect of Pollutant Concentration

In order to investigate the effect of the initial pollutant concentration on the adsorption efficiency, the adsorption reactions were investigated 30ºC and 0.2 g / 25 mL resin with initial pollutant concentrations between 20 - 80 mg L^{-1} at 120 rpm agitation were investigated. For all examined dyes, it was observed that almost 100% adsorption occurred. This means that the adsorption capacity of XAD-16 is quite high and it can be effective even at higher dye concentrations. Since similar behaviour was observed for all dyes, the effect of BCB initial concentration on adsorption onto XAD-16 is given as an example in Fig. 3. Although the initial concentration was quadrupled, it was observed that the entire dye was adsorbed on XAD-16.

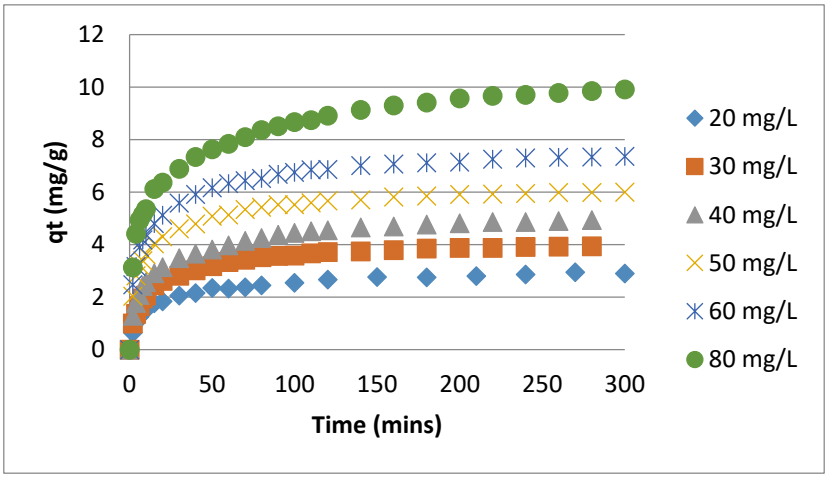

Figure 3. Effect of initial BCB concentration on adsorption onto XAD-16

Effect of Temperature and Thermodynamic Evaluation

In order to determine the effect of temperature on adsorption, experiments were carried out at four different temperatures in the range of 298 - 333 K. For all four dyes, the initial concentration was chosen as 20 mg L^{-1} and the optimum adsorbent amount was 0.2 g / 25 mL. All experiments were carried out at natural pH values. For all dyes, it was observed that the adsorption on XAD-16 increased linearly with temperature.

The thermodynamic parameters, ΔG^o, ΔH^o and ΔS^o, were calculated by using equations (7), (8) and (9). The data obtained for 303 K is shown in Table 2.

Table 2. Thermodynamic data for adsorption reactions

Dye	*ΔG° (J.mol^{-1})*	*ΔH° (J.mol^{-1})*	*ΔS° (J.mol^{-1}.K^{-1})*
BG	-1370.4	54239	183.53
AB	-1435.9	60205	203.44
MG	-4042.5	35863	131.70
BCB	-1856.4	51106	174.79

According to Table 2, Gibbs free energy, ΔG^o, was found to be negative for all dyes. This indicates that all dyes' adsorption onto XAD-16 occurs spontaneously. Positive ΔH^o values show that the processes are endothermic. This explains the increase in the adsorption efficiency obtained experimentally with temperature. The entropy values, which are positive values for all dyes show that the dyemolecules are randomly distributed on XAD-16 surface.

Effect of pH

In order to examine the effect of pH 25 mL of 20 mg L^{-1} dye solution at different pH values was added onto 0.2 g XAD-16 and adsorption reactions at 303 K were examined. pH adjustments were carried out using 0.1 M HCl and 0.1 M NaOH. The calibration solutions used to calculate the concentrations were also prepared at the relevant pH. The effect of pH on the adsorption of dyes on XAD-16 is given in Fig. 4. pH experiments were examined at intervals where λ_{max} value of each dye did not change.

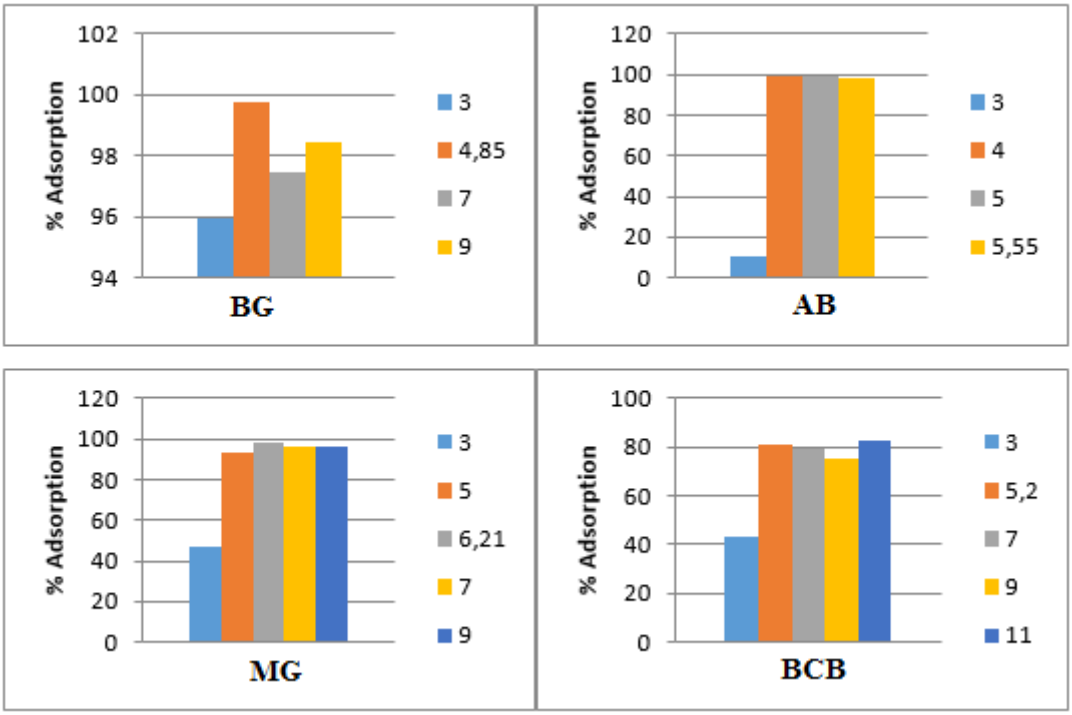

Figure 4. Effect of pH on the adsorption of the dyes on XAD-16

It was observed that the adsorption efficiency of the solutions was the highest at the natural pH values of the solutions. The adsorption of all the dyes examined at acidic medium (eg pH = 3) occurred in low amounts. The drastic decrease of AB adsorption was attributed to the protonation of the sulfonate groups in the AB structure and the reduction of the surface charge. No significant difference was observed in terms of adsorption percentage at other pH values

examined. For this reason, all experiments were carried out at the natural pH values of the dyes which are 4.85, 5.55, 6.21 and 5.2 for BG, AB, MG and BCB, respectively.

Adsorption Isotherms

In explaining the adsorption mechanism of the selected dyes on XAD-16, the Langmuir and Freundlich isotherms, the two most frequently used models, were used. The parameters obtained from the two isotherms are given in Table 3.

Table 3. Isotherm parameters for the adsorption of dyes on XAD-16

Isotherm	***Parameters***	***BG***	***AB***	***MG***	***BCB***
Langmuir	q_m	3,940	2,358	5,249	5,409
	K_L	3,793	1,187	8,676	1,672
	R^2	0,999	0,959	0,997	0,994
Freundlich	K_f	3,441	1,151	5,682	3,270
	n	2,152	1,474	4,100	4,141
	R^2	0,991	0,998	0,895	0,999

R^2 values were examined to decide which dye is suitable for which isotherm for adsorption on XAD-16. Accordingly, adsorption of BG can be evaluated by both Langmuir and Freundlich isotherms with similar asorption capacity values. It is also seen that Langmuir isotherm is more suitable for MG while Freundlich isotherm is more fitting for AB and BCB.

Adsorption Kinetics

Two different kinetic models were used to elucidate the adsorption mechanism of dyes on XAD-16 surface. These are pseudo first-order and pseudo second-order models. Kinetic parameters determined by using these two models are given in Table 4.

Considering the linear regression coefficients, it can be said that the adsorption of BG, AB and BCB dyes on XAD-16 follows pseudo-second order kinetic model. MG follows the pseudo-first order kinetic model for the concentration range studied. The same conclusion is reached in terms of the compatibility of the calculated adsorption capacity (q) values with the experimental q values. It can be concluded that the slowest adsorption on XAD-16 occurs for MG and BCB. It has been observed that the adsorption of AB on XAD-16 is very rapid compared to other dyes. These findings are in correlation with experimental results.

Table 4. Kinetic parameters for the adsorption of dyes on XAD-16

			Pseudo First Order			*Pseudo Second Order*		
Dye	C_0	q_{exp} ($mg.g^{-1}$)	q_1 ($mg.g^{-1}$)	k_1 (min^{-1})	R_1^2	q_2 ($mg.g^{-1}$)	k_2 ($g.mg^{-1}.min^{-1}$)	R_2^2
BG	10	1,299	1,073	0,1180	0,977	1,451	0,1759	0,997
	15	1,942	1,608	0,1142	0,978	2,129	0,0621	0,997
	20	2,936	1,728	0,0503	0,993	3,102	0,0500	0,995
	25	3,232	2,148	0,0573	0,965	3,483	0,0407	0,996
AB	10	0,903	0,383	0,0972	0,891	0,922	0,5325	0,999
	15	1,718	0,883	0,1246	0,902	1,696	0,3099	0,999
	20	1,878	1,397	0,2337	0,963	1,887	0,3508	0,999
	25	2,978	2,097	0,1760	0,965	3,026	0,1616	0,999
	30	3,362	2,171	0,1581	0,940	3,386	0,1433	0,999
MG	20	2,330	2,415	0,0530	0,995	1,793	0,0255	0,976
	25	3,152	3,280	0,0484	0,996	2,484	0,0194	0,961
	30	3,438	3,861	0,0471	0,989	3,041	0,0189	0,919
	35	4,431	4,819	0,0455	0,994	3,874	0,0205	0,978
	40	4,696	5,121	0,0385	0,992	4,138	0,0172	0,983
BCB	20	2,634	1,487	0,0294	0,973	2,751	0,0451	0,995
	30	3,948	2,145	0,0182	0,981	4,075	0,0223	0,999
	40	5,024	2,519	0,0128	0,970	5,165	0,0139	0,999
	50	6,023	2,637	0,0162	0,976	6,211	0,0104	0,999
	60	7,411	3,233	0,0145	0,946	7,572	0,0093	0,999
	80	10,048	4,910	0,0118	0,984	10,352	0,0065	0,999

CONCLUSIONS

In this work, we showed that XAD-16 resin can be used for the batch adsorption of a variety of dyes. By determining the isotherm, kinetic and thermodynamic parameters and elucidating the adsorption mechanism, this work is thought to be beneficial for future studies. Langmuir and Freundlich isotherms were drawn and the coefficients of the isotherms were calculated to determine the effectiveness of adsorbents and to elucidate the adsorption mechanisms of selected dyes. In order to determine the adsorption kinetics, the kinetic parameters obtained by applying pseudo-first order and pseudo-second order models were evaluated and was shown that almost all dyes, with the exception of MG, follow the pseudo-second order model. In addition to analyzing adsorption isotherms and kinetics, effects of adsorption parameters were also investigated. Using the experiments at different temperatures, the thermodynamic data of the reactions were calculated and it was determined that the reactions took place spontaneously and were endothermic.

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A CROSS-SECTIONAL STUDY ON KNOWLEDGE ABOUT HBV VACCINATION AMONG THE MEDICAL STUDENTS OF A TERTIARY MEDICAL COLLEGE AND ORGANIZATIONS INVOLVED IN VACCINATION AND AWARENESS

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ABSTRACT

Hepatitis is one of the alarming communicable disease in the world which is spread through the blood semen, saliva of an infected person. The agent is Hepatitis-B virus which is a DNA virus family of Hepadnaviridae. Hepatitis-B is a preventable disease by vaccination and has total four doses(first or initial dose, one and two months later from first dose and the last dose is taken after 1year from initial dose which is named as Booster dose).In EPI schedule total six vaccine preventable diseases namely measles, tetanus, polio, diphtheria, pertussis and tuberculosis are covered where hepatitis is not included. A cross sectional study was conducted among the medical students of northern Bangladesh in January, 2020 using a pretested self administrated structured questionnaire. $\square^2$ test was used to identify the knowledge about hepatitis and awareness of taking HBV vaccine. Total 253 medical students from different medical colleges under MBBS and BDS course participated.Among them 50.99% was male and 40.01% was female and 47.82% was from town and 52.17% was from village. From all participants 57.31% was vaccinated with full dose completed,26.29% didn't complete dose(Booster dose) and 13.44% never received vaccine, From the Unvaccinated persons 16.33% didn't know about the organisation from where vaccine is received and who knew about the organisation form where vaccine can take,more than 90% mentioned the name of SHANDHANI(A free blood donating and vaccination organisation).Along with HBV vaccination 53.78% could mention that they also completed EPI schedule,20.32% couldn't remember and 23.9% didn't complete EPI.Before taking vaccine 64.94% was go through screening procedure and 15.54% didn't know about screening. From our study we can say that we need more awareness for taking vaccine and government should include HBV in EPI.And also need to organise awareness program among the community people and students of all sectors. **Aim and objectives of this study:** The aim of the study was to identify the knowledge and prevalence of HBV vaccination among the current students of Rangpur Medical College, Rangpur,also a tertiary thousands beded government medical college of northern Bangladesh. This study is also to make the students aware and encouraging to receive vaccine in time and completing all doses in schedule time of vaccination.

INTRODUCTION

Hepatitis B virus, abbreviated HBV, is a partially double-stranded DNA virus, a species of the genus Orthohepadnavirus and a member of the Hepadnaviridae family of viruses This virus causes the disease hepatitis B.Viral infection by hepatitis B virus (HBV) causes many hepatocyte changes due to the direct action of a protein encoded by the virus,HBx and to indirect changes due to a large increase in intracellular reactive oxygen species (ROS) after infection. HBx appears to dysregulate a number of cellular pathways.HBx causes dysregulation

in part by binding to genomic DNA,changing expression pattern of miRNAs,affecting histone methyltransferase,binding to SIRT1 protein to activate transcription, and cooperating with histone methylase and demethylase to change cell expression patterns.HBx is partly responsible for approximate 10,000 folds increase in intracellular ROS upon chronic HBV infection, increase ROS can be caused,in part by localization of HBx decreases the mitochondrial membrane potential. In addition, another HBV protein, HBsAg,also increases ROS through interactions with the endoplasmic reticulum . The increase in reactive oxygen species (ROS) after HBV infection cause inflammation, which leads to further increase in ROS.ROS cause more than 20 types of DNA damage.Oxidative DNA damage can cause mutagenic.In addition, repair of the DNA. Epigenetic alterations and mutations may cause defects in the cellular mechinary that then contribute to liver disease. By the time accumuting epigenetic and mutation changes eventually cause progression to cancer,epigenetic alterations appear to have a larger role in this carcinogenesis than mutations.Only one or two genes,TP53 and perhaps ARID1A are metated in more than 20% fo liver cancers whule 41 genes each have hypermethylated promoters (repressing gene expression) in more than 20% of liver cancers, with seven of these gene being hypermethylated in more than 75% of liver cancers.In additions are also caused by HBx recruiting the DNA methytransferase enzymes,DNMT1 and/or DNMT3A to specific gene to loci alter their methylation level gene expression. HBx also alters histone acetylation that can affect gene expression. Several thousands protein-coding genes appear to have HBx-binding sites.In addition to protein coding genes,about 15 microRNAs and 16 Long non-coding RNAs are also affected by the binding of HBx promoters.Each altered microRNA can affect the expression of several hundred messenger RNAs.Hepatitis B virus is classified as the type species of the orthohepadnavirus,which contains eight other species. The genus is classified as part of the Hepadnaviridae family,which contains one other genus, Avihepadnavirus. Thia family of viruses have not been assigned to a viral order.Viruses similar to hepatitis B have been found in all apes(orangutans,gibbons,gorillas and chimpanzees), in old world monkeys(the woolly monkey hepatitis B virus),suggesting an ancient origin for this virus in primates.The virus divided into four major serotypes(adr,adw,ayr,ayw)based on antigenic epitopes present on its envelope proteins.These serotypes are based on a common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r).The viral strains have also been divided into ten genotypes (A-J) and forty subgenetypes according to overall nucleatide sequemce variation of the genome.Morphology of this virus is consists of, *HBsAg -Hepatitis B surface antigen was the first hepatitis B virus protein to be discovered.*HBcAg (HBeAg is a splice varient)-HBcAg is the main structural protein,*Hepatitis B virus DNA polymerase,*HBx,Hepatitis B virus protein HBx is small 154 amino acid long nonstructural and has an important role in HBV replication in HepG2

MATERIALS AND METHODS:

Study design and area of study :

A cross-sectional study was conducted among 253 medical students of Rangpur Medical College of five current batches. All current batches participated during data collection. A self-administrated pretested questionnaire was provided. The questionnaire has three parts and they are (1)demographic information -age, sex ,residence, study year, family income, present

residence (2)information about vaccination-screening, vaccinated/unvaccinated, dose completed or not completed (3)economic status related to vaccination.

Sampling techniques and sample size:

A single population proportion formula was used to estimate sample size. By considering 95% confidence level with Z value of 1.96 with a 5% margin error the sample size is measured. By calculation the ideal sample size was 384 and due to tight class schedule we could manage 253 participants.

Demographic Variables:

There are few variables and they are age, sex, study year, residence. Age group was divided into three categories and they are a age group of 18-20 years of age, 21-23 years of age and above 23 years of age group. Study year was considered as all current batches like first-fifth year and covered age groups. Male and female two groups of gender and residence was mainly considered as their academic and non academic residence like two groups who recently stay at home with family is non academic residence and who stay in campus residence is considered as academic residence thus we can compare the knowledge level who knew about Hepatitis after coming medical college and received vaccine.

Identify knowledge level:

Identification of knowledge about Hepatitis and Hepatitis B virus infection was determined by their answer on some questions we frequently asked them about Hepatitis-B infection. The questions included about hepatitis -B, mode of transmission, mode of contamination, screening procedure, total dose and duration. Total ten questions were included in the questionnaire. All the questions were true or false based and whom answered correctly got 1marks and for incorrect answer no point was given. According to their answer we determined their knowledge as who correctly answered 7-10/10 is considered as good knowledge, correctly answered 5-6/10 considered as moderate knowledge and below 5 correctly answered from total 10 are considered as poor in knowledge about hepatitis. In result section level of knowledge is presented by a pie chart which is shown on **chart-A**

Questionnaire of knowledge :

Total ten true false based questions were asked in the questionnaire to measure knowledge.

1.The easiest way to get hepatitis B through sharing equipment to inject drugs.

True False

2.The majority of people with chronic hepatitis B do not have any symptoms.

True False

3.Hepatitis B can be transmitted by air.

True False

4.Everybody with a positive hepatitis B antibody test has chronic hepatitis B disease (infection).

True False

5.There is medication to treat hepatitis B

True False

6.There is a vaccine for hepatitis B

True False

7.People who clear the hepatitis B virus, either spontaneously or after medical treatment can be infected again.

True False

8.Hepatitis B vaccine has total 4 doses.

True False

9.Booster dose is initial dose of hepatitis B

True False

10.Hepatitis B can be transmitted by syringe, blade, during receiving blood, by cutting sharp objects.

True False

Screening :

Screening is an important procedure before vaccination in case of Hepatitis B. When someone feel that he or she need to take vaccine, at first they have to do a test to ensure that they are already infected or not, and for confirmatory purpose they need to go through a test purpose and it's called screening. We asked each of the participants who received and receiving vaccine of they did screening before and asked them about screening procedure. The result of doing screening is shown in **chart-B**

Doses of vaccine received and reason of not received :

In case of Hepatitis B virus vaccine have total four doses, One is initial dose after screening and then second, third dose should take after one and two month interval of initial dose. Last dose or fourth dose should take one year after initial dose which called Booster dose. Who already received and receiving vaccine we collected the information which was included as a chart of doses of vaccine and calculated the total number of doses they received. Result of doses of received vaccine is shown on **chart-C 1 & 2**

Statistical analysis :

Statistical analysis was done by both SPSS version 20.0 and Microsoft Excel 2007 version. Chi square test was done to know the significance level. If the p value shows <0.05 it was considered as significant result of the variable.

RESULTS

Total 253 participant were participated in this study and from them 129were male and 124 were female in number ,in percentage 50.99% male and 40.01% female. According to present residence 217stay in hostel(institutional residence) and 36 stay with their family in percentage 86% stay in hostel(institutional residence)and 14% stay with family because they are local here, Age group was divided into three categories, from 18-20 age group there was 93in number and in percentage 36.75%,age group of 21-23 was 102 in number which is40.32% and age group above 23years of age group it was 58 students which is 22.29%.Total five current academic batches and intern students were participated and maximum number of interest group was from 1^{st},2^{nd} and 5^{th} year students.

Knowledge of the students were measured by a pretested questionnaire based on general idea about hepatitis B, according to correct and incorrect answer form two different groups like one group who are vaccinate and others were partially or not vaccinated.

Level of knowledge was divided into three groups and they are good knowledge, moderate knowledge and poor knowledge. Total ten questions about basic idea of Hepatitis B infection, way of transmission and agent by which hepatitis is transmitted, answer was true and false based question. Each correct answer was marked as 1 and wrong answer marked 0 and then calculated total number. Who answered 7-10correct answer was considered as good knowledge, who correctly answered >5 is considered as moderate in knowledge and below 5correct answer they were considered as poor knowledge. By calculating of knowledge level about Hepatitis-B virus 81% was declared as considered as good knowledge, 14% have moderate knowledge and 4%was considered as poor knowledge. Screening is a most important things to confirm about presence or absence of Hepatitis B in a human body. We frequently asked them about screening who already received vaccine because before taking vaccine screening have to done. People who did screening before receiving vaccine were 163 in number and 64.43%, 50 of them did not go through screening but received vaccine which is 19.76%.

From total participants the vaccinated are *n=145,57.31%* and they have completed all four doses, partially vaccinate or receiving vaccine or yet not completed all four doses are *n=66,26.09%* and people who do not received any dose are *n=34,13.44%.*

There were many organizations involved in making awareness among the medical students about infectious diseases and encouraging to receive vaccine in time, result of organizations involved in making awareness and vaccination program without profit are presented by histogram and shown on **chart E.** We found many names such as SHANDHANI, Medicine Club, Friends Foundation, Red Crescent, RBDC and many more. From them all maximum participants mentioned the name of organization from where received vaccine, it was SHANDHANI and which is a non profit Blood donating organization also involved in some vaccination program. Media from where they knew about Hepatitis-B and vaccination they mentioned from family, school, social media, campaign program, television and many more. Result of media is shown on **chart D**

Table:1

variables	n	%	p
male	129	50.99	
female	124	40.01	
			0.1
present residence			
hostel (institutional)	217	86	
with family	36	14	0.083
others	0	0	
permanent residence			
town	121	48.82	
outside the town	132	52.17	0.021
outside the country	0	0	
age group			
18-20	93	36.75	
21-23	102	40.71	0.582
>23	58	22.29	
study year			
first professional	141	55.57	
second professional	13	0.05	
third professional	11	0.04	0.001
final professional	71	28.06	
internship	17	0.06	
family income (monthly)			

<20,000	21	8.3	
20,000-40,000	152	60.07	0.092
>40,000	80	31.62	

Chart: A

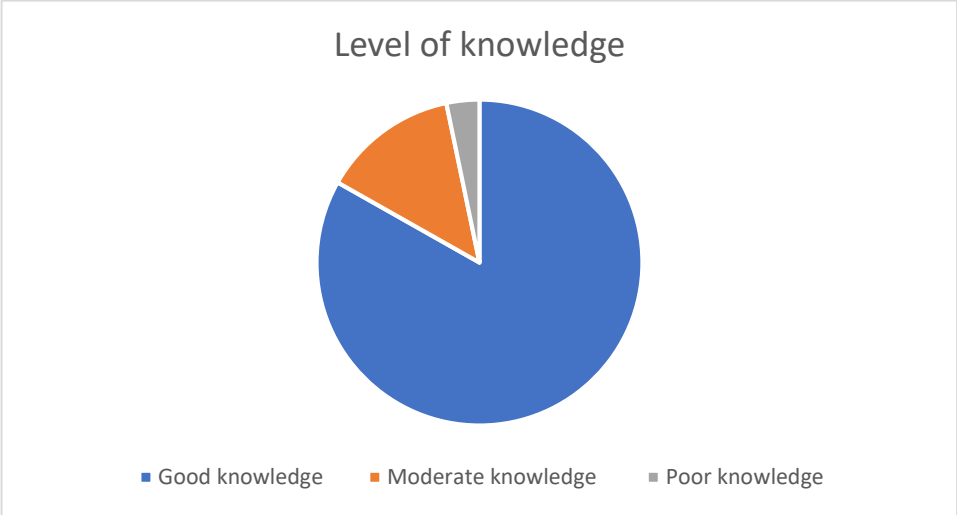


Table: ***response on knowledge based questions from the participants***

	Response	vaccinated (n,%)	partially (n,%)	p value
The easiest way to get hepatitis B through sharing equipment to inject drugs.	correct	71,48.96	58,53.70	0.9
	incorrect	74,51.03	50,46.29	
The majority of people with chronic hepatitis B do not have any symptoms.	correct	95,65.51	84,77.77	0.011
	incorrect	50,34.48	24,22.22	
There is medication to treat hepatitis B	correct	125,86.21	92,85.18	0.832
	incorrect	20,13.79	16,14.81	
Hepatitis B can be transmitted by air.	correct	134,92.41	78,72.22	0.087
	incorrect	11,7.58	30,27.77	

Everybody with a positive hepatitis B antibody test has chronic hepatitis B disease (infection).	correct	84,57.93	77,71.29	0.872
	incorrect	61,42.06	31,28.70	
There is a vaccine for hepatitis B	correct	93,64.37	68,62.96	0.214
	incorrect	52,35.86	40,37.03	
People who clear the hepatitis B virus, either spontaneously or after medical treatment can be infected again.	correct	79,54.48	83,76.85	0.007
	incorrect	66,45.51	25,23.14	
Hepatitis B vaccine has total 4 doses.	correct	115,79.31	101,93.51	0.01
	incorrect	30,20.68	7,6.48	
Booster dose is initial dose of hepatitis B	correct	101,69.65	99,91.66	0.08
	incorrect	44,30.34	9,8.33	
Hepatitis B can be transmitted by syringe, blade, during receiving blood, by cutting sharp objects.	correct	83,57.24	71,65.74	0.03
	incorrect	62,42.75	37,34.25	

Chart-B

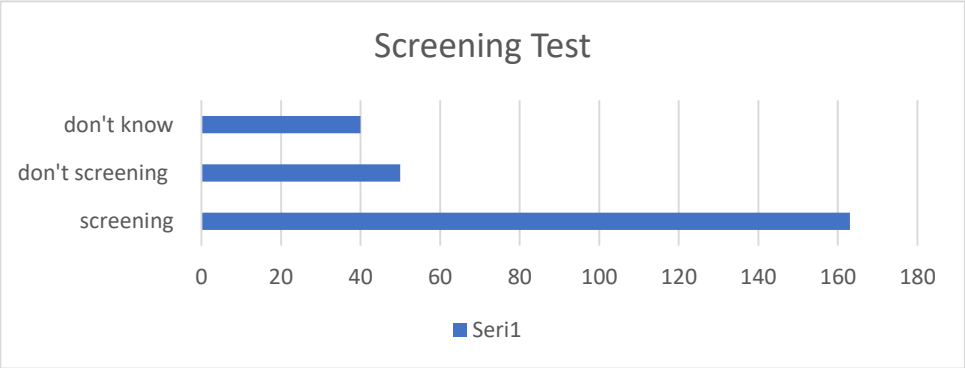


Chart: C 1

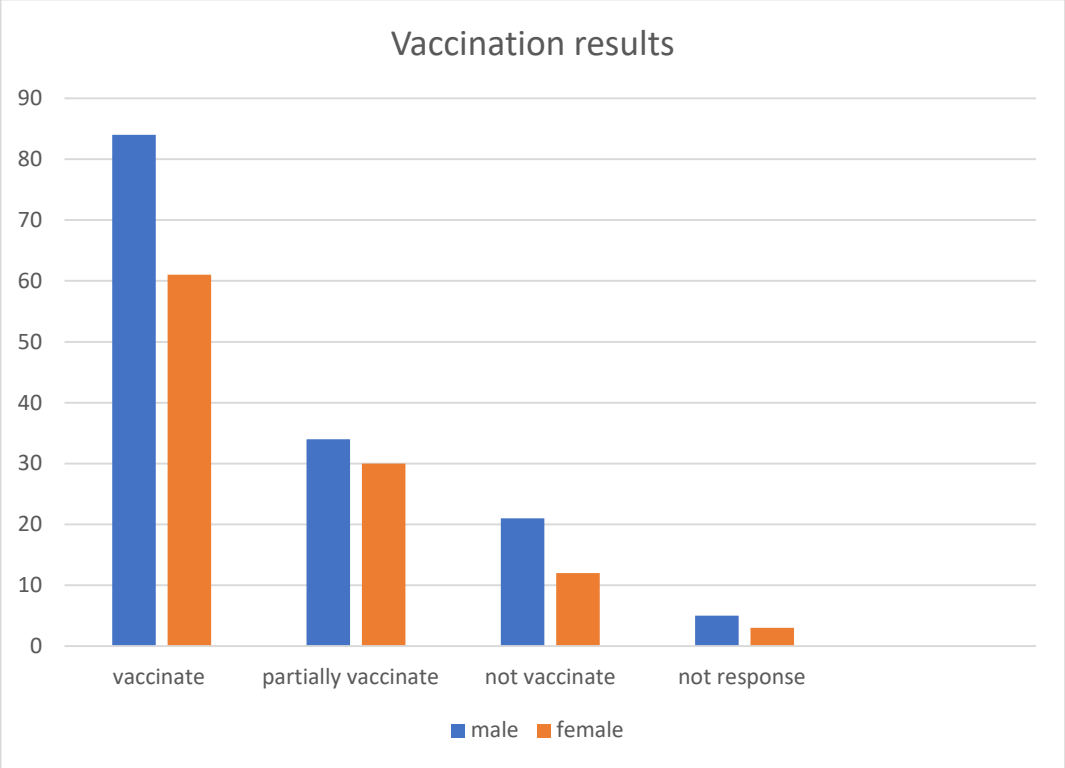


Chart: C 2

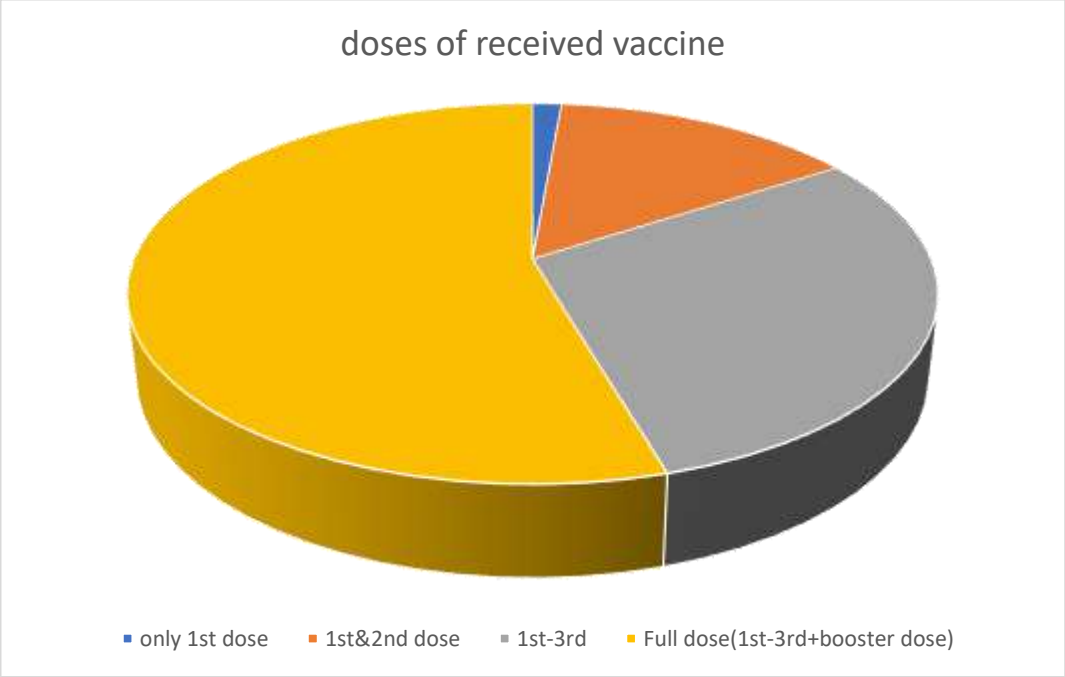


Chart: D

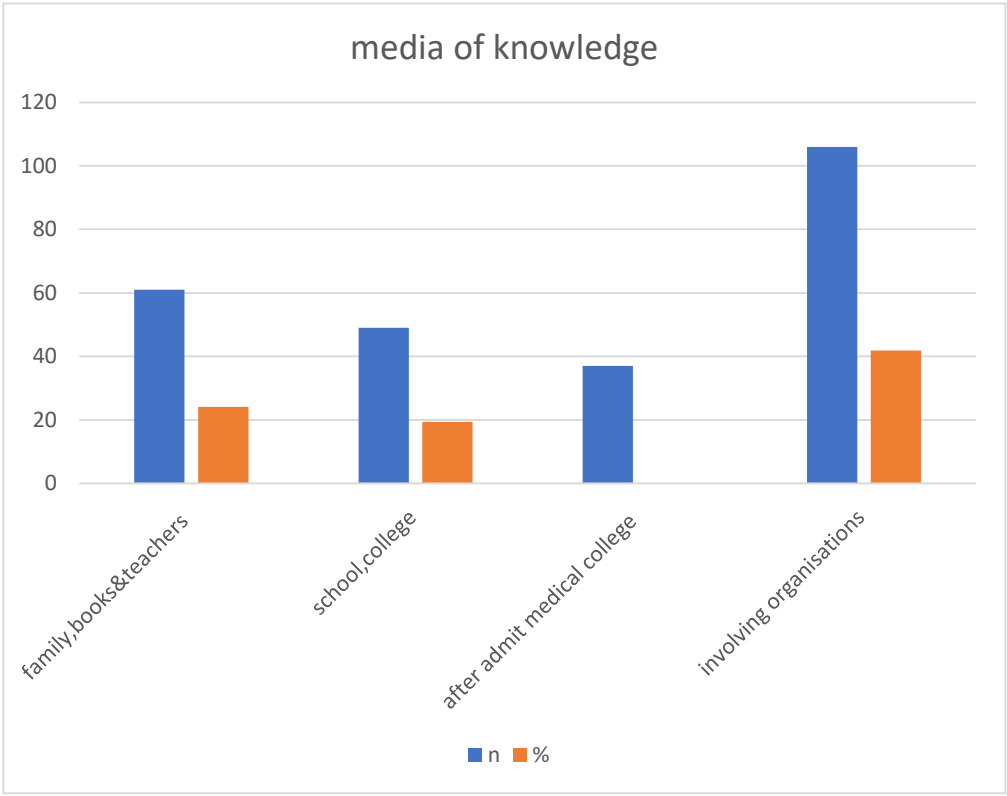
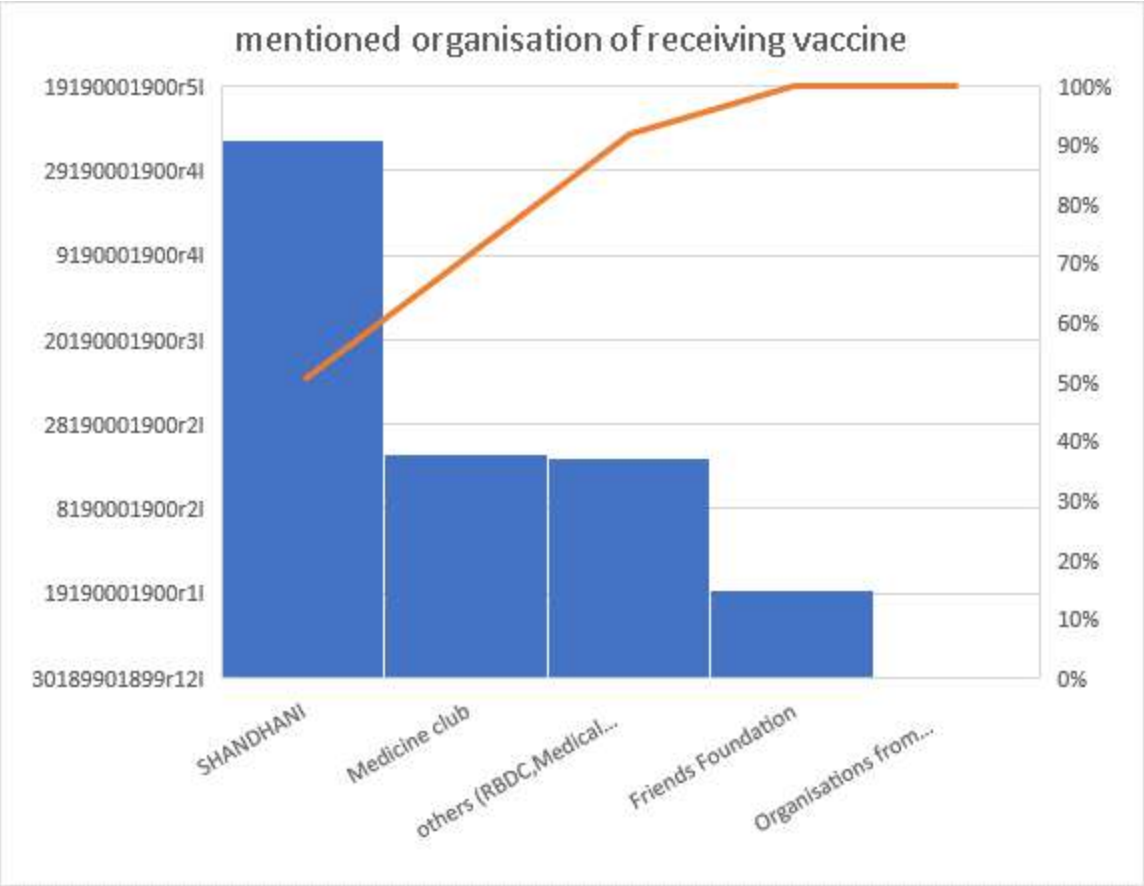

media of knowledge
120
100
80
60
40
20
0
family,books&teachers
school,college
after admit medical college
involving organisations
n
%

Chart: E



DISCUSSION

The study was done to find out the knowledge about Hepatitis B and importance of receive vaccine within appropriate age. Although there is no age limit to take vaccine but it is wise to take vaccine as early possible. We did a survey among the medical students of a tertiary medical college of northern Bangladesh. The survey was done on the medical students because Hepatitis is a medical term and it is must that sincere level should be significant in health science students. Vaccination awareness also be expected higher in this sector than we can compare the knowledge level and awareness of receiving vaccination percentage to other faculties in future study. We compared the knowledge level among different variables of the health science students. Knowledge about Hepatitis-B and vaccination we provided them ten questions on this topic and all the questions were true and false based answer. Each correct answer marked one and incorrect answer had no marks. Total ten marks were given to identify the knowledge level. Maximum scored 7 out of ten and which indicate a good knowledge. As we differentiate knowledge into three categories according to score they achieved. Who scored at least 7 correct answer was considered as good knowledge, who scored more than 5 and less than 7 is considered as moderate knowledge and less than 5 correct scorer considered as poor knowledge. And we found a good knowledge among the medical students. But they have poor idea and awareness about screening. Although screening is a very necessary part before receive vaccine.

Because already infected person of Hepatitis B can not get cure from the vaccine and he need to pre-vaccination preparation and treatment to be ready for proper vaccination outcome. In our study we also found some interesting thing of doses they received and who receiving but not at time. Hepatitis B vaccine has total four doses. Who received all four doses or who are complete vaccinated are 145 in number and 57.31% so, we can say that more than half are well vaccinated or completely vaccinated. Partially or who did not completed all doses (some have one or two or three doses of complete) are 66 in number and 26.09% so, we have to make aware that completing doses are very important. If someone don't received all the doses and it goes for many years they have chance for viral attack of hepatitis B. So, have to make awareness among the students about importance of final or booster dose receiving. We also tried to know about the vital media form where they knew about Hepatitis-B and importance of vaccination and we found different media such as television, radio, newspaper, social media, campaign, form family members and institutions. In case of medical students maximum of them have knowledge about Hepatitis-B from secondary school level and they received vaccine before coming medical college and that also a great news that they have awareness from very early stage of education life. So, we need to make aware among them about screening. We included in our survey the organizations that doing a great job in making awareness about vaccination program among medical students and others. Because this institutions are playing a vital role in vaccination awareness and encouraging to receive vaccine in time and in available cost. We think we should do more investigation and awareness outside of health science students and make more campaigns on vaccination to prevent people form hepatitis.

Limitations :

As in under graduate level in medical sector in our country we do not have any scope to do thesis or research work by our institutions so,it was very difficult to work. And It was a randomized question ask session and collection answers from thousands students and lack of volunteers, it was difficult to reach them and collect datas.The main problem was funding, here we didn't got any funding so,we had to manage all the cost from our pockets.

CONCLUSION :

The aim of this study was to make awareness for taking Hepatitis -B virus vaccine immediately to the health professionals and the students also.Because we know this virus can enter our body by blood and medical students when go to ward and OT sometimes they may injured by used needles,blades and can easily infected.So,it's very important to them to take vaccine to prevent themselves from hepatitis.

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Funding :

The main obstacles in our study to manage fundings. Because we could not manage fundings enough to do this study properly.Because in under graduate level we do not get any scope to do thesis or research nased work by institutions.And it was totally a self funded project by my professor who worked with me and provide physical and mentall help when needed.

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MANAGEMENT OF CROWN GALL DISEASE IN THE PRODUCTION OF FLOWER CUTTINGS IN KENYA

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ABSTRACT

Crown gall caused by *Agrobacterium tumefaciens* reduces yields and leads to significant loss of business through restrictions in trade on a wide variety of flower cuttings. The disease has no effective control measure and currently requires high use of synthetic chemicals. This research aimed to identify effective management strategies that minimize pesticide use in production of Asteraceae flower cuttings. Control products were evaluated individually and in combination in a greenhouse study and for suppression of bacterial growth when cultured on treated nutrient agar. Treatments included Sunflower oil, KSP-Colloidal Copper, KOBE-Chrysophanol parietin, Copper oxychloride and Electro-Chemical Activated water. Data on disease severity (galls) and incidence was collected weekly and analyzed using SAS software, by ANOVA and significantly different treatment means separated by Fisher's protected LSD test at $P \leq 0.05$. In the lab experiment Copper oxychloride was least effective and had the highest number of bacterial cells growing (>100) while ECA water, KSP and oil reduced bacterial growth. Combined ECA water and Oil in NA suppressed bacterial growth completely while Oil alone had than less 50 cells on average. KSP and oil combined resulted into high bacterial growth, suggesting antagonism between the two products. In the greenhouse, Copper oxychloride and KOBE were ineffective and did not cause drying of the galls nor reduce yellowing of plants. Oil was most effective and dried all galls (100%), followed by ECA water (80%) and KSP (50%).ECA water and oil combined was not significantly different ($P>0.05$) to either product when applied individually. Combined KSP and oil dried only 30% of the galls and 60% of the plants yellowed which was more than when the products were applied individually. No significant difference ($p>0.05$) was observed between treatments that had more than one strategy applied and those that only had one management strategy. Oil and KSP combined was not effective and seemed to antagonize each other. The most effective treatments were combination of ECA water and oil followed by oil alone and KSP alone. The study demonstrates that Crown gall disease can be effectively managed without use of chemical pesticides.

Key words: *Agrobacterium tumefaciens*, ECA water, KSP, Sunflower oil, KOBE, Copper oxychloride

INTRODUCTION

Kenya is the leading exporter of flowers to the European Union with a market share of approximately 38% (KFC, 2017). It exports cut flowers and flower cuttings mainly used for propagation. Flower cuttings production in Kenya faces challenges in the marketing stage because of crown gall disease caused by *Agrobacterium tumefaciens*. Although crown gall is

not a quarantine disease in Kenya, strict regulations in the markets require that flower cuttings be free of the disease. The disease causes great financial loss in the nursery when large numbers of plants are affected. So far, losses of up to 80% have been recorded in nurseries (Maina, et al, 2011).

Crown gall disease can move systemically throughout the root system and destroy the plants completely (Mutitu et al, 2012). So far, there has been no conclusive research on effective management strategies of crown gall in the production of flower cuttings. Chemicals have been largely used in the past for management of diseases, however, this is changing due to worldwide concern on the risk to the environment and health due to occupational exposure, and residues on food as well as in drinking water.

Flower producers have tried various strategies for managing the disease with little success and crown gall remains a major challenge in the production of flowers in East Africa (KFC, (2011). Limited research has been done to determine the effectiveness of various management strategies of crown gall on flowers and roses have been the main crop of interest in previous research. This has led to uncertainty among flower cuttings producers on what strategy to use in crown gall management; therefore, this study sought to determine the appropriate management strategies for Crown gall disease in the production of flower cuttings.

MATERIALS AND METHODS

Survey

The survey was carried out in Nairobi, Kiambu and Nakuru counties in selected flower cuttings producing farms. The persons responsible for phytosanitation were issued with a questionnaire to obtain the required information on Crown gall disease incidence, varieties affected, strategies used to manage the disease and their effectiveness.

There are twenty-four farms in total that produce flower cuttings in the three counties. All farms were targeted but only twenty farms were in production at the time of the survey, seven farms in Kiambu county, eight in Nakuru county and five in Nairobi county. The farms produce flower cuttings for sale while a few produce cut flowers, for example, roses for the retail market. Various types of flowers are produced, for example, Argyranthemum, Leucanthemum, Chrysanthemum and carnations like *Dianthus caryophyllus*. More than half of the farms have been in the business for over 10 years and vary in size between 8 and hectares.

Evaluation of individual management strategies against crown gall

The laboratory experiment was carried out in a flower cuttings farm in Kiambu County. The management products evaluated singly included chemical (Copper oxychloride), foliar solution (KSP), bio pesticide (KOBE), sunflower oil and ECA water.

Copper oxychloride contains 50% metallic Copper whose mode of action is contact and controls fungal as well as bacterial spots (Gitari, 2015). It was prepared by dissolving 1.5g, 3g and 6g in one litre of water. KSP is a foliar solution that contains colloidal Copper. KSP was prepared by dissolving 7.5ml, 15ml and 30ml in 1 litre of water. Both Copper oxychloride and KSP were obtained from local suppliers. KOBE is a bio pesticide that contains Chrysophanol Parietin from *Rheum officinale* plant. Its mode of action is contact and controls fungal as well as bacterial spots (UOH, 2014). It was purchased from a local supplier and prepared by dissolving 1ml, 2 ml and 4 ml in 1 litre of water. ECA water was obtained from a farm that generates it on site. It was prepared by dissolving 0.25ml, 0.5ml and 1ml in 1 litre of water.

To obtain crown gall innoculum, bacterial isolates were obtained from young, tender and milky galls on infected plants and washed using tap water. With the help of a sharp sterilized blade, the galls were diced into small cubes of approximately 2 millimeters. They were then surface sterilized in 3% sodium hypochlorite solution for fifteen minutes according to Schaad et al (2001) and rinsed in sterilized distilled water thrice to remove traces of Sodium hypochlorite. Ten cubes were crushed in 5 milliliters of sterile distilled water with the help of sterilized rod in sterile Petri dishes to form a suspension which was kept for 10 minutes to settle. The innoculum density was determined by use of serial dilution, plating and counting the number of colony forming units.

An innoculum density of 10^8 CFU/ml was immersed in 10mls of Copper oxychloride and left to settle. After one day, 0.5mls of the suspension was pippeted and serial dilution done. A 0.5ml volume of the diluted cell suspension was plated on nutrient agar medium, incubated in an incubator at 26^0C for 5 days and the colony growth studied. The same procedure was repeated using KSP foliar solution, KOBE bio pesticide and ECA water. To evaluate the effectiveness of vegetable oil against crown gall, a cell suspension of 10^8 CFU/ml was smeared on a slide with nutrient agar medium and the oil applied on it. After one day, colony growth was observed and number of cells determined. Bacterial growth on the slide was used to determine the number of bacteria before and after the control products were applied. This determined the effectiveness of the different products on the bacteria.

The treatments were, Control, $Cu_2(OH)_3Cl$ (1.5, 3 & 6gm/ltr), ECA water (0.25, 0.5 & 1ml/ltr), KOBE (1, 2 & 4 ml/ltr), KSP (7.5, 15 & 30ml/ltr) and oil.

The field study was carried out in a greenhouse in Kenyatta University, Nairobi County. Planting media was prepared using peat and pumice at a ratio of 25:75 and put into 3-litre plastic pots. Seedlings of Argyrathemum plants used in the experiment were obtained from a flower cuttings production farm grows them for export. The plants were prepared in a propagation chamber for 4 weeks before planting.

The treatments evaluated were Control, $Cu_2(OH)_3Cl$ (1.5, 3 & 6gm/ltr), ECA water (0.25, 0.5 & 1ml/ltr), KOBE (1, 2 & 4 ml/ltr), KSP (7.5, 15 & 30ml/ltr) and oil. Each treatment had three pots and was replicated three times.

When 1.5 months old, the plants were inoculated with 10^8 CFU/ml *Agrobacterium tumefaciens* at the root base using a hypodermic syringe. Inoculated plants were observed for two weeks to express disease symptoms. Application of products being evaluated started 12 days after symptoms appeared. The plants were evaluated weekly to determine the level of infection by studying the number of galls and chlorosis symptoms.

Application of the different management strategies was done once a week for all treatments and repeated weekly for four weeks.

Evaluation of combined management strategies against crown gall

In the laboratory, innoculum density of 10^8 CFU/ml was immersed in 10mls of ECA water and left to settle. After one day, 0.5ml volume of the suspension was serially diluted and 0.5ml of the diluted cell suspension plated on nutrient agar medium. The plates were incubated in an incubator at 26^0C for 5 days and the *Agrobacterium* cells growing counted. The same procedure was repeated with oil alone and KSP alone. The combined management strategies were evaluated as follows:

Treatment combination 1: ECA water and oil

An innoculum density of 10^8 CFU/ml was immersed in 10mls of ECA water and left to settle. After one day, 1ml of Oil was added to it, mixed and left to settle for 1 day. A 0.5ml volume of the suspension was serially diluted and 0.5ml of the diluted cell suspension plated on nutrient agar medium. The plates were incubated in an incubator at 26^0C for 5 days and the grown *Agrobacterium* cells counted.

Treatment combination 2: Oil and ECA water

An innoculum density of 10^8 CFU/ml was immersed in 10mls of Oil and left to settle. After one day, 1ml of ECA water was added to it, mixed and left to settle for 1 day. A 0.5ml volume of the suspension was serially diluted and 0.5ml of the diluted cell suspension plated on nutrient agar medium. The plates were incubated in an incubator at 26^0C for 5 days and the grown *Agrobacterium* cells counted.

Treatment combination 3: Oil and KSP

An innoculum density of 10^8 CFU/ml was immersed in 10mls of Oil and left to settle. After one day, 1ml of KSP water was added to it, mixed and left to settle for 1 day. A 0.5ml volume of the suspension was serially diluted and 0.5ml of the diluted cell suspension plated on nutrient agar medium. The plates were incubated in an incubator at 26^0C for 5 days and the grown *Agrobacterium* cells counted.

Treatment combination 4: KSP and oil

An innoculum density of 10^8 CFU/ml was immersed in 10mls of KSP water and left to settle. After one day, 1ml of oil was added to it, mixed and left to settle for 1 day. A 0.5ml volume of the suspension was serially diluted and 0.5ml of the diluted cell suspension plated on nutrient agar medium. The plates were incubated in an incubator at 26^0C for 5 days and the grown *Agrobacterium* cells counted.

In the greenhouse, the combined strategies were:

Treatment combination 1

ECA water was applied on the plants and galls then oil applied after one week. This was repeated for four weeks.

Treatment combination 2

Oil was applied on galls then ECA water applied to the plants after one week. This was repeated for four weeks.

Treatment combination 3

Oil was applied on galls then KSP applied on the plants after one week. This was repeated for four weeks.

Treatment combination 4

KSP was applied on the plants then oil applied on galls after one week. This was repeated for four weeks.

Treatments that had no combination were Control, Oil alone, ECA alone and KSP alone.

Data analysis

Data collected from survey was keyed in Microsoft excel 2013 and analyzed by use of SPSS software to indicate the variations in the farms with regard to Crown gall disease prevalence, effects and management.

In the laboratory, data was collected on bacterial growth after application of the different treatments. The number of bacterial cells grown after 24 hours was counted. In the greenhouse, data was collected weekly by counting the number of galls and number of chlorotic plants after application of *Agrobacterium* control products.

Data analysis was done by ANOVA using SAS software and treatment means were compared with Fisher's Protected Least Significant Difference (LSD) at probability level of 5%. The results were presented in graphs and tables.

RESULTS

Survey

The survey done indicated that crown gall disease was prevalent in 75% of the surveyed farms. It was noted that the farms where crown gall was not prevalent were not growing susceptible varieties. They were growing flowers like Gypsophila, *New guinea*, Verbena, Saxifraga and Osteospermum. The respondents identified five types of flowers susceptible to crown gall disease. These were: Argyranthemum, Leucanthemum, Chrysanthemum, Takazzi and Roses. Among the five, Argyranthemum was the most affected (50% of the flower farms).

The respondents indicated to use different methods to manage crown gall disease with the majority (35%) rouging infected plants. Twenty five percent practiced good hygiene practices and only 5% were using integrated management strategies that included maintaining high hygiene standards, use of biopesticides, rouging and chemicals. Only 5 percent each were using oil, KSP and KOBE biopesticide singly.

Out of the surveyed farms, 35% reported that integrated pest management is the best strategy for managing crown gall disease; 30% indicated high hygiene standards as the most effective while 15% did not choose any management strategy as the best because they do not grow susceptible varieties. Ten percent of the respondents indicated roguing as best strategy because once the plants are discarded, the innoculum source is eliminated, however, when the disease is widespread, roguing affects their plant density which affects production and supply to customers. Only 5% each were using KSP and oil and indicated them as best management strategies. From the responses, chemical use was not highly preferred.

Evaluation of individual management strategies against crown gall

Bacteria growth invitro

The laboratory experiment indicated that the culture medium with no treatment (control) had the highest number of *A. tumefaciens* cells (434). Of the different treatments, Copper oxychloride was the least effective in suppressing bacterial growth at the varying rates of - 1.5gm/ltr (292 cells), 3gm/ltr (244 cells) and 6gm/ltr (263 cells). The control and the three rates of Copper oxychloride were not significantly different ($p>0.05$). KOBE biopesticide treatments had the third highest number of cells 1ml/ltr (138 cells), 2ml/ltr (115 cells) and 4ml/ltr (128) with no significant difference between the rates. The lowest rate of KSP (7.5ml/ltr) had more cells (40) than the other rates 15ml/ltr (6 cells) and 30ml/ltr (2 cells). Two rates of ECA water 0.25ml/ltr (3 cells) and 0.5ml/ltr (3 cells) managed crown gall completely unlike the higher rate

of 1ml/ltr (18 cells). There was no significant difference between the KSP and ECA rates in suppressing bacterial growth ($p>0.05$). Oil was significantly more effective ($p<.0001$) in reducing growth of bacteria in the laboratory (grew 37 cells) compared to Copper oxychloride and KOBE. KSP, Oil and ECA water were not significantly different amongst themselves, but were significantly different from Copper oxychloride and KOBE ($p<.0001$)

Table 1: Effects of individual management products on bacterial cell growth

	Experiment 1		**Experiment 2**
Treatment	Mean of number of cells ± S.E		Mean of number of cells ± S.E
Cu2(OH)3Cl, 1.5gm/ltr	256.7±8.8b		326.7±17.6ab
Cu2(OH)3Cl, 3gm/ltr	177.0±4.7c		310.3±32.7b
Cu2(OH)3Cl, 6gm/ltr	204.7±15.8bc		321.3±21.9ab
ECA, 0.25ml/ltr	3.0±0.6f		3.0±1.0e
ECA, 0.5ml/ltr	3.0±0.9f		2.3±0.3e
ECA, 1ml/ltr	21.7±6.0f		13.7±2.0de
KOBE, 1ml/ltr	146.7±12.0cd		130.0±15.2c
KOBE, 2ml/ltr	130.0±15.3cde		100.0±5.8cd
KOBE, 4ml/ltr	130.0±5.8cde		126.7±3.3c
KSP, 15ml/ltr	6.7±2.4f		4.3±1.5e
KSP, 30ml/ltr	2.3±0.3f		2.3±0.3e
KSP, 7.5ml/ltr	43.3±20.3ef		36.7±12.0de
Use of oil	65.0±14.4def		9.3±5.4e
With no bacteria	0.0±0.0f		0.0±0.0e
with bacteria	466.7±120.2a		401.7±106a
P-value	<0.0001		<0.0001
LSD	93.935		87.193

Means followed by the same letter(s) within a column are not significantly different according to Fisher's LSD test at $P<0.05$.

Crown gall incidence on plants

In the greenhouse experiment, the different rates of Copper oxychloride were not effective and the galls did not dry. There was no significant difference between the Copper oxychloride treatments and the untreated control ($p>0.05$). ECA water (0.25ml/ltr and 1ml/ltr) were not effective on crown gall management and were not significant in how they managed the galls. KOBE (2ml/ltr) and ECA (0.25ml/ltr) were not effective in managing galls and were not significantly different ($p>0.05$).

KOBE (1ml/ltr and 4ml/ltr) and KSP 7.5ml/ltr treatments were not effective in managing crown gall and were not significantly different at $p>0.05$. Copper oxychloride and oil were significantly different in how they managed galls from all other management strategies at $p<0.05$ level of significance with a p value <.0001. ECA water (0.5ml/ltr) and oil managed galls very well and were not significantly different with a p value of <.0001. KSP (30ml/ltr) managed galls effectively and was significantly different from how ECA water (0.5ml/ltr) and oil managed the gall at 95% level of significance with a p value of <.0001.

Crown gall severity on plants

All the plants yellowed due to crown gall infection on plants treated with KOBE (1ml/ltr), which was not significantly different from the untreated control that had bacteria inoculation. More than three quarter of the plants yellowed in KOBE (4ml/ltr) treatment, in all Copper Oxychloride treatments and in KOBE (2ml/ltr) treatment indicating that the products did not manage crown gall effectively. Fifty percent of the plants yellowed in ECA water (1ml/ltr) treatment, KSP (7.5ml/ltr) treatment and KSP (15ml/ltr) treatment; indicating very little management of crown gall disease. Less than 50% plants yellowed in ECA (0.25ml/ltr) treatment and in vegetable oil treatment indicating a slightly better management of the disease. Less than quarter of the plants yellowed was observed in ECA water (0.5ml/ltr) showing effective management of crown gall disease. No plants yellowed in KSP (30ml/ltr) treatment which indicated very effective management of crown gall disease. There was no significant difference in the yellowing of plants in the control, Copper oxychloride and KOBE treatments at $p>0.05$ level of significance. ECA water (0.25ml/ltr and 1ml/ltr) and KSP (7.5ml/ltr and 15ml/ltr) had no significant difference in the number of plants with yellowing. ECA water (0.5ml/ltr) and KSP (30ml/ltr) did not show any significant difference in yellowing of plants at $p>0.05$ level of significance.

There was significant difference in yellowing of plants in Copper oxychloride treatments and all ECA water treatments, all KSP treatments and KOBE (2ml/ltr) at $p<0.05$ level of significance with a p value of <.0001.

Evaluation of combined management strategies against crown gall

Bacterial growth invitro

In the laboratory experiment, no bacterial cells grew when ECA water was applied first followed by oil treatment; or oil first followed by ECA water indicating effective management of crown gall invitro. No bacterial cells grew also for ECA water when applied alone. There was no significant difference between ECA water only application and combined application of ECA water and oil at $p>0.05$. There were an average of 27 bacterial cells growing when oil only was applied and 3 bacterial cells grew when KSP only was applied. Application of oil first followed by KSP led to growth of 105 bacterial cells while application of KSP first followed by oil led to growth of 107 bacterial cells which was more than in the KSP only and Oil only treatments. The control treatment had the highest number of cells (389) which was significantly different from all other treatments at $p<0.05$ with a p value of <.0001.

ECA only and KSP only treatments grew 0 and 3 cells on average respectively, which were not significantly different at $p>0.05$.

Table 2: Effects of combined management strategies on bacterial growth

Treatment	No. of cells. Exp. 1	No. of cells. Exp. 2	No. of cells. Exp. 3
Control	376.7±14.5a	386±33.0a	405.7±13.5a
ECA+Oil	0.0±0.0d	0.0±0.0c	0.0±0.0d
Oil+ECA	0.0±0.0d	0.0±0.0c	0.0±0.0d
Oil alone	41.7±6.0c	15.0±2.9c	23.3±4.4c
ECA alone	0.0±0.0d	0.0±0.0c	0.0±0.0d
KSP alone	3.0±0.6d	2.3±0.3c	2.7±0.9d
OIL + KSP	102.7±3.7b	101.3±9.6b	111.3±10.9b
KSP + Oil	111.7±4.4b	106.0±2.3b	103.7±6.9b
P-value	<0.0001	<0.0001	<0.0001
LSD	0	0	0

Means followed by the same letter(s) within a column are not significantly different according to Fisher's LSD test at P<0.05.

Crown gall incidence on plants

All galls dried when ECA water was first applied followed by oil; or oil first followed by ECA water indicating good management of crown gall disease. Application of Oil and ECA water separately dried the galls as well. There was no significant difference between combination of the products and applying them separately ($p>0.05$). One gall did not dry when KSP only was applied while only one gall dried when oil was first applied followed by KSP; or application of KSP first followed by oil which had no significant difference ($p>0.05$). Application of KSP only and the control were significantly different from the other treatments in the number of dried galls ($p<0.05$) level of significance with a p value of <.0001. Oil and ECA water showed excellent results in managing galls while combining KSP and oil did not give good results.

Crown gall severity on plants

Yellowing of plants was 100% in the control treatment. Application of oil first followed by KSP; or applying KSP first followed by oil had the second highest yellowing in plants. Only a quarter of plants yellowed when KSP only was applied. No yellowing of plants was noted when ECA water was first applied followed by oil; or application of oil first followed by ECA water treatment. Application of oil and ECA water separately did not have any yellowed plants as well. There was no significant difference between applying both products together or individually ($p>0.05$). Application of oil first followed by KSP; or KSP first followed by oil had 66% of the plants yellowing with no significant difference between the treatments ($p>0.05$). Application of KSP only and control treatments were significantly different from the other treatments in the number of yellowed plants ($p<0.05$).

DISCUSSION

Data collected on crown gall disease prevalence in Nairobi, Nakuru and Kiambu counties indicated 75% prevalence in the surveyed farms which were producing susceptible varieties. The results confirm that flower production in Kenya faces significant challenges with crown gall being one of the major diseases (Maingi et al, 2018). This indicates the need for more research to be carried out to identify better management strategies for the disease.

The survey indicated that there was no consensus among flower producers on an effective strategy for managing crown gall. High hygiene is very crucial in all activities in minimizing disease incidence and some farms were noted to adhere to such but others not. The surest way of controlling crown gall is through preventing introduction of infected nursery root stock (Gitari, 2015). Other hygiene measures to observe include maintaining clean working tools by cleaning every day with hypochlorite solution (Wilen, 2019), using a disinfectant when working on plants, ensuring work area is clean of any plant debris or other unwanted materials, disinfecting of hands before working on plants and discarding diseased plants as soon as possible to avoid cross contamination of other plants or work equipment. Planting in clean substrate is very key to sustaining a healthy crop, using sanitized irrigation water and avoiding areas with recent history of crown gall disease (Moore, 2013). Most of the hygiene measures are simple and cost-effective guidelines that need clear communication to be successful.

The evaluation of individual management strategies for crown gall indicated Copper Oxychloride and KOBE biopesticide to be ineffective. The treatments with the two products had the highest number of bacterial cells in the laboratory as well as the highest number of undried galls and more yellowing in plants in the greenhouse. Copper bactericides are used by more than 50% of flower farms to manage crown gall but do not manage to eliminate the disease. A study carried out on crown gall management in rose flowers indicated that there was temporal management of crown gall by Copper oxychloride because the galls regenerated after one month (Gitari, 2015). Farmers indicated that galls dry for a short while but regenerate after some time. Copper bactericides do not penetrate plants and therefore cannot manage crown gall effectively (Gitari, 2015). Copper based products have been used primarily to manage bacterial diseases, but scientists have found that continuous use of such products makes bacteria become resistant to them (Egel, 2014). They have been effective for crop disease management, however, high dependence on them poses serious threats to sustainable agricultural production; which has led to a worldwide pressure to restrict use (Lamichhane, et al, 2018). KOBE is a new product which many flower producers have not used and acts by activating a plants immunity by eliciting systemic acquired resistance (Onze, 2015). It has shown not to be effective in the management of crown gall in flower cuttings.

ECA water (0.5ml/ltr) showed good results in the management of crown gall, cells that grew in the laboratory were very few and galls dried completely with no yellowing of plants. The lower rate of 0.25ml/ltr was not effective as well as the higher rate of 1ml/ltr. ECA water contains chlorine which requires a pH range of 7.2 to 7.8. If the pH is altered, then its effectiveness is not observed (Adam, 2013). The higher ECA water rate (1ml/ltr) destabilized the pH. Chlorine requires a pH range of 7.2 to 7.8 for it to be most effective, If the pH gets too high, hypochlorous acid cannot form easily (Hadhazy, 2013). Chlorine is used widely in disinfection activities and in killing germs in drinking water, but many people have not used it in managing crown gall which it has shown to manage. Chlorine is lethal to bacteria because cell walls around bacteria have a net negative charge and are able to come into contact with it because it is neither positively not negatively charged; when inside the bacterium, chlorine disintegrates proteins making them loose their complex structures killing the cells of the microbes (Hadhazy, 2013).

ECA water can easily be produced onsite once the machine is purchased and the necessary salts making it an inexpensive product.

KSP contains both Copper and Silver which are lethal to microbes. Copper releases copper ions which are electrically charged and prevent cell respiration in microbes destroying DNA and RNA inside. Silver releases silver ions which are lethal to bacteria (Deardorff, 2014). KSP (30ml/ltr) managed crown gall effectively.

Sunflower oil managed crown gall effectively, galls dried completely, and no yellowing was observed on the plants. Oil manages crown gall by suffocation and prevents the galls from growing. Corn oil is able to manage crown gall and reduce the number of galls significantly (Gitari, 2015). Corn oil was noted to manage crown gall by drying plucked off galls and not regenerating in rose flowers (Gitari, 2015). Various oil types are capable of managing several plant diseases and have been used in bananas to manage Sigatoka disease (Calpouzos, 2001) and in rose flowers to manage crown gall. The advantages of using oil are excellent disease control, effectiveness at low dosages, good lead spreading and sticking properties, low cost and no toxicity to man and animals. The disadvantage is that oil application can be labour intensive depending on the area to be applied. Integrated disease management seeks to combine several strategies to manage a disease effectively. Combining ECA water and oil together showed good management of crown gall but was not significantly different from the individual products. Combining oil and KSP did not manage the disease well as compared to the individual products. They seemed to antagonize each other which could be because KSP contains Copper and Silver metals that promote oxidation affecting negatively the oil quality (Wiesman, 2009).

CONCLUSION

Based on the findings of the study, the following conclusions were made:

a. Crown gall is prevalent in most of the flower cutting growing farms
b. KSP, Oil and ECA water demonstrated effectiveness in the management of crown gall. Copper oxychloride and KOBE are not effective in crown gall management.
c. Combination of ECA and oil managed crown gall effectively.
d. Crown gall disease can be easily management without use of chemical strategies.

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DIFFERENTIAL SCANNING COLORIMETRY PATTERNS OF EXTRA VIRGIN OLIVE OIL AND REFINED OLIVE OILS

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ABSTRACT

In this study, thermal behaviors of extra virgin olive oil (EVOO) and refined olive oil (ROO) were determined. The phase transitions were monitored under crystallization and melting conditions between at -80^{o}C and +30^{o}C region. Three exothermic and two endothermic peaks were detected basically. The oil samples separate statistically from each other via ΔH (J/g) parameter. The onset temperature (T_{on}) did not show any significant impact (P>0.05) to differentiate oil type. It can be obviously concluded that differential scanning calorimetry (DSC) could be used as a powerful instrumental analysis method that does not require sample preparation to separate olive oil forms. For this reason, it can be said that fraudulent EVOOs mixed by refined olive oils can be easily determined with DSC.

Keywords: *extra virgin olive oil, refined olive oil, DSC, melting, crystallization*

INTRODUCTION

Making food fraud or misleading changes in the food formulation is strictly prohibited and a legal crime. In recent years, development of quality control methods based on various parameters have gained great importance for identifying adulteration, particularly using cheapest edible oils into various high quality virgin oils. Because of very high content of (~55-85%) oleic acid and its bioactive components (Ucuncuoglu & Kucuk, 2019), olive oil has a unique nutritional importance for humans. In the Mediterranean diet considered to be the most healthy diet model, virgin olive oil is recommended as the sole source of oil/fat (Ucuncuoglu & Sivri-Ozay, 2020). Nowadays, chromatographic, spectroscopic, thermal and structural advanced techniques or combinations of those could be used to detect adulteration in lipid science (Ucuncuoglu *et al.*, 2013; González-Domínguez *et al.*, 2019; Izquierdo *et al.*, 2020). Therefore, development of simple and rapid methods, and increasing number of routine analysis could make a great contribution to this matter. Fats and oils have a complex structure based on triacylglycerols (TAGs). The most noteworthy attributes of physical properties of fats and oils are associated with the solid-liquid phase change; i.e. melting or crystallization temperature. Onset and offset melting and cooling temperature, melting and crystallization enthalpy values can be examined by Differential Scanning Calorimetry (DSC). Nowadays, DSC is preferred to other similar calorimetric techniques, such as differential thermal analysis, because it has the advantage of providing a more direct measurement of the energy accompanying the physical and chemical changes studied (Cebula *et al.*, 1991; 1992).

The aim of this study was to detect any refined olive oil (ROO) addition into extra virgin olive oil (EVOO) using DSC via T_{on} temperature and enthalpy difference.

MATERIAL AND METHODS

Material

Extra virgin olive oil and refined olive oil samples was purchased from TARİŞ Olive and Olive Oil Agricultural Sales Cooperatives Union.

Methods

Thermal analysis

The thermal properties of oil samples were determined by using DSC Q20 (TA Instruments, New Castle, De, ABD) equipped with a thermal analysis data station Universal V4.5A version. About 8-10 mg of oil sample was placed in hermetically sealed aluminum pans (Cat.No.900793.901.). Indium (melting point 156.6°C, ΔH: 28.45 J/g) was used for calibration analysis. An empty aluminum pan was used as a reference. Chiavaro (2008) method was applied with slight modifications in this study. DSC runs (in triplicate for each oil) were performed from -80 to +30ºC at a scan rate of 5ºC/min. Based on the measured amount of heat energy absorbed by a sample during a run, the heat flow, the onset of melting (T_{on}) and enthalpy (ΔH) values were recorded.

Statistical analysis

Both the means and standard deviations (SD) were calculated using the SPSS (version 23.0) statistical software. The one-way analysis of variance (ANOVA) with the Duncan multiple comparison descriptive test, was performed to measure the significance level.

RESULTS

Thermal profile of EVOO

The cooling DSC curve presents two exothermic peaks (A and B) associated with two distinct crystallization events. The first one appears at T_{on} = -14.13ºC and can be mainly attributed to saturated fatty acids such as palmitic and stearic acid. The second exothermic peak has been shown at T_{on} = -42.27ºC corresponding to the phase transition of the low-melting highly unsaturated oil fraction. During heating, DSC curve shows two endothermic peak (C and D) at T_{on} = -9.94 and 2.82ºC followed by an exothermic one (T_{on} = -35.66ºC) (E). This probably indicates that a portion of the crystals formed during cooling melts and re-crystallizes into more stable polymorphic structures (**Fig 1**). **Table 1** also shows enthalpy difference. The maximum level of ΔH was detected under B and C region.

Thermal profile of ROO

During cooling, two exothermic peaks (A and B) associated with two distinct crystallization events. The first one appears at T_{on} = -15.02ºC and can be mainly attributed to saturated fatty acids such as palmitic and stearic acid. The second exothermic peak has been shown at T_{on} = -42.86ºC corresponding to the phase transition of the low-melting highly unsaturated fatty acids. Melting cycle presents two endothermic peak (D and E) at T_{on} = -9.89 and 3.05ºC followed by C, an exothermic one (T_{on} = -36.90ºC). This probably indicates that a portion of the crystals formed during cooling melts and re-crystallizes into more stable polymorphic structures (**Fig 2**).

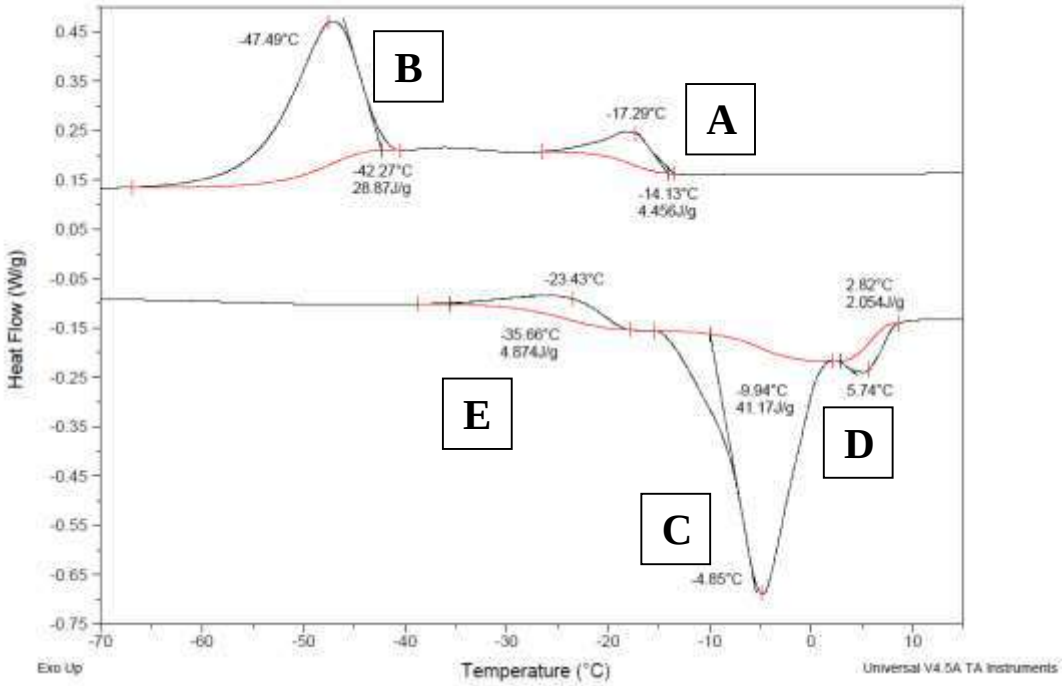

Figure 1. DSC full thermogram of extra virgin olive oil (EVOO)

Table 1. T_{on} temperature and ΔH values of EVOO

	EVOO				
	***Start* °C**	***T on Onset* °C**	***Max.* °C**	***Stop* °C**	***ΔH (Area) J/g***
A	-13,40	-14,13	-17,29	-26,42	4,46
B	-40,45	-42,27	-47,49	-66,82	28,87
C	-15,41	-9,94	-4,85	2,18	41,17
D	2,18	2,82	5,74	8,62	2,05
E	-38,77	-35,66	-23,43	-17,83	4,87

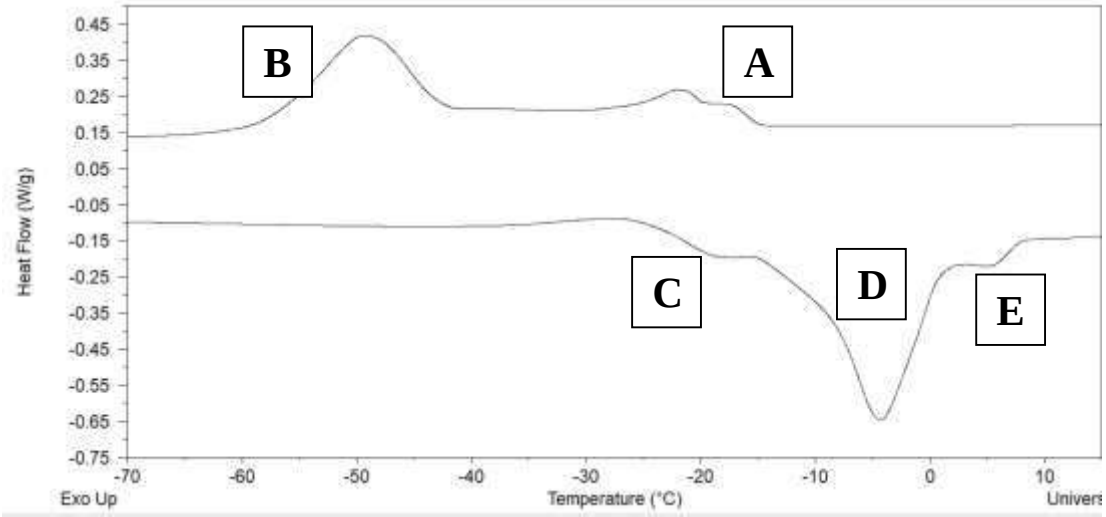

Figure 2. DSC full thermogram of refined olive oil (ROO)

Table 2 also shows enthalpy difference. The maximum level of *ΔH* was detected under B and D region for ROO.

Table 2. T_{on} temperature and ***ΔH*** values of ROO

	ROO				
	Start °C	***T on Onset °C***	***Max. °C***	***Stop °C***	***ΔH (Area) J/g***
A	-14,20	-15,02	-21,39	-30,38	6,39
B	-40,98	-42,86	-49,74	-68,30	26,96
C	-40,65	-36,90	-25,27	-17,69	7,28
D	-17,69	-9,89	-4,37	3,05	37,49
E	3,05	3,50	6,17	8,35	0,68

Cooling cycles of EVOO and ROO samples were compared at **Figure 3**. Moreover, melting cycles were given at **Figure 4**. It was so clear that the onset temperature was so close to each other and no significant difference on those (P>0.05). On the other hands, *ΔH* (J/g) showed statistical difference between EVOO and ROO. Described results were collected in **Table 3**.

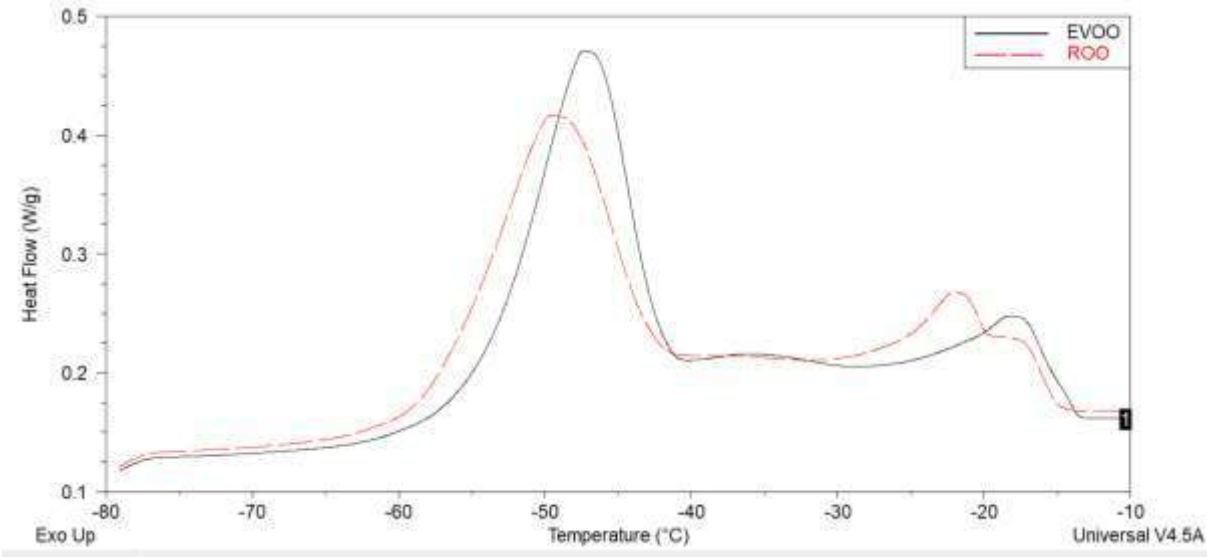

Figure 3. Comparing cooling profile of olive oils *(EVOO: extra virgin olive oils and ROO: refined olive oils)*

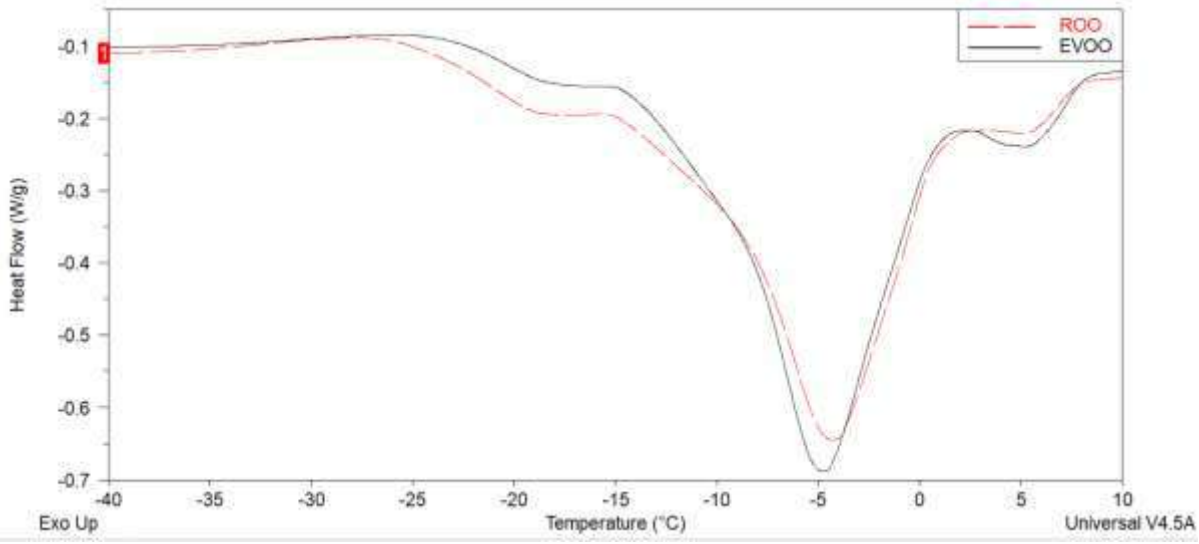

Figure 4. Comparing melting profile of olive oils *(EVOO: extra virgin olive oils and ROO: refined olive oils)*

Table 3. Comparison of two types of olive oil thermal characteristics

		T on* Onset °C	***ΔH** (Area) J/g***
A	EVOO	-14,13	4,46 b
	ROO	-15,02	6,39 a
B	EVOO	-42.27	28,87 a
	ROO	-42,86	26,96 b
C	EVOO	-35,66	4,87 b
	ROO	-36,90	7,28 a
D	EVOO	-9,94	41,17 a
	ROO	-9,89	37,49 b
E	EVOO	2,82	2,05 a
	ROO	3,50	0,68 b

**P>0.05; **P<0.05*

DISCUSSION

Differential Scanning Calorimetry (DSC) cooling/melting thermograms have been reported for studying various heat related phenomenon in oil by monitoring the changes in enthalpy (Chiavaro *et al.* 2007). In the present study the melting and crystallization points were determined as the onset temperature and ΔH values of the melting and crystallization peaks. In the DSC melting curves of oils and fats, complex features were not easily interpretable. This is a consequence of the known phenomenon of polymorphism of oils and fats that is strongly dependent on the thermal history of the sample. Conversely, the DSC crystallization curve, which is influenced only by the chemical composition of the sample, and not by the initial crystalline state, is more reproducible and simpler than the melting curve. Many studies have been conducted to investigate the thermal profile of various oils and fats products (Chiavaro, 2008).

Melting and crystallization, two commonly used physical events to characterize thermal behavior of oil samples, require the intake or release of thermal enthalpy. DSC is eminently suitable to determine these physical properties of oil samples. Thermal curves, as figured by DSC, were given in **Figures 1-4**. These results illustrate the complex nature of TAG in olive oil samples. This is a consequence of the known phenomenon of polymorphism of natural oils and fats that has interested researchers for many years. Due to the complexity of the recorded thermal events, all melting and crystallization points were recorded at the maximum and minimum of either endothermic or exothermic peaks. Overall, these transition parameters were given in **Table1-3**.

Every oil or fat has characteristic fatty acid and triacylglycerol profiles, which are unique to the type of oil and can be used in detecting adulteration. Different crystal forms can generate in lipid structure when edible oils were cooled below to the melting point (crystallization). This depends on the composition of TAGs in other words position of fatty acids (α, β or α') in ester structure of TAGs (Srivastava *et al.*, 2017).

CONCLUSIONS

DSC cannot provide any quantitative information about the chemical composition of olive oils. But, it provides useful information regarding the nature of the thermodynamic conversion that is linked with the oils transition characteristics from one physical state to another. These thermodynamic characteristics are sensitive to the complex structure of oil. This study found that one of the DSC parameter namely ΔH was sensitive indicator to identify types of olive oil.

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SELECTION OF MUTANT GROUNDNUTS (ARACHIS HYPOGAEA L.) SALINE-RESISTANT

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ABSTRACT

Two groundnuts (*Arachis hypogaea* L.) genotypes Kp29, Fleur 11 were evaluated to the effect of salinity stress The experiments were placed in a completely random design with three replicates to select the resistant genotype as a source for future research in our breeding programs. To evaluate the effect of salinity stress, we conducted two experiments, the first one focused on the seed's germination parameters, development, and growth under salt stress(NaCl) [0.0, 50, 100 and 150 mM]. The second experiment focused on the effect of saline soils on the physiological factors of plants.The results showed that salinity negatively impacts the germination, development of epicotyls, hypocotyls, root length and also physiological parameters of the mutatnts genotypes. Furthermore, Groundnut seeds of these two genotypes are differently sensitive to salinity.

Key words: *Arachis hypogaea.*L, mutation, resistance, salt stress

INTRODUCTION

Groundnut (Arachis hypogaea L.) is an important oilseed crop that plays an important role in the economy of many countries, especially the least developed and a nitrogen-fixing plant are essential for sustainable and economic agriculture [1]. Groundnuts are mainly planted in arid and semi-arid areas, where their growth and yield are limited by drought, and salinity [2]. Salinity reduces germination, seedling growth, dry matter production [3], and damages the photosynthetic mechanism [4]. The effects of salt stress on nitrogen fixation have been widely described in *fabaceae* [5].

The agriculture sector faces new challenges due to climate change, the population growth, and the pressure on agricultural land while cultivated plants are exposed to several types of environmental stress which affect plant growth and development, there by affecting crop productivity [6, 7]. The salinization of agricultural lands is one of the most critical factors threatening agriculture around the world in the near future [8, 9].

Improving varieties to be much more productive and adaptive is one of the foundations of modern agriculture. To face these new challenges, variety breeding programs must adopt new strategies to develop improved cultivars, the selection of tolerant varieties remains the only way to improve the functioning of plants in areas affected by salinity, this can lead to find a source of the gene as well as screening methods for a large number of tolerant genotypes.

We hope this approach will confirm the potential of irradiated groundnut plants to minimize yield losses. The selection of stress-tolerant genotypes capable of minimizing depressive effects On Yields Would Certainly Improve Agricultural Production In The Affected Areas.

MATERIAL AND METHODS

Plant material

Peanut cultivar seeds (*Arachis hypogaea ssp. Fastigiata*), variety KP29 VALENCIA TYPE and variety flower11 SPANISH TYPE irradiated by gamma-ray 100Gy, 150Gy, 200Gy, created by radioactive cobalt 60.

Design and experimental conditions

The research is based on two types of experiences. The first is focusing on germination parameters (germination percentage and germination rate), seedling growth and development (length, fresh and dry weight of the root, hypocotyl, and epicotyl) in vitro. The second experiment focused on the effects of salt soil on plant physiology (chlorophyll content and stomatal conductance). The seeds selected for the experiments are mature, uniform in size, shape, and without wrinkles. The peanut seeds were surface sterilized with mercury chloride (II) ($HgCl_2$) and washed with sterile distilled water.

In the first experiment, germination was carried out in sterile glass jars containing filter paper impregnated with different concentrations of NaCl with three replications and three seeds for each. In the second experiment, the peanut seeds were first sterilized, and germinated for 10 days, then transferred to the soil. The plants were transplanted into plastic pots with a cultivated plant in the middle of each pot filled to 2/3 of the soil and placed in natural conditions. Each variety contains three groups representing the dose of irradiation and a non-irradiated group, and in each group six repetitions for each treatment of salt stress.

Irrigation

The seedlings were irrigated with filtered water for a month, then treatment with salt water gradually imposed (to avoid osmotic shocks due to high concentrations), then increased to 50mM, 100mM, and 150mM. By watering with filtered water added with NaCl, the unstressed plants were irrigated with filtered water (control). The electrical conductivities of the soil, including control, were determined by a conductivity meter throughout the treatment period. In order to maintain constant levels of salt concentration in the soil the plants were irrigated with filtered water between treatments.

Parameters studied

Germination parameters: germination percentage (GP%): is noted after the 10th day, it is expressed by the following formula: GR = germinated seeds / total number of seeds x 100. Germination rate (GR): is determined by calculating the GP of the chronological evolution of the seed. Morphological parameter: 14 days after sowing, the root, hypocotyl, and epicotyl, were measured the lengths (cm).

Physiological parameters: The stomatal conductance is measured in the middle of the day using a portable porometer (leaf porometer model SC-1) on two well exposed leaves. The chlorophyll content was determined using a digital measuring device (Chlorophyll meter, type SPAD-502 Plus) on five well leaves and exposed on the middle part of the plant.

Statistical analyses

The data for each variety was analysed separately using SPSS Statistics v 25.0 software, using one-way ANOVA (ANOVA I) Fisher's Least Significant Difference Test (LSD), at level of probability at 5%, in summer used to test the differences between the treatments, in order to estimate if the level of saline stress/water stress, has a significant influence on the measured parameters.

RESULTS AND DISCUSSION

Generally the genotypes showed remarkable tolerance in saline conditions, a 100% germination for KP29 (150Gy) with concentrated 150mM and 100% for Fleur11 (100Gy) in 100mM and more than 70% in 150mM for the rest (100 and 200Gy). However the physiological parameters of mutated genotypes of peanut varieties under salt stress on the soil decrease when the concentration of salt increases, an indicator of the sensitivity of the plants to salt stress, even so there are some genotypes that tolerate the salinity 100Gy for fleur 11 and 200Gy for Kp29. This can be explained by the genetic modification that it was born in the previous generation because of the irradiation. These results clearly show that irradiation with gamma rays can lead to the improvement of peanut tolerance to abiotic stress.

CONCLUSIONS

Generation of plants that have undergone testing represents promising prospects, clearly indicates that there are mutated peanut genotypes that can withstand stress and could also be found in future breeding programs. These results constitute an important step in the evaluation protocol and the improvement of new varieties

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EFFECTS OF *BACILLUS SPECIES* ON SEED GERMINATION AND SEEDLING GROWTH OF SOYBEAN

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ABSTRACT

Some bacteria species can increase yield by promoting seed germination, plant growth and development, since they synthesize phytohormone and increase the availability of plant nutrients. This research was carried out to determine the effects of some Bacillus species on seed germination and seedling growth in soybean. Ten Bacillus species (*Bacillus simplex, Bacillus thuringiensis, Bacillus cereus* and *Bacillus subtilis* and 6 undiagnosed Bacillus species) were used. Surface sterilized soybean seeds were inoculated with bacteria in centrifuge tubes for 30 minutes. The inoculated seeds were germinated in growth chamber at a temperature of 25 °C for 8 days. At the end of the germination, germination rate, shoot length, root length, dry shoot and root weight were determined. The obtained results revealed that Bacillus spp, SZF135 had the highest germination rate, shoot length, root length, dry shoot and root weight. The results of the research showed that *Bacillus* species could be used to stimulate seed germination and seedling growth.

Key Words: Bacteria, *Bacillus* spp, germination, *Glycine max*, growth, soybean

INTRODUCTION

Soybean (Glycine max Merr.) has been cultivated since ancient times due to its higher protein and oil content. The World soybean production area was 121.53 million ha and production was 346.02 million metric tons in 2019. The 55.7 % of the world oil seed production comes from the soybean. The share of the soybean crude oil was 30 % in the total world crude oil production of 130.2 million tons. The South and North Americas have the largest soybean production areas, followed by Asia, Europe and Africa.

Plant growth promoting rhizobacteria (PGPR) and their products have been used to promote plant growth and development and protect plant health. They are a diverse group of bacteria species including Agrobacterium, *Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcus, Pseudomonas,* and *Serratia* (Bhattacharyya and Jha, 2012) that can be found in the rhizosphere, on root surfaces and in association with roots (Ahemad and Kibret, 2014; Lucas et al., 2014).

There has been growing interest for plant growth promoting rhizobacteria as supplements or alternatives to the synthetic chemicals to increase crop productivity. Microorganisms have been successfully used to promote seed germination of many crop species (Bashan, 1998). Rapid seed germination and vigorous seedling growth is very important for the successful establishment of soybean in the field. Previous studies demonstrated that PGPR have great importance to increase plant growth and enhance crop yields up to 57%, depending on the crop and bacteria species and races (Khalid et al. 1997; Asghar et al. 2014). Keeping these facts in view, in the current work we screened the Bacillus spp for their effect on seed germination and seedling vigor of soybean.

The seed inoculation with microorganisms has been applied as a new method to modify microbial populations around the crop to enhance crop yield. The stimulation of seedling

development by Bacillus spp has been attributed to the production of biologically active compounds. Studies on PGPR showed that PGPR involved in phosphate solubilisation, hydrocyanic acid, iderophores, chitinase, ammonia, and indole-3-acetic acid productions (Park et al. 2005; Ahmad et al. 2008). Plant growth and development is directly stimulated by nitrogen fixation (Lwin et al. 2012), phosphate solubilization, iron mobilization by microbial siderophores (Kafrawi et al. 2014), and secreting growth regulators such as indole acetic acid, cytokinin, and gibberellin (Beneduzi et al, 2008; Han and Lee, 2006; . Idris et al. 2007; Joo et al. 2005).

The *Bacillus* spp were then evaluated for their effect on soybean seed germination, seedling vigor. Our outcome suggests that *Bacillus* spp can be used in agriculture to stimulate seed germination in an eco-friendly manner.

MATERIAL AND METHOD

The experiment was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Erciyes University Turkey. The soybean (*Glycime* max L.) cultivar Asya was used in this study. The untreated seeds were obtained from ProGen Seed Co, Hatay, Turkey. The Bacillus spp were obtained from the Department of Agricultural Biotechnology, Agriculture Faculty, University of Erciyes, Kayseri, Turkey. The design of the experiment was completely randomized with six replications. Ten selected *Bacillus* spp were used in the experiment.

The seeds of soybean were exposed to *Bacillus* spp and were used in all possible combinations to determine the effects on germination traits and growth parameters for 10 days. Microbes used labelled.

Table 1. Applied *Bacillus* ssp and their codes

Code	Bacteria species
SZF32	*Bacillus thuringiensis*
SZF45	*Bacillus cereus*
SZF73	*Bacillus* spp
SZF86	*Bacillus* spp
SZF97	*Bacillus subtilis*
SZF120	*Bacillus* spp
SZF135	*Bacillus* spp
SZF147	*Bacillus* spp
SZF168	*Bacillus* spp
SZF194	*Bacillus* spp
Konrol	Control

Bacillus spp were grown in yeast manitol broth (YMB) and nutrient broth. Exponentially growing cells in shaken broth culture were inoculated. soybean seeds were surface sterilized by immerse in 3.5% Clorox solutio with a drop of Tween 80 for 30 minutes followed by three times washing with sterile water. After that, seeds were put in sterilized Petri dishes containing filter paper. After that, the seeds were soaked in various *Bacillus* spp. Water treated seeds were used as control. Twenty five seeds of both treated and controls were planted in sterilized plastic container containing sterile perlite and the plastic containers were kept in an incubator at 26°C for 120 h.

After soaking, the air-dried seeds were used for germination and the seedling percent emergence was calculated with the following formula:

$$\text{Percent of Emergence} = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds sown}} \times 100$$

Randomly selected 10 seedlings were taken from each container to measure root and shoot length. It was measured with a measuring scale and expressed in centimeters. Root and shoot length of the seedlings were measured after 10 days of germination.

The dry weigh of 10 seedlings were taken before and after dried out in oven at 60° C for 5 hours. These were measured by four-digit balance and expressed in milligram.

Analysis of variance (ANOVA) followed by the Least Significance Difference test was carried out to compare treatments using SAS software. Differences were considered to be significant at $p<0.05$.

RESULTS AND DISCUSSION

The seedling emergence rate of soybean seed was significantly ($p < 0.05$) affected from the treated the Bacillus spp (Figure 1). The highest seedling emergence rate was obtained from SZF135 with 79 % and the lowest was obtained from the Control treatment with 26%. Seedling emergence rate and seedling emergence speed are important seedling parameters since late emerging seedlings are under threat of soil born disease agents (Basra et al., 2003; Hadas, 2004). Our results showed that *Bacillus* spp coded SZF135 have a potential to use as a seedling emergence micro-agent for soybean. The concepts of uniform emergence and seedling shoot and root growth often appear in seed vigor descriptions (Dickson, 1980; Perry, 1980) and uniform emergence is involved in the description of seedling vigor adopted by the Association of Official Seed Analysts (McDonald, 1980). If uniform seedling emergence is an attribute of high quality seed, uniformity of emergence should be related to performance and quality seed yield.

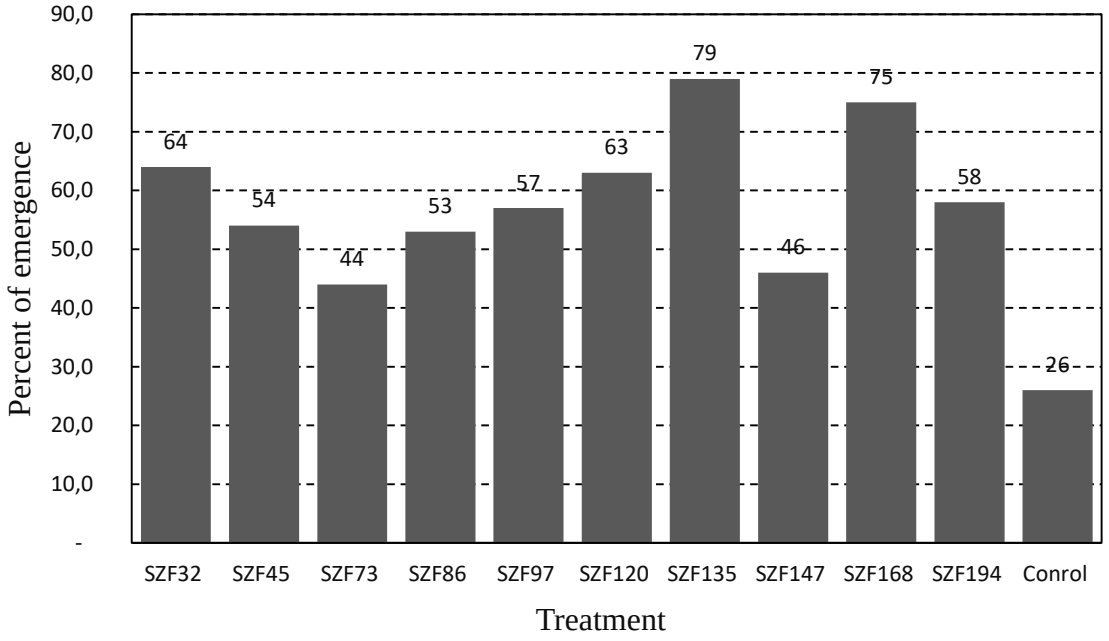

Figure 1. Effect of *Bacillus* spp on seedling emergence of soybean

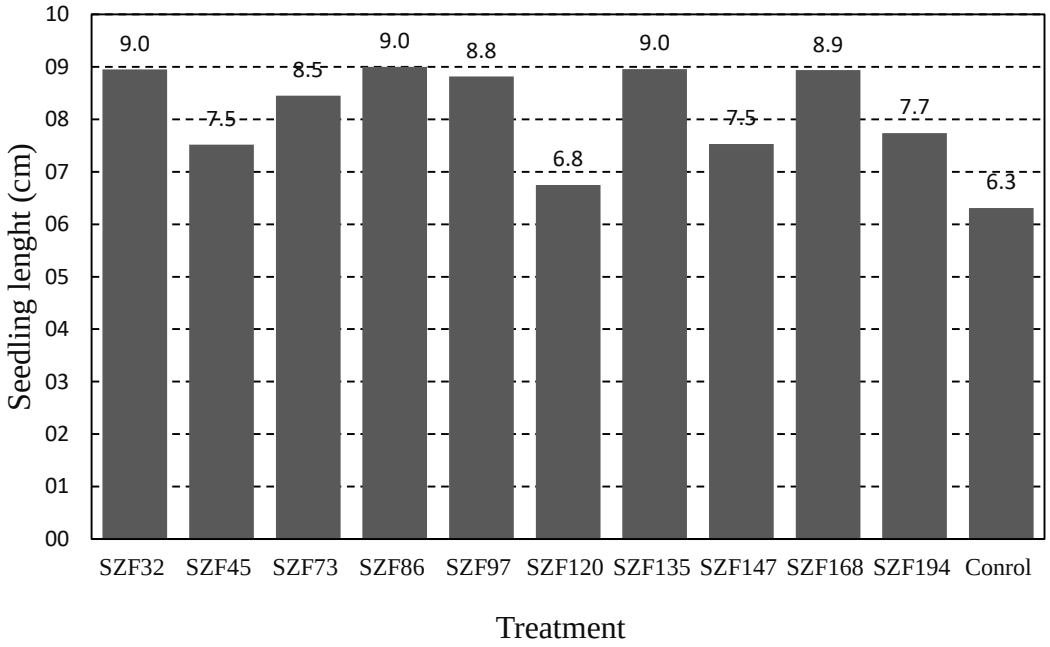

Figure 2. Effect of *Bacillus* spp on seedling length of soybean

Shoot length of soybean seedling had significantly ($p < 0.05$) affected from the *Bacillus* spp coded. Seedling shoot length varied between 6.3 and 9.0 cm Figure 2. The highest seedling shoot lengths were obtained from *Bacillus* spp coded SZF32, SZF86 and SZF135. Control had the lowest seedling shoot length. Rapid and uniform germination and emergence of vigorous soybean seedlings is key events to guarantee high plant performance that affects uniform plant development, yield and harvested seed quality. Wareing and Philips (1970) stated that when the young seedling photosynthesis becomes active, the power of the plant to synthesize new carbohydrate is undoubtedly dependent on the leaf number and size exposed to direct sunlight.

Root length of soybean seedling varied between 6.3 and 9.90 cm (Figure 3). The highest and the lowest root length values were obtained from control and SZF86, respectively. It is known that uniform seedling emergence is a significant parameter of seed vigor. Consequently, the determination of seedling length and seedling dry weight are considered for important seedling and vigor parameters. Seedling length differences due to seed reserves mobilization during the germination is an indicator of the extent and rapid emergence (Edge and Burris (1970), Gill and Delouche (1973). Therefore, it can be speculated that *Bacillus* spp helped to more mobilize the seed reserves during the germination.

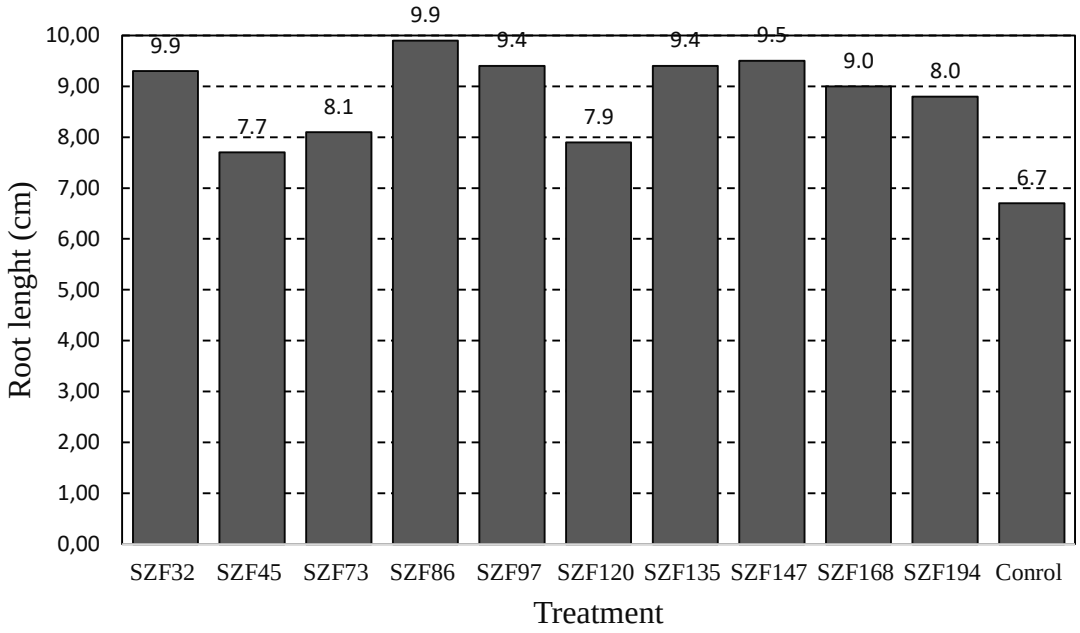

Figure 3. Effect of *Bacillus* spp on shoot length of soybean

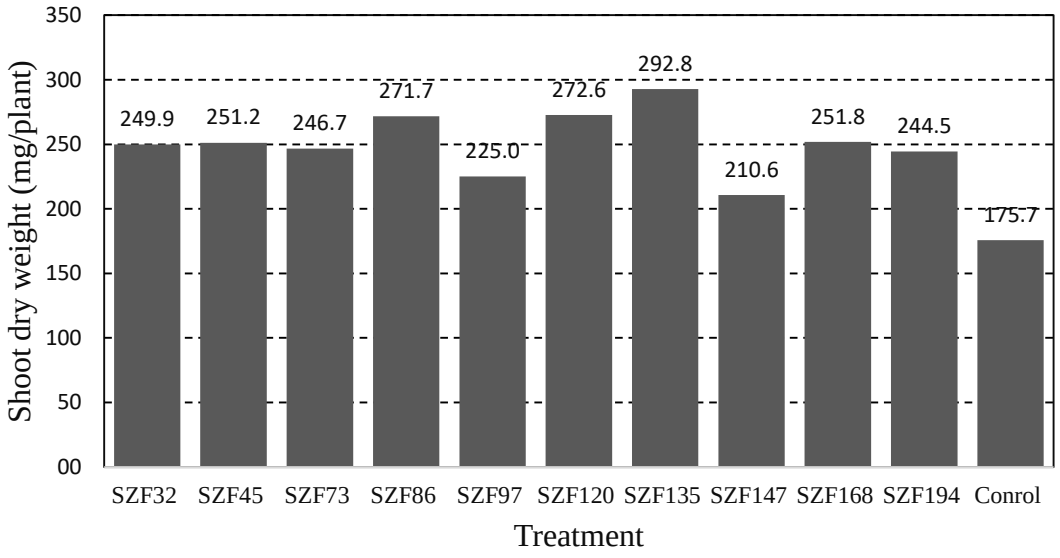

Figure 4. Effect of *Bacillus* spp on shoot dry weight of soybean

The treatment of *Bacillus* spp significantly ($p < 0.05$) affected shoot dry weight of soybean seedlings (Figure 4). The highest seedling shoot weight was obtained from SZF135 with 292.8 mg/plant and the lowest was obtained from the control treatment with 175.5 mg/plant

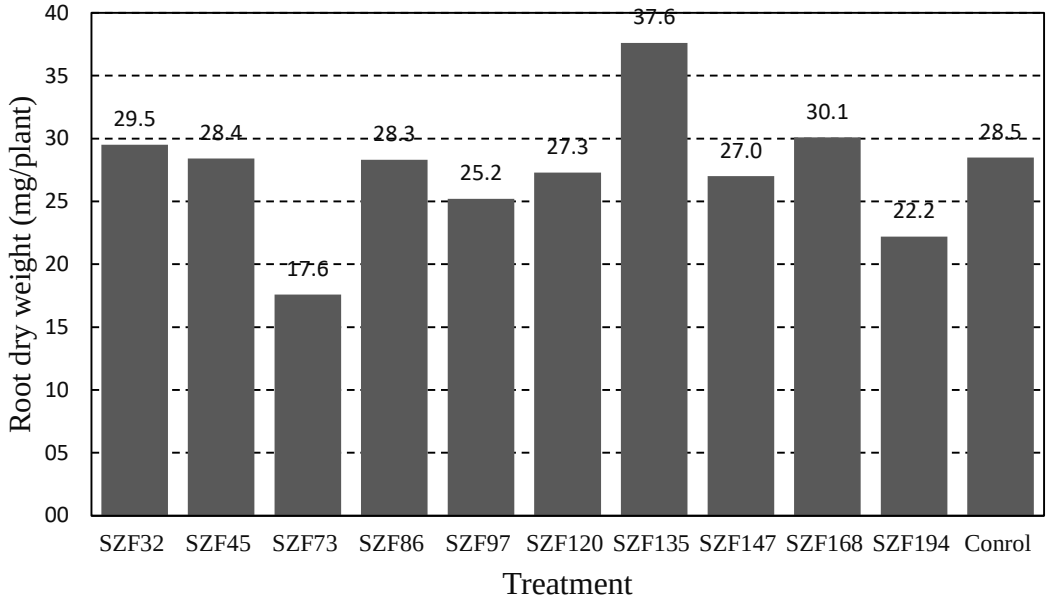

Figure 5. Effect of *Bacillus* spp on root dry weight of soybean

The application of *Bacillus* spp had significantly ($p < 0.05$) affected the seedling root weight of soybean (Figure 5). Dry root weight of soybean seedling varied between 17.6 and 37.6mg/plant. The highest and the lowest root length values were obtained from control and SZF135, respectively.

CONCLUSION

The current study showed *Bacillus* spp treated soybean seeds had higher seedling emergence, seedling shoot and root length, higher seedling shoot and root dry weights compared to control. Effective *Bacillus* spp could be used as a seed treating agent or seed coating agent for rapid germination and higher seedling vigor.

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USE OF PRINCIPLE COMPONENT ANALYSIS TO EVALUATE THE SURFACE AND GROUNDWATER QUALITY OF HASANAĞA STREAM BASIN

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ABSTRACT

Hasanağa Stream Basin is located in Edirne Province and it is one of the most significant sub – basin of Tunca River. In this research, surface and groundwater quality of Hasanağa Stream Basin were evaluated by using Principle Component Analysis (PCA). Water samples were taken from 17 stations (7 of them from surface water and 10 of them from groundwater) selected on the basin in winter seasons of 2019. Total of 8 variables including electrical conductivity (EC), total dissolved solids (TDS), salinity, turbidity, nitrate, nitrite, phosphate and sulphate were measured and PCA were applied to detected data in order to determine the effective factor on water quality. According to detected data, 2 factors named as "Agricultural Factor" and "Salinity Factor" explained 74% of the total variance.
Keywords: *Hasanağa Stream Basin, Surface – groundwater quality, Principle Component Analysis.*

INTRODUCTION

Multi-statistical techniques, which help the interpretation of complex data matrices to better understand the ecological status of the investigated water ecosystems, are being widely used in many countries for water quality assessment studies. Principle Component Analysis is the one of the most convenient multivariate statistical method that is being used commonly all over the world in order to evaluate the surface and groundwater quality of many different aquatic habitats (Shrestha and Kazama, 2007; Akın et al., 2010; Najar and Khan, 2012; Tokatlı, 2013; 2014; Tokatlı et al., 2014; Tokatlı and Helvacıoğlu, 2020).

Meriç River Basin is the most important watershed of Thrace Region of Turkey. Hasanağa Stream Basin is located in Edirne Province of Turkey and it is one of the most important sub–basin of Tunca River that is one of the main part of Meriç River Basin. Hasanağa Stream Basin is known to being adversely affected from agricultural and domestic discharges and surface - groundwater quality of the basin is decreasing day by day. The aim of this study was to assess the surface and ground water quality by using Principle Component Analysis.

MATERIAL AND METHODS

Sample Collection

In this study, surface water samples were collected from 7 stations located on the Hasanağa Stream Basin (3 of them were on the Sinanköy Stream, 3 of them were on the Korucuköy Stream and 1 of them was on the Hasanağa Stream) and groundwater samples were taken from 10 villages located on the Hasanağa Stream Basin in winter season of 2019. The coordinate information of the stations is given in Table 1 and the map of study area and selected stations are given in Figure 1 and Figure 2.

Table 1. Coordinates of selected stations

Stations		Villages	North	East	Stations		Potamic Name	North	East
Groundwater	**G1**	Büyükdöllük	41.759	26.601	Surface water	**S1**	Lalapaşa	41.837	26.733
	G2	Menekşesofular	41.763	26.640		**S2**	Sinanköy	41.812	26.698
	G3	Küçükdöllük	41.756	26.668		**S3**	Küçükdöllük	41.754	26.667
	G4	Kayapa	41.775	26.689		**S4**	Hanlıyenice	41.862	26.700
	G5	Korucu	41.791	26.655		**S5**	Sinanköy	41.824	26.680
	G6	Sinanköy	41.814	26.697		**S6**	Menekşesofular	41.762	26.643
	G7	Çömlekakpınar	41.839	26.645		**S7**	Hasanağa	41.724	26.624
	G8	Hanlıyenice	41.870	26.695					
	G9	Lalapaşa	41.839	26.736					
	G10	Hıdırağa	41.738	26.661					

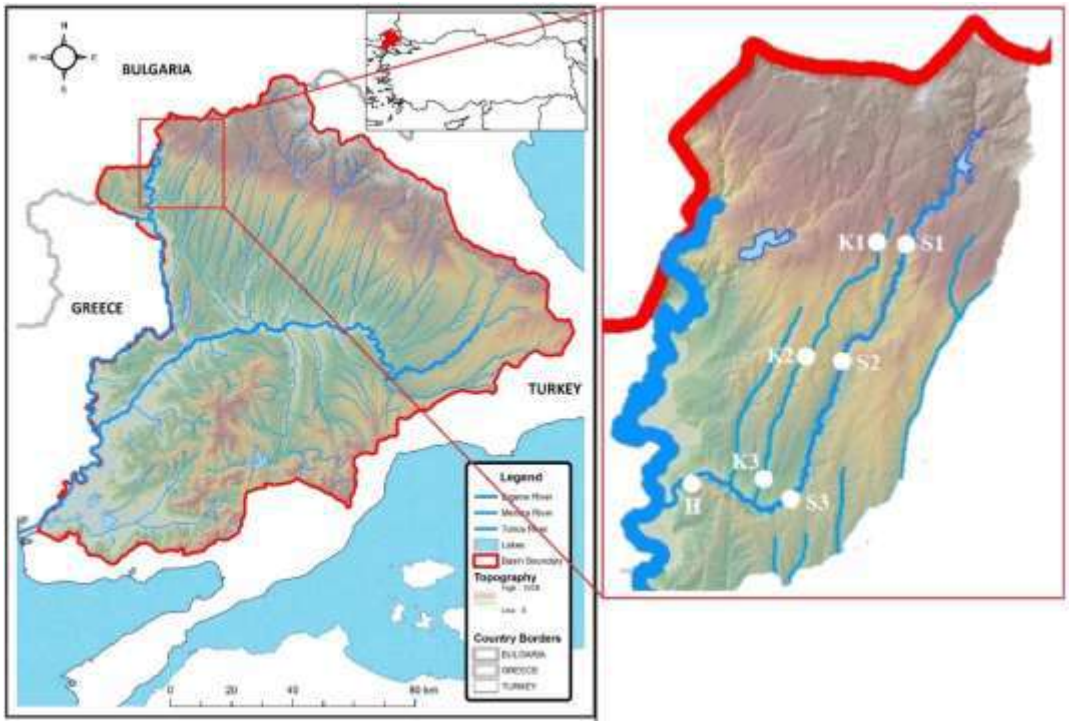

Figure 1. Hasanağa Stream Basin and surface water stations

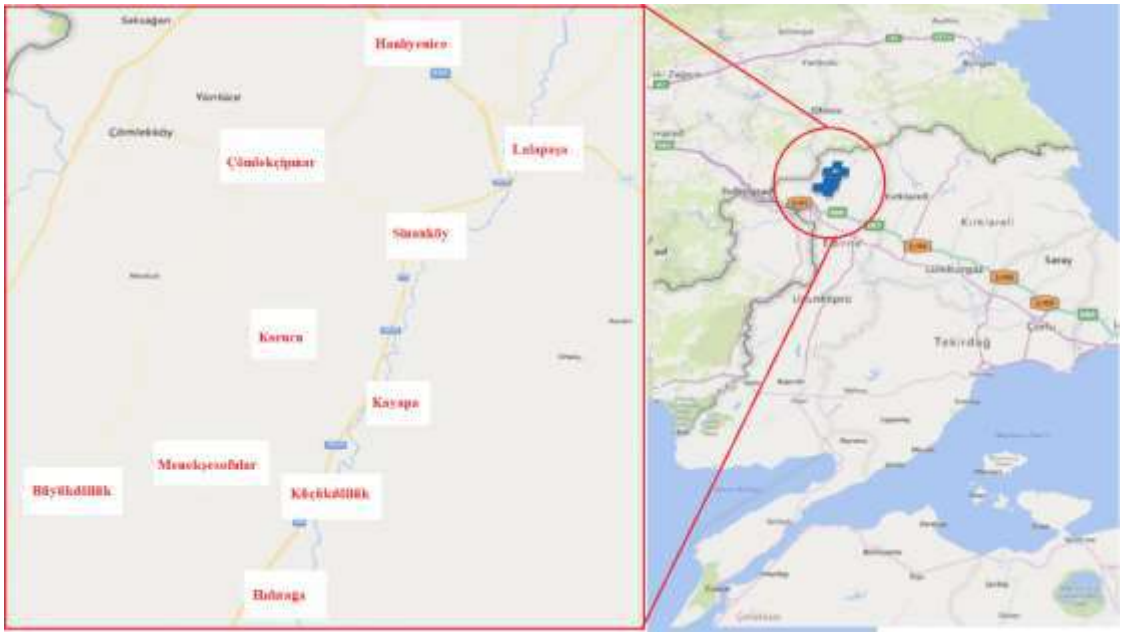

Figure 2. Ground water stations

Chemical and Statistical Analysis

Electrical conductivity (EC), total dissolved solids (TDS) and salinity variables were determined by using a multi – parameter device (Hach Lange – HQ40D) in the field studies; turbidity variable was determined by using a turbidimeter device (Hach Lange – 2100Q) in the field studies; nitrate (NO3), nitrite (NO2), phosphate (PO4) and sulphate (SO4) variables were determined by using a colorimeter device (Hach Lange – DR890) and by using a spectrophotometer device (Hach Lange – DR3900) in the laboratory studies.

Principle Component Analysis (PCA) was applied to detected data in order to the define the effective varifactors of water quality by using the "SPSS" package statistical program.

Results

Principle Component Analysis (PCA) was used to determine the effective varifactors on surface and groundwater resources of Hasanağa Stream Basin by using correlated variables. A total of 8 variables were used to detect the varifactors (n = 17 for all parameters). Result of KMO (Kaiser-Meyer-Olkin) test that presents the measure of sampling adequacy was 0.656 and this value means that, the sampling adequacy was enough for this application (>0.5) (Liu et al., 2003).

Eigenvalues higher than 1 were taken as criterion for evaluate the principal components (Table 3). According to rotated cumulative percentage variance, 2 factors explained 74.018% of the total variance (Table 2). Component plot in rotated space, which shows the related variables of 2 factors, is given in Figure 3 and the parameter loadings (> 0.5) calculated after rotation for 2 components are given in Figure 4.

Table 2. Total variances explained in PCA

Comp.	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Vari.	Cumul. %	Total	% of Vari.	Cumul. %	Total	% of Vari.	Cumul. %
1	4.076	50.944	50.944	4.076	50.944	50.944	**3.654**	**45.676**	**45.676**
2	1.846	23.073	74.018	1.846	23.073	74.018	**2.267**	**28.342**	**74.018**

Comp.: Component; Vari.: Variance; Cumul.: Cumulative

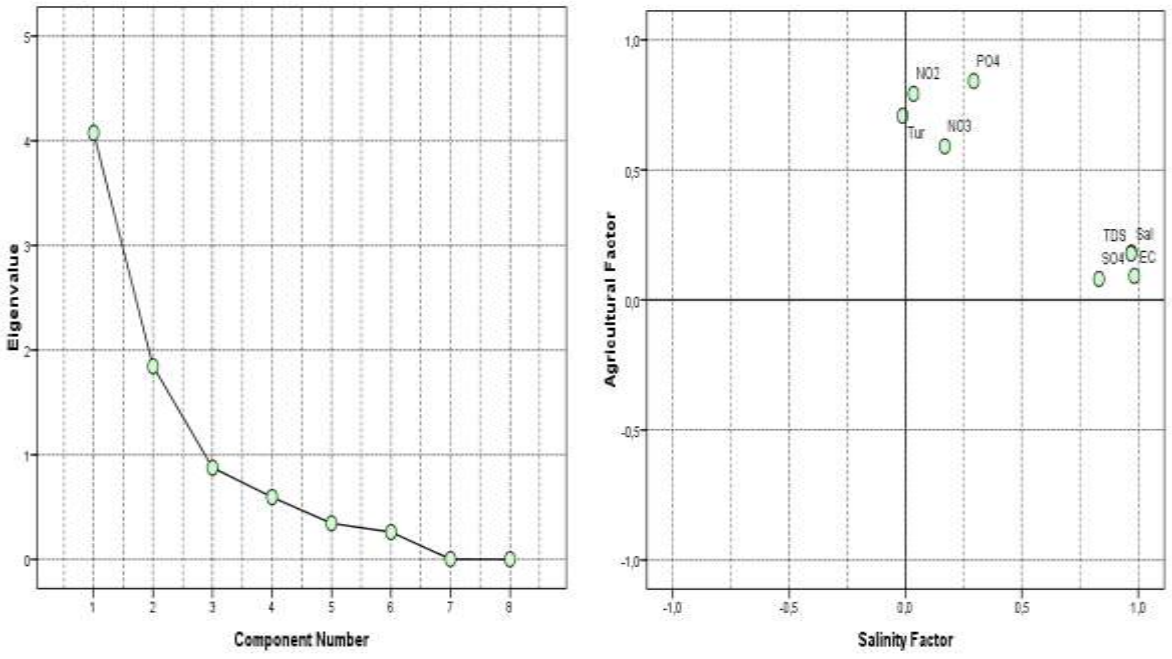

Figure 3. Scree plot of PCA (left) and component plot in rotated space (right)

First factor (F1), named as "Agricultural Factor" explained 45.676% of total variance and it was related to the variables of EC, TDS, salinity and sulphate parameters. All parameters were strong positively loaded with this factor.

Second factor (F2), named as "Salinity Factor" explained 28.342% of total variance and it was related to the variables of nitrate, nitrite, phosphate and turbidity parameters. Nitrate and nitrite parameters were strong positively and phosphate and turbidity parameters were moderate positively loaded with this factor.

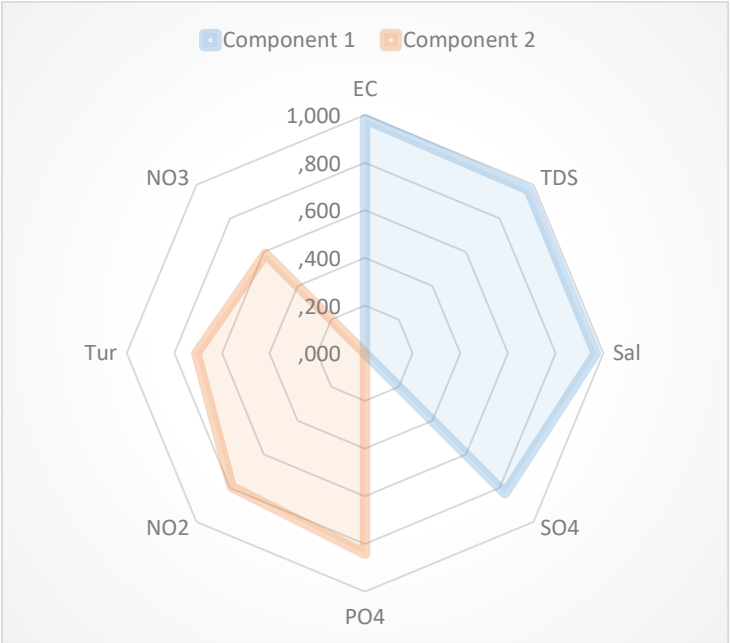

Figure 4. Rotated component matrix

Discussion

It is known that agricultural activities may significantly raise the levels of phosphate and nitrogenous compounds in close aquatic habitats to the agricultural lands (Wetzel, 2001; Manahan, 2011). According to results of PCA, "Agricultural Factor", which was explained 28% of total variance and strong-moderate positively related to the variables of nitrate, nitrite and phosphate parameters, was identified as an important effective component for the basin.

In a study performed in Uluabat Lake in Turkey, PCA was used to assess the surface water quality. According to results of this research, 3 factors explained 77% of total variance and as similar to the present investigation phosphate parameter was found as a significant contaminant for the lake (Iscen et al., 2007). In another study performed in Türkmen Mountain, groundwater quality was evaluated by using PCA. According to rotated cumulative percentage variance, 4 factors explain 79% of the total variance. As similar to the present study "Agricultural Factor" was found as an effective component on groundwater quality of Türkmen Mountain (Tokatlı et al., 2013).

CONCLUSIONS

A widely used multi-statistical method was used to evaluate the surface and groundwater quality of the Hasanağa Stream Basin by using a large number of physico – chemical data. The applied principle Component Analysis helped to identify the effective varifactors on the water quality and 2 effective factors were determined that were explained 74% of the total variance. In brief, multi-statistical techniques are necessary for a sophisticated environmental evaluation

especially both in surface and groundwater quality assessment studies and results of this study reveal the benefits of statistical approaches in freshwater evaluation studies.

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AN APPLICATION OF RIVER POLLUTION INDEX TO EVALUATE THE WATER QUALITY OF MERIÇ RIVER

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ABSTRACT

Meriç River is the most significant aquatic ecosystem of Thrace Region of Turkey. In this research, water quality of Meriç River was evaluated by using River Pollution Index (RPI). Water samples were taken from 2 stations (upstream and downstream) selected on the river in winter seasons of 2020. Total of 4 variables including dissolved oxygen (DO), biological oxygen demand (BOD), suspended solids (SS) and ammonium (NH_3) were measured and RPI were applied to detected data in order to assess the water quality. According to detected data, upstream of Meriç River has "Good" water quality characteristic and downstream of Meriç River has "Less polluted" water quality characteristic.
Keywords: *Meriç River, Water quality, River Pollution Index.*

INTRODUCTION

Environmental pollution is among the most important agenda items of the world especially in recent years. It is known that especially freshwater resources are significantly affected by developing technology and population growth. New methods are developed for the investigation of freshwater pollution and the multiple effects of many pollution parameters are evaluated using various indices. The River Pollution Index that is widely used all over the world is an effective water quality assessment tool (Akın et al., 2010; Najar and Khan, 2012; Chen et al., 2012; Tokatlı, 2013; 2014; Tokatlı et al., 2014; Wang et al., 2014; Tokatlı and Helvacıoğlu, 2020).

Meriç River Basin is the most significant fluvial ecosystem of Thrace Region of Turkey. But as in many aquatic habitats, Meriç River is known to being adversely affected from agricultural and domestic pollution. The aim of this study was to assess the water quality of Meriç River by determining some limnologic parameters and by using River Pollution Index.

MATERIAL AND METHODS

Sample Collection

In this study, water samples were collected from 2 stations located on the Meriç River in the winter season of 2020, when the rains and land runoffs are reached the highest level in the region. 1 of the selected sampling station was located on the upstream of Meriç River and 1 selected sampling station was located on the downstream of Meriç River. The topographic map of Meriç River Basin and selected stations on the river are given in Figure 1.

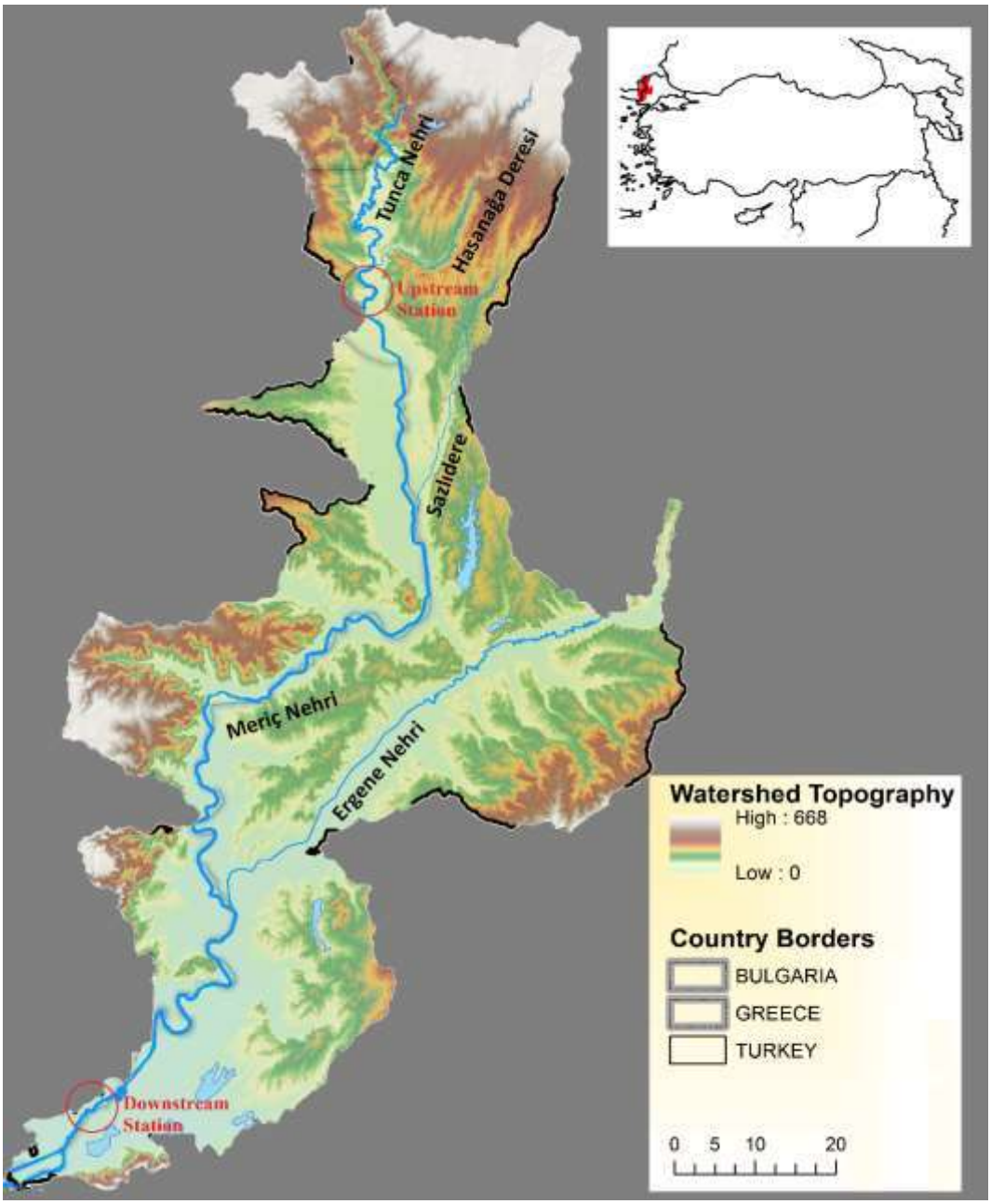

Figure 1. Meriç River and selected stations

Chemical Analysis

Dissolved oxygen (DS) parameter was determined by using a multi – parameter device (Hach Lange – HQ40D) in the field studies; suspended solids (SS) parameter was determined by using a colorimeter device (Hach Lange – DR890) in the laboratory studies; ammonium (NH_3) parameter was determined by using a spectrophotometer device (Hach Lange – DR3900) in the laboratory studies; biological oxygen demand (BOD) parameter was determined by using a BOD device (Hach Lange – BOD Track) in the laboratory studies.

River Pollution Index (RPI)

The RPI is a simple method for assessing river water quality, which is calculated by using levels of 4 parameters; dissolved oxygen (DO), biological oxygen demand (BOD), suspended solids (SS) and ammonium (NH_3). Each parameter is converted into a four-state quality score given in Table 1, and the RPI is calculated by using the following equation (Liou et al., 2004; Chen et al., 2012);

$$RPI = 1/4 \sum_{i=1}^{4} Si$$

Table 1. River Pollution Index scores

Parameters	Good	Less polluted	Moderately polluted	Highly polluted
DO (ppm)	>6.5	4.5-6.5	2.0-4.5	<2.0
BOD (ppm)	<3	3-5	5-15	>15
SS (ppm)	20	20-50	50-100	>100
NH_3 (ppm)	0.5	0.5-0.9	0.9-3.0	>3.0
Index Score (Si)	1	3	6	10
Sub-index Score	<2	2-3	3-6	>6

Results

Results of detected water quality parameters with the assigned index scores are given in Table 2. Results of River Pollution Index (RPI) scores of investigated stations selected on the Meriç River are given in Figure 2.

According to the results of monomial RPI, although the upstream of Meriç River has "Good" water quality characteristic in terms of all the index parameters, the downstream of Meriç River has "Less polluted" and "Moderately polluted" water quality characteristics in terms of NH_3 and BOD parameters respectively.

According to the results of multinomial RPI, the index scores were recorded as 1 for upstream and 2.75 for downstream. Therefore, the upstream of Meriç River has "Good" and the downstream of Meriç River has "Less polluted" water quality characteristics in general.

Table 2. Water quality parameters and index scores

Detected Parameters				
Stations	DO (ppm)	BOD (ppm)	SS (ppm)	NH_3 (ppm)
Upstream	10.47	2.9	5	0.061
Downstream	10.21	5.8	11	0.643
Index Scores				
Stations	DO	BOD	SS	NH_3
Upstream	1	1	1	1
Downstream	1	6	1	3

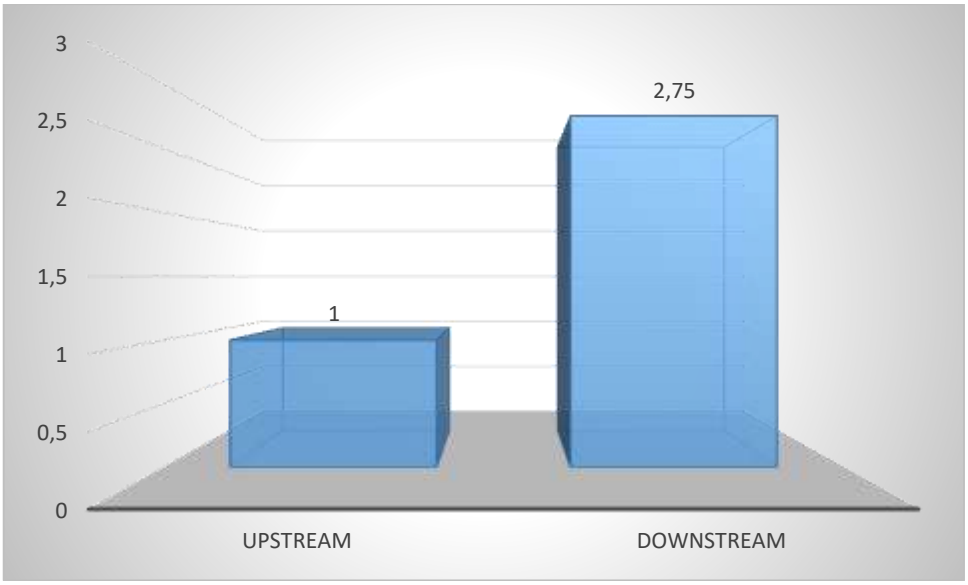

Figure 2. RPI scores of locations

Discussion

Ammonium is an intermediate product that occurs as a result of the breakdown of organic substances containing nitrogen in general and is not significantly toxic for most organisms. Organic matter degradation, organic fertilizer or chemical fertilization from inorganic ammonium, discharge of domestic and industrial wastewaters are important factors that increase the amount of ammonium in water, and a small amount of ammonium is found in oxygenated, clean waters. Another important indicator of biological activity and organic matter content in aquatic systems is the biological oxygen demand parameter (Wetzel, 2001; Manahan, 2011).

According to results of RPI, ammonium and BOD parameters were found as the most critical parameters for the Meriç River. It is thought that the intensive agricultural activities carried out in the river basin may be the reason of the detected quite high ammonium and BOD values detected especially in the lower basin.

Conclusions

In the present investigation, a widely used water quality assessment index was used to evaluate the surface water quality of Meriç River by determining some significant limnologic parameters including dissolved oxygen, biological oxygen demand, suspended solids and ammonium. The applied River Pollution Index helped to reveal the synergistic effect of multiple water quality parameters on the water qualities upstream and downstream of Meriç River. As a result of RPI, water quality of the upstream of Meriç River was found as "Good" level and water quality the downstream of Meriç River was found as "Less polluted" level in general. It was also determined that ammonium and BOD parameters were found as the most critical parameters for the Meriç River among the investigated parameters.

In brief, water quality assessment indices are necessary for a sophisticated environmental evaluation and results of this study reveal the benefits of using these indices in freshwater evaluation studies.

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IRRIGATION SCHEDULING OF WALNUT TREES WITH LEAF WATER POTENTIAL MEASUREMENTS

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ABSTRACT

The study was conducted under the drip irrigation method in 2018 and 2019 by creating three different irrigation treatments, which 75%, 100 and, 125% of the 5-day total reference evapotranspiration values calculated according to the Penman-Monteith method were applied. Soil moisture was monitored gravimetrically in the study. In the first year, evapotranspiration was measured between 577.70 mm and 723.75 mm according to the treatments. In the second year, evapotranspiration was measured between 584,21 mm and 816,31 mm. For irrigation scheduling, leaf water potential measurement, one of the measurement techniques based on plant monitoring, was carried out. In the study, two-year average leaf water potential measurement results ranged from -11.2 to -9.1 bar. As a result of the research, it can be suggested to apply irrigation at walnut trees at a pressure of approximately -10 bar. In addition, 100% irrigation treatment comes to the fore again due to water saving. The leaf water potential measurements over -10 bar in walnut trees indicate that significant losses can occur in yield and quality.

Keywords: Irrigation scheduling, drip irrigation, leaf water potential, walnut

INTRODUCTION

Thrace Region is under pressure due to dense population and industry. The already scarce water and land resources in the region are decreasing even more. Although agriculture is still the sector that uses water the most, this pressure directs farmers to irrigation systems with high irrigation efficiency and plants with high economic value.

Walnut is an important plant that has been used in nutrition and health since ancient times. In addition, it has become one of the plants with high economic profit in terms of other features used in the furniture, medicine, cosmetics and paint industry (Akça 2016).

Turkey has made significant progress over the last decade in the presence of walnuts. Thanks to government incentives, interest in walnut cultivation has increased in almost every part of our country. In the Thrace Region, it is seen that in the last decade, walnut cultivation has been made intensively in areas that are out of forest quality. In fact, walnut inventory, which was 90 683 hectares in 2010, increased to 111775 hectares in 2018. The annual walnut production in our country is around 225000 tons according to 2019 data, and the average yield per tree is 20 kg. With all these data, it is seen that our country ranks 4th in the world in terms of walnut cultivation area and production (Anonymous 2020a) (FAO 2018).

However, considering the scarcity of water resources in the region and especially the use of underground water resources for irrigation, it is seen that there are problems arising from irrigation in walnut cultivation. On the other hand, when we look at the irrigation studies in our

country, it is seen that there is no study on evapotranspiration and irrigation scheduling of walnut plants.

MATERIAL AND METHOD

The research was conducted in Tekirdağ Viticulture Research Institute, located 5.5 km from Tekirdağ city center, on an area of approximately 14 decares. The trial area is at 40 ° 59 ′ north latitude and 27 ° 29 ′ east longitude and its altitude is 44 m. The total agricultural area of the Institute is 979 decares and research and production activities continue in 91%. According to the long-term averages, the annual average temperature is 14.1 ℃ . In terms of monthly average temperatures, the coldest month is January with 5.0 ℃ and the hottest month is July and August with 24.0 ℃. Most of the average annual precipitation, which is 580.8 mm, occurs between October and April. Annual average relative humidity is 76.9%. The average value of the annual wind speed at 2 m height is 2.90 m / s.

Tekirdağ Viticulture Research Institute, where the research was conducted, consists of clay loam, slightly salty, less calcerous and low organic matter content.

Chandler, a variety with a yield rate of over 90% in the side branches, was used in the study. Fruit internal weight is 6.5 gr, internal rate is 49%. The perfection rate of light colored walnut is between 90-100% (Ramos 1998).

The dimensions of the experimental area are 72x192 m and its size is 13824 m^2. There are 9 parcels in total, 3 different irrigation water applications in each of the 3 blocks created in the study prepared according to the split plots trial pattern in random blocks. One parcel has a total area of 432 m^2, 24x32 m in size. 3 rows of trees were created in each parcel. There are 4 trees in each row created. Thus, there are 12 trees in each parcel. The distance between the tree rows and the tree row is 8x8 m. In all parcels created, one row of trees was excluded from the harvest parcel, taking into account the side effect. In all parcels created, 2 trees are marked as measurement trees.

Drip irrigation was used as an irrigation method in the study. The irrigation water collected in the storage pool was pumped with a pump and passed through the control unit consisting of hydrocyclone, sand-gravel filter tank and disc screen filters, and conveyed to the research area with a 6 atm pressure 50 mm outer diameter rigid PE pipe. Manometers placed at certain points are used to control the pressure occurring in the system. Manifold pipelines 40 mm outer diameter hard PE pipes are used. A double row lateral pipeline consisting of 16 mm outer diameter soft PE pipes was arranged in each row of trees within the parcels. Since the crown width of the walnut trees does not cover the plant between and above the rows, the whole row is not wetted. For this reason, a total of 8 pressure-regulated on-line drippers were installed, 4 on each lateral pipeline, considering the crown width per tree. Dripper flow rate has been selected as 4 L / h according to the principles stated in Yıldırım (2008), considering the structure of the soil and infiltration.

In the study, the experimental treatments were formed by applying different rates (75%, 100%, 125%) of the reference evapotranspiration values calculated over a 5-day period.

The following equation has been used in calculating the amount of irrigation water to be applied to experimental treatments.

$$I = ET_0 \times k \times P \times A$$

In equality;
I: Amount of irrigation water to be applied (L), ET0: 5-day reference evapotranspiration calculated by Penman Monteith method according to FAO 56, (mm), k: Coefficient required by the experimental treatments (75,100 or 125%), P: Cover percentage (%), A: Parcel size, (m^2).

In estimating the reference plant evapotranspiration using climate data, Allen et al. (1994), the method obtained by revising the Penman-Monteith method and defined as FAO-56-PM was used.

$$ET_0 = \frac{0.408\Delta + (R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_a - e_d)}{\Delta + \gamma(1 + 0.34u_2)}$$

In equality;
ET0: Reference evapotranspiration (mm / day), Rn: Net radiation on the plant surface (MJ / m2 / day), G: Heat flow in the soil (MJ / m2 / day), T: Average temperature (oC), u2: Wind speed at a height of 2 meters (m / s), ea: saturated vapor pressure (kPa), ed: real vapor pressure (kPa), Δ: slope of saturated vapor pressure curve (kPa / oC), γ: psychometric constant (kPa / oC) show the values.

Evapotranspiration values were calculated according to the effective root depth of the plant with the following water budget approach (James 1988). For this purpose, soil moisture was measured according to the dry weight percentage for each 30 cm soil layer at 90 cm soil depth in each treatment before irrigation application.

$$ET = I + P + Cp - Dp \pm Rf \pm \Delta S$$

In equality;

ET: Evapotranspiration (mm), I: Amount of irrigation water applied during the period (mm), P: Falling rainfall during the period (mm), Cp: The amount of water entry the root zone by capillary rise (mm), Dp: Deep leakage losses, mm, Rf: The amount of runoff (mm) entering and leaving the parcels, ΔS: The changes in the soil moisture in the root zone (mm).

The Cp value was not taken into account assuming that there was no ground water in the experimental area and there was no water entry to the plant root area by capillary action. In addition, since a pressure irrigation system was used, runoff amounts were ignored (Kanber 1997). Next substrate of soil was monitored for deep seepage losses.

In the research, a pressure chamber instrument was used to determine plant leaf water potential values. For the measurement of leaf water potential, two measurements were made as two leaves from a tree from all treatments in a block, in accordance with the principles given in Schollander et al. (1964).

RESULTS AND DISCUSSION

In the study, irrigation water was applied between 238.60 mm and 397.65 mm in the first year, and between 367.71 mm and 612.81 mm in the second year. Cumulative evapotranspiration was determined for each treatment (Figure 1). While 211.4 mm of precipitation fell in 2018, in 2019, 110 mm of precipitation was measured. In the first year, 658.20 mm of evapotranspiration value was measured from $I_{1.00}$. Evapotranspiration was obtained as 577.70 mm and 723.75 mm for $I_{0.75}$ and I1.25 treatment, respectively. When looking at the results of the evapotranspiration

in the second year of the experiment, the evapotranspiration value of 706.74 mm was obtained from the $I_{1.00}$ treatment where 100% of the measured ET0 value was applied. The evapotranspiration was measured as 584.21 mm for the $I_{0.75}$ treatment and 816.31 mm for the $I_{1.25}$ treatment. It was observed that the evapotranspiration increased with the increase of the amount of irrigation water applied in both years.

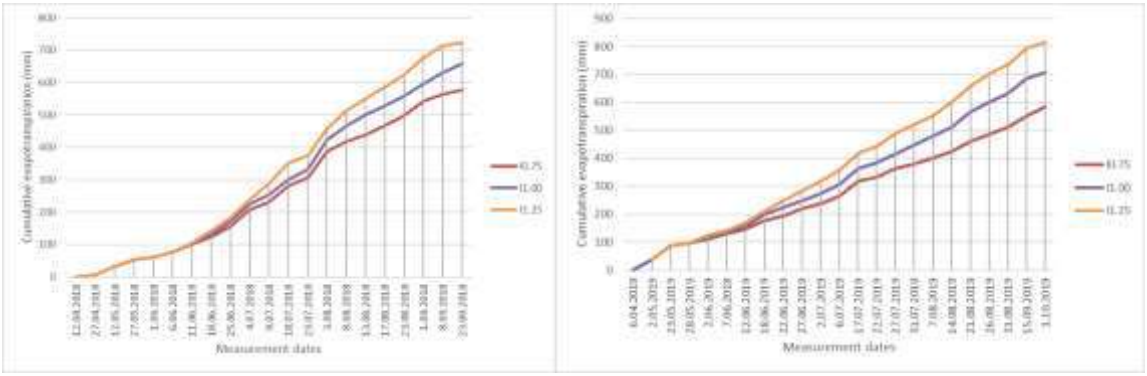

Figure 1. Cumulative evapotranspiration values obtained in the study (2018-2019)

Leaf water potential measurement results and averages obtained for two years from the experimental treatments in the study are given in Table 1,2,3. In both years, the highest values were obtained from the $I_{0.75}$ treatment. When examined Table 3 where the averages are given, the result was -11.2 bar for $I_{0.75}$ treatment, -10.3 bar for $I_{1.00}$ treatment, and -9.1 bar for $I_{1.25}$ treatment. From here, it is clearly seen that the results of YSP measurements decrease as the amount of irrigation water increases. Especially $I_{1.25}$ treatment has the lowest values.

Table 1. Midday Leaf water potential measurements obtained from treatments in 2018 (-Bar)

Measurement dates (Before irrigation)	Treatments					
	$I_{0.75}$		$I_{1.00}$		$I_{1.25}$	
	Örn. 1	Örn. 2	Örn. 1	Örn. 2	Örn. 1	Örn. 2
11 June	11,0	11,5	10,5	9,0	8,0	9,5
18 June	12,0	10,0	9,0	10,0	8,0	9,0
25 June	10,0	11,5	9,5	10,5	8,0	10,0
4 July	14,0	13,0	11,0	12,0	9,0	10,0
9 July	14,5	11,0	12,5	11,0	10,0	10,0
18 July	11,0	11,0	11,0	10,0	9,0	8,0
23 July	12,5	12,5	12,0	10,0	11,5	10,0
3 August	11,5	10,0	11,5	10,0	9,0	9,0
8 August	12,0	12,0	10,0	10,0	9,0	9,0
Average	11,0	11,5	10,5	9,0	8,0	9,5
General average	11,7		10,5		9,2	

Goldhamer et al. (1986) conducted a research by applying three different irrigation levels (100%, 66% and 33%) of ET measured in five-year-old chico walnut trees. They obtained the midday leaf water potential values measured from the treatment at 100% level between -7.5

and -10 bar. It has been stated that among the deficiency irrigation treatments, it is measured between -10 and -12 bar for 66% level and between -11 and -13 bar for 33% irrigation level. Cochard et al. (2002) explained in their study that the stomata were closed when the leaf water potential measurement value exceeded -16 bar. In the study conducted by Pinto (2014) on Chandler walnut trees in the USA, the lowest MSWP measurement was obtained from the treatment where pruning was intensive and irrigation was at the control level (average -5 bar). The highest MSWP value was measured as -11 bar for less pruning and deficit irrigation. Study results are similar to previous studies.

Table 2. Midday Leaf water potential measurements obtained from treatments in 2019 (-Bar)

Measurement dates (Before irrigation)	Treatments					
	$I_{0.75}$		$I_{1.00}$		$I_{1.25}$	
	Örn. 1	Örn. 2	Örn. 1	Örn. 2	Örn. 1	Örn. 2
2 June	8,5	8	9,5	9	8,5	8
7 June	9	11	9,5	10	8	8,5
12 June	11	11,5	10,5	10,5	9	9,5
27 June	11	12	10	11,5	10	9
2 July	12	12,5	8,5	11,5	10	9,5
Average	10,3	11	9,6	10,5	9,1	8,9
Generally average	10,65		10,05		9	

Table 3. Two-year average midday leaf water potential measurements on treatments (-Bar)

Year	Treatments		
	$I_{0.75}$	$I_{1.00}$	$I_{1.25}$
2018	11,7	10,5	9,2
2019	10,65	10,05	9
Avg.	11,2	10,3	9,1

CONCLUSION

It was observed that the leaf water potential values decreased with the increase of irrigation water applied. By evaluating the two-year results of the obtained midday leaf water potential measurements together, an average of -9 to -10.5 bar can be given as a range for starting irrigation. On average, this value can be suggested as -10 bar. Pressure values higher than this point have been measured for deficit irrigation treatment. It is predicted that this situation may adversely affect tree growth during the development years, and may have negative consequences in yield and quality values in maturity years.

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ANALYSIS OF CRISPR/CAS-SYSTEM OF PSEUDOMONAS AERUGINOSA STRAIN NCTC10728 AND SCREENING OF PHAGES THROUGH SPACERS OF CRISPR BACTERIA ARRAY BY BIOINFORMATICS TOOLS

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ABSTRACT

Background. Due to widespread and uncontrolled use of antibiotics in medicine and agriculture, many pathogenic bacteria have acquired multidrug resistance (MDR), on the basis of which "superbacteria" were formed. The problem of "superbacteria" (superbugs) has become relevant for health care around the world. The most dangerous for humans are “superbacteria” such as strains of *Pseudomonas aeruginosa,* which cannot be destroyed antibiotics of the carbapenem class. These strains made antimicrobial antibiotic therapy extremely difficult or practically impossible, which necessitated the search for alternative methods of treating bacterial infections caused by such "superbacteria." Among alternative strategies in the fight against bacteria with MDR can be the use of bacteriophages.

Aims. The purpose of our investigation is to search and to analyze the structural elements of CRISPR / Cas- systems in the genomic strain *Pseudomonas aeruginosa NCTC10728* to identify their structural properties and to assess the diversity of detected phage races detected through their CRISPR-arrays.

Materials and methods. This approach is demonstrated by means of a developed algorithm from search software bioinformatics methods.

Results. Analysis of the genome of the *P. aeruginosa strain NCTC10728* has showed that the CRISPR / Cas system identified in its structure is of type I-E. It was found that Cas genes and CRISPR arrays are located next to these CRISPR arrays. The spacer structures in the detected CRISPR arrays are identical to the phage protospacers, the hosts of which are bacteria of the following genera – *Arthrobacter, Mycobacterium, Gordonia, Streptomyces, Rhodococcus, Propionibacterium.*

Conclusions. The developed algorithm of program methods for locating CRISPR / Cas-systems allows determining the degree of resistance of bacteria to specific bacteriophages, which should ensure the effectiveness of targeted phage therapy of infections caused by pathogenic bacteria, including “superbacteria”.

Keywords: genome of *P. aeruginosa strain NCTC10728,* bioinformatics tools, CRISPR / Cas-system, spacers, repeats, protospacer, bacteriophages.

INTRODUCTION

Infections caused by bacteria with multidrug resistance (MDR) are a significant public health problem in the modern world [1; 2]. The consequence of this problem is the emergence of a class of "superbugs", which are already a great global threat to public health.

There are new factors of the endless adaptation for bacteria such as: increasing numbers of their population, amazing plasticity of genetic material, facilitate the exchange of genetic information between completely different species. Accordingly, their infectious activity and aggressiveness against hosts sensitive to them also increase, that becomes a global problem not only for healthcare, but for almost all sectors of agriculture and industrial food production [3; 4].

It is already recognized that the uncontrolled use of antibiotics in medical institutions, livestock and food production are the main sources and cause of the appearance of "superbugs" [5]. It has conducted research, which assess the level of antibiotic consumption from 2000 to 2015 in different countries and it has shown that antibiotic consumption during this period increased by 65% [6]. A number of predictive studies show that with this level of antibiotic usage and the development of MDR pathogenic bacteria in the world, annual mortality by 2050 can reach almost 10 million people. At the same time, investments will cost: up to 3.5% of its total GDP or up to $ 100 trillion per year [7].

Due to the problem of the emergence and development of MDR by bacteria, a special study has conducted to determine the most resistant among them to the modern antibiotics. As a result, a group represented by the following bacterial pathogens has isolated: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp.* – (by the first letters of their names: *ESKAPE*).

Pathogens of this group are responsible for the most nosocomial infections and are able to "avoid" the biocidal action of many antimicrobial agents [8]. Carbapenem-resistant pathogens of *A. baumannii, P. aeruginosa, K. pneumoniae* and *Enterobacter spp.* form the first category of critical priorities. Such a threatening tendency to form new variants of "superbacteria" requires the urgent search and creation of new antibacterial drugs with characteristics superior to the previous ones. Therefore, a lot of pharmaceutical companies have begun to invest more in the development of non-bacterial anti-infective drugs. So, today various variants of peptidomimetics of hydrophobic peptides, as well as synthetic preparations based on nanoparticles, are already being synthesized [9].

There are alternative strategies in the fight against bacteria with MDR, f.e. bacteriophages. The phages used for therapy have many advantages, such as high host specificity, low doses for treatment, and rapid propagation within the host bacteria. The advantage is that they develop a new infectivity in bacteria and can gain superiority over them, since their mutation rate is much higher than that of their hosts [10].

A number of studies on the use of *in vitro* phagotyping technology have been shown to be effective as antibacterial agents against biofilm forms of bacteria of the ESKAPE group in recent years [11]. So, today in the treatment of bacterial infections, phage cocktails have been used, consisting of a combination of phages that act against various types of bacteria. It was also proposed to combine them with antibiotics, in which they could exhibit a synergistic effect for the destruction of bacterial biofilms. It has shown that the combination of PEV20 phage and the ciprofloxacin antibiotic have a synergistic effect in vitro against *P. aeruginosa*. In another study using the phage OMKO1 with ceftazidime against *P. aeruginosa* strains, it has revealed that this combination led to the successful treatment of complications caused by this

"superbacterium". Proposed combination therapy approach is still in a state of immaturity and the data obtained in *in vivo* models are insufficient [12].

Therefore, the search for new approaches continues. In addition to the mentioned variants of phage destructors of pathogenic bacteria, other innovative approaches are also proposed. One of them is our proposed idea of screening highly specific phage races through the structures of CRISPR / Cas bacterial systems, which are capable of targeted lysis of "super bacteria". It has mentioned in other our publications [13; 14; 15; 16; 17].

CRISPR / Cas-systems (Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR-associated proteins, or short palindromic repeats regularly located in groups with CRISPR-associated proteins) are the oldest "adaptive immunity" systems in bacteria. It allows integrating DNA fragments of bacteriophages and plasmids (spacers) into certain areas of the bacterial genome, which gives bacteria subsequent resistance to these phages and plasmids upon repeated infection [18].

Our approach is based on the use of the most modern technologies: comparative genomics, bioinformatics, PCR diagnostics and sequencing. Thus, at the first stage we consider it necessary to conduct studies of the structural and functional characteristics of CRISPR / Cas-systems. This will serve as a platform for the subsequent development of screening technology for targeted phage races capable of strain-specific lysis of the pathogen.

MATERIALS AND METHODS

The gene of the strain *Pseudomonas aeruginosa NCTC10728* is taken for our investigation. It has downloaded from the GenBank database (NZ_LR134342.1). This strain for decoding the full genome has purchased from the Pasteur Institute (Tehran, Iran) [19].

To search and to study loci of CRISPR / Cas-systems of this bacterium, we developed an algorithm using bioinformation software methods. This algorithm has used to detect all variants of cas-genes of CRISPR / Cas systems, CRISPR-arrays, phage structures detected by complementarity with spacers from various phage databases. To identify cas-genes, the following programs were used: MacSyFinder (Macromolecular System Finder, ver. 1.0.2) [20] and CRISPI: a CRISPR Interactive database (http://crispi.genouest.org). The search and decoding of CRISPR-arrays have made by: 1) CRISPR R Tool (http://www.room220.com/crt/); 2) CRISPI: a CRISPR Interactive database (http://crispi.genouest.org); 3) CRISPRFinder (http://crispr.upsud.fr/Server); 4) CRISPRDetect (http://brabtools./CRISPRDetect. html). To screen phage structures detected by complementarity with spacers from various phage databases have used: Mycobacteriophage Database (http://phagesdb. org/blast) and Phages database (http://www.phantome.org) – CRISPRTarget: explore targets of CRISPR RNAs.

RESULTS

The studied gene of the *P. aeruginosa* strain *NCTC10728* has a relatively large size equal to 6363395 nucleotide bases (b.p.) and contains 5.903 genes. As a result of the bioinformation search, the developed program algorithm in the genome of this strain has revealed two loci of CRISPR / Cas-systems classified as type-I-E. Genes encoding Cas-proteins of the I-type CRISPR / Cas-system were also detected and visualized (Table 1).

Table 1: Structural and functional characteristics of cas, csm-genes in the genome of the strain *Pseudomonas aeruginosa NCTC10728*

№	Sequence Id	Position	System	Protein length(aa)	Score	i-evalue	Profile coverage	Sequence coverage	Begin match	End match
1	Cas2_I-E	806	CAS	360	43.5	3.3e-12	0.55	0.53	133	321
2	Cas1_I-E	1686	CAS	448	40	3.8e-11	0.57	0.44	0.44	343
3	Cas6_I-E	2245	CAS	506	25.3	7,6e-5	0.54	0.38	0.38	336
4	Cas5_I-E	806	CAS	360	43.5	3.3e-12	0.55	0.53	133	321
5	Cas7_I-E	1686	CAS	448	40	3.8e-11	0.57	0.44	0.44	343
6	Cse2_I-E	2245	CAS	506	25.3	6,2e-4	0.54	0.38	0.38	336
7	Cse1_I-E	1686	CAS	748	47	32.9e-11	0.67	0.34	0.32	131
8	Cas3_I	2245	CAS	806	20.2	6,44e-3	0.54	0.11	0.22	213

Note: Sequence Id- the name of the protein in the whole genome sequence presented in the Gen Bank databases; Position- the position of the test protein relative to other *P.aeruginosa* proteins; Protein length- amino acid sequence length; Score- profile match metric (hmmer); Profile coverage- percentage overlap of amino acid profiles of cas-proteins with the studied amino acid sequence; Sequence coverage- percent overlap of the studied amino acid sequence with the amino acid profile of cas- proteins; Begin match- the starting position at which the amino acid profiles match; End match-the end position at which the amino acid profiles match.

The detected two CRISPR-arrays have contained inter-spacer repeats, the consensus sizes reach up to 29 b.p. (fig. 1).

Fig. 1. Consensus repeat structures in the genome of P. *aeruginosa* strain *NCTC10728* (A is the general consensus repeat structure for both CRISPR-arrays, B is the consensus repeat structure for CRISPR-array 1, C is the consensus repeat structure for CRISPR-array 2).

Both CRISPR-arrays contain 10 spacer sequences, with the locus positions of the CRISPR-array 1 in the genome 863733-864372 b.p., and CRISPR-array 2 – 873607-874187 b.p.. As a result of bioinformation analysis of the nucleotide structures of the identified CRISPR-arrays' spacers, phage races have screened from the NCBI database, which are complementary to these

spacer sequences (Table 2). It is identified belonging of *Pseudomonas aeruginosa NCTC10728* through complementary identical protospacers of the detected phage races to the following bacterial hosts from the genera: *Arthrobacter, Mycobacterium, Gordonia, Streptomyces, Rhodococcus, Propionibacterium.*

Table 2: Spacer structures in CRISPR-arrays of *P. aeruginosa* strain *NCTC10728* and bacteriophages detected by them

№	**Spacer CRISPR-array 1**	**Complementary bacteriophages**	**№GenBank**
1	ATCACGGACCAGAACCGCTTT CTGAGTGCGGT	*Arthrobacter phage Xenomorph*	MK919473
2	GTGGCAGGATGAAGCCGGCGC CGGCCGACTCG	*Mycobacterium phage Edugator*	MF185719
3	TCGGAATACTCCTGGGCGTCCT TACCAGGGAA	*Gordonia phage William*	MK801721
4	CCGTATCTGAATATTTTTGCAC GCATGCTCAT	*Mycobacterium phage Shedlock Holmes*	KR080206
5	TTCATCGGTGATACTGAAGCT AATGCTGCTGTG	*Streptomyces phage Saftant*	MN204498
6	TATCCGGACTGGCGGCGACCG ATTCCGCTCGA	*Arthrobacter phage Tank*	KU160669
7	CAGGCCTGAACCAGTCCGAGC TAGCCCGGCGC	*Streptomyces phage YDN12*	KP876465
8	ATCGCGGAGAGAAGGGCCTGG TCGAGACGGTA	*Streptomyces phage Daudau*	MF766045
9	TCGCCGGTGCGGCCGTGGGGC TACGAGCGGCC	*Rhodococcus phage Finch*	MG962366
10	GTGATGGAGCGGACCGCCCCG AGCACCGCAGA	*Mycobacterium phage Herbertwm*	MN224566
1	CGCGTCGAGGCCCTGGAGGCA GAGAAAGGCAA	*Microbacterium phage Tyrumbra*	MN175603
2	AGCTTCCTCATCCCGAAATGTC GTGATTGGAG	*Propionibacterium phage B3*	KX620749
3	TGCACGTCCAACCCGTTCTTCG CAACCGTCTC	*Gordonia phage Ghobes*	NC_031028
4	GAGCTCAAGCTGGGTTCCCTC GATCAACTGATC	*Streptomyces phage Keanu*	AL645882
5	GTACAGGCCGCGATTATCACT GTCAATACGTC	*Mycobacterium phage Xula*	MN234195
6	CTGATCCTCAACGACCGCACA GTTACTCAGTAC	*Microbacterium phage MonChoix*	MK894437
7	TAGTCCTGGCGGATATCGCTG CACCAGTGCAT	*Gordonia phage Lucky10*	KU963256
8	CCTGCATCAGATGCGGCCTCCT TCAGGCGGGC	*Gordonia phage Yeezy*	KU963249
9	ATGGAAGACCCAACCCGCGGC CAGGGGTGGGG	*Gordonia phage EpicDab*	MK660712
10	GTATCGACATCGATGGTGACC AGGCTGGTCC	*Streptomyces phage Daudau*	MF766045

An analysis of the diversity of phage associations identified through spacer structures in CRISPR-arrays of the studied strain according to the ecological and geographical characteristics and the spectrum of their bacterial hosts has carried out (Table 3).

Table 3: The structure of the diversity of phage races detected through the spacers of the identified CRISPR arrays of the *P. aeruginosa* strain *NCTC10728*

№	**Complementary Phages**	Size of phage's genome (b.p.)	Type of host bacteria	Insulation source bacteria	Country isolation
	Complementary Phages CRISPR- array 1				
1	*Arthrobacter phage Xenomorph*	58690	*A.sp. ATCC 21022*	soil	USA
2	*Mycobacterium phage Edugator*	63344	*M.smegmatis mc2 155*	soil	USA
3	*Gordonia phage William*	50678	*G.terrae 3612*	soil	USA
4	*Mycobacterium phage Shedlock Holmes*	61081	*M.smegmatis mc2 155*	soil	USA
5	*Streptomyces phage Saftant*	48883	*S.griseofuscus ATCC 23916*	soil	USA
6	*Arthrobacter phage Tank*	67592	*A.sp. ATCC 21022*	soil	USA
7	*Streptomyces phage YDN12*	56528	*S.griseus subsp. griseus ATCC 10137*	soil	USA
8	*Streptomyces phage Daudau*	50602	*S.xanthochromogenes NRRL B-5410*	soil	USA
9	*Rhodococcus phage Finch*	138896	*R.erythropolis RIA 643*	soil	USA
10	*Mycobacterium phage Herbertwm*	51546	*M.aichiense ATCC 27280*	soil	USA
1	*Microbacterium phage Tyrumbra*	53975	*M.paraoxydans NRRL B-14843*	soil	USA
2	*Propionibacterium phage B3*	35948	*P.freudenreichii TL110*	cheese	France
3	*Gordonia phage Ghobes*	45285	*G.terrae 3612*	soil	USA
4	*Streptomyces phage Keanu*	-	-	-	-
5	*Mycobacterium phage Xula*	48540	*M.smegmatis mc2 155*	soil	*SA*
6	*Microbacterium phage MonChoix*	41670	*M.foliorum NRRL B-24224 SEA*	soil	USA
7	*Gordonia phage Lucky10*	42979	*G.errae 3612*	soil	USA
8	*Gordonia phage Yeezy*	51884	*G.terrae 3612*	soil	USA
9	*Gordonia phage EpicDab*	16658	*G.neofelifaecis NRRL 59395*	soil	USA
10	*Streptomyces phage Daudau*	50602	*S.xanthochromogenes NRRL B-5410*	soil	USA

On the one hand, the ecological and geographical diversity of the identified phage races and their bacterial hosts is not very wide. On the other hand, it presents both a large range of genome sizes of these phages (from 16658 to 138896 b.p.), and a variety of identified phage races assigned to 14 phage species.

DISCUSSION

This may indicate, that the CRISPR / Cas-systems of the *P. aeruginosa NCTC10728* strain are functionally active, which, accordingly, allows it to maintain its adaptive potential. Also, the representation of such a variety of phage associations may indicate the expression activity of the spacer structures of its CRISPR-arrays.

Thus, in a number of studies, it has shown, that the presence of a functional CRISPR / Cas-system in the bacterial genome, potentially interferes with the acquisition of plasmids or phages (prophages). They carry antibiotic resistance genes, and simulations, helps support the high sensitivity of these bacteria to antibacterial drugs [21]. Let's use an example, a decrease in antibiotic resistance due to the possible destruction by the CRISPR/Cas-system of those conjugative plasmids that provided this resistance, has shown by *S.epidermidis*. This strain, possessing these properties has lost high resistance to antibiotic agents and has acquired high epidemic potency and virulent qualities.

Thus, based on the results obtained through the use of bioinformation technologies, it has found that the developed software algorithm makes it possible to identify the loci of CRISPR / Cas-systems in bacterial genomes, as well as to assess the degree of their resistance to foreign genetic elements (detected bacteriophages, plasmids). It is also possible to identify and detect both spacer's structures in the CRISPR-arrays of the studied strain and inter-spacer repeats.

The programs, which we use, made it possible to determine the structures and positions of cas-genes and the type of CRISPR / Cas- system of the bacteria to IE type. Phages have been identified through spacers of CRISPR-arrays of the studied *P. aeruginosa* strain. Further analysis of the identified phages through their protospacer structures complementary to the identical spacer sequences of CRISPR / Ca- systems will allow us to assess the degree of resistance of this strain to the identified specific phage races.

The developed algorithm of bioinformatics software methods for locating CRISPR / Cas - system, which has tested in this study, can also be used on other decrypted genomes of many bacteria. This makes it possible to carry out similar search studies on a large sample of bacterial genomes, which will be important both as well for a comparative analysis of the diversity of structures of CRISPR / Cas-systems, as for determining phage race profiles through CRISPR-arrays. From this information, it is also possible to determine the degree of resistance of bacteria to bacteriophages specific to them, which is important for the development of technology for targeted phage treatment of infections caused by pathogenic bacteria in the nearest future.

CONCLUSION

The bioinformatics tools demonstrate promising possibilities for carrying out studies of the structure, functioning, and evolution of CRISPR / Cas-systems in "superbacteria" and other species. The unique structure of the revealed structures of CRISPR / Cas systems in the studied *P. aeruginosa* strain *NCTC10728* indicates the diversity of genes and arrays, that make up the pathogen genome. The genetic differences of the defense system within one bacterial species may be significant in terms of creating promising therapies for infections caused by "superbacteria". It is possible, that the unique structure of the revealed structures of CRISPR / Cas – systems of the *P. aeruginosa* strain *NCTC10728* is explained by its adaptability to intraspecific and interspecific relationships. In recent years, thousands of genomes of many types of bacteria, bacteriophages, plasmids, including *P. aeruginosa* strains, have been deciphered. Therefore, the algorithm developed for programmatic search for loci of CRISPR / Cas-systems can be used on other decrypted genomes of many bacteria. It can be seen, data that the establishment of the spacer's structure in the detected CRISPR-arrays of the *P. aeruginosa* strain *NCTC10728* allows determining the degree of resistance of bacteria to specific

bacteriophages, which can be used to develop a technology for targeted phage treatment of infections caused by pathogenic bacteria, including “superbacteria”.

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CLIMATE CHANGE IN TURKEY

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ABSTRACT

The present study aimed to investigate the climate change inducted by global warming in Turkey. The reasons for climate change experienced due to global warming could be categorized in two groups: natural and artificial reasons. Natural causes of global climate change include magnetic solar storms, deviations in the axis of the Earth, and the increase of the kurtosis of the Earth orbit around the Sun, the El Nino effect, and artificial causes (anthropogenic activities) include fossil fuel consumption, greenhouse gases, and the reduction of the ozone layer. The increase the density of atmospheric greenhouse gases such as Carbon Dioxide (CO_2), Methane (CH_4), chlorofluorocarbon gases (CFC-11, HCF-C22, CF4, etc.) and Nitrogen (N_2O) is the main source of global warming. The carbon dioxide emissions in Turkey are concentrated in two industries: the energy sector (86.3%) and industrial processes (13.4%). Methane emissions are mainly due to agriculture (62.30%) and energy industry activities (21.30), waste emissions (16.4%), while N_2O emission sources include agricultural activities (71%), waste emissions (15.1%), energy sector (10.7%) and industrial processes (3.3%). When compared to 1990, CO_2 emission per capita has increased about 1.5-fold today, which is a serious warning to take precautions. Consequently decrease in *Marmaris salamander, Otis tarda, Karaman grasshopper, Turkish red dragonfly* populations and *Eğirdir longsnout scraper, European eel, Acıgöl sailton pupfish*, and *marine turtle* reproduction decreased to extinction levels evidenced that global warming and climate change are observed in Turkey.

Keywords: Global warming, climate change, species diversity, Turkey.

INTRODUCTION

Although climate change has been an ongoing phenomenon throughout history, it was never as rapid as it is today. In recent years, several atmospheric events occurred in many regions of the world unique in violence, affects, duration and location. These changes also threaten the life on earth and socioeconomic development. Various parameters trigger climate change (Saraçoğlu, N., 2018).

Although the causes of climate change are generally anthropogenic, there are also natural causes: The solar effect, the Earth's precise movement, and the El Nino Effect. The sun generates cosmic radiation. Excessive cosmic radiation increases cloud cover, alters the soler radiation, and leads to an increase global temperature. Serbian scientist Milankovic explained the Precision Movement of the Earth. It was demonstrated that the Earth's orbit around the sun slightly flatten every 95000 years. It was also reported that there is a linear shift in Earth's axis every 41000 years and a circular deviation every 23000 years. This is one of the reasons for the linear global warming. The El Nino Effect entails 2-5^{0}C higher than normal sea surface temperatures in the tropical eastern Pacific Ocean between 1990 and 1998. The temperatures

increased in record levels during 1997 and 1998. It was suggetsed that the main effect of global warming in 1998 was El Nino (Saraçoğlu, N., 2018).

After the industrial revolution in the 18th century, mechanization and technological advanced led to the consumption of fossil fuels in energy generation, currently called traditional energy sources. When fossil fuels are burned, they release CO_X, SO_X, NO_X, CH_4, H_2O, and O_3 gases, called the greenhouse gases. These gases released by the burning of fossil fuels increase the atmospheric air temperature and lead to global warming and climate change. The most important environmental problem induced by the use of fossil fuels in energy generation is the CO_2 emissions. Furthermore, other harmful emissions that lead to acid rain and destruction of the ozone layer, include sulfur oxides (SO_X), and nitrogen oxides (NO_X).

2.1 The Industries with the Highest Impact of Greenhouse Gas Emissions in Turkey:

It was accepted that Carbon dioxide (CO_2), Methane (CH_4), Nitrous Oxide (N2O), Hydro-fluoride carbons (HFCs), Perfluoro carbons (PFCs), Sulfur-hexea fluoride (SF6) are the main factors that lead to the greenhouse effect. In Turkey, greenhouse gases are released during various processed that include:

Forest Fires: Although forests are usually carbon consumers, forest fires release carbon dioxide, methane, carbon monoxide, nitrogen dioxide and other nitrogen oxides to the atmosphere.

Power Plants: They lead to air pollution due to NO, CO and high CO_2 emissions based on the raw material.

CFCs: The common use of CFCs (Chlorofluorocarbons) include refrigerants, car air conditioners, insulation material, perfumes and deodorants, and they react with the ozone in the ozone layer and lead to the breakdown of ozone and destruction of the ozone layer.

Paddy Fields: Nitrous oxide (N_2O) gas is released by nitrification and denitrification in paddy fields. In addition, methane (CH_4) gas emissions are high due to anaerobic breakdown. Paddy cultivation is among the major agricultural activities in the world and Turkey.

Fertilization: N_2O is released when chemicals are added to fertilizers to increase crop yield.

Cattle Husbandry: Methane (CH_4) is released by manure stored in pastures.

Landfills: It was determined that regular old landfills without removal facilities have high levels of methane emissions.

Factory Chimneys: PAH, SO_2, CO, NO_x and vapor are released by the factories based on the raw material and manufacturing process employed in the factories when the chimneys are not controlled and routinely cleaned, the filters are not routinely changed, or the compounds are not burned or half-burned.

Vehicle Exhausts: It is known to contain carbon monoxide (CO), hydrocarbons (HC) and nitrous oxide (NOx), which are dangerous for human health and the environment, in addition to harmless substances such as vapor, carbon dioxide and nitrogen dioxide.

The most effective greenhouse gases include CO_2, CH_4 and N_2O, respectively. It is known that the greenhouse effect of methane is 25 times more than nitrogen oxide and 298 times more than carbon dioxide; however, CO_2 emissions is considered to be the main cause of global warming due to the increase in CO_2 emissions every day (Lallanilla M., 2019).

The most effective greenhouse gases include CO_2, CH_4 and N_2O, respectively. The industrial distribution of the greenhouse gases in Turkey are presented in Figures 1, 2, and 3.

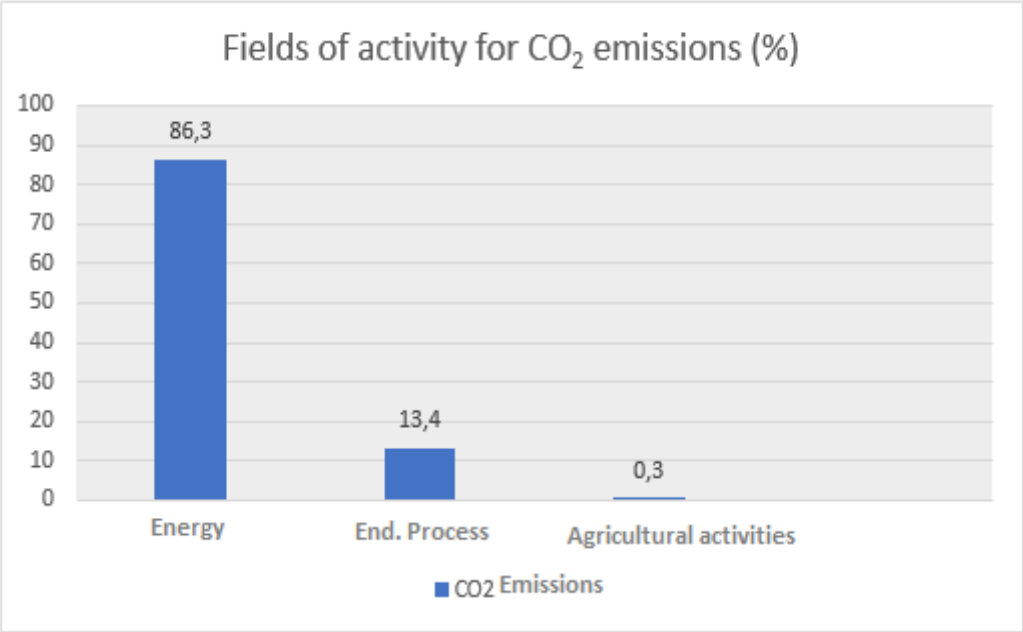

Figure 1: CO_2 Emissions Based on Industry

The analysis of CO_2 emissions based on industry revealed that the power plants ranked the first (86.3%). Based on carbon dioxide emissions, industrial processes ranked second (13%) and agricultural activities ranked the last (0.3%). The highest CO_2 emissions are produced by the power plants. Since the power plants, which are directly affect the exponential increase of the greenhouse gases in the atmosphere, employ fossil fuels as raw material, the CO_2 emissions are at very high levels. CO_2 emissions in industrial processes, on the other hand, represent the gases released due to the substances processed in factories and released from the factory chimneys (http://www.tuik.gov.tr/, 12.02.2020).

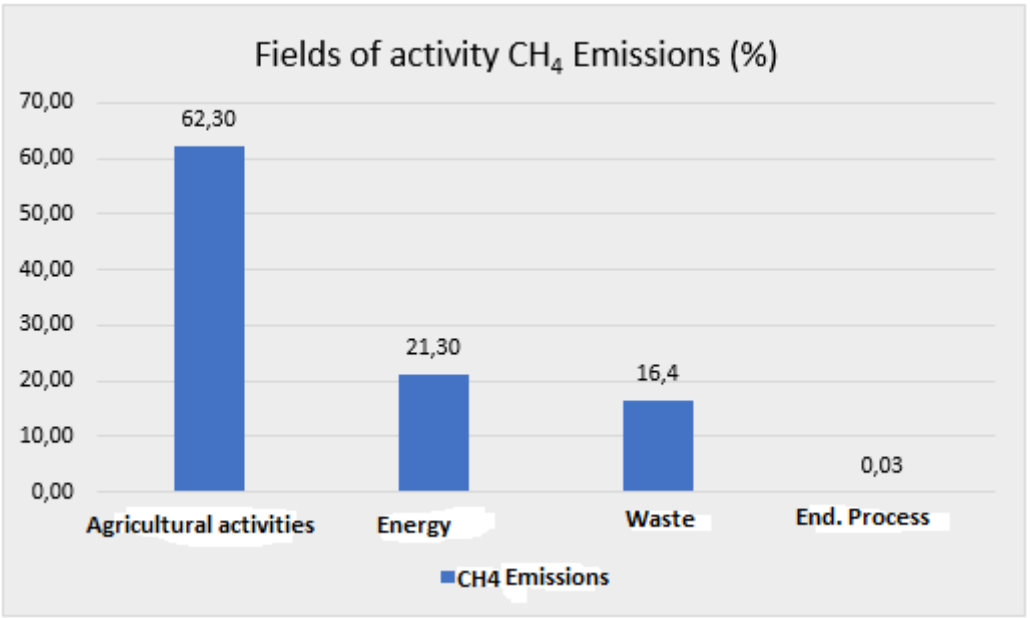

Figure 2: CH_4 Emissions Based on Industry

The CH_4 emission data based on industry revealed that agricultural activities ranked the first (62.30%). It was followed by energy activities (21,30%), waste-induced emissions (16,4%), and industrial processes (0.03%). The highest methane emissions was observed in agricultural activities due to animal husbandry activities and paddy cultivation. The main factor behind methane emission, the greenhouse effect of which is known to be 25 times more than carbon dioxide, is the decay of organic matter in an oxygen-free or low-oxygen environment (http://www.tuik.gov.tr/, 12.02.2020).

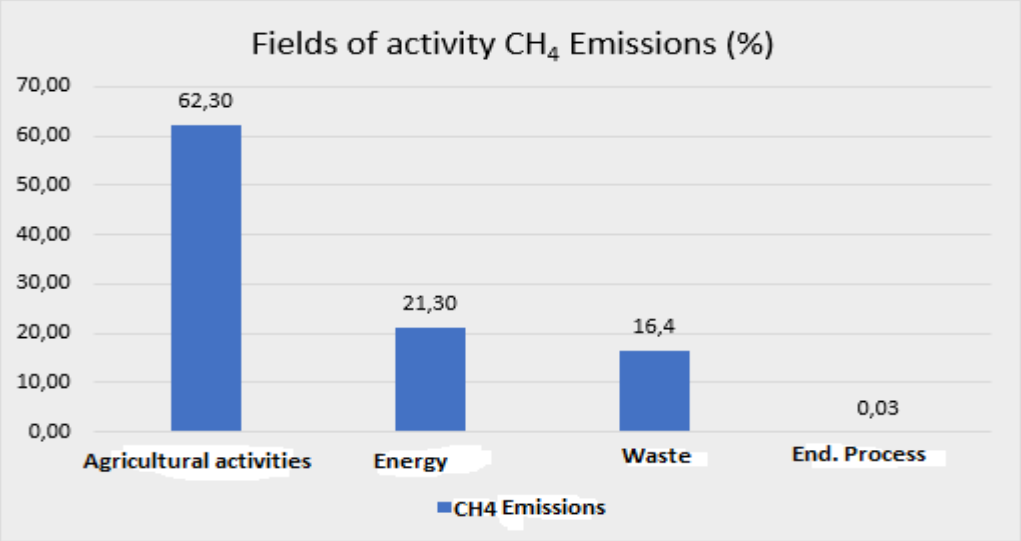

Figure 3: N_2O Emissions Based on Industry

The analysis of N_2O emissions based on industry revealed that the agricultural activities led to the highest emission rate (71%). Nitrous oxide (N_2O) emissions are observed in agricultural activities due to fertilization. Various chemicals are included in fertilizers to improve crop yield. Thus, increasing N_2O emissions. The agricultural activities were followed by waste (15.1%), energy industry (10.7%), and industrial processes (3.3%) (http://www.tuik.gov.tr/, 12.02.2020).

Measures to reduce greenhouse gas emissions: Individual measures to prevent climate change include the preference of public transportation, non-packaged products, washing with water at lower temperatures, turning the electronic devices off completely, preparing the picnic contents at home, and collection of recyclable waste separately.

Turkey has abundant renewable energy reserves when compared to other countries. Turkey has advantages due to the number of sunny days, wind potential, hydroelectric energy potential, and geological and geographical location on an active tectonic belt, leading to geothermal potential. Preference of more sustainable energy sources instead of fossil fuels would contribute to the reduction of greenhouse gas emissions in the country.

Potential Impact of Climate Change on Turkey

Climate change led to reduced snowfall, reduction of evening temperatures, variable climatic events, droughts, floods caused by sudden precipitation, early onset and late termination of the summer season when compared to previous years, etc. in Turkey. As a result, corn, paddy, wheat, oat, barley, rye, pea, sugar beet, chickpea, lentil, pistachio, sunflower, cotton, olive, grape, soy, sesame, fig, and nut yields have reduced (Güventürk, A., 2013).

Due to increasing soil and temperatures in continental climate regions, *Marmaris salamander, Otis tarda, Karaman grasshopper, Turkish red dragonfly* populations decreased and *Eğirdir*

longsnout scraper, European eel, Acıgöl sailton pupfish, and marine turtle reproduction decreased to extinction levels due to the increase in sea and freshwater temperatures.

CONCLUSION

Industrialization, population growth, fossil fuel use, deforestation, depletion of the ozone layer, destruction of the environment, uncontrolled and unplanned urbanization are among the important causes of climate change in Turkey.

Instead of the old public transportation vehicles, new generation vehicles powered by renewable energy should be used, green areas should be improved, residences with thermal insulation should be preferred and the waste should be recycled. Greenhouse gas emissions have increased due to fossil fuel consumption. Renewable energy sources such as geothermal, wind, solar energy and hydroelectricity that exist in Turkey should be preferred instead of traditional energy generation methods that utilize fossil fuels.

Persistence on current conditions could accelerate events such as droughts, desertification and erosion, agricultural production potential could decrease, new issues could be added to the present water resource problems in Turkey, limiting drinking and tap water supplies, coastal areas may submerge, destroying the existing flora and fauna in these areas and increasing the prevalence of viral and bacterial epidemics.

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CONSUMER PERCEPTION OF FRESH MEAT QUALITY IN TIRANA

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ABSTRACT

The quality of meat products differs from intrinsic and extrinsic indications so that it can be establish in accordance with preferences of consumer. This survey examines the consumer confidence, attitude and behavior towards the quality of fresh meat in Tirana. The source of information and data were collected from a detailed questionnaire using random interviews 367 persons in different areas in the city of Tirana. Chi squared or Fisher's exact tests was utilized to analyzed relationships between two categorical variables. A significant relationship between education, income and frequency of meat consummation was observed. Place of origin (51.6%) was the most extrinsic factor that consumer prefer more while colour (49.2%) was the most important intrinsic cues.

Keywords: meat quality; Tirana consumers' perception; survey.

INTRODUCTION

Meat is an important component in the daily diet of a large proportion of society and is regarded as a valuable food from a nutritional perspective (Fayemi & Muchenje 2012). Chemically meat is composed of four major components including water, protein, lipid, carbohydrate and many other minor components such as vitamins, enzymes, pigments and flavour compounds (Lamber et al., 1991). The present demand of consumers for receiving information on food quality and safety, determines in great measure the characteristics of the food chains and the strategies to develop by the industry (Garcia et al., 2004). Nowadays, food safety is an issue that regards credence and trust on food products, which is becoming more and more important for consumer (Brunso et al., 2002). As result of the recent food crises, consumer feels more concerned with food quality and safety, demanding more transparency in the food-chain and more information on the diverse qualitative characteristics of foods (nutritious value, origin, way of production, etc.) (Ventura-Lucas, 2004). The latest food scandals such as BSE, Foot and mouth disease, the Dioxin crisis in Belgium, Horse meat (2013) and Listeriosis in Spain (2019) have significantly increased the consumer incredulity and suspicion in meat products. Food safety perception not only differs between countries, but also may appear dissimilar within a single country.

Patterns in meat consumption are unpredictable and it is clear that changes are occurring in the way consumers behave towards food (Grunert, 2006). Food choice is a complex phenomenon affected by changing preferences and many variables. Especially meat represents an unpredictable task which is influenced by many factors such as knowledge, previous experience, extrinsic and intrinsic cues. Despite our knowledge on the type and importance of meat quality attributes, consumers still have difficulty in accurately predicting experienced

quality by perception at the point of purchase (Grunert et al., 2004). When a consumer evaluates a product alternative that may satisfy the same need, desire or want, he integrate the perceptions of the alternatives into an overall judgement, or attitude, about the attractiveness of each product alternative (Steenkamp and Trijp, 1989).

Consumption of meat in Albania has almost tripled compared to the 1990s, passing from 20 kg to nearly 60 kg per capita (Instat 2017). Nevertheless, these level still remains lower compared to the EU countries. This research intent to investigate deeply perceptions, attitude, that mostly affect consumer behavior when they choose meat products in the city of Tirana. The main focus was understanding consumer opinion, knowledge and demands above quality of meat products. The paper presentation includes a comprehensive information on the research methodology and final results. At the same time these publication intents to discover findings and recommendations for meat industry to develop products in accordance with consumer expectations and preferences.

MATERIALS AND METHODS

2.1. Study Site

The study was carried out in the capital of Albania (Tirana), which is located in the center of Albania. Tirana is the biggest city in Albania with a population of 906,166 as of 2018, makes for 31.84% of Albania's population (Instat 2018).

2.2. Selection of Respondents

The proposed format was considered in order to understand the habits, trends and attitudes towards food safety and meat quality before purchase. A random sampling strategy of 367 consumers (48.8% Male and 51.2% Female) from the city of Tirana was utilized in the study to fulfill the objectives of the study. The survey data were collected through face-to-face interview methods by the first author using survey instruments which they prepared reviewing the earlier studies (Grunert 2006). The purpose of the questionnaire was clearly explained to the consumer before the interview were addressed.

2.3. Data collection

In order to get detailed information the survey questionnaire compromise 11 questions, divided in three different parts. The questionnaire paid a special attention on consumer profile, consumption habits, knowledge of certification and differentiation, beef purchase process, knowledge of beef traceability, beef consumption, and a general question. The first part was related with the gender, age, education and incoming, while on second part information on, type of meat they prefer most, frequency on meat consume and place of purchase. The third part of the questionnaire was focused in the evaluation of the extrinsic and intrinsic quality cues.

2.4 Extrinsic and intrinsic quality cues

To evaluate extrinsic and intrinsic quality cues was utilized a scaling method with five alternatives in different scores range from 1-“very important” to 5-“totally unimportant”. Extrinsic cues were focused on place of origin, price, transability and packaging while intrinsic were focused on colour, flavour, tenderness and marbling.

2.5. Statistical analysis

First, descriptive statistics was used to present date as mean, standard deviation (SD), range or number and percentage. Then, Chi-square Goodness was used to compare the observed distribution with the expected probability distribution (assuming all categories equal) while the

relationships between two categorical variables were analyzed by Chi squared or Fisher's exact tests. In addition, z-tests was used to compare column proportion. A P-value < 0.05 was considered statistically significant All analyses were performed using SPSS version 25.0 statistical analysis software (IBM Inc., Chicago, IL, USA).

RESULTS

Table 1 represent demographic data of participants. A total of 367 people participated in the study. There was no difference in the proportion of males and females: 48.8% were males (n=179) and 51.2% were females (n=188; P>0.1). The ages of the participants ranged from 19 to 74 years with a mean±SD age of 44 ± 14 years. Most were 29-48 years old (P<0.001), with high school (39.2%) or university education (58.9%; P<0.001). Most of the participants had a (87.9%, n = 323) had monthly income between 17 and 2000 mije lek.

Table 1. Demographic data of participants

Parameter		**Count (N=367)**	**N %**	**P value**
Gender	**Male**	179	48.8%	0.639
	Female	188	51.2%	
Age	**19-28 ys**	61	16.6%	**0.017**
	29-38 ys	89*	24.3%	
	39-48 ys	89*	24.3%	
	49-58 ys	58	15.8%	
	>58 ys	70*	19.1%	
Education	**Primary school**	7	1.9%	**<0.001**
	High school	144*	39.2%	
	University	216*	58.9%	
Month income	**Until 17 mije lek**	9	2.5%	**<0.001**
	From 17-50 mije lek	97*	26.4%	
	From 50-100 mije	140*	38.1%	
	From 100-2000 mije	86*	23.4%	
	Up to 200 mije	35	9.5%	

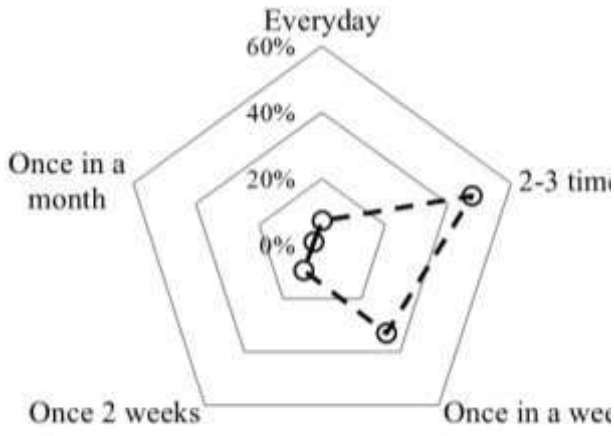
* higher observed number respect to expected (all categories equal)

Most of the participants consumed meat 2-3 times per week (P<0.001) and almost half preferred calf meat (47.1%; P<0.001; Table 2/Figure 1). Over 80% consumed fresh meat (P<0.001), purchased at the butcher (43.1%) or in the village (45.0%; P<0.001).

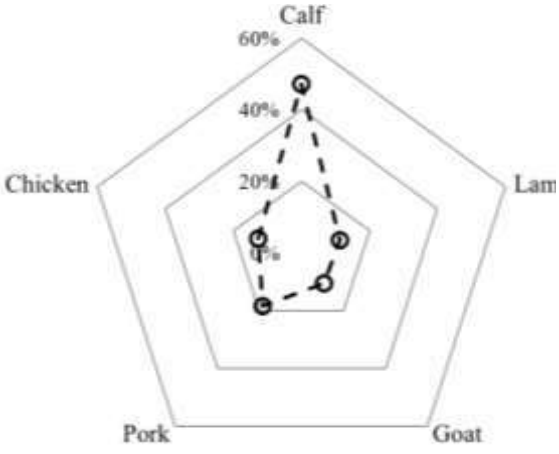
Table 2. Frequencies of dietary patterns associated with meat intake

Parameter		Count (N=367)	N %	P value
Consumption per week	**Everyday**	27	7.4%	**<0.001**
	2-3 time/week	175*	47.7%	
	Once in a week	121*	33.0%	
	Once 2 weeks	35	9.5%	
	Once in a month	9	2.5%	
Consumption per animal species	**Calf**	173*	47.1%	**<0.001**
	Lamb	41	11.2%	
	Goat	39	10.6%	
	Pork	67	18.3%	
	Chicken	47	12.8%	
Consumption per kind of product	**Fresh**	319*	86.9%	**<0.001**
	Frozen	14	3.8%	
	Packaging	23	6.3%	
	By-products	11	3.0%	
Where consumers prefer to buy meat	**At the butcher**	158*	43.1%	**<0.001**
	Supermarket	30	8.2%	
	Minimarket	14	3.8%	
	In village	165*	45.0%	

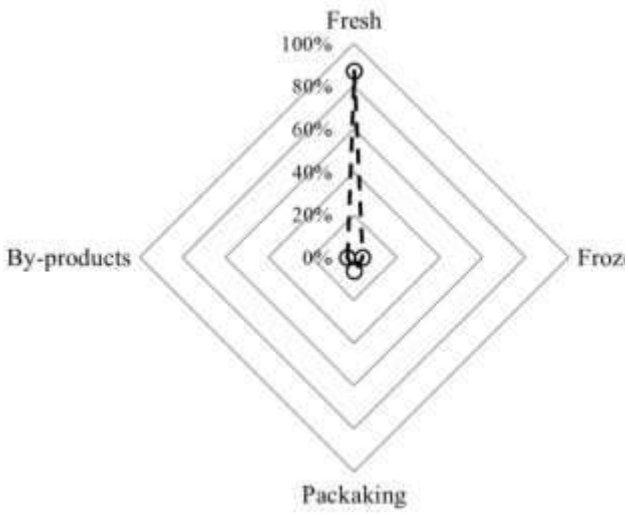
* higher observed number respect to expected (all categories equal)



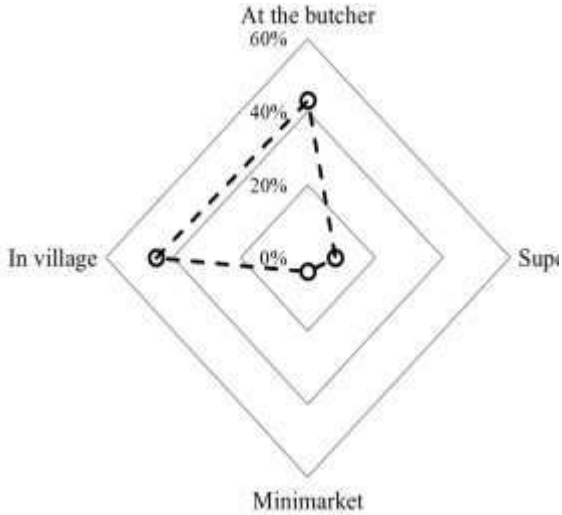
Consumption per week



Consumption per animal species



Consumption per kind of product



Where consumers prefer to buy meat

In particular, a greater proportion of men than female claimed to eat meat every day. For simplicity, age was classified into 3 levels (young adult: 18-34 ys; middle-aged adult: 35-54 ys; senior adult: >54). Participant aged between 35 and 54 have eaten more often meat (every day, 10.9%, or 2-3 time/week, 53.8%) compared to senior adult. Conversely, a greater proportion of senior adults ate meat once per weeks (18.9%) compared to younger participants.

Table 3. Associations between gender of participants and dietary patterns associated with meat intake.

		Sex		
Eating practices		**Male**	**Female**	**P value**
Consumption per week	**Everyday**	21_a (11.7%)	6_b (3.2%)	**0.006**
	2-3 time/week	82_a (45.8%)	93_a (49.5%)	
	Once in a week	52_a (29.1%)	69_a (36.7%)	
	Once 2 weeks	17_a (9.5%)	18_a (9.6%)	
	Once in a month	7_a (3.9%)	2_a (1.1%)	
Consumption per animal species	**Calf**	84_a (46.9%)	89_a (47.3%)	0.924
	Lamb	21_a (11.7%)	20_a (10.6%)	
	Goat	17_a (9.5%)	22_a (11.7%)	
	Pork	35_a (19.6%)	32_a (17.0%)	
	Chicken	22_a (12.3%)	25_a (13.3%)	
Consumption per kind of product	**Fresh**	155_a (86.6%)	164_a (87.2%)	0.931
	Frozen	6_a (3.4%)	8_a (4.3%)	
	Packaging	12_a (6.7%)	11_a (5.9%)	

	By-products	6_a (3.4%)	5_a (2.7%)	
Where consumers prefer to buy meat	**At the butcher**	75_a (41.9%)	83_a (44.1%)	0.802
	Supermarket	13_a (7.3%)	17_a (9.0%)	
	Minimarket	8_a (4.5%)	6_a (3.2%)	
	In village	83_a (46.4%)	82_a (43.6%)	

Table 4. Associations between age of participants and dietary patterns associated with meat intake

Eating practices		**Age (years)**			
		18-34	**35-54**	**>54**	**P value**
Consumption per week	**Everyday**	3_a (2.5%)	17_b (10.9%)	$7_{a,b}$ (7.8%)	**<0.001**
	2-3 time/week	63_a (52.1%)	84_a (53.8%)	28_b (31.1%)	
	Once in a week	44_a (36.4%)	43_a (27.6%)	34_a (37.8%)	
	Once 2 weeks	9_a (7.4%)	9_a (5.8%)	17_b (18.9%)	
	Once in a month	2_a (1.7%)	3_a (1.9%)	4_a (4.4%)	
Consumption per animal species	**Calf**	58_a (47.9%)	76_a (48.7%)	39_a (43.3%)	0.092
	Lamb	13_a (10.7%)	18_a (11.5%)	10_a (11.1%)	
	Goat	8_a (6.6%)	23_b (14.7%)	$8_{a,b}$ (8.9%)	
	Pork	19_a (15.7%)	27_a (17.3%)	21_a (23.3%)	
	Chicken	23_a (19.0%)	12_b (7.7%)	$12_{a,b}$ (13.3%)	
Consumption per kind of product	**Fresh**	100_a (82.6%)	143_b (91.7%)	$76_{a,b}$ (84.4%)	0.242
	Frozen	7_a (5.8%)	2_b (1.3%)	$5_{a,b}$ (5.6%)	
	Packaging	10_a (8.3%)	7_a (4.5%)	6_a (6.7%)	
	By-products	4_a (3.3%)	4_a (2.6%)	3_a (3.3%)	
Where consumers prefer to buy meat	**At the butcher**	49_a (40.5%)	69_a (44.2%)	40_a (44.4%)	0.184
	Supermarket	14_a (11.6%)	7_b (4.5%)	$9_{a,b}$ (10.0%)	
	Minimarket	4_a (3.3%)	4_a (2.6%)	6_a (6.7%)	
	In village	54_a (44.6%)	76_a (48.7%)	35_a (38.9%)	

Instead, the monthly salary affects several aspects associated with meat intake (Table 5). Participants with the highest monthly salary ate meat more often (everyday: 17.4%, 2-3 time/week: 65.3%; P<0.001), more fresh (92.6%) and less frozen (0.8%) products, ate less

chicken (8.3%; P<0.05), and preferred to buy at the butcher (46.3%) or in the village (49.6%) than in the supermarket or minimarket (P<0.001). positive connection was observed regarding the level of incoming, higher were the salary upper was the quantity of meat consumed. Significant differences were also noticed between levels of incoming and type of meat. Persons with higher income consume fresh meat, while people with lower income consume more often frozen meat. At the same time people with higher income prefer more the beef meat

Table 5. Associations between monthly salary and dietary patterns associated with meat intake

		Month income (miji lek)			
Eating practices		**<50**	**50-100**	**>100**	**P value**
Consumption per week	**Everyday**	2a (1.9%)	4_a (2.9%)	21_b (17.4%)	**<0.001**
	2-3 time/week	14_a (13.2%)	82_b (58.6%)	79_b (65.3%)	
	Once in a week	62_a (58.5%)	43_b (30.7%)	16_c (13.2%)	
	Once 2 weeks	23_a (21.7%)	10_b (7.1%)	2_c (1.7%)	
	Once in a month	5_a (4.7%)	1_b (0.7%)	$3_{a,\,b}$ (2.5%)	
Consumption per animal species	**Calf**	47_a (44.3%)	68_a (48.6%)	58_a (47.9%)	0.372
	Lamb	11_a (10.4%)	14_a (10.0%)	16_a (13.2%)	
	Goat	7_a (6.6%)	16_a (11.4%)	16_a (13.2%)	
	Pork	21_a (19.8%)	25_a (17.9%)	21_a (17.4%)	
	Chicken	20_a (18.9%)	$17_{a,\,b}$ (12.1%)	10_b (8.3%)	
Consumption per kind of product	**Fresh**	82_a (77.4%)	125_b (89.3%)	112_b (92.6%)	**0.030**
	Frozen	8_a (7.5%)	$5_{a,\,b}$ (3.6%)	1_b (0.8%)	
	Packaging	11_a (10.4%)	7_a (5.0%)	5_a (4.1%)	
	By-products	5_a (4.7%)	3_a (2.1%)	3_a (2.5%)	
Where consumers prefer to buy meat	**At the butcher**	45_a (42.5%)	57_a (40.7%)	56_a (46.3%)	**0.001**
	Supermarket	16_a (15.1%)	10_b (7.1%)	4_b (3.3%)	
	Minimarket	9_a (8.5%	$4_{a,\,b}$ (2.9%)	1_b (0.8%)	
	In village	36_a (34.0%)	69_b (49.3%)	60_b (49.6%)	

Table 6 reports the frequency with which participants rated the importance of statements about their dietary patterns associated with meat intake. All the factors listed in the table were rated as important or very important for many participants (P<0.001). Place of origin (51.6%) is most important cues for consumers in Tirana follow by a narrow difference in meat colour (49.1%). However, there was also more than 20% of the participants judging price, packaging, aroma, tenderness, presence of fat as Neither important nor unimportant. On the other side meat packaging and labelling have the higher percentage (12.6%) as extremely unimportant.

Table 6. The frequency (number and percentage) with which participants rated the importance of statements about their dietary patterns associated with meat intake.

Statement	Very important	Important	Neither important nor unimportant	Unimportant	Extremely unimportant
How do you evaluate the origin of the meat (national vs imported meat)	196* (51.6%)	78* (20.5%)	41 (10.8%)	46 (12.1%)	19 (5.0%)
How do you evaluate the price of the meat	62 (16.3%)	139* (36.6%)	113* (29.7%)	48 (12.6%)	18 (4.7%)
How do you evaluate the transability of the meat	168* (37.8%)	139* (31.2%)	89 (20.0%)	26 (5.8%)	23 (5.2%)
How do you evaluate the packaging of the meat	118* (31.1%)	97* (25.5%)	89* (23.4%)	28 (7.4%)	48 (12.6%)
How do you evaluate the colour of the meat	187* (49.2%)	102* (26.8%)	71 (18.7%)	14 (3.7%)	6 (1.6%)
How do you evaluate the flavour of the meat	147* (38.7%)	73 (19.2%)	101* (26.6%)	41 (10.8%)	18 (4.7%)
How do you evaluate the tenderness of the meat	97* (25.5%)	114* (30.0%)	89* (23.4%)	44 (11.6%)	36 (9.5%)
How do you evaluate the presence of marbling in meat	79* (20.7%)	93* (24.4%)	104* (27.3%)	71 (18.6%)	34 (8.9%)

* higher observed number respect to expected (all categories equal).

DISCUSSION

This survey analyzed the behavior, frequency, preference, knowledge and attitudes of consumers towards meat quality. Based on surveyed consumer gender have a strong influence where male consume often meat than female. The majority of the people in the city of Tirana (52.1%) prefer to consume meat at least 2-3 time in a week. Beef meat was the first preference choose for (43.3%) the consumer from Tirana follow by pork 23,3% and chicken 13,3%. Pork and poultry meat is cheaper compered to beef meat and price can strongly affect consumer's decision during meat purchase. High correlation in incoming levels and types of meat types were noticed were people with higher income consume more fresh meat in front of people with lower income who consume more frozen meat (P value 0.030). Nevertheless, the income level doesn't influence the selection of the type of meat (P value 0.372).

This survey show that the majority of consumers prefer to bay their meat at village 45.0% and butcher shop 43.1 %, while only 8.2% prefer to buy their meat in supermarkets. Often consummators in Tirana prefers to bay meat products directly households in village or to their relatives in order to ensure quality, taste and safety. Buy meat directly at local vendors is part of traditional behavior not only for meat but also for other products. Especially consumers

believe that the quality of meat is higher from the mountainous clean environment where animals are left free in green pastures. Specialized local butchery was the second preferred place to buy meat. This may be related with the fact that consumers prefer to buy meat from the trusted place where they usually can develop relationship with the seller. Butchers and independent retailers are by far the most trusted source for information on the safety of meat (Becker et al., 2000).

Country of origin was the most important extrinsic attribute, while these studies point out the strong importance for the consumers to buy domestic meat. In fact, 72.1 % of the consumer's think that country of origin is either very important or important. A research from (Imami *et al* 2011 and Wongprawmas, et al 2018) found same results for the place of the origin in a study for the lamb meat in Central and Southwest urban Albania and Kosovo. Origin was the most important extrinsic attributes for five European regions (Bernues, 2003). Origin of meat has also been pointed out as an indicator of meat safety (Becker, 1999; Cowan, 1998; Latouche et al., 1998; De Andrade 2016). These study display that people with higher incomes prefer to bay meat directly on farms. Here, the high regard for this attribute seemed to be linked to the value of 'locality', or the 'consumer sense of belonging', as expressed by (De Cicco, Van der Lans, and Loseby 2001). The high request of consumers to bay domestic meat shows the opportunity for meat industry to become even more important, especially beef market shows great potential to expand even more.

Meat traceability was the second most valued extrinsic cues for 69% of the consumers in the city of Tirana. With respect to traceability characteristics, there is a distinction between functional and process attributes, the former referring to the intrinsic opportunities of the systems, i.e. the ability to organize the chain more efficiently, monitor the chain, and assess individual responsibilities (Gellynck, Xavier, and Wim Verbeke 2001). With the rapid development of information technology, the traceability systems increase important being integral part of labelling schemes. However, only 11 % of the consumers considered not important or extremely not important the traceability at the moment of the meat purchase. These percentages can be associated with insufficient knowledge or incredulity over the application of this system in the country. Almost 30% of the persons think that price was neither important nor unimportant at the moment of the purchase. The elevated percentage of the people with high income and education level of the interview persons can be the main factor in benefiting from these results. Actually, Tirana represent the most developed and rich city in Albania composed from comprehensive terms of population in incoming, education and religion. This phenomenon suggests that economic variables are becoming less suitable indicators in describing segments of consumers, whereas nationality, cultural level, age, place of residence, lifestyle, etc. are increasingly important (Dagevos & van Gaasbeek, 2001; Issanchou, 1996; Wandel & Bugge, 1996). Color was the most important intrinsic visual cues for the consumers in the city of Tirana where 49.2% think it is very important and 26.8% considered an important factor. The color of the fresh meat is of the utmost importance in meat marketing since it is the first quality attribute seen by the consumers who it is as indication of freshness and wholesomeness (Troy *et al* 2010). Consumers relate red–purple color with freshness and brown color with lack of freshness (Carpenter, Cornforth, & Whittier, 2001; Faustman & Cassens, 1990; Issanchou, 1996). For consumers colour is equal to meat quality, nevertheless these merit is not well related with meat quality. Colour preferences differentiate among consumers within the same country and is affected from the intensity and tonality of the color.

Food label is an important source of relevant information for consumers' meat buyer when evaluating different alternatives. Using label information can be considered as an important and effective instrument that influence consumer purchase behaviors and judge, before buy it

However, 43.4% show mistrust in the meat labeling as a process, while many persons think that meat labeling is easy to remove or adulterated. Falsified meat products represent a serious problem for many country and the scandal of horse meat in beef burgers is a clear indication. A lack of trust among consumers remains a significant issue for meat industry in Albania and impressive action will be necessary to increase the credibility in the minds of consumers. Meat flavor stems from lipids and water-soluble components. Although hundreds of volatile compounds have been identified in cooked meat, only a small subset of molecules have been proven to be of sensory significance (Cerny and Grosch, 1992,1993; Rochat et al., 2007). Flavour depends on intrinsic and extrinsic factors (i.e., species, genetics, sex, feeding regimen, and management practices) (Maughan & Martini, 2012; Melton, 1990). In these study 57,9 of the consumers classified flavor as very important or important attributes while 26.6% of consumers considered it as neither important nor unimportant. These high percentages may be associated with the fact consumer sensory attributes frequently are considered simultaneously without a completely segment regarding juiciness, tenderness, and flavor.

Only 45.1 % of the consumer prefer the presents of the fat in meat and these data indicate that consumers from Tirana have a poor preference for marbling. It must be acknowledged that consumers constantly don't have the accurate information regarding marbling which is often confused with subcutaneous and visceral fat. Marbling is the visible fat present in the interfascicular spaces of a muscle and the architecture of the muscle influences the pattern of fat deposition such that looseness of the fascicular organization generally parallels the quantity of interfascicular lipid present (Kauffman & Marsh, 1987). Tenderness, juiciness, mouthfeel, flavor development, and delivery are highly dependent on the level of marbling in meat (Corbin et al., 2015; Lorido et al., 2015; Frank et al., 2016a). Marbling fat contains more oleic acid and less stearic acid than subcutaneous adipose which effects not only the palatability of beef but also has a positive health aspect (Troy *et al* 2016).

CONCLUSIONS

The study defines red meat as a significant component for a balanced diet and beef meat was the first choice the most of the consumers. Individual meat consumption is a reflection of incoming level, education and physiological factors. The results of the survey determined that country of origin and color were the most important cues for consumers in the city of Tirana, while shopping meat. In this context, the national brand of meat has a great potential to increase rapidly in future. Therefore, these results should be considered from policy makers for incentive helping the development of the meat industry as an important source of profit.

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CORRELATION BETWEEN THE MULTIFRACTAL STRUCTURE AND CRYSTALLINE PROPERTIES OF SYNTHESIZED BI DOPED ZNO NANOPOWDERS.

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ABSTRACT

Semiconductor oxides have received growing interest in recent decades, for their application in several fields including the manufacture of varistor devices. In this paper we investigate one of the best materials to obtain excellent varistor device, it is ZnO material. To do this pure and Bi doped ZnO nanopowders have been synthesized at different sintering temperatures by a soft chemistry method, the sol-gel route.

Investigations were made by XRD and AFM to determine structural properties and morphological grainsizes of Bi phases. The electrical characteristics J(E) were measured to correlate these results with the varistor effect. XRD spectra confirmed the würtzite structure and the presence of many transition phases for each sintering temperatures. On the other hand AFM images allowed us to study the location of different phases, their morphology and the size of particles. Statistics on the distances between grains and the mean surface for each sintering temperature has been recorded.

Keywords: *Varistors, Sintering temperature, Transition phases.*

INTRODUCTION

ZnO presents several interesting performances in various applications such as piezoelectricity [1], electronics, optics and medicine. Such properties have already, permitted the use of ZnO in the fabrication of several devices, especially in solar cells to manufacture transparent conductive electrodes, and in opto-electronics to manifacture blue emitting diodes [2]. On the other hand, ZnO is the ideal substrate for the epitaxy of GaN layers [3]. But the notable properties of ZnO are its direct band gap: 3.37 eV at room temperature and high transparency in the 0.4–2 mm optical wavelength range. ZnO possesses also a high exciton bonding energy of 60 meV, which is much higher than the values of other wide band gap materials widely used, such as ZnSe (20 meV) and GaN (21 meV). This large exciton binding energy provides excitonic emission more efficiently even at high temperature. Zinc oxide (ZnO) is considered as one of the best of these materials to manufacture good varistors and to lead to promising applications [5]. The manufacture of good varistors using this material is therefore very suitable [6].The main objective of this study is to prepare ZnO nanopowders doped with 1%mol Bi for varistor applications and sintered at different temperatures by a sol-gel technique. The sol-gel technique has been used for its easy preparation of samples and low cost.

Experimental details

To synthesize undoped and Bi doped ZnO nanopowders, following steps were under taken; first, 0.15 M zinc acetate dehydrate [$Zn(CH_3COO)2,2H_2O$] and 0.25 M citric acid are dissolved in 100 ml of ethylene glycol and well mixed together, secondly, the obtained solution is stirred at 70 °C for 1 hour with the help of a magnetic stirrer to obtain a transparent gel. During stirring a small amounts of bismuth (1%mol) is added to the zinc solution. Finally, a very fine and thin powders are obtained after calcination at 500°C.

RESULTS

XRD analysis

The figure 1 shows the XRD spectra of pure ZnO and 1% mol Bi varistors sintered different temperatures for the 2θ values ranging from 20° to 160°. In addition, different phases appeared for each sintering temperature: ZnO phase, β-Bi_2O_3 liquid phase, α-Bi_2O_3, and δ-Bi_2O_3. Some works [7, 8] report that Bi_2O_3 system has at least four polymorphs α, β, γ, and δ. We note that γ-Bi_2O_3 phase exhibit a centered cubic structure which is obtained during the cooling phase of δ-Bi_2O_3. Moreover, W. Onreabroy et al. [8] have reported that, it is difficult to distinguish tetragonal β-Bi_2O_3 or cubic γ-Bi_2O_3 phases from respective isostructural ZnO–Bi_2O_3 compounds in this type of varistor.

The size of the grains is an important parameter to know their dimension scales. In the goal to calculate the grain size of the samples, the Debye-Scherer equation is used.

It can be said also that the grain size increases when the sintering temperature increases and reaches a maximum value of 42 nm at 800 °C (Table1). Then the grain size decreases for higher temperatures. So it is important to note that this size keeps always a nanometric dimension.

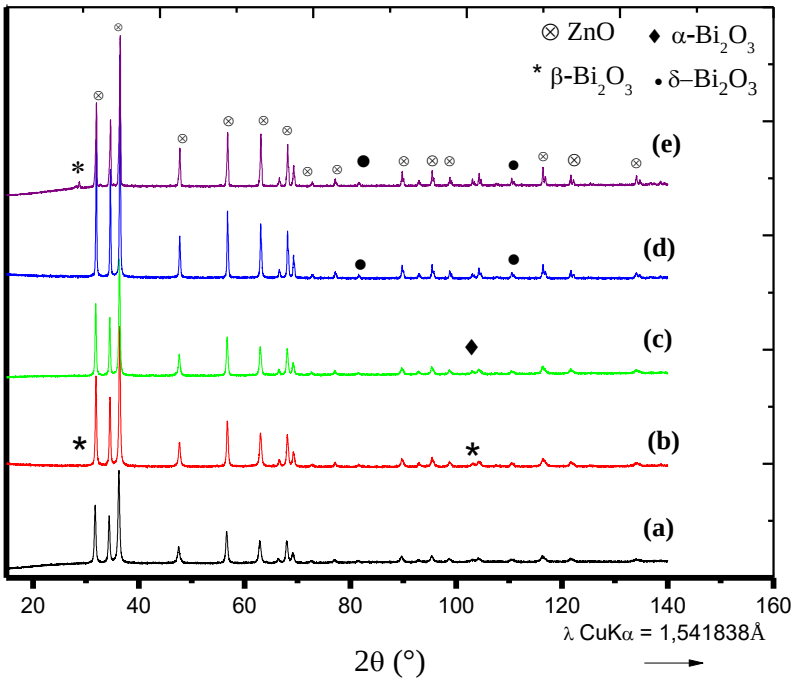

Fig.1 XRD patterns of pure and doped 1% mol Bi varistors sintered at different temperatures ((a) pure ZnO, (b) T=650°C, (c) T=730°C, (d) T=800°C and (e) T= 900°C).

Table 1 X Ray analysis of pure and sintered ZnO varistors

Sample (sintering temperature)	Structure	Detected phases	2θ position	(hkl)	β (rad)	D (nm)
Pure ZnO	Hexagonal	ZnO	32°-110°	(101)	0.00125	76
650 °C	Tetragonal	β-Bi_2O_3	90°	(101)	0.00234	58
730 °C	Monoclinic	α-Bi_2O_3	70-110°	(101)	0.00232	56
800 °C	Tetragonal	β-Bi_2O_3	75°-110°	(101)	0.00192	42
900 °C	Cubic	δ-Bi_2O_3	78°-112°	(101)	0.00218	51

AFM analysis

The figure 2 shows the two-dimensional (2D) AFM images of the ZnO varistor surfaces sintered at different temperatures. All samples exhibit a set of agglomerated grains distributed in a homogeneous and continuous surface without cracks. The images show clearly the presence of grain boundaries and secondary phases for the sintered samples. The characterization of surface samples can be highlighted with the topographic measurement. The surfaces can be quantitatively analyzed using an image analysis software (Gwyddion) to get different topographic informations about our sintered material.

The correlation of surface heights can be performed in terms of correlation functions. There are two forms of correlation functions, the autocorrelation function (ACF) and the height–height correlation function (HHCF) [9]. The grain size distribution is measured from several AFM images and the statistics is analyzed. The Height-Height Correlation Function (HHCF) of the AFM images can provide insight into the growth mode and the distribution of grains. The average height differences between any two points of the AFM image separated by a distance r are described by the function g(r). The HHCF can be modelized by:

$$g(r) = 2\sigma^2(1 - e^{-\left(\frac{r}{\xi}\right)^{2\alpha}})$$

where σ is the root mean square surface roughness, α is the Hurst parameter, and ξ is the correlation length. The Hurst parameter measures the short-range roughness and describes the grain surface structure. The resulting lateral correlation coefficients ξ is a measure for the surface domain size analysis. This coefficient was averaged to obtain statistically the relevant information. α can be connected to the fractal dimension of the surface with D = 3 - α. For α close to 1 the surface is smooth.

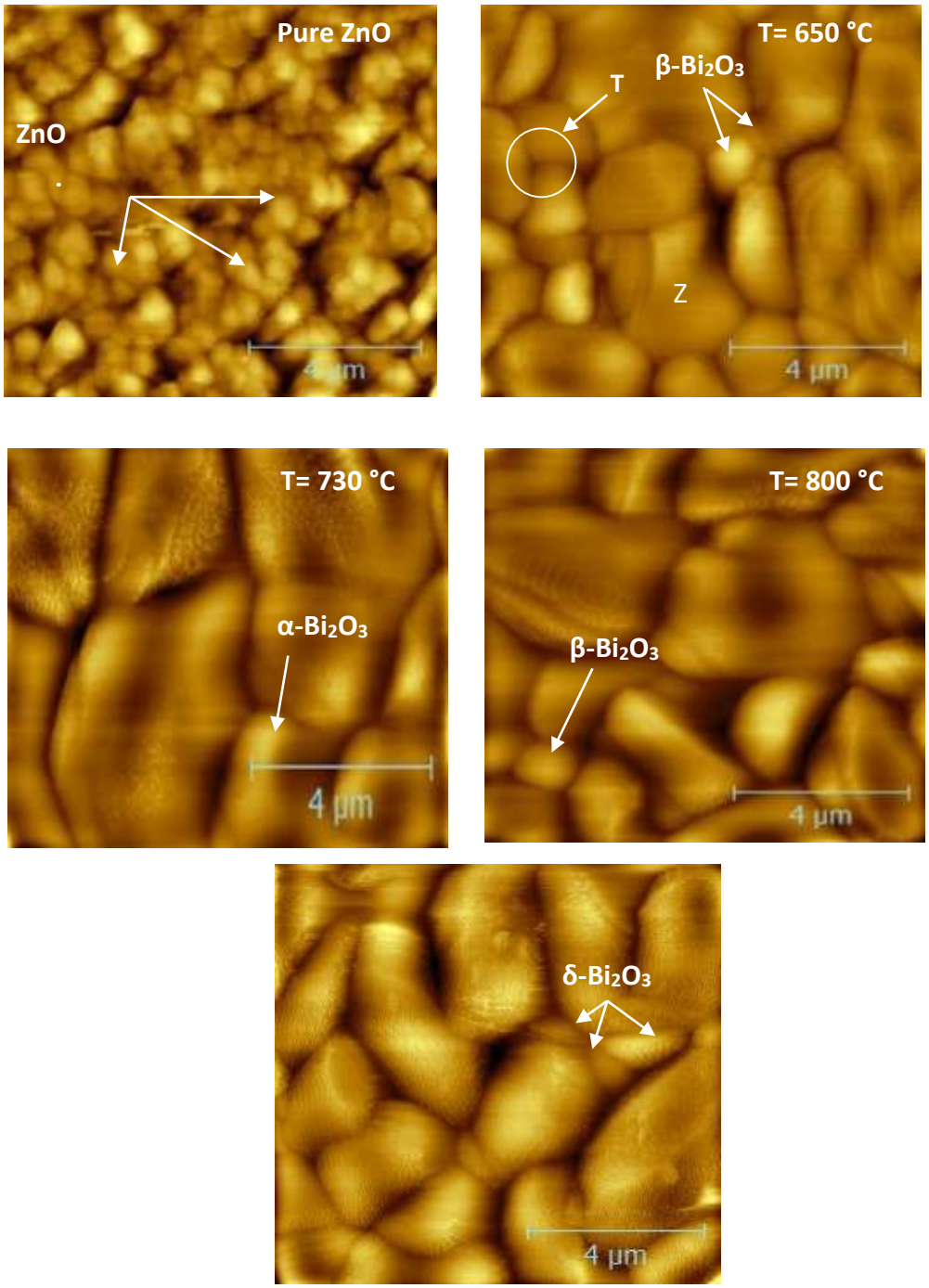

Fig.2 Surface topographies of the ZnO varistor surfaces for different sintering temperatures.

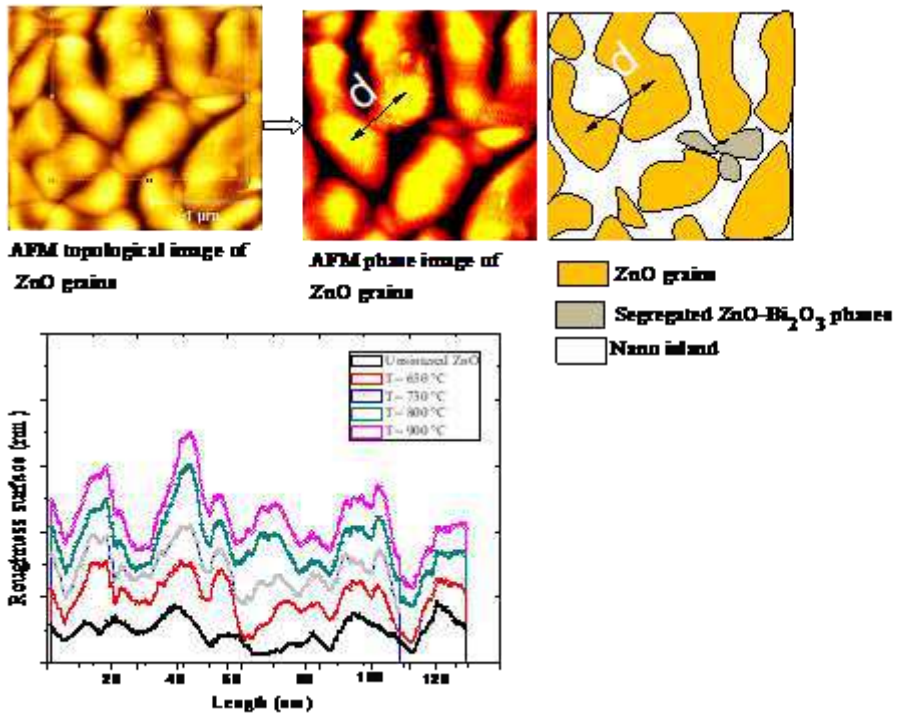

Fig.3 . Distribution of surface roughness.

The growth of grains formed the sample surfaces and contains inserted spaces between them as shown in Fig.4. The formation of nano-island structures observed in the images of the sintered varistors indicate that there is no change in the growth orientation of grains and transition phases during the sintering process. This result confirms the homogeneity of the structures.

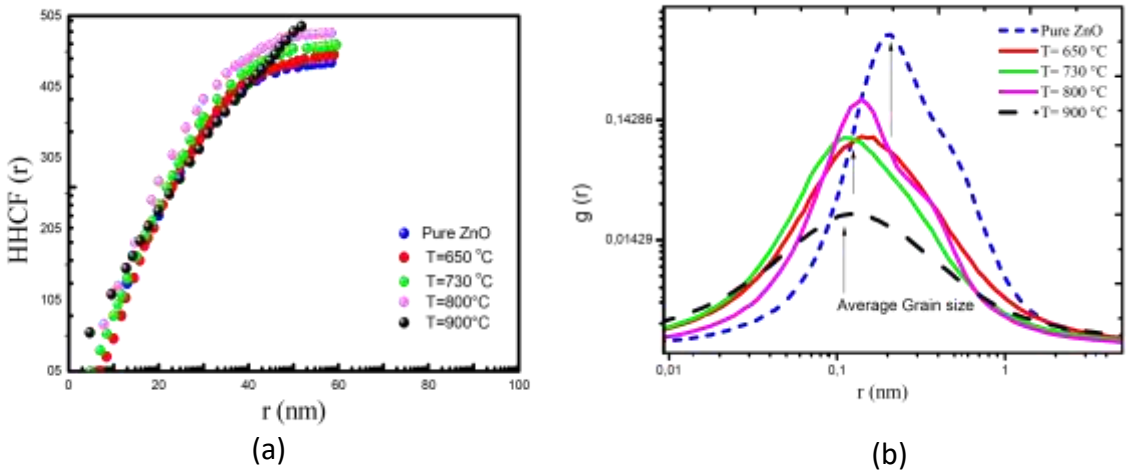

Fig.4. a) Height-height correlation functions of unsintered and sintered ZnO varistors, b) Correlation functions from simulated rough profile including a grain size of the sintered ZnO varistors.

Table .2 Calculated σ, α, ξ D, d and Average area of the sintered samples at different sintering temperatures

	Samples				
	Pure ZnO	T= 650 °C	T= 730 °C	T= 800 °C	T= 900 °C
σ (nm)	98	245	216	186	128
α	0.635	0.652	0.976	0.835	0.983
ξ (nm)	23	92	83	79	52
D (nm)	2.36	2.89	2.02	2.16	2.017
Average area (nm^2)	523	425	123	128	625
d (nm)	10	32	20	40	75

The calculated average surface roughness (σ) of pure ZnO is small for pure ZnO. It becomes more important for the sintered samples. Its value is in the range between 245 nm for the ZnO varistor sintered at 650 °C to 128 nm for the varistor sintered at 900 °C and decreases with the temperature. The height fluctuations of the AFM topographies occur at different length scales. In consequence, the frequencies of the height fluctuations, which is the Hurt parameter, provides a quantitative and unique measure of the surface roughness. This parameter quantifies the relative tendency of a height fluctuations along a line either to regress strongly to the mean value or to cluster in a direction. The Hurt parameter has values between 0 and 1. Values closer to zero indicate a jagged, rough surface whereas values close to 1 indicate a smooth surface. One can observe that the fitted Hurst parameter value (α) is close to 1 for T = 900 °C and increases from 0.635 to 0.983 nm when the temperature is increased from 650 °C to 900 °C, as it is summarized in the table 2. The analyzes also showed that for a pure ZnO sample, the larger grains are associated with a low surface roughness, a low value of the Hurst coefficient than the sintered samples (Fig.4a). However, it is important to note that, according to α values, the surface of the sintered varistors becomes more and more smooth and uniform as the temperature increases as it is shown in the figure 5 and the values of the Hurst coefficient tend to 1. In this case we can say that ZnO matrix grains contained crystallites that could be indexed to the different transition phases of ZnO-Bi_2O_3 binary varistors. As we observe, the cross sectional micrograph of Fig.4, the ZnO varistor sintered at 730 °C shows that the surface has less dense grain structures. The surface morphology of our varistors therefore strongly depends on the temperature. Numerous profiles of random size (drawn from a Gaussian distribution fitting curve) are joined. The pair correlation function of such a profile is calculated, and the size of the profile is then defined as the grain size in Log-Log scale as shown in Fig. 4b. On the other hand, the correlation length of the samples (ξ) as a function of the sintered samples (92 nm, 83 nm, 83 nm,79 nm and 52 nm for the sintered samples at 650 °C, 730 °C, 800 °C and 900 °C, respectively). The explanation of these results may be due to the decreasing of the grain size with increasing temperatures.It is indeed observed that the size of the grains decreases with the increase of temperature, which appears as a catalyst for the growth of the grains, we can say that the transition phases comes to be placed at the grain boundaries causing the compression of these, or that the appearance of these phases slow the germination and the growth of the grains and consequently their sizes [10].

Statistics on the distances between grains and the mean surface for each sintering temperature can also be measured through image analysis and data processing. It is known that the maximum peak to valley height roughness is the vertical distance between the highest and lowest points in the evaluated length/area and describes the overall roughness of the surface as reported by B.R. Kumar et al. [11], so we can determine the distance between grains from Figure 3. As shown Tab.2, the distance between grains increases with increasing temperatures and reaches

its highest value at nearly 75 nm at 900 °C sintered ZnO varistor while the lowest is obtained for pure ZnO, we deduce that the grains growth are actually made up of agglomerates in the presence of transition phases of: α, β, and δ held together in the ZnO-Bi_2O_3 binary varistors during sintering process. They diffuse and occupy the exact site in the crystal lattice and grains in the surface. In addition, a significant difference occurs in the average surface for pure and sintered ZnO varistors, where the sintering varistor at 900 °C exhibits a higher value of average surface, this is probably due to the rapid growth of the secondary transformed phases of δ-Bi_2O_3 at grain boundary, another possibility is that the high temperature can stimulate the migration of grain boundaries of our varistors and cause the coalescence of more grains during the annealing processes as confirmed by Lin et al. [12].

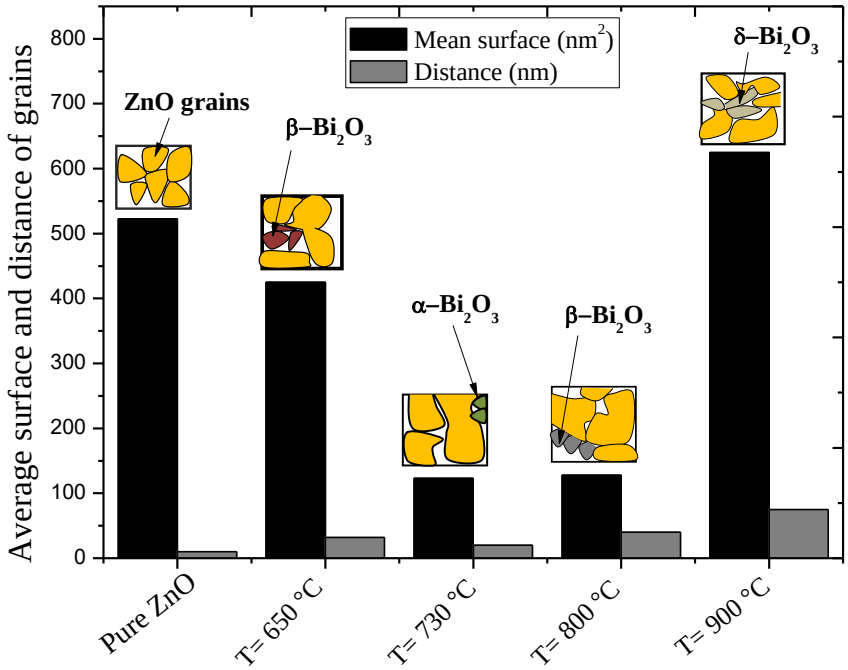

Fig.5 Average surface and distance between grains as a function of temperatures

CONCLUSIONS

Pure and 1%Bi doped zinc oxide nanopowders have been synthesized by sol–gel route. This is a simple and inexpensive method permitting to obtain a very small grain size powders. The obtained powders have been characterized by means of XRD and AFM to determine, respectively, their structural and morphological characteristics. All the obtained powders follow the würtzite structure .These powders are constituted by very small grains. The AFM images show the formation of nano-island .

The well-defined obtained morphology and the different nanostructures (hexagonal, tetragonal, monoclinic and cubic) in our study are important and present a better activity for the manufacturing of varistor application.

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BACILLUS RHIZOBACTERIA ASSOCIATED TO *PHOENIX DACTYLIFERA* OF HYPER-ARID AND SALINE AREA IN ALGERIA, PROMOTE TWO COWPEA PLANTS GROWTH

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ABSTRACT

The objective of this study was to determine the diversity of Plant growth promoting rhizobacteria (PGPR) of the genus *Bacillus* that produce extracellular hydrolytic enzymes and the effect of their inoculation in two ecotypes of cowpea from southern Algeria. The decimal soil dilution technique was carried out on tryptic soy agar preceded by heat pre-treatment to select *Bacillus* strains associated with the rhizosphere of *Phoenix dactylifera* growing in hypersaline and arid soils of Algeria (In Salah, Tamanrasset). The activity of six extracellular bacterial enzymes and three PGP traits were explored. The effect of inoculation of these strains on the growth of cowpea plants was assessed on the basis of biometric and physiological parameters. In this regard, 13 strains of halophilic *Bacillus* were isolated. These bacteria are able to grow optimally in media containing 3-15% salts and in one case up to 20-25% salts. These strains have been identified on the basis of their phenotypic characteristics as well as members of the genus Bacillus. Halophilic rhizobacteria are a source of hydrolytic enzymes such as amylase, cellulase, caseinase, lipase, lecithinase, gelatinase and urease. Inoculation of these strains into cowpeas has shown positive results on growth. Based on these results, we conclude that halophilic and halotolerant *Bacillus* strains belong to the rhizocompetent bacterial community associated with date palm in saline and arid soils. *Bacillus* PGPRs have been shown to be very effective strains for improving the growth and development of the cowpea plant.

Key-words. *Bacillus, rhizosphere, cowpea, phoenix dactylifera, salinity*

INTRODUCTION

Salinity is a natural feature of ecosystems in arid and semi-arid regions due to very low rainfall but can also be induced by irrigation with saline water. The salinity effect is always more pronounced in the rhizosphere due to increased water absorption by plants as a result of transpiration (Ibekwe et al., 2010), where different halophilic and halotolerant bacterial species can be found. Moreover, the rhizosphere is a narrow ground area adjacent to the plants (inside and around the roots) where high microbial activity is characterized (Sorensen, 1997; Kennedy, 1999) which simulates plant growth (Kloepper and Beauchamp, 1992; Boehm et al., 1993).

Soil salinity is a major factor in microbial selection because salt can reduce bacterial diversity. Therefore, Donn Kushner (1989) is defined the most commonly categories used: (i) extreme halophiles (optimal growth in salt concentrations of 2.5 to 5.2 M), (ii) extreme limit halophiles (optimal growth in salt concentrations of 1.5 to 4.0 M), (iii) moderate halophiles (optimal growth in salt concentrations of 0.5 to 2.5 M), and (iiii) halotolerant microorganisms that do not have an absolute need for salt for growth, but tolerate many salt concentrations that are often very high (considered extremely halotolerant if the growth interval extends above 2.5 M salt). In this perspective, different ecological niches have been explored for the isolation and characterization of halophilic and halotolerant bacteria which include the rhizospheric soil of different cultivated plants. In fact, halophilic bacteria are a heterogeneous physiological group of microorganisms belonging to different genera and capable of developing optimally in media containing a wide range of NaCl (3-15 %) (Ventosa et al. 1998). In addition, saline environments support taxonomically diverse bacterial groups, which have modified physiological and structural characteristics under prevailing saline conditions. The majority of these bacteria can synthesize compatible specific organic osmolytes such as glutamine, proline and glycine betaine and some of them accumulate inorganic solutes such as Na^+, K^+ and Mg^{2+}, in order to osmo-regulate themselves (Zahran, 1997). Moreover, rhizobacteria can produce several compounds including enzymes, nitric oxide, osmolytes, siderophors, organic acids and antibiotics (Dimkpa et al., 2009). In addition, halophilic or halotolerant non-pathogenic rhizobacteria can increase plant growth under aridity stress factors including salinity. Inoculation with Plant growth promoting rhizobacteria is the biological way to replace the use of chemical fertilizers, pesticides in agriculture practices (Benaissa, 2019).

The date palm (*Phoenix dactylifera*) is considered as a xerophytic and halophilic species at the same time or the conditions of difficult aridity do not pose a problem for its cultivation. Therefore, The study of the rhizobacterial community associated with plants naturally adapted to cope with extreme saline conditions could lead to several knowledge outputs: (i) understanding the plant-microbe interaction under saline conditions, (ii) defining the mechanisms underlying plant growth with promotion under saline stress, and (iii) identifying bacterial strains to design organic fertilizers for agriculture practice in arid and saline lands (Mapelli et al., 2013).

To the best of our knowledge, there are no studies conducted on the halophilic bacteria isolated from arid and saline soil of Algeria, associated with *Phoenix dactylofiera* rhizosphere. We hypothesize, that *Bacillus* group of halophilic and halotolerant rhizobacteria can have a potential to enhance plant growth and to cope with aridity conditions. Therefore, the main objectives of this study were to characterize halophilic and halotolerant *Bacillus* group of rhizobacteria with hydrolase activities and Plant Growth traits on inoculation of two cowpea plants.

MATERIAL AND METHODS

1.1.Sample site and collection

The soil samples are randomly collected from three sites of palm grove located in arid area of Algeria (In Salah) (27° 11′ 55,69″ North, 2° 26′ 45,29″ East) during December 2018 (Figure 1). The soil texture and physicochemical analysis (Table 1) were performed as described previously (Mathieu & Pieltain, 2003; Mathieu & Pieltain, 1998). Soil Classification (WRB): Soil is classified in the Group of Arid Hapylic Solonchaks (FAO, 2006). The first few centimeters of the soil top layer are removed with a sterile spatula, about 50 g of soil is collected in a sterile container from a depth of about 15 cm and transported to the laboratory in an ice box set at 4 °C.

Figure 1. Geographical location of the palm grove in the In Salah region (Algeria, Google Earth, 2020) - *Situation géographique de la palmerie de la région d'In Salah (Algérie, Google Earth, 2020)*

Table 1. Texture and physicochemical parameters of soil research - *Texture et paramètres physico-chimiques du sol de la recherche*

Parameters	Results
pH	7.13
EC $_{1:5}$ (ds/m)	2.6
OM %	0.1
Calcium Carbonate $CaCO_3$	0%
Texture (Particle-Size)	Sandy loam

1.2.Isolation of halophilic and halotolerant *Bacillus* strains from the rhizospheric soil

Soil dilution plate technique was performed on Tryptic Soy Agar complemented with cycloheximide (40 mg/ml) and nalidix acid (10 mg/L) and supplemented with NaCL (0 %, 15 %) to allow isolation of halotolerant or/and halophilic bacteria. To increase the selectivity of the *Bacillus* isolation medium, a thermal pretreatment (10 mn at 80° C) of soil dilution is carried out, in order to select bacterial spores and eliminate all vegetative forms.

Therefore, to find out if the strains that are grown on the initial 15 % NaCl medium are halophilic or halotolerant, the Nutrient Broth medium (NB) was used, adjusted to NaCl concentrations (0 %, 3 %, 20 %, 25 % and 30 %). Halotolerant strains are considered if they are grown on both media (0 and 15 % NaCl), moderate halophilic strains are those grown only on the medium at (3 %-15 %) of NaCl, while extreme halophilic strains are grown on media at (15 %-30 %) of NaCl (Table 2).

Table 2. Types of microorganisms by Kushner (1978) - *Types de microorganismes selon Kushner (1978*)

Microorganism	Optimum NaCl concentration
Non-halophilic	< 1.17 %
Weak halophilic	1.17-2.93 %
Moderately halophilic	2.93-14.63 %
Extremely halophilic	14.63-30.45 %
Halotolerant	1.17-30.45 %

1.3.Screening for hydrolytic activity

The activity of six extracellular enzymes was investigated under non-saline and saline (5 % NaCI) conditions. The hydrolysis test of gelatin was performed as described by Egamberdiyeva

(2004) modified, using a nutritient broth supplemented with 50 g/l gelatin powder as a solidifying agent. Ureolytic activity is revealed on Christensen urea agar medium (peptone 1 g, dextrose 1 g, sodium chloride 5 g, potassium phosphate monobasic 2 g, urea 20 g, phenol red 0.012 g, agar 15 g). Casein hydrolysis is tested on Mueller Hinton agar (MH) supplemented with 10% skimmed milk (Castro-Escarpulli et al., 2003) and amylolytic activity was detected on Tryptic soy agar (TSA 1/10) with 1% starch added (Delarras, 2014). Cellulose hydrolysis is demonstrated on agar medium with crushed pulp as a source of cellulose (Lesel et al., 1985). Lecithinase was revealed on an ordinary nutrient agar supplemented by an emulsion of egg yolk and distilled water (2 ml / 20 ml) (Delarras, 2007). All media recommended for the study of exo-enzyme production were modified by adding NaCl (10 % for halotolerant strains and 20 % for halophilic strains).

1.4.Screening for Plant Growth promoting activities

Molecular nitrogen fixation is tested on the Jensen medium, a free nitrogen medium. Growth on this medium after being transferred ten times in the same medium reflects the ability of bacteria to fix nitrogen (Haahtela et al., 1983). The ability of strains to produce hydrocyanic acid (HCN) is achieved by the Lorck method (1948). The qualitative solubilization activity of phosphate has been tested on the NBRIP (National Botanical Research Institutes Phosphate) medium (Nautiyal, 1999).

1.5.Phenotypical characterization of halophilic isolates

The *Bacillus* strains were phenotypically characterized using standards procedures of Gram © and spore position and physiological tests as catalase and oxidase complemented with several biochemical tests as respiratory type, Voges-Prauskeur, Methyl red, Arginine Di-hydrolase, Ornithin Decarboxylase and sugars' assimilation (mannitol, glucose, fructose and saccharose). All the strains were preserved in nutrient broth added with 20 % glycerol at -80° C.

1.6.Assay of *Bacillus* isolates on cowpea plants growth

The seeds of two cowpea ecotypes (P10 and P14) from Tamanrasset region (Table 3), were surface sterilized by brief rinsing in 95 % ethanol, then rinsed with distilled water for 5 minutes three times. Then, the seeds were evenly distributed over the surface of a sterile absorbent paper in petri dishes. The boxes were sealed with film paper and incubated in an oven set at 28° C. The seedlings with a full widened cotyledons were selected to be inoculated. Then, 24-hour cultures of *Bacillus* inoculants were first prepared by growing a colony in 10 mL of nutritious broth.

Table 3. Geographic characteristics of two cowpea ecotypes selected for inoculation - *Caractéristiques géographiques de deux écotypes de niébé sélectionnés pour l'inoculation.*

Ecotype	Origin	Longitude	Latitude	Altitude (m)
P10	Igléne (Abalessa)	4°5E	22°53N	630
P14	Tit (Tamanrasset)	5°14E	22°58N	1174

Thus, the germinated seeds were misled in bacterial cultures for about 30 minutes. Three inoculated seedlings were directly implanted into a soil previously sterilized at 120° C/ 2 h. The pots were then kept under light with a 16-hour photoperiod under laboratory conditions. Plant growth was monitored and compared to controls grown without cultured bacteria. For this purpose, biometric parameters are measured and photosynthetic pigments concentrations were determined according to Lichtenthaller (1987), and the relative water content of the leaf was determined by the method described by Barrs (1968).

1.7.Statistical analysis

The data was subjected to statistical analysis using the Microsoft Excel 2010 program. All values are the mean ± ES (standard error) of three replicates of a single sample for each experiment. The obtained data have been submitted to Unidirectional analysis of variance (ANOVA) using the Statistical Analysis System (XLSTAT) version 2016. 02. and differences between individual means were considered significant at P <0, 05. The averages are compared by the LCD test. A Principal Component Analysis (PCA) are also established.

RESULTS

In this study, we focused on the diversity of halophilic and halotolerant *Bacillus* species of *Phoenix dactylofira's* rhizosphere and the evaluation of their hydrolytic, PGP and application potentials.

1.8.Bacteria characterization

Thirteen strains of *Bacillus* genera have been isolated in the rhizosphere of date palm (RP 1 to RP 13). The results of the salt tolerance properties revealed that among the 13 bacterial isolates tested, only four isolates could not grow in the presence of 3 % NaCl (Figure 1). Thus, only one species is classified as moderately halophilic bacteria (Table 5). 61,53 % (8/13) of isolates that may have developed on media added between 5 and 15 % salt, but have optimal growth on normal media with only 0.9 % NaCl, are considered as halotolerant strains, a significant difference with moderately halophilic bacteria according to the classification proposed by Kushner (1985).

Morphological and chemotaxonomic analyses exposed that all isolates are rod-shaped, Gram positive, aerobic, sporulating, catalase positive and oxidase variable (Table 4). Then, all isolates were identified as members of the genera *Bacillus*. Halophilic, non-halophilic or halotolerant strains of *Bacillus* (n=13) isolated from the date palm rhizosphere represent an infinite fraction of the soil microbial community.

All isolates reveal a combination of hydrolytic activities under normal growing conditions (0.9 % NaCl). It appears that almost all strains had the six hydrolytic activities tested (amylase, cellulase, lipase, protease and lecithinase). At the same time, in the presence of salt (15 % NaCl), the enzymatic activities decrease in particular those of urease, amylase and cellulase (Table 4).

Table 5. Types and Plant Growth Promoting effects of *Bacillus* strains from *Phoenix dactylifera's* rhizosphere - *Types et effets de promotion de la croissance des plantes des souches Bacillus de la rhizosphère de Phoenix dactylifera*

Code	Phosphate solubilization	HCN production	Nitrogen fixation	Type
RP1	+	+	+	Halotolerant
RP2	+	+	+	Non-halophilic
RP3	+	-	+	Halotolerant
RP4	+	+	+	Non-halophilic
RP5	-	-	+	Halotolerant
RP6	+	-	+	Halotolerant
RP7	-	-	+	Halotolerant
RP8	+	+	-	Halotolerant
RP9	+	+	+	Moderately Halophilic
RP10	+	-	+	Non-halophilic
RP11	+	-	-	Non-halophilic
RP12	-	+	+	Halotolerant
RP13	-	+	+	Halotolerant

Table 4. Morphological and biochemical characterization of *Bacillus* isolates associated to *Phoenix dactylifera*'s rhizosphere growing in hyper-arid area of Algeria - *Caractérisation morphologique et biochimique des isolats de Bacillus associés à la rhizosphère de Phoenix dactylifera poussant dans la zone hyper-aride d'Algérie*

Code / Tests	RP1	RP2	RP3	RP4	RP5	RP6	RP7	RP8	RP9	RP10	RP11	RP12	RP13
Form	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Grouping mode	chain	chain	chain	chain	chain	chain	chain	chain	chain	chain	chain	chain	chain
Gram	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Spore position	TD	CD	CD	CND	CND	TND	CD	TD	CND	CD	TND	TD	CND
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxydase	+ve	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
Respiratory type	ANAF	ANAF	AS	ANAF	ANAF	ANAF	ANAF	ANAF	ANAF	ANAF	AS	ANAF	ANAF
Voges-Prauskeur	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Methyl red	-ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Arginine Di-hydrolase	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Ornithin Decarboxylase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Sugar assimilation:													
-Mannitol	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Fructose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Saccharose	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Hydrolytic activities in presence of 0.9 % NaCl:													
-Urease	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
-Gelatinase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
-Caseinase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Amylase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Cellulase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Lecithinase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Hydrolytic activities in presence of 15 % NaCl :													
-Urease	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve
-Gelatinase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
-Caseinase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

-Amylase	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
-Cellulase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
-Lecithinase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

DT: deformant terminal, TND: terminal non-deformant, CD: central deformant, CND: central non-deformant, ANAF: aero-anaerobic facultative, AS: aerobic strict

1.9. *Bacillus* strains improves cowpea growth

In order to study the in vitro effects of *Bacillus* isolates on two varieties of cowpea, different biometric, physiological and biochemical parameters were evaluated of inoculated and non-inoculated plants (RP 0: control) grown in soil. On the 15 th day after being inoculated under laboratory conditions, cowpea seedlings were observed and photographed to identify the best growth plants (Figure 2).

*Bacillu*s isolates shown to increase plants growth of both cowpea varieties studied after inoculation process (Figure 1). For example, the two-cowpea plants inoculated with RP 7, RP 8, RP 10 and RP 12 grow better compared to only 30-40 % of controls (Table 5). The analysis of variance also indicated that each of the biometric and physiological parameters recorded a highly significant difference from one strain to another for the two accessions studied (Figure 3).

Root and stem lengths were measured in the absence and presence of inoculation. Table 3 shows that the stem sizes in the control are 8 cm and 10 cm in P14 and P10 respectively to a maximum of 10 cm in inoculated seedlings. Concerning the results obtained for the root length part, it is noted that the plants inoculated with the majority of strains are smaller compared to the control. On the other hand, the fresh weights of shoot and root appear to be greater than the control (Table 6) only for a few strains (RP 1 to RP 7) for ecotype P14 and strains RP 4 to RP 13 for ecotype P10.

The relative water content does not seem to be higher in inoculated plants in the majority of cases (Table 6), although it is more interesting with RP 9 and RP 8 strains in P14 and P10 respectively. The carotenoid content is higher in inoculated plants in the majority of cases even if it is more interesting with RP 5, 6, 7, 8 and RP 12 strains in P14 and all strains in P10. However, it is interesting to note that the total chlorophyll content is higher in plants inoculated by all strains.

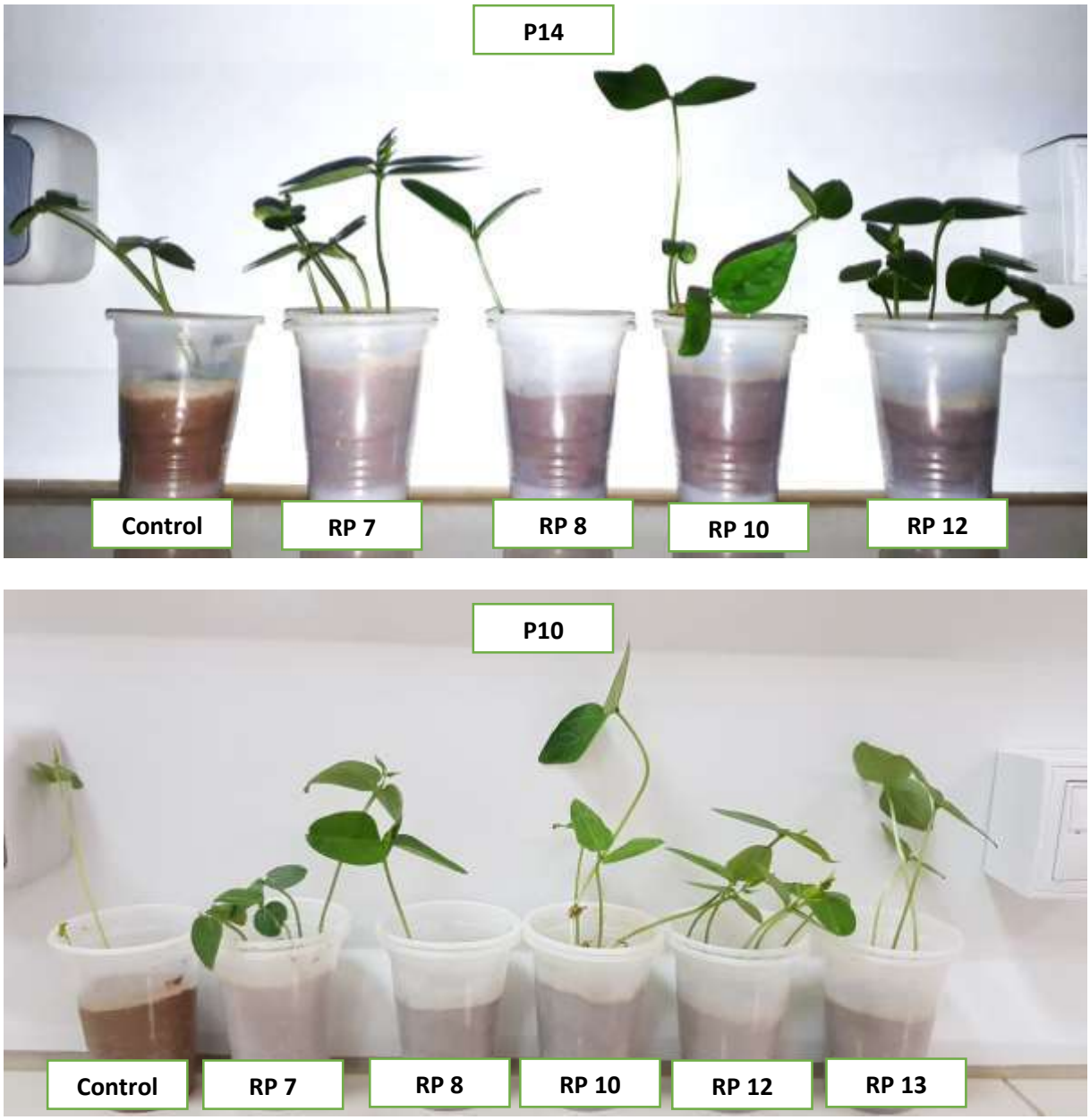


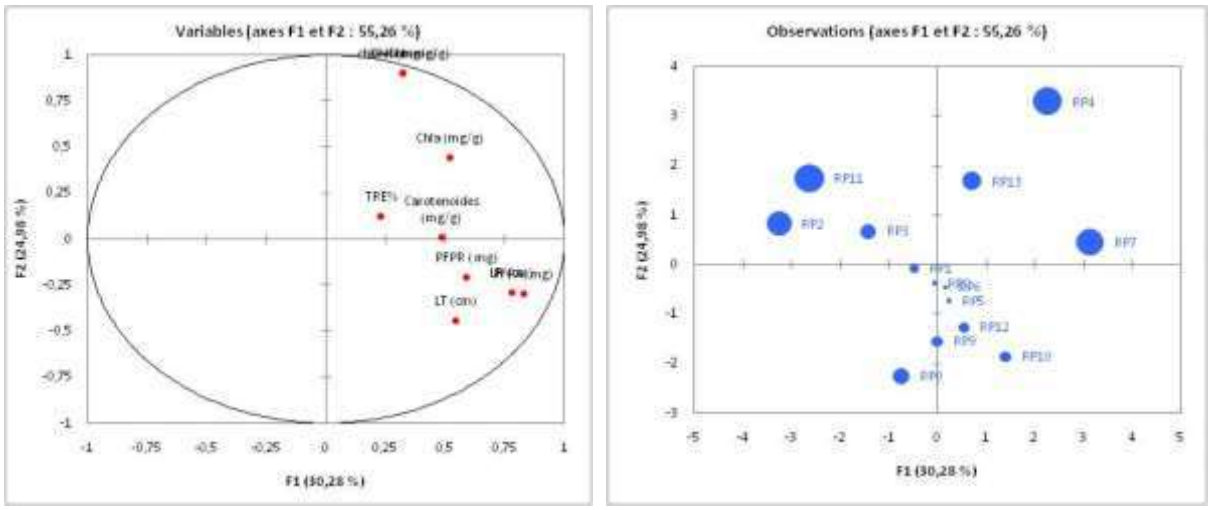
Figure 2. Inoculation effect of high-performant *Bacillus* strains (RP 7, RP 8, RP 10, RP 12 and RP 13) on the growth of the two-cowpea ecotypes (P14 and P10) from Tamanrasset region (south Algeria). Control plants showed less growth than inoculated ones - *Effet d'inoculation de souches de Bacillus très performantes (RP 7, RP 8, RP 10, RP 12 et RP 13) sur la croissance des deux écotypes de niébé (P14 et P10) de la région de Tamanrasset (sud de l'Algérie). Les plantes témoins ont montré une croissance inférieure à celle des plantes inoculées.*

Table 6. Effect of *Bacillus* PGPRs inoculation on stem and root length, fresh weight of shoot and root, photosynthetic pigments and relative water content of cowpea plants in laboratory experiment - *Effet de l'inoculation de Bacillus PGPRs sur la longueur des tiges et des racines, le poids frais des pousses et des racines, les pigments photosynthétiques et la teneur relative en eau des plants de niébé dans une expérience de laboratoire.*

Accessions	Strains	FWSP (mg)	FWRP (mg)	SL (cm)	RL (cm)	RWC %	Chla (mg/g)	Chlb (mg/g)	Chla+Chb(mg/g)	Caroten
P10	RP0 (control)	209± 91cd	112,33± 28,5de	8,03±1,51abc	5,73±1,02ab	52,07±13,71ab	0,206±0,69b	0,244±0,28de	0,795±0,03e	0,200±0,
	RP1	327± 39b	327± 15,94a	8,13±1,24abc	5,23±0,64abc	53,58±18,80ab	0,456±0,19ab	0,24±0,1de	0,687±0,18e	0,456±0,
	RP2	385,33± 29ab	257,66±15,13 b	9,36±2,53ab	6,06±1,40abc	50,81±15,65ab	0,477±0,22ab	0,498±0,42cde	0,777±0,12e	0,150±0,
	RP3	298± 0,57bc	124,33± 13,65cde	7,56±1,09abc	4,33±0,63abcd	48,38±10,32ab	0,456±0,13ab	0,095±0,13e	0,655±0,25e	0,184±0,
	RP4	311± 79,26b	168,33±19,62 c	6,96±1,47bc	4,46±0,89bcd	46,98±8,21ab	0,654±0,45a	1,11±0,26a	1,474±0,52abc	0,107±0,
	RP5	387± 1ab	174,66±28,29 c	_8,1±1,47abc	4,46±0,89bcd	48,01±5,71ab	0,368±0,05ab	0,225±0,07de	0,775±0,04e	0,240±0,
	RP6	470± 36,59a	131,66± 37,2cd	10±1,04a	3,46±0,45de	56,84±1,70ab	0,7±0,18a	0,430±0,55de	0,796±0,14e	0,328±0,
	RP7	433± 3,77a	122± 19,07cde	8,13±4,10abc	4,16±1,15cde	41,26±10,5b	0,616±0,39ab	0,165±0,13de	0,868±0,26de	0,371±0,
	RP8	76,33± 80,99e	76,33± 17,32e	9,23±1,87ab	3,40±0,17de	51,56±12,39ab	0,301±0,07ab	0,381±0,10cde	1,224±0,13bcd	0,240±0,
	RP9	104,66± 67,03e	104,33± 5,85de	10,03±0,90a	3,2±0,26e	59,96±17,61ab	0,309±0,1ab	0,526±0,07cde	1,309±0,18bc	0,192±0,
	RP10	84,33± 88,96E	84,33±4,61 E	7,63±0,41abc	3,33±0,37e	45,76±9,66ab	0,529±0,19ab	0,668±0,21bc	1,599±0,3ab	0,192±0,
	RP11	107,66± 62,44e	107,66± 19,85de	8,30±1,58abc	3,96+1cde	41,35±5,27b	0,323±0,21ab	0,983±0,22ab	1,758±0,05a	0,061±0,
	RP12	123,33± 29,77de	123,33±29,36cde	5,81±0,07c	3,63±0,8de	41,35±5,27b	0,498±0,14ab	0,423+0,01cde	1,262±0,09bc	0,253±0,
	RP13	84,33E± 20,55	84,33±16,25 E	7,43±1,56abc	3,5±0,6de	67,84±27,86a	0,563±0,05ab	0,550±0,10cd	1,200±0,34cd	0,121±0,
P14	RP0 (control)	255±61ef	237,66±12,01bcd	10,60±3,50a	5,6±0,9bcd	84,41±5,40abc	0,206±0,05ab	0,264±0,07bc	0,264±0,07e	0,049±0,
	RP1	255±42,67ef	193,33±20,55cde	8,33±0,95abcd	4,6±0,79cde	80,76±6,61bcd	0,456±015ab	0,667±0,18bc	0,667±0,29cde	0,203±0,
	RP2	122,33±63,7g	80±18,02f	4,3±1,47f	2,7±0,43ef	57,14±12,36d	0,456±0,25ab	0,565±0,64bc	0,565±0,30cde	0,180±0,
	RP3	259,33±43	80±18,02F	4,8±0,15ef	4,83±0,15cd	87,60±3,97abc	0,477±0,01ab	0,663bc	0,663±0,02cde	0,134±0,
	RP4	304±70 cdef	324,66±15,58ab	7,73±2abcde	5,66±1,4bcd	80,42±6,09bcd	0,654±0,12a	1,862±0,36a	1,862a	0,330±0,
	RP5	280±90,5def	396±1a	6,5±0,7cdef	6±0,5bc	89,04±2,72ab	0,368±0,03ab	0,570±0,07bc	0,570±0,15cde	0,143±0,
	RP6	204±34,1fg	325±27,9ab	5,4±0,52def	6,96±1,61ab	66,11±7,37bcd	0,700±0,03a	0,351±0,54bc	0,352±0,73e	0,228±0,
	RP7	461,66±32,18a	271,33±14,73bc	8,33±0,76abcd	8,33±0,55a	88,45±6,79ab	0,616±0,01ab	1,094±0,03abc	1,094±0,61b	0,325±0,
	RP8	306,66±40,41cdef	246±7,76bcd	7,33±1,15bcdef	5,4±±0,36bcd	110,65±10,16a	0,301±0,25ab	0,66±0,03bc	0,666±0,14cde	0,157±0,
	RP9	394,33±5,85abc	241±12,50bcd	9,9±3,51ab	4,9±0,96cd	57,62±11,23d	0,309ab	0,528±1,01bc	0,528±0,12de	0,133b
	RP10	423,66±4,61ab	174,33±15,50de	8,8±2,60abc	5,9±2,68bc	62,31±8,79cd	0,529±0,56ab	0,213±01,01c	0,213±0,57e	0,782±0,
	RP11	139,33±19,8g	137±9,29ef	4,8±2,08ef	2,53±0,45f	78,25±17,31bcd	0,323±0,06ab	1,014±0,03abc	1,014±0,21bcd	0,166±0,
	RP12	380,66±29,3abcd	380±16,52a	7,2±1,32bcdef	4,8±2,42cd	90±3,25ab	0,498±0,62ab	0,326±0,03bc	0,326±0,01e	0,156±0,
	RP13	344,33± 16bcde	181±29,53cde	8,43±2,25abcd	3,76±0,25def	89,17±1,92ab	0,563±0,18ab	1,232±0,69ab	1,232±0,03b	0,307±0,

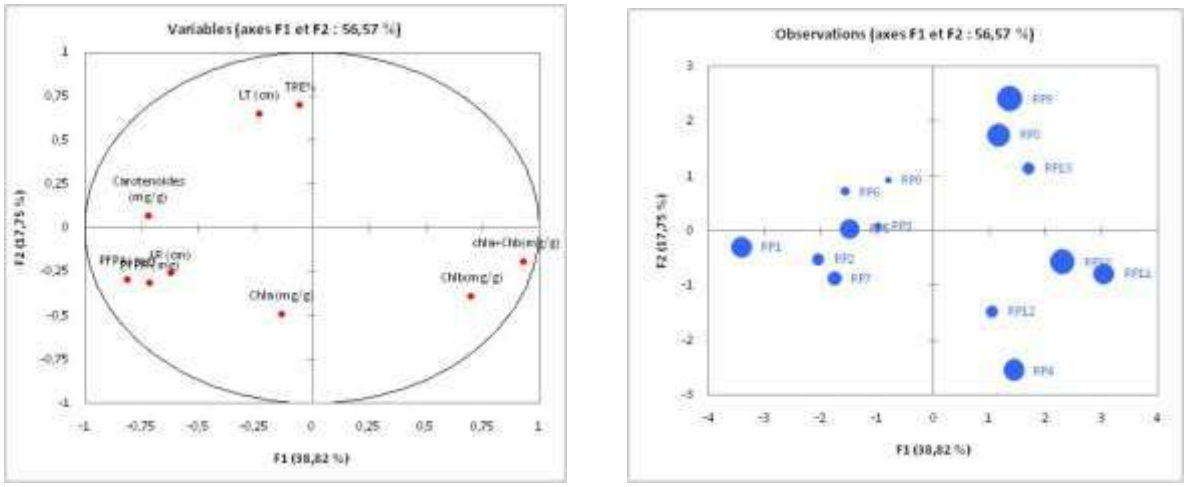
SL: stem length; **RL:** root length; **FWSP:** fresh weight of the air part; **FWRP:** fresh weight of the root part; **RWC:** relative water content; **Chl:** chlorophyll

A principal component analysis (P.C.A.) was performed for all strains. The plane formed by the first two factorial axes were shown a correlation circle (Figure 3). All the variables are well represented in this factorial plane since their correlations with the axes are relatively important (the projections are close to the correlation circle). The first two axes retained of the Principal Component Analysis alone explain nearly 55.26 % of the variability.



(a) **(b)**

Figure 3. Principal Component Analysis (PCA) [a: Correlation circle between variables, b: projection of PCA results on a factorial plane (F1 -F2)] of the inoculation effect of *Bacillus* strains on cowpea ecotype P10 growth - *Analyse en composantes principales (ACP) [a : Cercle de corrélation entre les variables, b : projection des résultats de l'ACP sur un plan factoriel (F1 -F2)] de l'effet d'inoculation des souches de Bacillus sur la croissance de l'écotype P10 du niébé.*



(a) **(b)**

Figure 4. Principal Component Analysis (PCA) [a: Correlation circle between variables, b: projection of PCA results on a factorial plane (F1 -F2)] of the inoculation effect of *Bacillus* strains on cowpea ecotype P14 growth - *Analyse en composantes principales (ACP) [a : Cercle de corrélation entre les variables, b : projection des résultats de l'ACP sur un plan factoriel (F1 -F2)] de l'effet d'inoculation des souches de Bacillus sur la croissance de l'écotype P14 du niébé.*

The individuals' projection on the factorial plane defined by axes 1 and 2 showed a fairly large distribution along the plane (Figure 3). Axis 1 accounted for 30.28% of the variation and associated the characters on the positve side: RWC, chlorophyll a, chlorophyll b, Chlorophyll a+b and carotenoids and FWAP, FWRP, RL, SL. Axis 2, which explains 24.98% of the variation, is not defined by FWAP ,

FWRP , RL , SL. Axis 1 isolated the following strains on the negative side: RP 6 and RP 1, RP 5, RP 2, RP 12, RP 9 and RP 10, and on the positive side by the strains RP 4 , RP 13.

Axis 2 is defined on the positive side by the strains RP 7 and RP 10. On the other hand, the axis negative part is defined much more by the strains RP 11, RP 3, RP 2.

The interpretation that can be made of this graph is the following: the first factorial axis seems to oppose the strains with the highest chlorophyll a , chlorophyll b , chlorophyll a +b. Comparatively, it is the RP 4 strain. The strain RP 7 give the highest carotenoid content seems to characterize the second factorial axis.

The correlations circle for the plane formed by the first two factorial axes is shown in figure 4, all the variables are well represented in this factorial plane since their correlations with the axes are relatively important (the projections are close to the correlations circle). The first two axes alone of the Principal Component Analysis explain nearly 56.7% of the variability; they have been retained.

The projection of individuals on the factorial plane defined by axes 1 and 2 that showed a fairly large distribution along the plane:

- Axis 1 isolated on the negative side the following strains: RP 12 and RP 4. On the positive side, the strains RP 13, RP 8, RP 9 and the control were isolated.
- Axis 2 is defined on the positive side by the RP 11 and RP 10 strains. On the other hand, the strains RP 7, RP 3, RP 1, RP 2, RP 5 and RP 6 defined much more the axis negative part.

The interpretation that can be made of this graph is the following: the first factorial axis seems to oppose the strains with high stem length and water content and very low chlorophyll content, comparatively to RP 11 strains. The second factorial axis is characterized by strains RP 11 and RP 1 respectively with: [high chlorophyll a and chlorophyll a +b content] and [fresh weight (FWAP, FWRR) and the highest carotenoid content].

DISCUSSION

We studied the distribution of halotolerant cultivable aerobic *Bacillus* trains in *Phoenix dactylifera's* rhizosphere growing in arid and saline soil from Algerian Sahara. The bacterial isolates were characterized through hydrolytic and PGP activities and inoculation potential. Moreover, isolated bacteria are likely to offer new opportunities for biotechnological applications in agro-ecological systems, especially in arid areas. This present work showed a high diversity among the *Bacillus* isolates.

Our ecological study in arid and hypersaline environments reveals a great diversity of moderately halophilic bacteria with hydrolysis potential of a fairly diversified organic material. In the same line, Rhizobacteria isolated from saline soils are able to grow at different salinity levels (Tripathi et al., 1998). Consequently, the growth of halotolerant microorganisms associated with plant roots would lead to better fertility in saline soils (Hallman et al., 1997).Therefore, the sporulation capacity of this bacterial genus promotes ubiquity on the one hand and survival in very diverse environments on the other. Surviving and evolving in environments with high salt concentrations is a real advantage in the rhizosphere, another reason for the interest of this study is the diversity of bacteria recognized mainly by their antibiotic production: *Bacillus* (Stabb et al., 1994; Jamil et al., 2007). The soil nature has an impact on the bacterial community diversity, indeed, the date palm rhizosphere soil in our study is hyperarid and saline, two unfavourable conditions for the growth of microorganisms in normal times. In recent years, research on halophilic and halotolerant bacteria of the genus *Bacillus* has exploded in different ecological niches and several new species have been discovered (Gugliandolo et al., 2003; Cerritos et al., 2008; Carrasco et al., 2007; Yumoto et al., 2005; Lim et al., 2006).

Enzymes from halophilic sources are expected to have optimal activity under extreme conditions. For this reason, the possibility of having a wide variety of moderate halophiles producing extremozymes will be of great help for biotechnological applications, particularly in the agricultural field. Thus, it would be very important to select enzyme-producing halophyte bacteria or these would have optimal activity at different salt concentration. However, our study showed that the enzymatic activity of our isolates decreases in the presence of 15% NaCl, which implies that salinity can pose a certain ecological challenge for production of certain extracellular enzymes. Indeed, some of the bacterial isolates were able to grow at 5 % NaCl but failed to express hydrolytic activity at the same level. The persistence of bacteria under saline conditions. Moreover, It has recently been shown that halotolerant bacteria of the genus *Bacillus* produce industrially important hydrolases and their enzymatic activities are more diverse.

Thus, Enzyme-active bacilli have already been isolated in saline soils (Zahran et al., 1992) or salt marshes (Weisser & Truper, 1985), with bacteria showing significant enzymatic activity under saline stress (10 % NaCl). In this study, most of the *Bacillu*s isolates were able of producing hydrolysis enzymes. The genus *Bacillus* is well known as an enzyme producer. Many industrial processes use species belonging to this genus for the commercial production of enzymes (McConnell et al., 1986). It is interesting to note that the combined hydrolytic activities were detected in some strains which could have an application for biotechnological purposes. On other hand, the production of hydrolytic enzyme reflects a good adaptation of halophilic rhizobacteria to harsh environnemental conditions and establish theirselfs in competition for the colonization of the plants rhizopshere. For this reason, it would be very important for these rhizobacteria to exhibit hydrolytic activity under salinity conditions. Indeed, halophilic bacteria are a potential source of extracellular hydrolases like proteases with a wide array of industrial applications (Shivanand & Mugeraya, 2011).

The cowpea (*Vigna unguiculata*) is one of the most important food crops of the fabaceae family in arid regions. The importance of this plant is linked to its leaves and seeds, which are edible and rich in protein (Borget, 1989). Therefore, the development of a microbial strain with beneficial activities to promote cowpea production is a great need, especially in arid regions. Crop yield improvement by inoculation of PGPR strains of the genus *Bacillus* has been observed in pot experiments, which is in agreement with many workers which includes the species *B.megaterium* (Zou et al., 2010), *B.circulans* and *B.cereus* (Tilak & Reddy, 2006), *B.subtilis* (Mena-Violante & Olalde-Portugal, 2007). Thus, the use of certain *Bacillus* strains capable of maintaining and developing on the root system has significant beneficial effects on inoculated plants. The genus *Bacillus* is very widespread in the rhizosphere, many of whose species have been listed as Plant Growth Promoting Rhizobacteria. Indeed, *Bacillus* species used as biofertilizers probably have a direct effect on plant nutrition, growth and health.

CONCLUSIONS

The isolation and characterization of halophilic and halotolerant *Bacillus* strains has increased knowledge of the rhizocompetent bacterial community associated with date palm in saline and arid soils. That said, it would be interesting to study the biological properties of these microorganisms in order to understand how they adapt to salinity in the first place and to exploit their potential applications in the second place. Furthermore, the isolates resistance to the physico-chemical parameters of the hyperarid Algerian ecosystem of In Salah is a first step to select an effective Promoting Growth Plant Rhizobacteria capable of supporting plant growth under soil and climatic conditions in these extreme climatic conditions.

Therefore, our second investigation focus on the PGP effects of isolated halophilic and halotolerant bacteria on inoculation of cowpea plants. *Bacillus* PGPRs have proven to be a very effective strains in terms of improving cowpea plant growth and development. However, further research should be conducted in field trials in several arid and semi-arid locations and on several crops to provide clear evidence of their usefulness.

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ANNEX

DESCRIPTIVE STATISTICS

Ecotype P10

Variable	Observations	Minimum	Maximum	Moyenne	Ecart-type
FWAP (mg)	42	90,000	478,000	290,262	107,222
FWRP (mg)	42	60,000	449,000	233,476	107,627
SL (cm)	42	3,400	14,000	7,336	2,465
RL (cm)	42	2,000	9,400	5,179	1,878
RWC %	42	50,000	147,540	80,141	19,608
Chla (mg/g)	42	0,013	1,219	0,461	0,254
Chlb (mg/g)	42	0,005	1,909	0,552	0,526
chla+Chb (mg/g)	42	0,104	1,864	0,715	0,518
Carotenoids (mg/g)	42	0,015	1,860	0,235	0,281

Ecotype P14

Variable	Observations	Minimum	Maximum	Moyenne	Ecart-type
FWAP (mg)	42	53,000	540,000	242,976	149,768
FWRP (mg)	42	53,000	356,000	142,762	73,829
SL (cm)	42	3,500	12,100	8,196	1,843
RL (cm)	42	2,900	7,500	4,240	1,124
RWC %	42	33,700	100,000	50,413	13,268
Chla (mg/g)	42	0,220	1,600	0,865	0,302
Chlb(mg/g)	42	0,017	1,425	0,467	0,356
chla+Chb(mg/g)	42	0,377	1,880	1,084	0,404
Carotenoids (mg/g)	42	0,013	0,618	0,218	0,132

Correlation matrix (Pearson (n)) :

Variables	FWAP (mg)	FWRP (mg)	SL (cm)	RL (cm)	RWC %	Chla
FWAP (mg)	**1**	0,367	**0,662**	**0,589**	0,177	
FWRP (mg)	0,367	**1**	0,273	**0,576**	0,273	
SL (cm)	**0,662**	0,273	**1**	0,359	0,061	
RL (cm)	**0,589**	**0,576**	0,359	**1**	0,179	
RWC %	0,177	0,273	0,061	0,179	**1**	
Chla (mg/g)	0,210	0,179	-0,229	0,380	-0,169	
Chlb(mg/g)	0,031	0,013	-0,056	-0,055	0,231	
chla+Chb(mg/g)	0,031	0,013	-0,056	-0,055	0,233	
Carotenoids (mg/g)	0,461	-0,095	0,180	0,247	-0,294	

Values in bold are different from 0 at significance level alpha=0.05

Bartlett's sphericity test:

Khi² (Observed value)	138,955
Khi² (Critical value)	50,998
DDL	36
p-value	< 0,0001
alpha	0,05

Test Interpretation :

H0: There is no correlation significantly different from 0 between the variables.

Ha: At least one of the correlations between the variables is significantly different from 0.

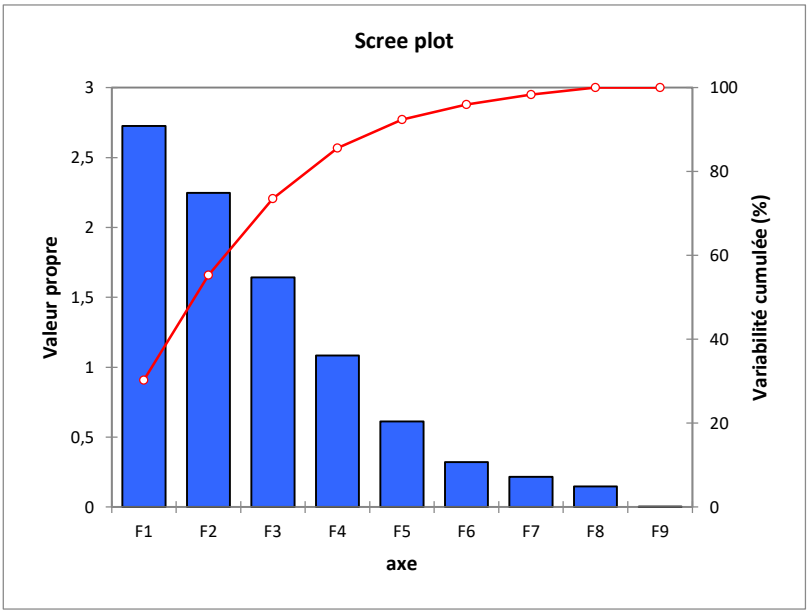
Since the calculated p-value is below the significance level alpha=0.05, the null hypothesis H0 must be rejected, and the alternative hy

The risk of rejecting the null hypothesis H0 when it is true is less than 0.01%.

Principal Component Analysis :

Eigenvalues:

	F1	F2	F3	F4	F5
Eigenvalues	2,726	2,248	1,643	1,085	0,612
Variability (%)	30,284	24,977	18,256	12,053	6,802
% cumulated	30,284	55,261	73,517	85,571	92,373



	F1	F2	F3	F4	F5
FWAP (mg)	0,503	-0,199	-0,020	0,276	0,177
FWRP (mg)	0,359	-0,141	0,306	-0,450	-0,427
SL (cm)	0,330	-0,296	0,140	0,569	-0,333
RL (cm)	0,474	-0,196	0,025	-0,336	0,045
RWC %	0,141	0,081	0,605	-0,044	0,713
Chla (mg/g)	0,315	0,294	-0,400	-0,417	0,042
Chlb(mg/g)	0,197	0,601	0,129	0,183	-0,156
chla+Chb(mg/g)	0,197	0,601	0,130	0,183	-0,154
Carotenoids (mg/g)	0,294	0,002	-0,572	0,203	0,340

Variable coordinates :

	F1	F2	F3	F4	F5
FWAP (mg)	0,831	-0,299	-0,025	0,288	0,139
FWRP (mg)	0,593	-0,212	0,392	-0,469	-0,334
SL (cm)	0,544	-0,444	0,180	0,593	-0,261
RL (cm)	0,783	-0,293	0,032	-0,350	0,035
RWC %	0,233	0,122	0,775	-0,046	0,558
Chla (mg/g)	0,521	0,441	-0,513	-0,434	0,033
Chlb(mg/g)	0,325	0,901	0,165	0,191	-0,122
chla+Chb(mg/g)	0,325	0,900	0,166	0,191	-0,120
Carotenoids (mg/g)	0,486	0,003	-0,733	0,211	0,266

Correlations between variables and factors :

	F1	F2	F3	F4	F5
FWAP (mg)	0,831	-0,299	-0,025	0,288	0,139
FWRP (mg)	0,593	-0,212	0,392	-0,469	-0,334
SL (cm)	0,544	-0,444	0,180	0,593	-0,261
RL (cm)	0,783	-0,293	0,032	-0,350	0,035
RWC%	0,233	0,122	0,775	-0,046	0,558
Chla (mg/g)	0,521	0,441	-0,513	-0,434	0,033
Chlb(mg/g)	0,325	0,901	0,165	0,191	-0,122
chla+Chb(mg/g)	0,325	0,900	0,166	0,191	-0,120
Carotenoids (mg/g)	0,486	0,003	-0,733	0,211	0,266

IS THE EXPLOITABLE BIOMASS (B) VULNERABLE TO CHANGES OF NATURAL MORTALITY (M) BY AGE? CASE OF THE ROUND SARDINELLA

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ABSTRACT

A proper estimation of biomass is critical for environmental and decision making to conserve fish stocks. This work aims to explore the vulnerability of biomass estimation to the variability of natural mortality M by age. 2460 individuals of all sexes, sizes ranging from 7.25 to 21.75 cm, were studied. Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners in the central region of Algeria between Tenes and Bejaïa. Descriptive statistics were performed using FISAT II and VIT4Win. The results generated two different scenarios: first, the current total biomass (Bc) was estimated at 8686.6 tons for M = constant = 0.51 yr^{-1} ; secondly total biomass was estimated at Bc = 10636.4 tons for M = variable by age n, M_n, [M_1 = 1.130 ; M_2 = 0.791 ; M_3 = 0.721 ; M_4 = 0.606 ; M_5 = 0.545 yr^{-1}]. Evaluating Bc with constant M showed a decrease of 1914.19 tons compared to biomass assessment with Mn. Judging by these results, considering that mortality M varies with age has a great impact on biomass evaluation. Naturally, juvenile fish are exposed to larger predation mortality than an adult fish. This difference in predation mortality may be quite significant. Finally, we recommend adjusting the catch effort factor F_C = 1 to $F_{0.1}$ = 0.54 for M variable. This precautionary approach would permit long-term renewal of the stock of *S. aurita* in the central region of the Algerian coast (increasing sea biomass from 10632.91 to 14848.9 tons).

Keywords: *Sardinella aurita, Algeria, Biomass (B), Mortality (M), Stock assessment.*

INTRODUCTION

Fishing in Algeria constitutes an economic activity that provide not only a food source for the population but also thousands of jobs for the Algerians. Fishing has to be controlled and handled according to a sustainable approach to maintain this resource. Thus, fish stocks biomass needs to be constantly evaluated while making sure that the values obtained are the most representative of the reality. In literature many authors as Benamar (2011), Amponsah (2013) and many others, conducted fish stock biomass assessment with the assumption that natural mortality (M) is a constant value (M_{cte}) that isn't linked to fish age. However, it seems that fish mortality (M) varies with age according to Garrido (2016) and Quattrocchi and Maynou (2017), its value is the highest at the youngest age and diminishes with the aging of the fish This can be explained by juveniles, eggs and fish larvae being the most prone to predation and less resistant to environmental conditions than adult fish. This work aims to underline the impact of natural mortality values used when assessing fish stock biomass, in the case of *Sardinella aurita* (Valenciennes, 1847), a small pelagic fish of the family of clupeoids. To do so, we compared the biomass assessed while considering natural mortality as a constant (M_{cte}) with the biomass

calculated using natural mortality as a variable (M_n). This study was conducted in the central region of the Algerian coast between Tenes and Bejaïa.

MATERIAL AND METHODS

Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners situated in the central region of the Algerian coast, 2460 *Sardinella aurita* individuals of all sexes were sampled and each eviscerated and weighed (W_{ev}) and measured from the extremity of the fish muzzle to the end of the caudal fin (TL). Sizes were arranged into length classes ranging from 7.25 to 21.75 cm, with a class width of 0.5 cm. The age-length key was then calculated using Bhattacharya's (1967) method following the recommendation of the DYNPOP working group of the CIESM (Abella *et al.*, 1995 ; Aldebert and Recasen, 1995 ; Alemany and Oliver, 1995 and Campana, 2001 in Bouaziz, 2007). The estimation of the age-length key using Bhattacharya's method was performed on the software FISAT II version 1.2.2 (Gayanilo and Sparre, 2005). We then used the software VONBIT_ALXL (2012) to assess the von Bertalanffy's (1938) growth parameters (L_∞, K and t_0), which is a fish growth model translating into the equation: $L_t = L_\infty(1 - e^{-K(t-to)})$.

We then used the growth parameters obtained to calculate the mortality of the fish stock (M_{cte}) using Djabali's *et al.*, (1993) natural mortality equation:

$$\mathrm{Log}_{10} M = -0{,}0278 - 0{,}1172\ \mathrm{Log}_{10} L_\infty + 0{,}5092\ \mathrm{Log}_{10} K$$

To calculate mortality for each age ($M_n, n = \text{age}$), we first calculated the K for each age using von Bertalanffy's model equation as follows:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$$
$$L_t = L_\infty - L_\infty e^{-K(t-t_0)}$$
$$(L_t - L_\infty) = -L_\infty e^{-K(t-t_0)}$$
$$\ln(L_\infty - L_t) = \ln\left(L_\infty e^{-K(t-t_0)}\right)$$
$$\ln(L_\infty - L_t) = \ln L_\infty + \ln e^{-K(t-t_0)}$$
$$\ln(L_\infty - L_t) - \ln L_\infty = -K(t - t_0)$$
$$K = \frac{\ln L_\infty - \ln(L_\infty - L_t)}{t - t_0}$$
$$K = \frac{-\ln\left(\frac{L_\infty - L_t}{L_\infty}\right)}{t - t_0}$$

We calculated L_∞ for each age ($L_{\infty n}$) using Taylor's (1962) in Bouaziz (2007) equation:

$$L_\infty = \frac{L_{max}}{0{,}95}$$

And then we used Djabali's *et al.*, (1993) natural mortality equation cited above to calculate mortality for each age (M_n) as the following:

$$\mathrm{Log}_{10} M_n = -0{,}0278 - 0{,}1172\ \mathrm{Log}_{10} L_{\infty n} + 0{,}5092\ \mathrm{Log}_{10} K_n$$

To calculate fishing mortality (F) we first calculated total instantaneous mortality rate (Z) using FISAT II, and then we calculated fishing mortality (F) using the following equation:

Knowing Z=F+M therefore F=Z-M

We also calculated the length weight relationship ($W_t = a\ Lt^{b}$) constants a and b.

After having calculated VIT4Win inputs, that said, mortality F, M_n, M_{cte} and the von Bertalanffy's (1938) growth parameters (L_∞, K and t_0), we ran the software VIT4win 1.3 (Lleonart and Salat, 2011) to assess current (Bc) biomass using M_n and M_{Cte}.

RESULTS AND DISCUSSION

The estimation of the average length by age resulted of the following:

Age (year)	Length (cm) ± Standard deviation	Population (NI)	Population %	Separation index (S.I.)
1	10.59 ± 0.960	889.75	36.20%	n.a
2	14.44 ± 1.290	1309.76	53.28%	2.280
3	17.25 ± 0.510	186.28	7.58%	2.130
4	19.11 ± 0.370	68.04	2.77%	2.110
5	22.75 ± 0.420	4.34	0.18%	2.290

Table 5. The average length by age for the population of *Sardinella aurita* sampled using FISAT II.

SI > 2 at all ages, therefore, the separation index is significant. This means that, the decomposition of size frequencies of our sample reflects representative results.

Bhattacharya's method performed on the software FISAT II 1.2.2 provided us with the decomposition of our sample into five (05) cohorts with a median length of 10.59, 14.44, 17.25, 19.11 and 22.75 cm for the ages 1, 2, 3, 4 and 5 respectively.

According to the table 1, age 1 and 2 are the most present in our sample, constituting 36.2% and 53.3% respectively. On the other hand, cohorts of age 3, 4 and 5 constitutes only around 10% of our sample, therefore it is likely that ages 1 and 2 are the most caught by seiners in the study zone.

The calculation of L_∞ and K (growth parameters of the whole sample):

By entering the age-length values to the VONBIT_ALXL (2012) we obtained the following growth parameters, L_∞ = 53.16 cm, K = 0.08 year^{-1}, t_0 = -1.838 year, which we consider as aberrant. This can be explained by L_∞ not being close to the value of L_{max} (the maximal length of the sample, which is 22.8 cm) as it should according to Pauly and Moreau (1997) and Bocar *et al.,* (2019), also K in this case has a low value that doesn't represent the growth speed of small pelagic fish which has a fast growth (Pauly et Moreau, 1997 and Bocar *et al.,* 2019), as for t_0 the value obtained doesn't make sense regarding this specie firstly because the later has a low longevity (05 years in this case), moreover, this value of t_0 seemed aberrant when compared to the values of t_0 obtained in other studies (Tsikliras et *al.*, 2005 ; Bouaziz, 2007 ; Apostolidis and Stergiou, 2014 ; Benamar, 2019), these results can be the effect of a non-regular growth of the fish caused by ecosystemic disturbances, this non-regular growth would then translate into an irregular growth curve (in other words a growth graphic with non-distinguishable curvature), which in turn distort the growth values obtained. To correct the growth parameters obtained we added the values age = 0 and TL = 0 to the age-length key, when using VONBIT_ALXL (2012), doing so, we considerately improved the curvature of the growth graphic, as a result, we obtained growth parameters that are largely more representative of the specie in reality, that are: L_∞ = 23.54 cm, a value close to L_{max} = 22.8 cm, K = 0.48 year^{-1} a value representative of the fast growth of small pelagic fish, t_0 = -0.043 year a t_0 that concords with literature (Tsikliras et *al.,* 2005 ; Bouaziz, 2007 ; Apostolidis and Stergiou, 2014 ; Benamar, 2019).

The calculation of asymptotic length (L_∞) for each age ($L_{\infty n}$, asymptotic length for each age n) using Taylor (1962) in Bouaziz (2007) equation and K_n using von Bertalanffy's model equation provided us with the following results:

Table 6. Values of $L_{\infty n}$ (asymptotic length) and K_n (curvature parameter of the von Bertalanffy growth function) calculated for each age.

Age classes	$L_{\infty n}$ (cm)	K_n (year^{-1})
1	12.11	1.99
2	16.32	1.06
3	18.42	0.91
4	20.53	0.66
5	24.21	0.57

Results of the calculation of the mortality M_{cte} and M_n using Djabali's *et al.,* (1993) natural mortality equation with K_n and $L_{\infty n}$ results for each age (table 2):

$M_{cte} = 0.51$ year^{-1}

$M_1 = 1.13$, $M_2 = 0.79$, $M_3 = 0.72$, $M_4 = 0.61$, $M_5 = 0.54$

The results obtained show that mortality varies with age, it is the highest at age 1 and then diminishes with age (rapidly at first and then diminishes with a slower pace) which concords with Garrido (2016) and Quattrocchi and Maynou (2017).

Concerning length weight relationship ($W_t = a\,Lt^{b}$) constants a and b, we obtained the following:

a = 0.0045 and b = 3.123

The results of biomass assessment using VIT4Win 1.3:

Critical age and size of the virgin stock (mean size and age of the cohort having the highest biomass) with M_n were 2.20 year, 15.5 cm meanwhile with M_{cte} were 2.63 year, 17 cm. Opposingly mean age and size of the current stock didn't shift much (1.37 year, 11,21 cm for M_n and 1.43 year, 11.54 cm for M_{cte}). The same conclusion was drawn for the current stock critical age and length which were 1.63 year, 13 cm for M_n and 1.73 year, 13.5 cm for M_{cte}.

Concerning exploitable biomass, we used the virtual population analysis (VPA) feature in VIT4Win software to obtain the results presented in the table 3. Using natural mortality as a constant (M_{Cte}) the current biomass obtained was Bc = 86866.6 tons (biomass Bc = (Bc/R)*R, R is the number of recruits). On the other hand, when assessing biomass using mortality as a variable (M_n) we obtained Bc = 10636.4 tons, a value different from Bc calculated with M_{cte} by 1949.8 tons.

Table 7. Yield and biomass parameters obtained by the VIT4Win 1.3, for round sardinella, *Sardinella aurita*, of the central region of the Algerian coast.

Parameters for M_{Cte}					Parameters for M_n				
	Factor	Y/R (g)	B/R (g)	SSB/R (g)		Factor	Y/R (g)	B/R (g)	SSB/R (g)
F(0)	0	0	41.024	34.093	F(0)	0	0	20.929	15.967
F(0.1)	0.400	9.202	18.918	12.681	F(0.1)	0.540	5.176	10.152	5.657
F_{MSY}	0.700	9.808	13.205	7.394	F_{MSY}	1.200	5.664	6.521	2.485
F_C	1	9.585	10.092	4.675	F_C	1	5.640	7.272	3.103
Number of recruits: 860736484.88					Number of recruits: 1462652863.53				

Factor = Factor of effort, Y/R = Yield per recruit, B/R = Biomass per recruit, SSB/R = Spawning stock biomass per recruit, F(0) = Factor of non-fishing, F(0.1) = factor of fishing mortality rate at which the marginal yield-per-recruit is only 10 percent of the marginal yield per recruit on the unexploited stock, F_{MSY} = factor of fishing mortality rate corresponding to the maximum sustainable yield, F_c = factor of current effort fishing.

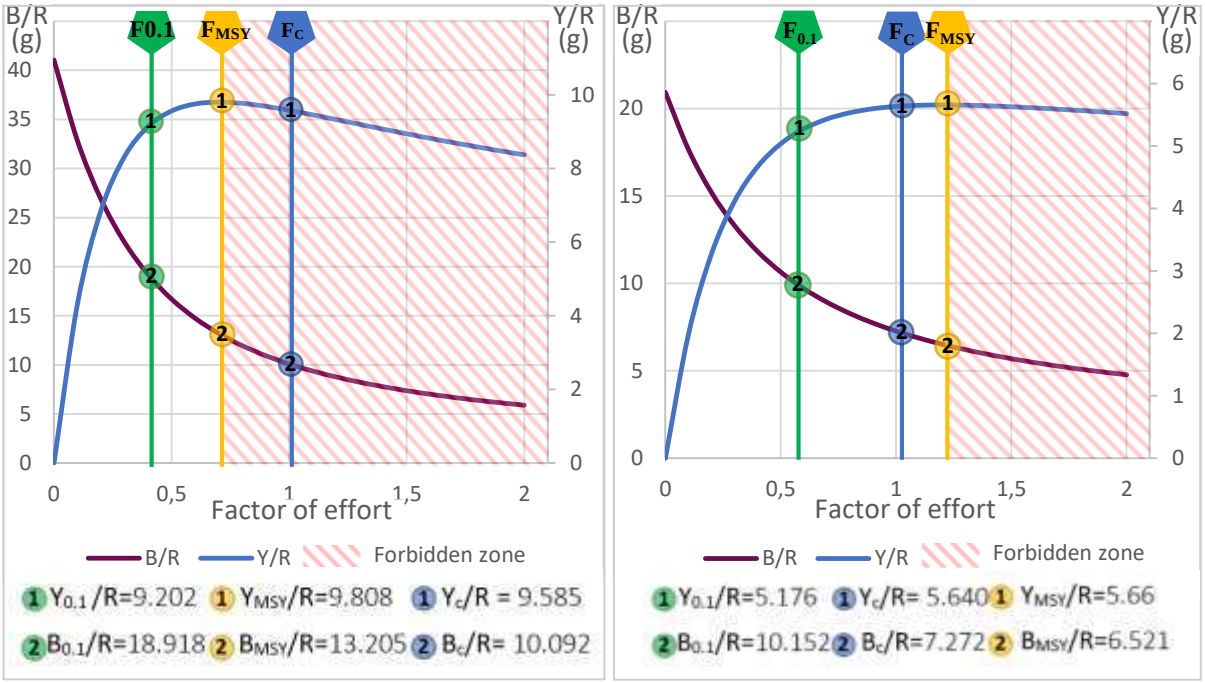

Figure 1. Yield per recruit (Y/R) and biomass per recruit (B/R) according to the effort factor with constant natural mortality (M_{cte}) (left) and with variable natural mortality (M_n) (right).

At the first glance on the graphics in figure 1 we can already see that the results differ according to the natural mortality used, we can also see that with M_{cte} the current factor effort (F_c) exceeds the maximum sustainable yield effort factor (F_{MSY}) and is positioned in the forbidden zone. On the other hand with M_n the current effort factor is positioned before the maximum sustainable yield and thus it is not in the forbidden zone, moreover, according to the values with M_{cte}, $F_{0.1}$ = 0.400, this value imply that to maximise a sustainable exploitation we would have to reduce fishing effort by 60% (to increase Bc to 16337.6 tons), that said, with M_n, $F_{0.1}$ = 0.54, so to have a sustainable exploitation we would need a reduction of 46% of fishing efforts (to increase the biomass to Bc = 14848.9 tons).
To investigate the occurrence of an overexploitation we used the Froese and Proelss (2012) criteria, and obtained the following, for constant natural mortality (M_{cte}) the results showed an overfished and overfishing state (dark grey), on the other hand, with variable natural mortality (M_n) the results showed a not overfished and not overfishing state (light grey).

CONCLUSIONS

According to the previous results biomass assessment is largely sensitive to mortality values. By comparing biomass assessment using M_{cte} with biomass obtained using M_n we obtained an estimated shift of the biomass value of 1949.8 tons, (Bc = 8686.6 tons with M_{cte} to Bc = 106364.4 tons with M_n). Additionally, results obtained led to different stock evaluations and even different suggestions. Consequently, while using M_{cte} the results pointed that the stock was in a state of overfishing and overfished, while when using M_n, the results showed a state of not overfished and not overfishing. Also, when using M_{cte} the results suggested a fishing effort reduction of 60%, while with M_n the results suggested a reduction of fishing effort of 46% (to increase biomass to Bc = 14848.9 tons). Since mortality values changes with age and

considering the impact of the natural mortality used on the biomass assessment, we therefore suggest the use of variable natural mortality when conducting similar procedures.

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STUDY OF NATURAL PREVALENCE OF VARIATIONS ASSOCIATED WITH RESISTANCE TO NS5A INHIBITORS IN TREATMENT NAIVE HCV GENOTYPE 1A PATIENTS IN PAKISTAN

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ABSTRACT

In the last decade, the prevalence rate of hepatitis C virus has increased in Pakistan. According to WHO report of July 2019, 10 million people in Pakistan are infected with hepatitis C. Among HCV genotypes, genotype 1a shows more hindrance to treatment as compared to other genotypes of HCV. In recent years, Direct acting Antiviral Agents (DAA) has been launched as a standard anti-viral therapy in the Pakistan. HCV shows high mutation rate, due to which, the possibility of the emergence of drug resistant variants may results into the treatment failure. The aim of this study was to find the potential variations in NS5A region of HCV genotype 1a that can show the resistance to DAA during the treatment of naive HCV patients of Pakistan. To achieve our objectives, serum samples were collected from HCV 1a treatment naive patients. Domain I of NS5A gene was amplified and purified. Sequencing was performed and these sequences were analyzed for potential variation. The results showed that Pakistani sequences were polyphyletic since they were grouped into two different clades with different possible ancestors. The nucleotide distance between Pakistani sequences ranged from 0.01 to 0.15. The average distance between all sequences was 0.1 ± 0.01. Evolutionary, the distance between amino acid sequences was also calculated. The results showed that the range of amino acid substitution per site between Pakistani sequences was 0.01-.25. The overall mean distance between amino acid sequences was 0.17 ± 0.02. The number of variations were observed in Pakistani sequences. One of the amino acid mutations is Q30R which was previously reported in genotype 1a. This variation was significantly associated with daclatasvir resistance in previous studies. The prevalence of Q30R mutation was 33% in our study, indicating possible resistance to daclatasvir treatment.

Keywords: *Prevalence, HCV genotype 1a, NS5A, Direct acting Antiviral Agents (DAA), Q30R*

INTRODUCTION

Hepatitis is an infection causing pathogen that greatly damages the liver (Barnes et al., 2009). Long lasting infections of liver and hepatocellular carcinoma are mainly caused by HCV (Hepatitis C Virus) (Kanda et al., 2013). It damages nearly 3 to 4 million people (Berenguer M. et al., 2001) annually. Among the victims, almost 27% individuals have cirrhosis while nearly 25% individuals have hepatocellular carcinoma (Alter, 2007).

HCV is prevalent in certain countries for example, in the Europe almost 25 million people are victimized by this infectious disease (Rockstroh et al., 2012) and in Pakistan, the commonness of HCV is 5 %. (Kamal and Nasser, 2008). The most common genotype of HCV in the whole world is genotype-1a and 42.6% of HCV patients are infected by just genotype-1a and from them, only one third (1/3) cases are present in East Asia (Messina et al., 2015). Interferon alpha and ribavirin as a treatment has side effects and less successful rate. The recent drugs called Direct Acting Antiviral (DAA) have maximum success rate with least side effects. DAA are those kinds of medicines, which target specific encoded proteins of HCV and results in the viral life cycle disruption (Jacobson et al., 2011, Poordad et al., 2011). Tough many infected persons became healthy ones after treatments in their early stages of infection, but HCV is the activated

reason of deaths. There are no proper antiviral medicines or vaccines by which HCV infection could be controlled 100 % (Shepard et al., 2005).

In this study the potential variations in NS5A region of HCV 1a genotype are identified that can confer resistance to DAA in treatment naïve HCV patients in Pakistan.

LITERATURE REVIEW

In the recent times, tiny molecules are discovered that can directly attack on any part of HCV life cycle. These molecules revolutionize the cure procedure of long-lasting HCV infections Vermehren et al. (2012). These small molecules are collectively called Direct Acting Antivirals (DAA) and are consist of variety of inhibitors of non-structural (NS) 3/4A protease, NS5B polymerase and NS5A protein. HCV genotype-1a is treated by using two NS3/4A protease inhibitors named boceprevir and telaprevir and both are used in the combination with pegylated interferon and ribavirin. In this way, the frequency of their successful treatment could be expanded by 20% to 30%. But most of DAA are in the clinical trial stages. The use of DAA agents as drugs for HCV treatment would improve the treatment ratio in HCV infected persons Hofmann and Zeuzem (2011). This is expected that in coming days, license would be given to the two drugs which would be used as a triple therapy with PEG-IFN-α and ribavirin by the severe HCV infected patients and that is expected in the USA, Europe and many other countries. Zhang (2016) studied Boceprevir and Telaprevir are the first generations of DAAs and these are used for protease inhibition. In 2011, these were approved for clinical purpose. When Boceprevir or Telaprevir were included in standard PEG-IFN/ribavirin then the treatment rate was extended from 45% to 70% in patients of genotype-1. Second generation of DAAs replaced the first generation in 2013 and these are more active and less harmful. Moreover, the treatment responses of HCV can be improved by using new anti-HCV agents and most of those are at the initial stages of their developmental processes Zeuzem (2008). The names of these anti-HCV agents are protease inhibitors which are specifically used against the NS3-4A protease, HCV polymerase inhibitors in which both nucleoside and non-nucleoside are present and cyclophilin inhibitors.

Poveda et al. (2014) examined the presence of resistance in HCV virus against DAA agents and that was achieved by selecting the mutations at the different points in NS3 protease, NS5B polymerase and NS5A proteins. Hayashi and Takehara (2006) studied in the start of 1990s, interferon monotherapy was introduced and from then antiviral treatment for long lasting HCV has intensely progressed. At present, pegylated IFN and ribavirin are used in combination as a standard treatment. The cure period and reaction to the treatment, all are according to the different genotypes of HCV. For the successful reduction of 50% virus, 48 weeks of this therapy combination is needed for 1a genotype treatment, although the persons infected with genotype 2 requires 24 weeks for viral reduction by 80 or 90%. Asselah et al. (2018) studies at present IFN-free direct acting antivirals combination can treat HCV infected persons and can treat more than 95% of HCV even after 8 to 12 weeks of cure process. For fully inhibition of HCV from the whole world, there are some ways which must be adopted, and these are increased screening, linkage to care.

AIMS AND OBJECTIVES

The aim of this study was to identify potential variations in NS5A region of HCV 1a genotype that can confer resistance to DAA in treatment naive HCV patients of Pakistan.

MATERIALS AND METHODS

Sample Collection

Serum samples of HCV genotype 1a patients were collected from Genome Centre in eppendrofs and stored at -20 before proceeding to RNA extraction. All the samples were of genotype 1a and treatment naïve.

HCV Viral RNA Extraction

HCV RNA was extracted by using FavoRPrep Viral Nucleic Acid Extraction Kit following the standard procedure. Pangenotypic degenerate Primers were used for the amplification of NS5A gene of HCV genotype1a.Primer sequence along with the melting temperature and product size is given in the (Table 3.1).

cDNA Synthesis: cDNA was synthesized by using "Reverted cDNA Synthesis Kit" and procedure of cDNA synthesis.

Table 3.1: Primers sequences for the amplification and sequencing of NS5A region of HCV 1a.

Sr. No.	Primer Name	5'-3' Sequence	Length (bases)
1	NS5A OF	gcctcactgtaacccagctc	20
2	NS5A OR	cccgtccaattctgtgaaaa	20
3	NS5A IF	taagggacatctgggactgg	20
4	NS5A IR	ccgccttatctccacgtatt	20

Gene Specific Primers

Typically, cDNA is primed from total RNA using either a polyT primer that is directed against the poly-A tail of mature mRNAs or random hexanucleotides. If the sequence of a particular gene of interest is known, a gene-specific primer can be used to copy and amplify the transcripts of a single gene.

Protocol of cDNA Synthesis

After thawing, mixing and briefly centrifuging the components of the kit, Stored on ice. the following reagents were added into a sterile, nuclease- free tube on ice in the indicated order:

Primer 10Pm (gene specific or random hexamer primers)	2µL
Template RNA	9µL
H2O	1µL
Total	12µL

Then after mixing, centrifuging, and incubating all the mixture it was put in PCR for 5 minutes at 65°C. Then chilled all mixture on ice. The following mixtures were added in the indicated order:

5X Reaction Buffer	4µL
RiboLock RNase Inhibitor (20 U/µL)	1µL
10Mm dNTPs Mixture	2µL
RevertAid M-MuLV RT (200 U/µL	1µL
Total	8µL

The following reaction profile for PCR reaction is same for cDNA synthesis from either of two primers which are gene specific primers or random hexamer primers.

Temperature (°C)	Time (Minutes)
25	5
42	60
70	5

PCR Amplification of NS5A gene of HCV 1a: Target amplification portion: 637bp of NS5A genotype 1a of HCV was amplified by using Nested PCR and their details of reaction cycles are given below:

1st Round PCR Reaction

cDNA was used as a template for the 1st round of PCR reaction and its amount of 4µL was used for 20µL reaction. The composition of first round of PCR reaction is given below:

10X PCR Buffer	2.0 Ml
25Mm MgCl2	2.4 µL
2.5Mm dNTPs	2.0 Ml
Primer 1 (NS5A F1)	2.0 Ml
Primer 2 (NS5A R1)	2.0 µL
dH2O	4.6 µL
Taq DNA Polymerase (5U/ µL)	0.4 µL
RT PCR Product	4.0 µL
Total Reaction Volume	20 µL

Reaction Profile

The reaction profile of 1st round PCR is as

94°C for 4 minutes (Initial Denaturation)
94°C for 30 seconds
50°C for 30 seconds
72°C for 1 and half minutes } 35 cycles
72°C for 10 minutes
4°C for ∞

2nd Round PCR Reaction

Reaction products of 1st round of PCR were used as a template for 2nd round of PCR reaction. The composition of second round of PCR reaction for 20 µL is given below:

10X PCR Buffer	2.0 µL
25Mm MgCl2	2.4 µL
2.5Mm dNTPs	2.0 µL
Primer 1 (NS5A F1)	2.0 µL
Primer 2 (NS5A R1)	2.0 µL
dH2O	4.6 µL
Taq DNA Polymerase (5U/ µL)	0.4 µL
RT PCR Product	4.0 µL
Total Reaction Volume	20 µL

Reaction Profile

Reaction profile for 2nd round of PCR is given below:

94°C for 4 minutes (Initial Denaturation)
94°C for 30 seconds
55°C for 30 seconds
72°C for 1 and half minutes
72°C for 10 minutes
4°C for ∞

35 cycles

Detection of amplified PCR product (Gel electrophoresis)

1.7% agarose gel was made for the visualization of DNA product under UV transluminescence.

Targeted DNA bands were noted which were compared with ladder of 50 bps.

DNA Band Excision

After visualizing DNA band in the UV light, the next step was cutting the target band by using sterilized surgical cutter and placed the cut gel band in the labelled Eppendorf and stored at 4°C for further use.

Purification of DNA

DNA bands were separated from gel for their further analysis by using Vivantis Nucleic acid extraction kit.

Protocol of separation of DNA from gel is given below which was followed from procedure given along with kit.

Before the start of DNA elusion process, on water bath and set it to 50°C.

Gel Electrophoresis

Gel electrophoresis was already did for DNA visualization in UV light. Then DNA band was cut and placed in the labelled Eppendorf. Weighed the empty Eppendorf on the electric balance and then also weighed Eppendorf that had gel piece in it.

Solubilization of agarose

Firstly, determined the net weight of gel slice and then added Buffer GB according to the volume of gel slice (A gel slice of mass 0.1g would have a volume of 100µl). Then centrifuge the gel and buffer containing ependroff so it was ensured that gel was present at the bottom of the ependroff. Then incubated the ependroff in the pre-heated waterbath so that all gel slice would be completely melted. Ependroff was votexed after few minutes to ensure that all gel was completely melted.

Loading to column

Then the sample was transferred into column that was linked with a clean collection tube with its bottom. Then centrifuged it for 2 minutes at 10,000 x g. That step was also repeated for those samples that were remained from step 2.

Column washing

Then 650 µl of wash buffer was added in the column and then centrifuged it for 1 minute at 10,000 x g. Then discarded the flow through. It was ensured that ethanol must be added in the wash buffer before use.

Column drying

Then next step of DNA purification is the column drying. In it firstly centrifuged the column at 10,000 x g for 1 min so that residual ethanol could be removed. That step was completed so that all traces of ethanol as a residual ethanol could be effective for the DNA quality and might be subsequently used for inhibition of enzymatic reactions.

DNA elution

The last step of DNA purification is the DNA elusion. In this step column was placed into the clean microcentrifuge tube and then 30 to 50µl Elution Buffer was directly added onto the column membrane and stand that for 2 minutes. Then centrifuged the column at 10,000 x g for 1 minute so that DNA could be eluted. Then stored that DNA at 4°C.

Sequencing

Purified samples were sent Applied Biosciences for sequencing. Sequencing was done by using Sanger Sequencing Method.

Sequence Alignment and Analysis

Forward and reverse Primers were adopted for sequencing of large portion of gene and multiple-sense and anti-sense primers were used for that purpose and those were performed in isolated reactions of sequencing. Then consensus sequences were made for each fragment from all those sequences which were produced from all reactions. A specific software name as BioEdit sequence alignment editor (v 7.2.5) was used to generate consent sequences. Then all the sequences were given into the NCBI and accession numbers were allotted to all of sequences.

Sequence Data Set

For computing the genetic distances, software named MEGA 5 was used. Phylogenetic tree was constructed by using Neighbor Joining Method. Boot strap 100 was used for calculating the strength of tree.

For phylogenetic analysis of sequences, a sequence data set of 33 sequences was generated by downloading sequence from NCBI (National Center for Biotechnology Information). The sequences ID, along with along with their region of isolation and accession number is given in the Table 3.2. Phylogenetic analysis was done using the Neighbor Joining Method in MEGA software version 5 (Tamura *et al*., 2011). Nucleotide and amino acid distances were calculated by using maximum likelihood method in MEGA software (Tamura *et al*., 2011).

Potential Resistance mutations

All the sequences from Pakistan including the sequences generated in this study were aligned by using Clustal W Alignment (Thompson *et al*., 2003) and potential resistance mutations were identified.

Table 3.2: Sequences included in the Analysis

NO	Virus	Acession numbers	Country	Year of collection
1	HCV-1a	KT735187	France	2016
2	HCV-1a	KF667819	Italy	2014
3	HCV-1a	KF667818	Italy	2014
4	HCV-1a	KF667817	Italy	2014
5	HCV-1a	KF667816	Italy	2014
6	HCV-1a	HQ113761	USA	2016
7	HCV-1a	HQ113758	USA	2016
8	HCV-1a	HQ113757	USA	2016
9	HCV-1a	HQ113756	USA	2016
10	HCV-1a	HQ113688	USA	2016
11	HCV-1a	HQ113687	USA	2016
12	HCV-1a	HQ113686	USA	2016
13	HCV-1a	HQ113685	USA	2016
14	HCV-1a	AB520610	Japan	2010
15	HCV-3a	GQ300882	Pakistan	2016
16	HCV-3a	KC415749	Pakistan	2013
17	HCV-5a	KT756267	Pakistan	2016
18	HCV-1b	DQ859970	China	2016
19	HCV-3b	MG456214	USA	2017
20	HCV-3b	MG455796	USA	2017
21	HCV-2a	DQ859969	China	2016
22	HCV-1a	HQ891277	Pakistan	2016
23	HCV-1a	HQ891276	Pakistan	2016
24	HCV-1a	HQ891275	Pakistan	2016
25	HCV-1a	HQ891274	Pakistan	2016
26	HCV-1a	HQ891273	Pakistan	2016
27	HCV-1a	HQ891272	Pakistan	2016
28	HCV-1a	HQ891271	Pakistan	2016
29	HCV-1a	HQ891270	Pakistan	2016
30	HCV-1a	HQ891269	Pakistan	2016
31	HCV-1a	HQ891268	Pakistan	2016

RESULTS

Demographic Data

Total 18 samples of Hepatitis virus genotype 1a were collected from Genome Center Lahore. The information about the age, gender and area of infected patients are given in the Table 4.1. In collection of demographic data total number of men and females were 9 and 9 respectively. The minimum and highest age of infected male patients were 15 and 60 years. Similarly, minimum and maximum ages of female infected patients were 18 and 55 years from collected demographic data.

The HCV infected patients were from different areas of Pakistan and those were Lahore, Gujranwala, Swat, Sahiwal, Sargodha and Peshawar. HCV distribution among different genders is also shown by a graph in (Figure 4.3).

Table 4.1: Demographic Data of HCV Patients

Samples	Age (years)	Gender	Area
1	37	Male	Lahore
2	15	Male	Lahore
3	45	Male	Peshawar
4	35	Male	Lahore
5	18	Female	Lahore
6	42	Female	Peshawar
7	60	Male	Swat
8	32	Female	Lahore
9	52	Male	Gujranwala
10	53	Male	Sahiwal
11	19	Male	Sargodha
12	20	Female	Lahore
13	55	Female	Gujranwala
14	25	Female	Lahore
15	45	Female	Faisalabad
16	32	Female	Peshawar
17	30	Female	Lahore
18	40	Male	Gujranwala

PCR Amplification of NS5A/1a

PCR was run by using gene specific primers. Targeted portion of NS5A 1a was of almost 637 bps. Ladder of 50 bps was also run on the gel along with samples so that better results would be achieved by comparing samples DNA band with the specific band of the ladder. Targeted DNA bands run on gel and then visualized in the UV light by using UV transilluminator were shown in (Figure 4.4 and Figure 4.5). Both of those pictures are shown that size of targeted DNA bands was more than 500 bps so those are seen above the ladder band of 500 bps and below the ladder band of 750 bp.

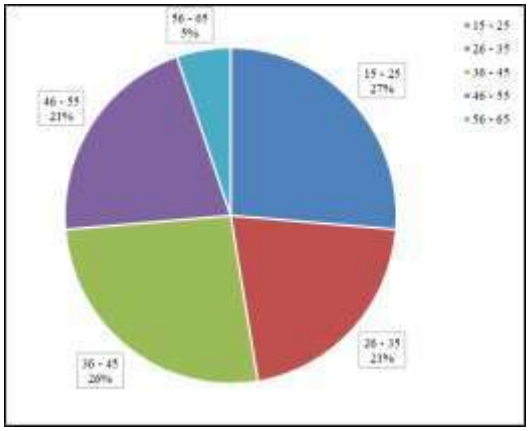

Fig 4.1: HCV 1a distribution among different ages

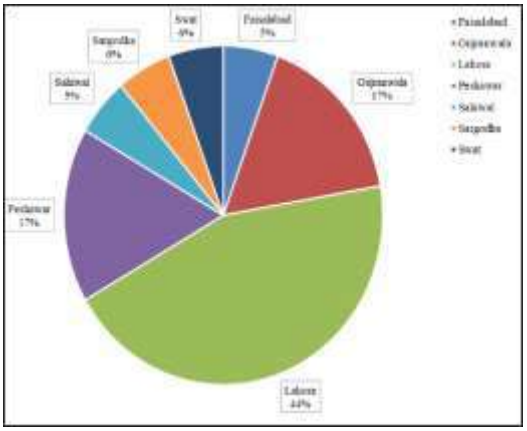

Fig 4.2: HCV 1a distribution among different cities of Pakistan

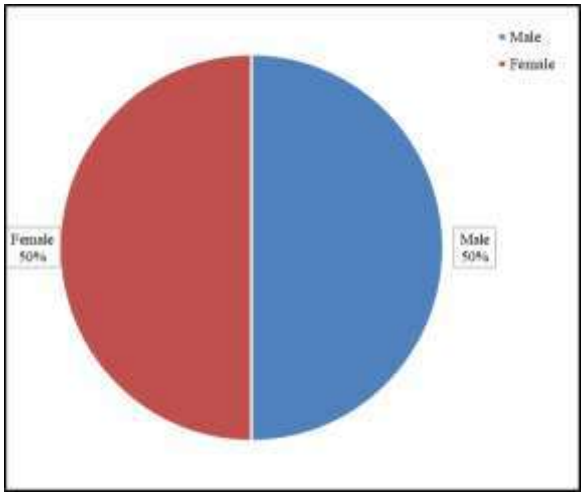

Fig 4.3: HCV 1a distribution among different genders

DNA products were separated from gel by the process of gel elusion. Then eluted DNA products were again run on the gel for confirmation of our desired DNA product size. Eluted DNA product run on the gel shown in the (Figure 4.6).

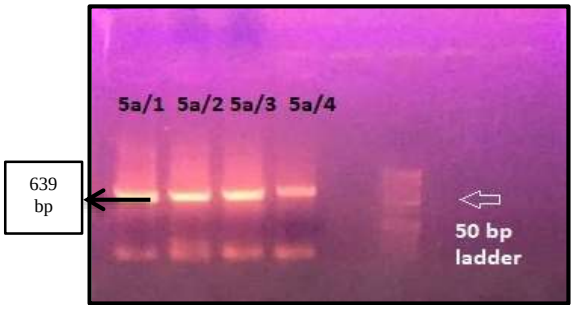

Fig 4.4: PCR amplification of NS5A gene of HCV 1a gene.

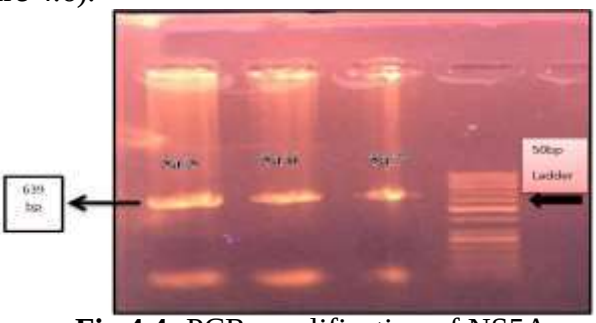

Fig 4.4: PCR amplification of NS5A gene of HCV 1a gene

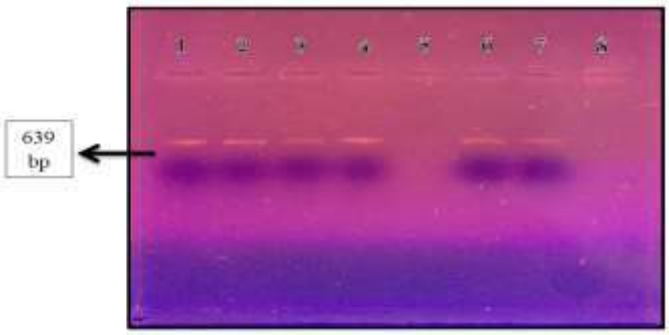

Fig 4.6: DNA Elusion Products of NS5A 1a genotype of HCV.

Figure 4.4 reveals that in the lane 1, 2, 3 and 4 PCR product of NS5A gene is shown. In lane 6 is a ladder of 50bp. Figure 4.4 reveals that in the lane 1, 2 and 3 PCR product of NS5A gene is shown. In lane 4 is a ladder of 50bp while eluted DNA samples were shown in the lane 1, 2, 3, 4, 6 and 7 as illustrated in figure 4.6.

Nucleotide Sequence Chromatogram and BLAST Results

Sequences generated were read and analyzed through BioEdit. Chromatograms showing peaks of different colors representing specific nucleotides are shown in Figure 4.7 and 4.8. FASTA sequences were then aligned with reference sequence (NC-0041021) using BLAST tool (Basic Local Alignment Search Tool). BLAST results are shown in (Figure 4.9 and 4.10).

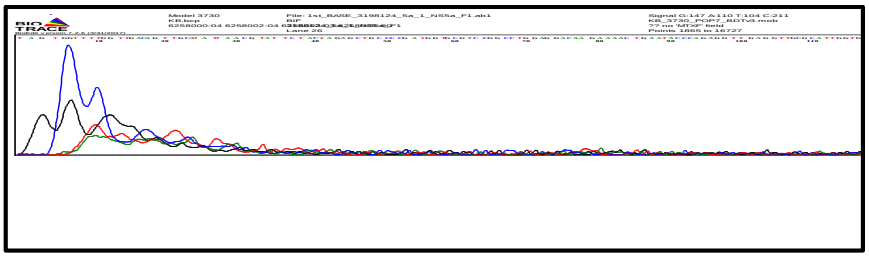

Fig 4.7: Chromatogram of sample 1

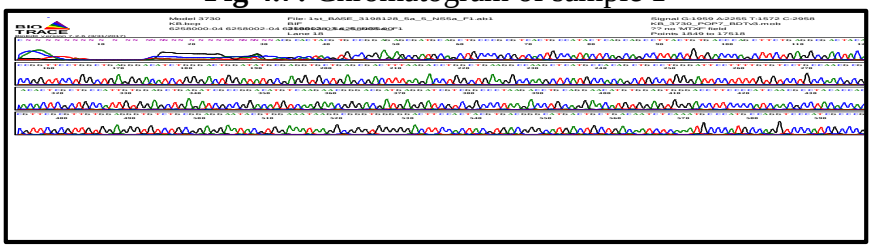

Fig 4.8: Chromatogram of sample 2

```
Query  32   ACGCACTACGTGCCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTT  91
            |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  49   ACGCACTACGTGCCGGAGAGCGATGCAGCCGCCCGCGTCACTGCCATACTCAGCAGCCTC  108

Query  92   ACTGTGACCCAGCTTCTGAGGCGACTACACCAGTGGATAAGCTCTGATTGCACCACCCCA  151
            ||||| |||||||| |||||||||||| || ||||||||||||||| || || ||||| |||
Sbjct  109  ACTGTAACCCAGCTCCTGAGGCGACTGCATCAGTGGATAAGCTCGGAGTGTACCACTCCA  168

Query  152  TGCTCCGGTTCCTGGCTGAGGGACATCTGGGACTGGATATGCGAGGTGCTGAGCGACTTT  211
            |||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  169  TGCTCCGGTTCCTGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGCTGAGCGACTTT  228

Query  212  AAGACCTGGCTGAAGGCCAAGCTCATGCCACAGCTGCCTGGGATTCCTTTTGTGTCCTGC  271
            ||||||||||||||| |||||||||||||||| |||||||||||||| ||||||||||||
Sbjct  229  AAGACCTGGCTGAAAGCCAAGCTCATGCCACAACTGCCTGGGATTCCCTTTGTGTCCTGC  288

Query  272  CAACGCGGGTACAGAGGGGTCTGGCAAGGAGATGGCATTATGCACACTCGCTGCCATTGT  331
            || |||||||| || |||||||||| |||||| ||||||||||||||||||||||| |||
Sbjct  289  CAGCGCGGGTATAGGGGGGTCTGGCGAGGAGACGGCATTATGCACACTCGCTGCCACTGT  348

Query  332  GGAGCTGAGATCGCCGGACATGTCAAGAACGGGACGATGAGGATCGTCGGCCCTAAGACC  391
            |||||||||||| | |||||||||| ||||||||||||||||||||||| |||| ||||
Sbjct  349  GGAGCTGAGATCACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACC  408

Query  392  TGCAGGAACATGTGGAGTGGGACCTTCCCCATCAACGCCTACACCACGGGCCCCTGTACC  451
            ||||||||||||||||||||||| |||||||| ||||||||||||||||||||||||||||
Sbjct  409  TGCAGGAACATGTGGAGTGGGACGTTCCCCATTAACGCCTACACCACGGGCCCCTGTACT  468

Query  452  CCCCTTCCTGCGCCGAACTATACGTTCGCGTTGTGGAGGGTGTCTGCGGAGGAATACGTG  511
            ||||||||||||||||||||||| ||||||| ||||||||||||||| ||||||||||||
Sbjct  469  CCCCTTCCTGCGCCGAACTATAAGTTCGCGCTGTGGAGGGTGTCTGCAGAGGAATACGTG  528

Query  512  GAAATAAGGCGGGTGGGGGACTTCCACTACGTGACGGGCATGACTGCTGACAATCTCAAA  571
            || |||||||||||||||||||||||||||||  |||| ||||||| |||||||||| |||
```

```
Sbjct  529  GAGATAAGGCGGGTGGGGGACTTCCACTACGTATCGGGTATGACTACTGACAATCTTAAA  588

Query  572  TGCCCATGCCAGGTCCCATCGCCCGAATTCTTCACAGAATTGGACGGG  619
            ||||| |||||| ||||||||||||||| ||||||||||||||||||
Sbjct  589  TGCCCGTGCCAGATCCCATCGCCCGAATTTTTCACAGAATTGGACGGG  636
```

Figure 4.9: Chromatogram and BLAST results of sample 1

```
Query  4    ATGAACAGGCTGATAGCGTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTACGTG  63
            |||||| |||| ||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATGAACCGGCTAATAGCCTTCGCCTCCCGGGGGAACCATGTTTCCCCCACGCACTACGTG  60

Query  64   CCGGAGAGCGATGCAGCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTGACCCAG  123
            |||||||||||||||| |||||||||||||||||||||||||||||||||| ||||||
Sbjct  61   CCGGAGAGCGATGCAGCCGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCAG  120

Query  124  CTTCTGAGGCGACTACACCAGTGGATAAGCTCTGATTGCACCACTCCATGCTCCGGTTCC  183
            || |||||||||| || |||||||||||||| || || ||||||||||||||||||||||
Sbjct  121  CTCCTGAGGCGACTGCATCAGTGGATAAGCTCGGAGTGTACCACTCCATGCTCCGGTTCC  180

Query  184  TGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGCTGAGCGATTTCAAGACCTGGCTG  243
            |||||||||||||||||||||||||||||||||||||||||||||| || ||||||||||||
Sbjct  181  TGGCTAAGGGACATCTGGGACTGGATATGCGAGGTGCTGAGCGACTTTAAGACCTGGCTG  240

Query  244  AAGGCCAAGCTCATGCCACAACTGCCTGGGATTCCTTTTGTGTCCTGCCAACGCGGGTAT  303
            || |||||||||||||||||||||||||||||| |||||||||||||| |||||||||
Sbjct  241  AAAGCCAAGCTCATGCCACAACTGCCTGGGATTCCCTTTGTGTCCTGCCAGCGCGGGTAT  300

Query  304  AAGGGGGTCTGGCAAGGGGATGGCATTATGCACACTCGCTGCCATTGTGGAGCTGAGATC  363
            | |||||||||||| ||| || |||||||||||||||||||||| ||||||||||||||||
Sbjct  301  AGGGGGGTCTGGCGAGGAGACGGCATTATGCACACTCGCTGCCACTGTGGAGCTGAGATC  360

Query  364  GCTGGACATGTCAAGAACGGGACGATGAGGATCGTCGGCCCTAAGACCTGCAGGAACATG  423
            |||||||||||||| |||||||||||||||||||||||| |||| ||||||||||||||||
Sbjct  361  ACTGGACATGTCAAAAACGGGACGATGAGGATCGTCGGTCCTAGGACCTGCAGGAACATG  420

Query  424  TGGAGTGGGACCTTCCCCATCAACGCCTACACCACGGGCCCCTGTACCCCCCTTCCTGCG  483
            |||||||||||| |||||||| |||||||||||||||||||||||| ||||||||||||
Sbjct  421  TGGAGTGGGACGTTCCCCATTAACGCCTACACCACGGGCCCCTGTACTCCCCTTCCTGCG  480

Query  484  CCGAACTATACGTTCGCGTTGTGGAGGGTGTCTGCGGAGGAATACGTGGAAATAAGGCGG  543
            |||||||||| ||||||| ||||||||||||||| ||||||||||||| |||||||||
Sbjct  481  CCGAACTATAAGTTCGCGCTGTGGAGGGTGTCTGCAGAGGAATACGTGGAGATAAGGCGG  540

Query  544  GTGGGGGACTTCCACTACGTGACGGGCATGACTGCTGACAATCTCAAATGNCC  596
            ||||||||||||||||||  |||| |||||| |||||||||| ||||| ||
Sbjct  541  GTGGGGGACTTCCACTACGTATCGGGTATGACTACTGACAATCTTAAATGCCC  593
```

Figure 4.10: Chromatographic and BLAST results of sample 2

Nucleotide distance

All the Pakistani NS5A sequences were aligned together through Clustal W multiple alignment and genetic distance between nucleotide sequences was computed. Nucleotide distance between sequences was calculated using Maximum Likelihood method. The analysis involved 13 nucleotide sequences. Evolutionary analyses were conducted in MEGA 6 (Tamura *et al*., 2013). Bootstrap test of 1000 replicates were applied. The results showed that nucleotide distance between Pakistani sequences ranged from 0.01 to 0.15. The average distance between all sequences was 0.1 ± 0.01 (Table 4.2).

Amino acid distance

Evolutionary distance between amino acid sequences was also calculated using Maximum likelihood method conducted in MEGA software. Results showed that range of amino acid substitution per site between Pakistani sequences was 0.01-.25. The overall mean distance between sequences was 0.17 ± 0.02 (Table 4.3).

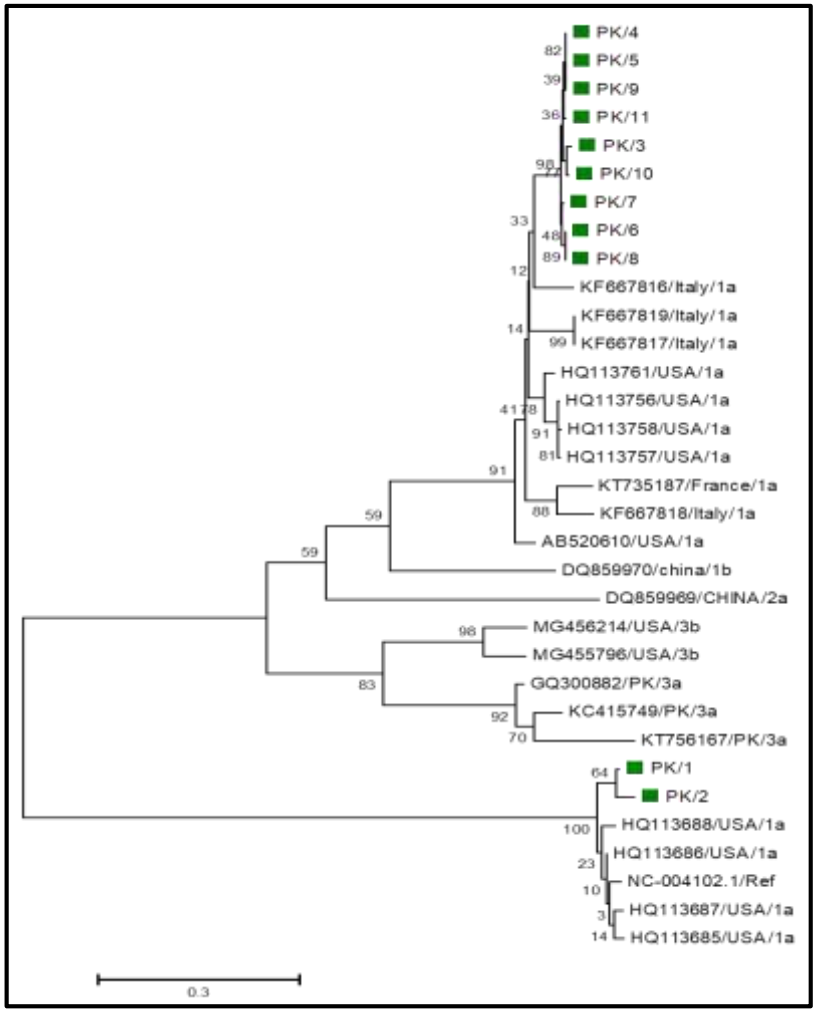

Fig 4.11: Phylogenetic tree generated using Neighborhood Joining Method of NS5A sequences

The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. The analysis involved 33 nucleotide sequences. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013).

Table: 4.2: Estimates of Nucleotide distance between Pakistani sequences

	NC-004102.1/Ref	PK/1	PK/2	PK/3	PK/4	PK/5	PK/6	PK/7	PK/8	PK/9	PK/10	PK/11	PK/12
NC-004102.1/Ref		0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
PK/1	0.07		0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02
PK/2	0.06	0.02		0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.02	0.02
PK/3	0.13	0.11	0.10		0.01	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.02
PK/4	0.11	0.10	0.09	0.09		0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.02
PK/5	0.10	0.09	0.08	0.11	0.10		0.02	0.01	0.02	0.02	0.02	0.02	0.01
PK/6	0.13	0.12	0.11	0.08	0.10	0.13		0.02	0.01	0.02	0.02	0.02	0.02
PK/7	0.11	0.10	0.09	0.12	0.10	0.01	0.14		0.02	0.02	0.02	0.02	0.00
PK/8	0.11	0.09	0.08	0.02	0.08	0.10	0.06	0.11		0.02	0.02	0.01	0.02
PK/9	0.15	0.14	0.13	0.11	0.10	0.11	0.12	0.11	0.09		0.02	0.01	0.02
PK/10	0.14	0.12	0.11	0.11	0.08	0.10	0.12	0.10	0.10	0.10		0.01	0.02
PK/11	0.12	0.12	0.11	0.10	0.02	0.11	0.10	0.11	0.09	0.09	0.08		0.02
PK/12	0.11	0.10	0.09	0.12	0.10	0.01	0.14	0.00	0.11	0.11	0.10	0.11	

Table: 4.3: Estimates of amino acid distance between Pakistani sequences

	PK1	PK2	PK3	PK4	PK5	PK6	PK7	PK8	PK9	PK10	PK11	PK12
PK1		0.01	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.03	0.03
PK2	0.01		0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.03	0.03
PK3	0.15	0.15		0.03	0.04	0.03	0.04	0.02	0.04	0.04	0.04	0.04
PK/4	0.12	0.12	0.17		0.03	0.04	0.04	0.03	0.04	0.03	0.02	0.04
PK/5	0.11	0.12	0.20	0.18		0.04	0.01	0.03	0.04	0.04	0.04	0.01
PK/6	0.15	0.15	0.15	0.19	0.23		0.04	0.03	0.04	0.04	0.04	0.04
PK/7	0.13	0.14	0.22	0.20	0.03	0.25		0.04	0.04	0.04	0.04	0.00
PK/8	0.10	0.11	0.04	0.14	0.17	0.11	0.19		0.03	0.04	0.04	0.04
PK/9	0.22	0.22	0.21	0.22	0.22	0.24	0.23	0.18		0.04	0.04	0.04
PK/10	0.21	0.21	0.21	0.17	0.22	0.22	0.23	0.19	0.21		0.03	0.04
PK/11	0.15	0.15	0.18	0.07	0.19	0.21	0.22	0.18	0.19	0.15		0.04
PK/12	0.13	0.14	0.22	0.20	0.03	0.25	0.00	0.19	0.23	0.23	0.22	

Potential Resistance Mutation in Pakistani NS5A Sequences

Amino acid substitutions are one of the reasons of mutations formation in the protein. Due to the formation of such a kind of mutation, the resulted proteins are usually unable to continue their targeted functions so that they would perform some other kind of functions due to changes in their function as a result of mutations in them.

The alignments of amino acids in the reference sequence, sample sequences and other collected sequences of 1a are shown in the (Table 4.4) and that table is represented the positions of different amino acids at the same positions in the different sequences.

Several potential resistance mutations were observed when all the sequences were aligned together. There were total 53 amino acid positions which showed variations. Some notable variations are S17A, K24E, T21S, Q30R, I34F, R44K/M/G, V46A, R48Q/K, D50N, I52F,

C59W, A61V, E62G, T64A/P, M72I, R73K, G76A, R78K/I/G/M/K, R81K, L101F/L, P102F, P104Q, N105K, K107T/N/I/Y, A109G/P, W111G, E116K, E117K, V119L, E120K, I121K/L, R122G, R123G, D126N, F127S, H128Q/V, V130L/E, S131T/R/N, M33I, T134A/S, T135A, K139N, C142V/S, Q143R, I144V, P147L, E148D/N, F149L, F150Y, E152R/K, L153P, D154T/E and G155A.

One of the amino acid change Q30R has previously been reported in the 1a genotype. This variant was significantly associated with the Daclatasvir resistance in some studies (Nakamoto *et al*., 2014).

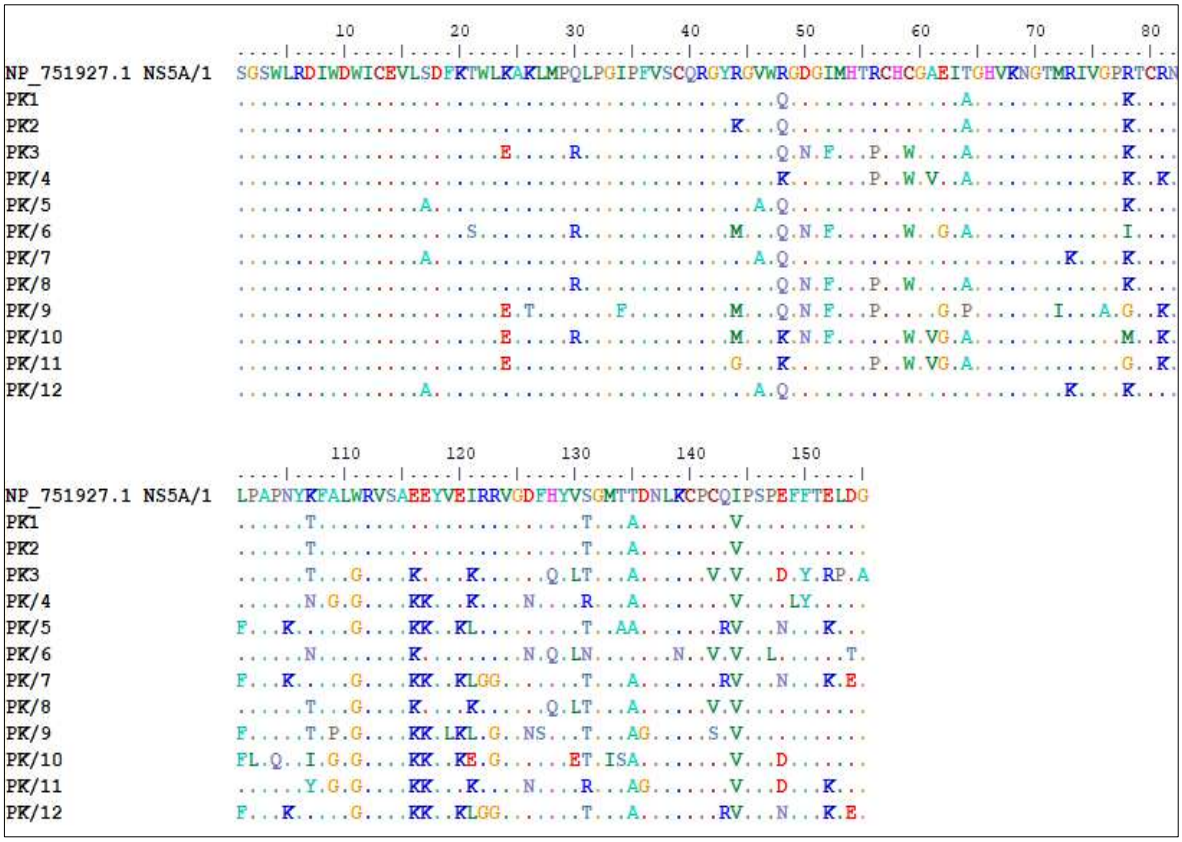

Fig 4.12: Multiple alignment of Pakistani amino acid sequences and reference sequence. Amino acid positions that are conserved are shown in the form of dots. Amino acids are represented in the form of symbols

Table 4.4: Potential resistant mutations

Amino acid position	Ref	PK1	PK2	PK3	PK/ 4	PK/ 5	PK/ 6	PK/ 7	PK/ 8	PK/ 9	PK/ 10	PK/ 11	PK/ 12
17	S	-	-	-	-	A	-	A	-	-	-	-	A
21	T	-	-	-	-	-	S	-	-	-	-	-	-
24	K	-	-	E	-	-	-	-	-	E	E	E	-
30	Q	-	-	R	-	-	R	-	R	-	R	-	-
34	I	-	-	-	-	-	-	-	-	F	-	-	-
44	R	-	K	-	-	-	M	-	-	M	M	G	-
46	V	-	-	-	-	A	-	A	-	-	-	-	A
48	R	Q	Q	Q	K	Q	Q	Q	Q	Q	K	K	Q
50	D	-	-	N	-	-	N	-	N	N	N	-	-
52	I	-	-	F	-	-	F	-	F	F	F	-	-

59	C	-	-	W	W	-	W	-	W	-	W	W	-
61	A	-	-	-	V	-	-	-	-	-	V	V	-
62	E	-	-	-	-	-	G	-	-	G	G	G	-
64	T	A	A	A	A	-	A	-	A	P	A	A	-
72	M	-	-	-	-	-	-	-	-	I	-	-	-
73	R	-	-	-	-	-	-	K	-	-	-	-	K
76	G	-	-	-	-	-	-	-	-	A	-	-	-
78	R	K	K	K	K	K	I	K	K	G	M	G	K
81	R	-	-	-	K	-	-	-	-	K	K	K	-
101	L	-	-	-	-	F	-	F	-	F	L	-	F
102	P	-	-	-	-	-	-	-	-	-	F	-	-
104	P	-	-	-	-	-	-	-	-	-	Q	-	-
105	N	-	-	-	-	K	-	K	-	-	-	-	K
107	K	T	T	T	N	-	N	-	T	T	I	Y	-
109	A	-	-	-	G	-	-	-	-	P	G	G	-
111	W	-	-	G	G	G	-	G	G	G	G	G	G
116	E	-	-	K	K	K	K	K	K	K	K	K	K
117	E	-	-	-	K	K	-	K	-	K	K	K	K
119	V	-	-	-	-	-	-	-	-	L	-	-	-
120	E	-	-	-	-	K	-	K	-	K	K	-	K
121	I	-	-	K	K	L	-	L	K	L	E	K	L
122	R	-	-	-	-	-	-	G	-	-	-	-	G
123	R	-	-	-	-	-	-	G	-	G	G	-	G
126	D	-	-	-	N	-	N	-	-	N	-	N	-
127	F	-	-	-	-	-	-	-	-	S	-	-	-
128	H	-	-	Q	-	-	Q	-	Q	-	-	-	-
130	V	-	-	L	-	-	L	-	L	-	E	-	-
131	S	T	T	T	R	T	N	T	T	T	I	R	T
133	M	-	-	-	-	-	-	-	-	-	I	-	-
134	T	-	-	-	-	A	-	-	-	-	S	-	-
135	T	A	A	A	A	A	-	A	A	A	A	A	A
139	K	-	-	-	-	-	N	-	-	-	-	-	-
142	C	-	-	V	-	-	V	V	V	S	-	-	-
143	Q	-	-	-	-	R	-	-	-	-	-	-	R
144	I	V	V	V	V	V	V	V	V	V	V	V	V
147	P	-	-	-	-	-	L	-	-	-	-	-	-
148	E	-	-	D	-	N	-	-	-	-	D	D	N
149	F	-	-	-	L	-	-	-	-	-	-	-	-
150	F	-	-	Y	Y	-	-	-	-	-	-	-	-
152	E	-	-	R	-	K	-	-	-	-	-	K	K
153	L	-	-	P	-	-	-	-	-	-	-	-	-
154	D	-	-	-	-	-	T	E	-	-	-	-	E
155	G	-	-	A	-	-	-	-	-	-	-	-	-

DISCUSSION AND CONCLUSION

The results showed that Pakistani sequences were polyphyletic since they were grouped in two different clades with different possible ancestors. The results showed that nucleotide distance between Pakistani sequences ranged from 0.01 to 0.15. The average distance between all sequences was 0.1±0.01. Evolutionary distance between amino acid sequences was also calculated. Results showed that range of amino acid substitution per site between Pakistani sequences were 0.01-0.25. The overall mean distance between sequences was 0.17±0.02. The other kinds of mutations were also analyzed in analysis procedure and those were included nucleotide substitutions, amino acid substitutions and synonymous and non-synonymous mutations. For the analysis of all those mutations, different software was used including Bioedit, Expasy, Mega and NCBI nucleotide Blast. Nucleotide and amino acid substitutions were detected by using all previously described software. Amino acids which were different at same positions in different samples were noted manually by comparing each position of each sequence with the position of the reference sequence amino acids. Mutations were found at many positions in different samples for instance noted mutations were 30QR, 44RK/M/G, 48RQ/K, 107KT/N/I, 148ED/N, 131ST/R/N and many other were also noted manually.
In this study, Q30R mutation is observed in 4 out of 12 sequences and this mutation was compared with the already present mutations in the literature which would show resistance to drug action on effected persons. In literature many other mutations were also already reported which would show resistance against drug action on the infected persons and those would be M28T, Q30R, Q30H, L31M and P32 (Nakamoto et al., 2014). Apart from this mutation, several changes were observed in sequences, indicating the possible resistance to Daclatasvir. In order to find out the association of these mutations and drug resistance, further investigation and treatment follow up is needed. Prevalence of Q30R is 33% in our study.

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OIL CONTENT AND FATTY ACID COMPOSITION OF APRICOT VARIETIES

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ABSTRACT

Apricot (*Prunus armeniaca* L.) fruit seed contains up to 54% oil, 23% protein and 35.26% carbohydrate, and 4.71% crude fiber. A total of 54 apricot varieties were tested for oil content and oil compositions. Oil content was determined using the Soxhlet extractor and the fatty acids in oil were analyzed by a GC-MS. Oil content of apricot verities varied between 26.4 and 58.6%. The highest oil content was obtained from Gül03 and the lowest was obtained from Cansa Dresova varieties. The major fatty acid components of tested apricot varieties were palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid. More researches are needed to develop new products from apricot oil for cosmetic and other purposes.

Key Words: Apricot, oil content, oil composition, *Prunus armeniaca*

INTRODUCTION

Apricot (*Prunus armeniaca* L.), a member of the Rosaceae family, is widely distributed in the world. Apricots concussion is strongly recommended due to its important nutritional properties; since it is a good food source for elimination of vitamin A and trace element deficiencies, anemia, physical and mental fatigue, depression, neurosis, stress, etc. (Iordanescu and Micu, 2012). Turkey is the world's largest apricot producing country. The apricot production in the world is about 4.000.000 tons, and 800.000 tons in Turkey (Fao, 2018). Except for the Black Sea and high plateaus of the East Anatolian Region, it is grown all part of Tukey. Malatya, Erzincan, and Iğdır provinces are the leading apricot producer provinces (Guleryuz et al 1999). Most apricot cultivars are for drying (Paydas et al., 1992; Bas et al., 2001). Currently, apricot cultivars from Greece, France, Italy, Spain, and USA are introduced to Turkey and are under evaluation (Polat, and Caliskan, 2013). Some fruit seeds such as cherry, apricot, citrus and apple can be used as sources of oils. Apricot seed kernel is an important source of oil and protein (Nout et al., 1995). Apricot kernel has up to 54% oil, 23% protein and 35.26% carbohydrate, and 4.71% crude fiber (Alpaslan and Hayta, 2006, Ali et al. 2011). Apricot kernel oil is a rich source (94.4%) of unsaturated fatty acids rich in oleic acid (66.2%) and linoleic acid (28.2%). Sweet kernel is mainly consumed without processing as an important source of dietary protein, oil and fiber. The apricot oil is popular as body oil and massage oil and is known for its ability to penetrate the skin without leaving an oily feel. Apricot kernel oil is used in paint and varnish formulations, surface coatings and oleo-chemicals, and as oils for cosmetic purposes (Hauhout-Helmy 1990). Apricots kernel has mainly two types sweet and bitter based on taste of the kernel. Due to its medicinal value, there is an unexpected demand for the apricot oil in recent years. The purpose of this study were determine oil content and fatty acid ratio of apricot cultivars and genotypes.

MATERIALS and METHODS

The apricot kernels were crashed in a mill and dried to 9% moisture. Kernel seed oil content was determined in ten samples from each genotype and cultivar. Oil content was determined using the Soxhlet extractor (Sigma-Aldrich, Germany) with diethyl ether by

weighing 3 gram of dried sample. After 3-hour extracting with ethyl ether, the defatted sample was kept in an air oven at 45 °C for 12 hours and then the weight was used to calculate the oil content. The fatty acid composition analysis was performed by GC-MS-QP2010 (Shimadzu, Japan gas chromatography). The chromatographic operation parameters was fame ionization detector 250 °C; Rtx-5Sil MS column (30 m×0.32 mm×0.25 μm); carrier gas: helium; and flow rate 1.0 mL/min. The profile of the column temperature was as follows: increased from 90 °C to 265 °C at 5 °C/min and maintained for 5 min. The operation parameters of the MS analysis were: ion bombardment source: electron impact ion source; ion source temperature: 200 °C; electron multiplier voltage: 70 eV; scanning system: full scan; and solvent delay: 2.0 min. The fatty acids were identified by comparison of the peaks with those of the fatty acid standards analyzed under the same conditions. The relative content of fatty acids was calculated by the area normalization method.as follows

RESULTS and DISCUSSION

The Seed kernel oil content of apricot genotypes and cultivars were given in Table 1. Seed kernel oil content of apricot genotypes and cultivars were varied between 26.4 and 58.6%. The highest seed oil content was obtained from apricot cultivar Gül03 and the lowest was obtained from cultivar Cansa Dresova followed by Royal (Table 1). Similar seed kernel oil ratios for apricot was reported by (Femenia et al. 1995). The majority of the apricot genotypes and cultivar had oil content greater than 40%. Although apricot is not produced for its kernel oil its oil had a great usage and application range as food or for industrial use (Nout et al., 1995; Hauhout-Helmy, 1990). Apricot seed kernel has higher oil contents than the oil yield of some commercially produced oil crops.

Table 1. Seed kernel oil contents of apricot genotypes and cultivars.

No	Cultivar	Oil content (%)	No	Cultivar	Oil content (%)
1	İsmail Ağa	55.0	30	295	46.3
2	Ordubat Benzeri	54.0	31	14	44.6
3	Gü103	58.6	32	Çöloğlu	53.0
4	Luiset	46.6	33	Hacıhaliloğlu	51.6
5	Royal	29.6	34	EB	44.6
6	Turfanda Eski Malatya	32.3	35	X1 Zerdali	41.0
7	92-23-01	50.0	36	Tokalıoğlu Erzincan	51.3
8	Hasanbey 3	54.0	37	Akçadağ Güney	54.0
9	Kabaadı	52.6	38	Tokalıoğlu Yalova	40.3
10	Mahmut Ölmez	56.6	39	Hungarian Best	47.3
11	Ziraat Okulu	40.0	40	Ağerik	50.6
12	23	54.0	41	Adilcevaz 1	47.0
13	Roxana	51.0	42	Şekerpare Benzeri	52.0
14	Markul eski	38.3	43	Kishnewsky	42.0
15	1927	31.3	44	Cansa	48.3
16	Artvin PA	55.0	45	Adilcevaz 5	49.3
17	Sivas PA	42.3	46	Mahmudun eriği	52.6
18	Şam	38.0	47	06	55.3
19	Karacabey 1	48.6	48	Alkaya	51.3
20	Harleyne	43.0	49	Adilcevaz 2	43.6
21	Gü 50	43.3	50	Soğancı	52.0
22	03	46.0	51	Şekerpare	48.3

23	Adilcevaz 3	43.0	52	Aprikoz	50.6
24	13	53.3	53	Çekirge 52 Edirne	49.6
25	Çataloğlu	46.3	54	Ordubat	44.6
26	İmraha	45.6	55	İri bitirgen	38.7
27	11	57.0	56	Zard	32.4
28	1298	52.6	57	Cansa dresova	26.4
29	Albayrak	46.6			

Only seed oil of selected apricot cultivars was used for their fatty acid composition analysis. Fatty acid components of selected apricot cultivars and genotypes were given in Table 2.

Table 2. Fatty acid components of apricot cultivars and genotypes

Retention Time	Carbon number	Component	Area (%)											
			1	2	3	4	5	6	7	8	9	10	11	12
8.204	C16	Palmitic acid	5.3	7.8	5.38	7.5	6.2	9.3	6.2	7.5	7.2	6.6	6.2	6.3
8.368	C16	Palmitoleic acid	2.6	1.41	2.21	1.4	0.8	1.2	1.24	1.4	1.6	1.6	0.8	0.8
9.409	C18	Stearic acid	3.7	1.91	3.60	1.4	3.4	3.3	3.3	3.8	1.5	1.6	1.1	1.1
9.518	C18	Oleic acid	65.0	64.4	63.19	67.3	66.8	67.2	67.2	66.1	70.0	69.2	67.7	67.2
9.803	C18=	Lineloic acid	23.2	24.4	22.9	22.2	22.7	23.1	23.0	22.3	20.1	21.0	24.0	23.9

1=İsmail Ağa; 2=Ordu Benzeri; 3=Gül103; 4=Luiset; 5=Royal; 6=Turfanda Eski Malatya; 7=92-23-01; 8=Hasanbey; 9=Kabaadı; 10=Mahmut Ölmez; 11 Ziraat Okulu; 12=28

When fatty acid composition of selected genotypes and cultivars were considered oleic acid ratios varied between 57.39 and 69.22% and the genotype Mahmut Ölmez had the highest oleic acid ratio followed by Ziraat Okulu (Table 2). Linoleic acid ratios varied between 21.06 and 25.76%. The highest linoleic acid ratio was obtained from genotype Kabadı and the lowest was obtained from genotype Mahmut Ölmez. Palimitic acid, palmitoleik acid and stearik acid are the other major fatty acids detected in the apricot seed kernel oils of tested genotype and cultivars (Table 2). Seed oil fatt acid compositions of apricot genotypes and varieties were similar with results of Sebedio et al. (1987) and Cosge et al. (2007).

Apricot seed kernel contains higher amount of potassium and magnesium minerals essential amino acids, various vitamin B groups. It was showed that apricot kernel oil had high unsaturated fatty acids, especiallyoleic acid and linoleic acid (Femenia et al., 1995; Lazos, 1991; Gupta, 2012). It was reported that bitter apricot seed kernel oils had high fatty acid contents (Hassanein, 1999). Sebedio et al. (1987) and Cosge et al. (2007) reported that chemical properties of apricot kernel oil could be a good replacement for olive oil. The apricot seed kernel oil has similar mineral composition as the oil crops in terms of phosphorous, copper, zinc, iron and potassium contents.

CONCLUSIONS

The work presented here shows that apricot cultivars and genotypes had oil content varied between 26.4 and 58.6%. The highest oil content was obtained from cultivar Gül03. The lowest was obtained from cultivar Cansa Dresova. The major fatty acid components were palmitic acid, palmitoleic acid, stearic acid, oleic acid and linoleic acid. More researches are needed to develop new products from apricot oil for cosmetic and other purposes

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MICROBIOLOGICAL SAFETY AND QUALITY OF MOZZARELLA CHEESE, PRODUCED BY TWO BUSINESS OPERATORS IN THE TIRANA CITY

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ABSTRACT

Dairy products are an excellent growth medium for a wide range of microorganisms. This can be a reason for reduced shelf life. The quality and safety of fresh cheese such as Mozzarella are strictly related to their microbial content. In particular, some microbiological parameters have to be checked for safety: *E. Coli* (its concentrations are indicators of the hygienic state of the dairy products); *Salmonella spp* and *Listeria monocytogenes* (as safety indicators). In this study, these indicators were evaluated in one hundred cheese samples Mozzarella, originating from two business operators in Tirana city.The study was conducted in the period 2013-2017. The results of the analytical test, based on Ordinance n. 234/2014 "On approval of regulation on microbiological criteria on food " Commission Regulation (EC) 2073/2005, showed 16/100 (16%) positive samples for *E.Coli*, while for *Salmonella.spp* and *Listeria monocytogenes* positive samples were not found.

Keywords: *Mozzarella, E.coli, Salmonella.spp, Listeria monocytogenes, food safety*

INTRODUCTION

The presence of pathogens in dairy products is a major concern in public health (Sobrinho et al., 2012). Cheese consumption has been associated with foodborne disease outbreaks in different parts of the world, since natural cheeses can support the growth of microorganisms including foodborne pathogens (Varga., 2007; Choi et al., 2016). Certain categories of cheeses made from unpasteurised milk, such as fresh soft and soft-ripened varieties, can be considered like potentially risky foods because it is possible for pathogenic bacteria to contaminate the final product via different paths: milk contamination in dairy farms; cross contamination during cheesemaking; contamination at the ripening process; microbial contamnation during storage process (Kousta et al., 2010). As a result, pathogens may grow to levels where they can cause human illness. It is important for cheesemakers to manage microbiological risks during cheesemaking to protect public health.

Moderate and high humidity cheeses are good environment growth of food pathogens. Pasteurization is the safest method to destroy bacterial pathogens commonly reported in raw milk. In recent years the microorganisms *Salmonella spp., L.monocytogenes,* and *E. coli O157:H7* have been mainly connected with foodborne illness outbreaks linked to cheeses produced after milk pasteurization treatment. (Center for Science in the Public Interest, 2013; Centers for Disease Control and Prevention, 2013). S.aureus has not been often associated with foodborne illness outbreaks linked to cheese, even though this pathogen is generally linked to

foods, such as cheese, which are often hand manipulated during processing and packaging (Centers for Disease Control and Prevention, 2010; U.S. Food and Drug Administration, 2013)

Escherichia coli represents a hygienic indicator microorganism, reflecting faecal contamination. In reference to specific virulence factors and phenotypic characteristics, these bacteria have different groups such as enterohemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), and so on. Globally, E. coli O157:H7 serotype is responsible for foodborne disease outbreaks (Martin N. H et al., 2016).

MATERIALS AND METHODS

Sample Collection

The study was conducted in the 2013-2017 period. one hundred cheese samples Mozzarella originating from two business operators in Tirana city were evaluated.
Sampling of Mozzarella cheese samples was performed according to ISO 707:2008 milk clause 16 (IDF 50: 2008) Milk and dairy products - Sampling instructions. Samples were collected and transferred to the laboratory for bacteriological examination.

Sample Analysis

Mozzarella cheese samples were analyzed using reference methods for the microbiological parameters, *E. coli*, *Salmonella* spp. and *L. monocytogenes*.

Escherichia coli

Escherichia coli were enumerated according to: *E.coli* enumeration, ISO method 16649-2. A 10-g portion of the sample was placed in stomacher bag, 90 ml of sterile BPW was added, and the suspension was batter in a Stomacher 400 (Seward Medical, London, UK) for 1 min at maximum speed. Serial dilutions (10^{-1} to 10^{-4}) in BPW were prepared, and petri dishes were inoculated by the pour plate technique. 25 mL of molten and tempered (45^0 C) Tryptone bile x-glucuronide medium (TBX; Biolife) was added to 1.0 ml of each serial dilution, and the plate was agitate gently. After solidification plates were inverted and incubated aerobically for 24 h at 44 ±1 celsius grade. From plates with greater than 10 but less than 300 colonies of confirmed colonies (blue/green coloured) were enumerate. The final result as CFU per gram was obtained as the arithmetic mean of the results from the two consecutive agar plates multiplied by the dilution

Salmonella

For Salmonella qualitative determination,was usedpart one of ISO 6579. Twenty five grams of Mozzarella chesse were aseptically aliquot for *Salmonella spp* detection. Then, each aliquot was homogenized and was incubated at 37^0 C for 18 ± 2 h with Buffered Peptone Water Muller-Kauffman Tetrathionate-Novobiocin broth (MKTTn; Biolife) medium was used. One ml of BPW for each sample was transferred to 10 mL MKTTn medium and were incubated at 37 for 24 ± 3 h and 0.1 mL of incubated BPW was added to 10 mL of RVS and be incubated at 41.5^0 C for 24 h. A loopful from each incubated RVs and MKKTn culture was streaked on the dry surface of Xylose Lysine Deoxycholate agar plate (XLD; Biolife) and Hectoen Enteric Agar plate (HEA; Oxoid) and incubated at 37^0 C for 24 ± 3 h. The plates were examined for the presence of typical colonies of Salmonella spp. and then subculture was performed on Nutrient Agar (NA, Biolife) to get isolate culture. Suspected colonies were confirmed by conventional biochemical methods (API 20 E; bioMerieux) and serological confirmation was performed by slid agglutination test using standard polyvalent antisera for O antigen group, Vi and H antigen (phase I and II).

Listeria monocytogenes

ISO 11290 part one was applied to all the samples analyzed. Aliquot of 25 g from representative sample was added to 225 mL of Half Fraser broth base (HFB; Biolife, Italy) and the mixture was incubated at 30^0 C for 24 ± 1 h. For the first enrichment step, a loopful was treaked over two selective plates, respectively, to Agar Listeria acc. to Ottaviani & Agosti (ALOA, Bioife, Italy) and to Oxfortd selective agar (Biolife, Italy) and incubated at 37^0 C for 25 ± 1 h to ALOA and 30^0 C for 48 ± 2 h to Oxford medium. For the second enrichment step, 0.1 mL of the first enrichment culture was transferred to a tube with 10 mL of the Fraser broth and the mixture was incubated at 37^0 C for 48 ± 2 h. This second enrichment culture was streaked on ALOA and Oxford agar and incubated respectively at 37^0 C for 25 ± 1 h and 30^0 C for 48 ± 2 h. Typical colonies were transferred onto tryptic soy yeast extract agar (TSYEA) and incubated at 37 °C for 24 h. Strains were identified using the API Listeria biochemical system.

RESULTS

In the present study, Mozzarella cheese samples (n=100) were analysed for *Salmonella* and *L. monocytogenes* as safety index and *E. coli* as food hygienic indicators. The microbiological quality and safety of examined cheese samples assessed using criteria limits in the EU regulations (Commission regulation (EC) No 2073/2005) and Albanian standards for food (Order.234.dt.20.05.2014 amendment of 261).

The results of the analytical test, showed that 16/100 (16%) are positive samples for *E.coli.* The obtained results are shown in Table 1 and Graph 1, respectively by years. Trend of the average value of *E.coli* load in the Mozzarella cheese matrix during the period 2013-2017 is given in Graph 2, as well as the presentation of typical blue, blue-green colonies of *E.coli* b-glucuronidase positive on chromogenic Tryptone Bile X- Glucuronide medium (TBX), plate counting, shown in photo 1. Meanwhile, we note that Salmonella and *L. monocytogenes* were not present in any of the tested samples.

Table 1. The results of the analytical test: *E.coli*

Sample type	N. of samples analyzed	Year 2013	Year 2014	Year 2015	Year 2016	Year 2017
Mozzarella (soft cheese)	100	**1**/15 (0.67)	**8**/22 (0.364)	**3**/28 (0.107)	**2**/17 (0.118)	**2**/18 (0.11)

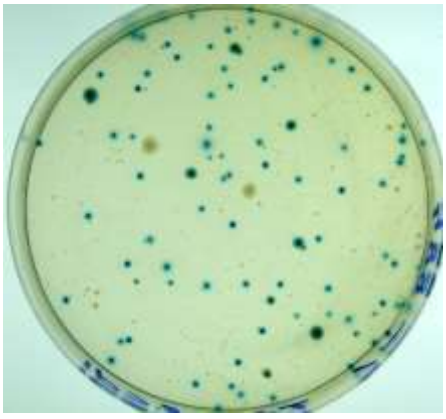

Photo 1. Presentation of typical blue, blue-green colonies of *E.coli* b-glucuronidase positive on chromogenic Tryptone Bile X- Glucuronide medium (TBX), plate counting.

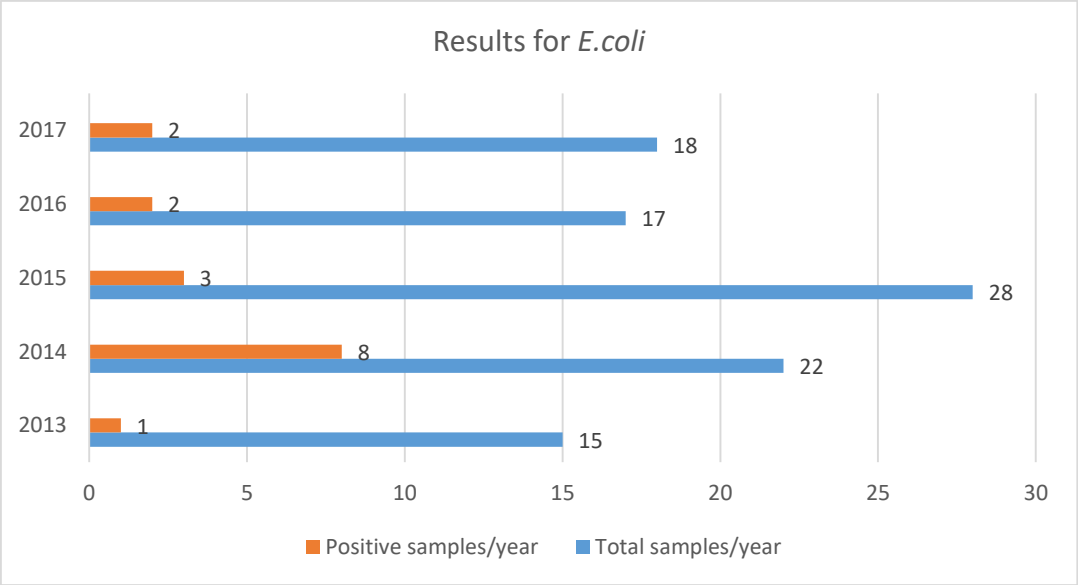

Figure 1 Graphic presentation of results, by years: *E.coli*

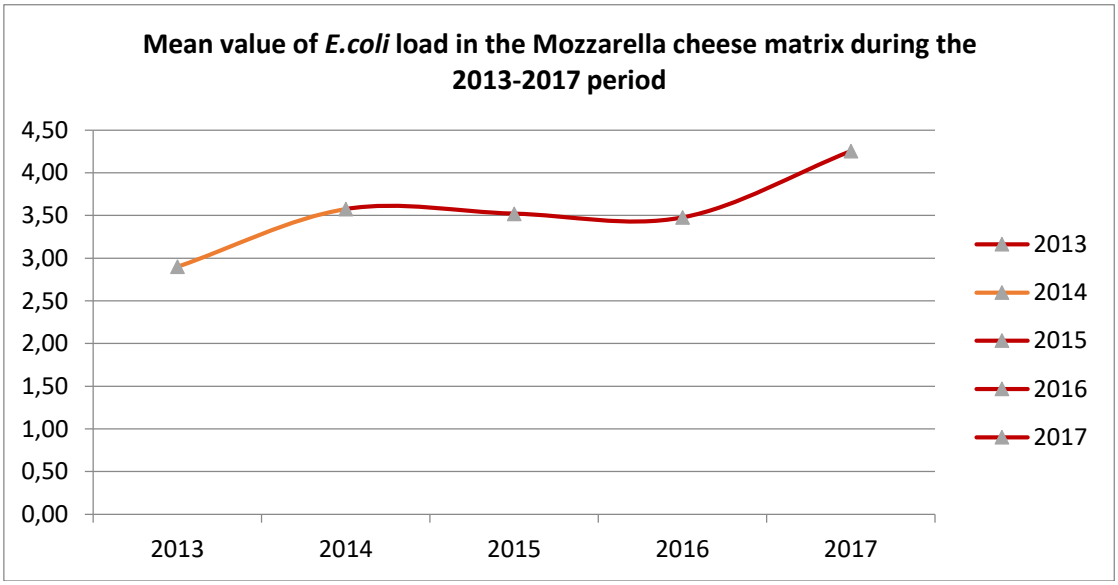

Figure 2 Trend of the average value of E.coli load in the Mozzarella cheese matrix during the period 2013-2017

DISCUSSION

This implication with *E.Coli* in 16 Mozzarella cheese samples may have resulted from the properties of soft cheese like the high water activity and low acidity as well as improper sanitation that is common route for pathogenic bacteria to contaminate the cheese during cheese-making process (Gould et al., 2014).

In establishing EU Community microbiological criteria, the EU believes that such criteria should: enhance food safety, be feasible in practice and be based on scientific risk assessment. ICMSF (The International Commission on Microbiological Specifications for Foods) guidance was considered in establishment of microbiological criteria for cheese in EC Regulation 2073/2005 (Donnelly C., 2018). EU microbiological criteria for cheese and milk intended for cheese making are risk based and differ depending upon whether cheese has been made from heat treated versus raw milk.

Cheese related foodborne illnesses have been generally linked to soft cheese or cheese made from raw or unpasteurized milk, but rarely in hard cheese. Hence, manufacturing process and distribution and storage process should be controlled appropriately to prevent foodborne illness by these cheeses. In addition, microbial risk assessment including predictive model for exposure assessment also should be importantly counted for microbiological safety assurance (Choi K-H et al., 2016)

Microbiological contaminants in the dairy processing environment are important sources of finished product contamination. Risk reduction efforts should be placed on the identification of reservoirs of pathogens such as *Listeria monocytogenes* in the production system and the development of practices that reduce pathogen spread and minimise of the potential for cheese contamination (Donnelly C., 2018)

Referring to the data of the IPARD II Program for Albania, for the dairy processing industry, the tendency of increasing investments in dairy processing plants is emphasized - to improve the buildings and equipment to avoid cross-contamination (Programi IPARD II., 2015)

CONCLUSIONS

The application of *E. coli* boundaries provides a scientifically meaningful standard in cheese made from heat-treated milk as E. coli will not survive after heat treatment. Its presence in cheese made from heat-treated milk as are the samples in the study, indicates recontamination. The action required in this case of unsatisfactory results includes improvements in production hygiene and raw material selection (Cretenet et al., 2011).

Absence of *Salmonella*.spp and *L. monocytogenes* in Mozarella cheese samples, clearly showed the efficiency of the pasteurization process applied to kill both microorganisms. Evaluation of microbiological criteria in dairy products is a necessary step to ensure the effectiveness of the HACCP plan.

A survey of the microbiological quality of raw milk specifically intended for soft cheese production in Albania may provide an assessment of the overall quality of raw milk used for cheese production and identification of areas where improvements can be made.

As consumers demand increased access to locally produced, high quality foods such as cheeses, promoting food safety will be key to sustaining the Albanian cheese industry.

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SYNTHESIS OF TIO_2 NANOPARTICLES VIA DIFFERENT METHODS: STRUCTURAL, OPTICAL AND PHOTOCATALYTIC PROPERTIES

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ABSTRACT

TiO_2 photocatalysis is an advanced oxidation process to eliminate organic pollutants in water or air. These pollutants are converted to small molecules such as CO_2, H_2O at the end of the process. The method is based on the usage of UV-A light and TiO_2 as a semi-conductor together. TiO_2 is the most popular and used photocatalyst due to its unique properties such as chemical inertness, being low-cost, non-toxicity, having good photostability and having high oxidative power.

In this study, TiO_2 nanoparticles (TiO_2 NP) were sythesized by two methods namely, an acid-catalyzed and a modified sol-gel method without any impurity. The prepared photocatalyst samples were characterized by FTIR, XRD, ESEM-EDX, UV-DRS, and BET techniques. The photocatalytic performances of the photocatalysts were determined by examining the degradation reaction of 4-nitrophenol under UV-A light. The results indicated that the preparation method affects the structural, optical and photocatalytic properties of the synthesized TiO_2 photocatalysts. ESEM micrographs of the synthesized TiO_2 photocatalysts showed almost spherical particles. A higher photocatalytic performance was obtained for the TiO_2 NP sample synthesized by modified sol-gel method.

Keywords: *4- nitrophenol, photocatalysis, sol-gel, TiO_2 nanoparticles.*

INTRODUCTION

TiO_2 photocatalysis is a popular advance oxidation process concerning a great attention as an effective wastewater treatment. The basis of this process is the formation of photogenerated charge carriers on the surface of TiO_2 with UV light irradiation. Thus, an electron is transferred from the valence band to the conduction band of TiO_2 and forming a positive hole in the valence band. This accumulated electron/hole pair reacts with H_2O and O_2 or OH^- to generate reactive oxygen species such as hydroxyl radicals. Subsequently, hydroxyl radicals react with the adsorbed pollutants and mineralized them to CO_2 and H_2O (Bora and Mewada, 2017; Byrne et al., 2018; Khaki et al., 2017).

In literature, a significant number of studies have been performed to remove organic pollutants in water by using TiO_2 photocatalysis (Birben Nazmiye et al., 2016; Birben et al., 2017; Birben et al., 2015; Gurkan et al., 2017; Gurkan et al., 2012; Shaham-Waldmann and Paz, 2016; Turkten and Cinar, 2017; Turkten et al., 2019). It is well known that surface area, crystallite phase composition are important parameters affecting TiO_2 photocatalysis. The differences between these parameters are directly related to synthesis and processing routes (Testino et al., 2007).

In this study, we investigated the effects on the structural, optical, and photocatalytic properties of the synthesized TiO_2 photocatalysts by two diffrent sol-gel preparation routes namely, an acid-catalyzed and a modified sol-gel method. The synthesized TiO_2 photocatalysts were characterized by FTIR, ESEM-EDX, XRD, UV-DRS, and BET spectroscopic techniques. Hence, crystal phase composition, crystallte particle size, surface area, and morphology were determined. Moreover, the photocatalytic activities of the synthesized TiO_2 photocatalysts were investigated in the degradation of 4-nitrophenol (4-NP) under UV-A light.

Material and Methods

Materials

Titanium(IV) isopropoxide (TIP) was purchased from Aldrich. Absolute ethanol, glacial acetic acid, and 4-NP were obtained from Merck. Distilled water was used to prepare solutions.

Synthesis of TiO_2 Nanoparticles

TiO_2 NP was synthesized by an acid-catalyzed sol-gel method (TiO_2 ACSG) (Kaneko et al., 2001; Kitayama et al., 1998). Briefly, 25 mL of TIP was added to 75 mL acetic acid solution (80 wt%) under continuous stirring for 2 h at 50^{o}C. A transparent sol was obtained. After agitation and extensive washings, TiO_2 ACSG was dried at 105^{o}C for 24 h. Finally, the obtained white powder was calcinated at 500^{o}C for 5h. TiO_2 NP was synthesized by modiefied sol-gel method (TiO_2 MSG) as described in our previous work (Turkten and Cinar, 2017).

Characterization Techniques

Fourier transform infrared (FTIR) spectra of the TiO_2 NP samples were perfomed by Perkin Elmer Spectrum One spectrometer. The measurements of nitrogen adsorption/desorption isotherms were recorded on using a Quantochrome Nova 2200e. ESEM-FEG/EDAX PhilipsXL-30 instrument was used for the examination of morphologies of the prepared TiO_2 NP samples. The X–ray powder diffraction (XRD) patterns were recorded on a Rigaku-D/MAX-Ultima diffraction spectroscope using Cu Kα radiation (λ=1.5418 Å). Perkin Elmer Lambda 35 spectrometer was used to obtain the UV-visible diffuse reflectance spectra (UV-DRS) of the photocatalysts. The photocatalytic degradation experiments of 4-NP were investigated by using an Agilent 8453 UV–Visible spectrophotometer.

Photocatalytic Degradation Experiments

The photocatalytic degradation experients of 4-NP was investigated using TiO_2 NP samples under UV-A light. A double-jacket pyrex photoreactor connected with a water bath was utilized for photocatalytic experiments. 5x8 W blacklight fluorescent lamps were used as the light source. 600 mL solution containing 4-NP (1.0×10^{-4} mol/L) with TiO_2 NP photocatalyts was suspended in an ultrasonic bath for 30 minutes in dark. The amount of the photocatalyst used was 0.2 g/100 mL. The prepared 600 mL voume suspension was stirred mechanically. 10 mL samples were taken with appropreciate time intervals and were filtered immediately to separate TiO_2 through 0.45 μm Millipore HA filters.

Results

FTIR

FTIR spectra of the synthesized TiO_2 NP samples are presented in Fig. 1. The spectra for the TiO_2 NP samples have a wide peak in the region 3500–2400 cm^{-1} corresponding to O–H stretching mode of OH groups. The peak at ~1626 cm^{-1} is related to the bending mode of adsorbed H_2O (Mino et al., 2016; Yalçın et al., 2010). In the spectral region below ~970 cm^{-1}, the peaks are assigned to Ti–O stretching and O–Ti–O bending vibrations (Yalçın et al., 2010).

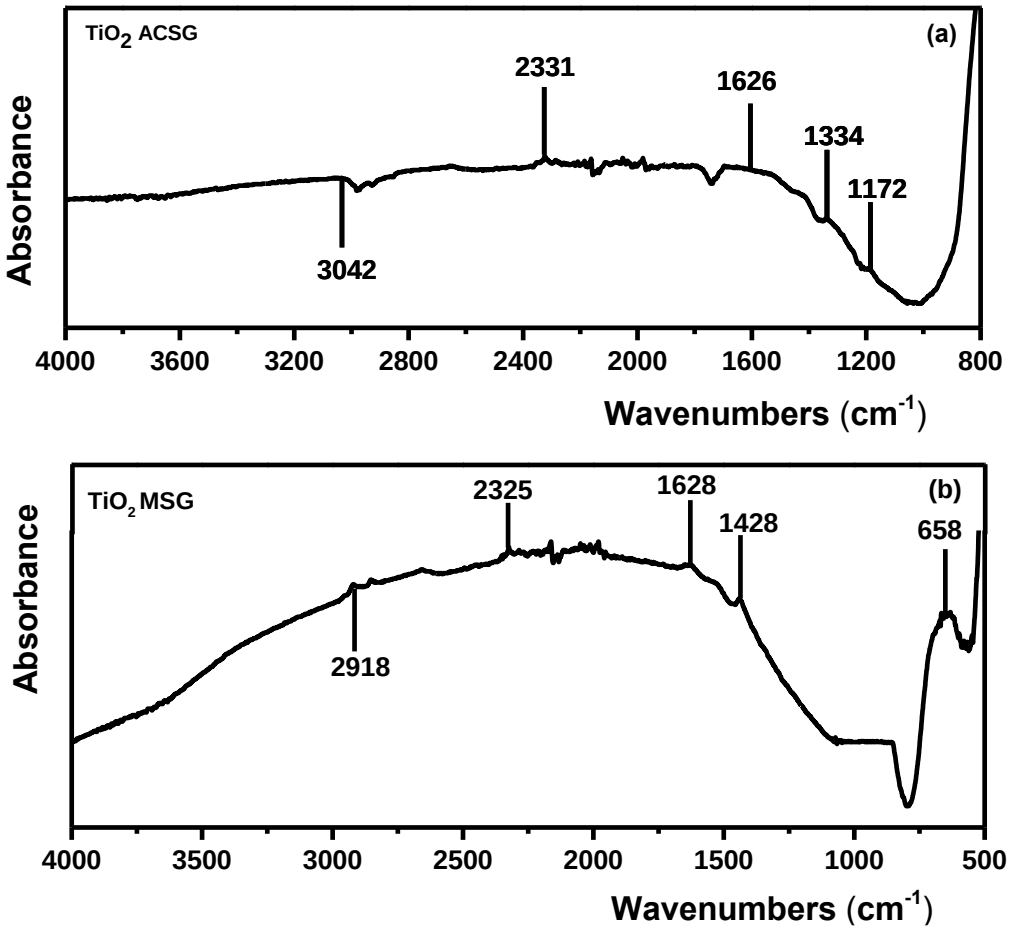

Fig. 1. FTIR spectra of TiO_2 NP samples, (a) TiO_2 ACSG, (b) TiO_2 MSG.

Figure 2 displays XRD diffractograms of the TiO_2 ACSG and TiO_2 MSG samples. The spectra were investigated in the range 2-70^o following MDI-JADE6 library. The diffractogram of TiO_2 ACSG sample indicates only the existence of anatase phase while TiO_2 MSG sample contains both anatase and rutile phases. In Figure 2 (a), the most intense peak at 25.30^o is the characteristic (101) anatase peak. Other peaks at 37.81^o, 38.64^o, 48.14^o, 54.02^o, 55.29^o, 62.89^o and 68.80^o correspond to (1 0 3), (0 0 4), (2 0 0), (1 0 5), (2 1 1), (1 1 8) and (1 1 6) planes of anatase. As it can be seen from Figure 2 (b), a minor peak at 25.7^o is the characteristic (110) rutile peak. The other rutile peaks are at 36.27^o and 41.29^o correspond to (1 0 1) and (1 1 1) planes of rutile.

Crystallite size (d) was determined using the Scherrer equation;

$d=(0.9\ \lambda\ 180)\ /\ (\Pi FWHM_{hkl}\cos\theta)$ where, $FWHM_{hkl}$ is the full width at half maximum of an hkl peak at θ value. The crystallite sizes calculated from diffraction plane (1 0 1) of anatase. The calculated crystallite sizes were 19 nm and 33 nm for TiO_2 ACSG and TiO_2 MSG samples, respectively.

Fig. 3 shows the ESEM micrographs and EDX spectra of TiO_2 ACSG and TiO_2 MSG samples. As it can be seen, both TiO_2 nanoparticles consist of nearly spherical particles with a slight agglomeration. The EDX spectra contain Ti and O as the principal components. No impurities are detected in EDX spectrum.

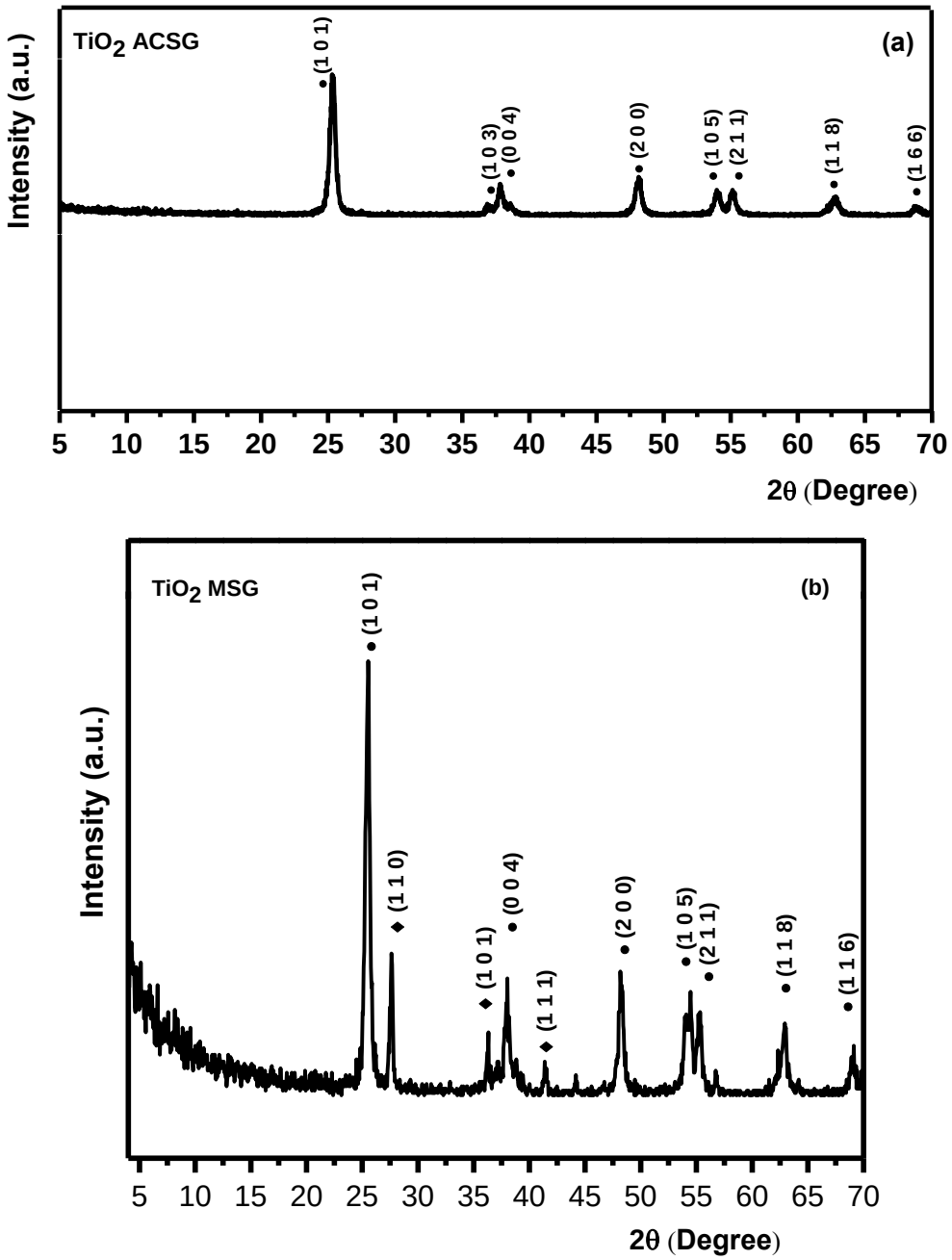

Fig. 2. XRD diffractograms of TiO_2 NP samples, (a) TiO_2 ACSG, (b) TiO_2 MSG (● anatase, ♦ rutile).

The band-gap energies of the samples were calculated by the Kubelka-Munk formula, where R is the reflectance read from the spectrum.

$$F(R) = \frac{(1-R)^2}{2R}$$

With the use of Tauc equation a graph $[F(R).h\nu]^n$ vs $h\nu$ (photon energy and n = 1/2) was plotted (Kuvarega et al., 2011). The calculated band-gap energies were derived from the intersection of the Tauc's linear portion extrapolation with the photon energy axis. The obtained band-gap energies and the corresponding wavelengths for TiO_2 ACSG and TiO_2 MSG samples are 3.05 eV at 406 nm and 2.98 eV at 416 nm, respectively.

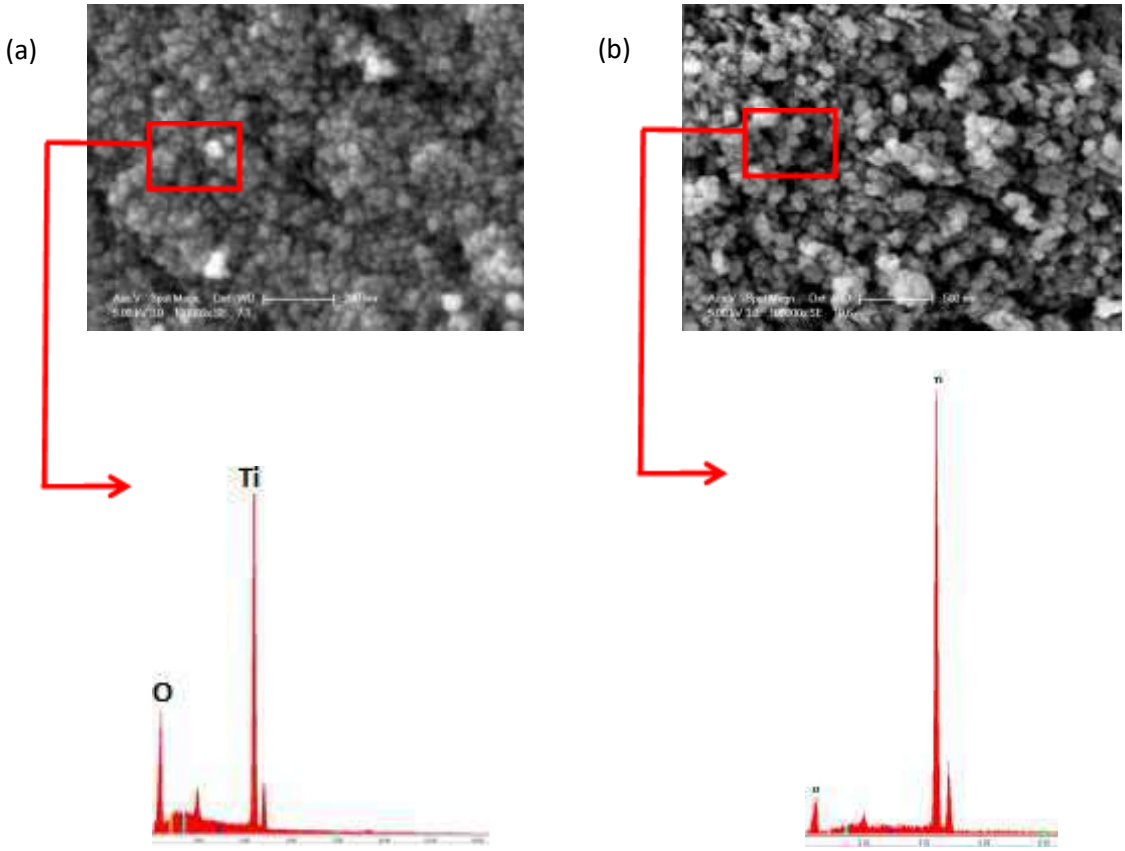

Fig. 3. SEM micrographs and EDX spectrums of TiO_2 NP, (a) TiO_2 ACSG, (b) TiO_2 MSG

BET surface area for TiO_2 ACSG and TiO_2 MSG samples were calculated to be 34 and 45 m^2g^{-1}, respectively using a multi-point BET technique.

Photocatalytic activities of the TiO_2 ACSG and TiO_2 MSG samples were determined by investigating the kinetics of the degradation of 4-NP under UV-A light irradiation. There was no noticeable loss of 4-NP when the irradiation was performed in the absence of TiO_2. In non-irradiated experimental condition, there was a minor loss, approximately 3%, due to adsorption onto TiO_2 particles. On the other hand, in the presence of TiO_2 ACSG and TiO_2 MSG photocatalysts, a fast degradation of 4-NP occurred by irradiation. 25% of 4-NP was removed in 120 min. as compared to 57% removal with TiO_2 MSG.

Conclusions

TiO_2 NP samples were synthesized by two methods namely, an acid-catalyzed and a modified sol-gel method. The results revealed that crystallite particle size and crystallite phase composition was altered in the synthesized TiO_2 NP samples owing to synthesis route. TiO_2 ACSG sample was only containing anatase phase, while TiO_2 SMG sample indicated anatase with a trace amount of rutile. Moreover, the surface area and optical properties were slightly different. This situation affected the photocatalytic activity of TiO_2 NP samples. TiO_2 SMG sample showed a better photocatalytic activity compared to TiO_2 ACSG sample. In conclusion, the preparation method affected the structural, optical and photocatalytic properties of the synthesized TiO_2 NP photocatalysts.

Acknowledgements:

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INVESTIGATION OF THE EFFECTS OF ZINC FERTILIZER APPLICATION ON YIELD AND PROTEIN IN FABA BEAN (*VICIA FABA* L.)

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ABSTRACT

More than three billion people worldwide face the problem of mineral deficiency in nutrition. The human body needs protein and minerals in every period of its life. Grain protein ratio is between 18.0-31.6%, it is included in the human nutrition of the group of legumes. (Vandemark G.J. et al., 2018). Adequate zinc uptake can be achieved by fertilizing plants from the soil or through leaf fertilization at different stages of growth. (Çakmak, 2008). (Sevinç et al., 2017). This study was conducted in the trial field of Aydın Adnan Menderes University Agriculture Faculty in 2019-2020. In this study, the use of zinc fertilizer, 2 different doses (0 kg / da and 3 kg / da) in the form of ZnSO47H2O, the effects of yield and yield on 3 different cultivars of faba bean (Salkım, Kıtık 2003 and Filiz 99) and the protein content were examined. As a result, the effects of fertilizer application of zinc on 100 seed weight, pod weight, harvest index and grain yield are significant. On the other hand, the effect of zinc fertilizer application on the first pod height, pod number, plant weight and grain protein content were found to be statistically nonsignificant.

Key words: *Faba bean, zinc, yield, protein*

INTRODUCTION

Edible legumes constitute an important plant group in both human and animal nutrition. When grown in rotation with other crops, under certain environmental conditions, they can improve soil fertility and reduce the incidence of weeds, diseases and pests (Mwanamwenge et al., 1998). One of the most important inputs of crop production is fertilization. With fertilization, plant growth and development are closely related to quality products. (Dinesh et al., 2014). Since legumes can bind the free nitrogen of the air, the need for nitrogen fertilization is low. Intensive agriculture in the territory of the region causes a decrease in soil fertility (Tosun, 1981). They are also beneficial in terms of sustainability of agricultural production and environmental protection as they increase soil fertility by binding the free nitrogen of the air to the soil.

About 1/3 of the world's soil is zinc deficient (Çakmak ve ark., 2017). Adequate zinc intake can be achieved through fertilization of plants from the soil or through foliar fertilization at different stages of growth (Çakmak, 2008). Considering the important relationship between human health and agriculture, it is revealed that feeding with products with high zinc content has a positive effect on zinc deficiency, thus on human health (Joy ve ark., 2017). It is an important fact that the more quality products it can produce, the healthier it will be. Zinc deficiency in

humans has been determined to be caused by zinc deficiency in agricultural soils and therefore zinc deficiency in agricultural products (Çakmak et al., 2017). Zinc deficiency has been detected in the soils where chickpea is cultivated in the world. In addition, it is observed that zinc intake by the plant is limited in alkaline soils (Broadley et al., 2007). Zinc intake in plants has some physiological functions. Zinc deficiency is one of the most important factors limiting plant yield. In addition, carbohydrate metabolism is negatively affected indirectly as it is negatively affected by sugar transport and starch biosynthesis. In addition, RuBisCO, chlorophyll synthesis and chloroplast structure are affected by zinc deficiency, which is due to insufficient supply of zinc in photosynthetic reactions. There is an interaction between zinc intake and macro elements such as phosphorus, nitrogen, calcium, magnesium and potassium (Kryvoruchko, 2017). Compared to many other products, chickpeas are among the products that are sensitive to zinc deficiency (Brennan ve ark., 2001). Zinc application increases the number of nodules and dry weight of nodules as well as increasing the yield and quality of chickpeas (Khan et al. 2003, Kayan et al., 2015, Das et al., 2012). In this study, the effect of zinc fertilizer application on grain yield and yield components on different broad bean varieties was investigated.

MATERIAL AND METHODS

Material

Salkım, Filiz 99 and Kıtık 2003 faba bean varieties registered by Aegean Agricultural Research Institute were used in the study.

Methods

The trial was planted in three replications in a randomized plot trial pattern on the 4th of October in 2020. The plot area was 9 m^2 (1,8x5) with 6 rows. Fertilization was made at 3 kg / da N and 6 kg / da P2O5 before sowing.

Zinc fertilization was applied in 2 doses as control 0 kg/da and 3 kg/da Zn2SO4 fertilizer. Zinc fertilizer application was carried out in 2 periods, pre-flowering and flowering.

Plant height, number of branches, first pod height, pod number per plant, seed number per pod, number of seeds per plant, hundred seed weight, dry seed yield and seed protein ratios were measured in 10 plants randomly selected from each plot, and the data obtained were analyzed with JMP statistical analysis program

RESULTS

The results obtained from the research were evaluated statistically and the results are presented in table 1 and table 2.

Table 1. Plant morphological characteristics of faba bean varieties

Cultivars	Plant Height (cm)	Number of branches per Plant	First pod Height (cm)	Pod Number per Plant (adet)	Seed per Pod (adet)	100 Seed Weight. (g)	Pod Height (cm)
Salkım	132,7	4,56	43,2	14,3	3,90	132,7	13,1
Filiz 99	138,8	4,58	44,4	10,7	4,05	96,1	12,7
Kıtık 2003	128,3	4,41	39,3	13,0	4,70	142,4	18,0
LSD_C	4,42*	ns	ns	ns	ns	16,9	1,55
LSD_A	ns	ns	ns	ns	ns	13,82	1,12
LSD_{C*A}	ns	ns	8,86	ns	ns	22,83	ns

C:Cultivar A:Application C*A:interaction of cultivar and application * significant (*P<0,05*) ns:nonsignificant (*p>0,05*)

Plant Height

It was determined that the interaction between cultivar and application was nonsignificant on plant height but mean values was between 128.3 and 138.8 cm. Filiz 99 has the longest plant height with 138.8 cm. The same results preoviusly obtained by El-Gizawy and Mehasen, 2009; Bozorgi et al.,2011, Weldua et al., 2012.

Number of Branches

It was determined that the interaction between cultivar and application was nonsignificant on number of branches while the highest mean value was obtained from Filiz 99 variety. El-Hosary and Mehasen, (1998), El-Masri et al. (2002) and Atiia et al., (2016), foliar application with micronutrients had significantly increased yield attributes of faba bean. Salem et al. (2014) showed positive effect of micronutrients spraying on the yield and yield attributes of faba bean except branch number per plant.

First Pod Height (cm)

It was determined that the interaction between cultivar and application was significantly affected on first pod height. It was seen that the average values varied between 39.3 and 44.4 cm. Filiz 99 had the highest mean value (44,4) and similar significant effects were reported by Usama et al. (2013) and Salem et al. (2014).

Pod Number per Plant

Average values for the number of pods per plant vary between 10.7 and 14.3. the interaction between cultivar and application was nonsignificant affect on pod number per plant. According to mean values the highest average value was obtained from Salkım.

Number of Seed/Pod

The varieties were examined in terms of the number of seeds per pod and it was determined that their mean value varied between 3.90 and 4.70. While there was no statistically significant difference between applications and varieties, the highest average value was obtained from Kıtık 2003 variety.

100 Seed Weight

It was determined that the interaction between cultivar and application was significantly affected on 100 seed weight. It was determined that the average values varied between 96,1 and 142,4. In terms of average values, one hundred grain weight of Kıtık 2003 variety was measured as the highest (142.4 gr).

Pod Height

It was determined that the interaction between cultivar and application was nonsignificant. In the experiment, pod height values of the varieties were measured and average values between 12.7 and 18.0 cm. The highest mean value was obtained from Kıtık 2003 variety.

Table 2. Plant morphological characteristics of faba bean varieties

Cultivars	Harvest Indeks	Seed Yield (kg/da)	Seed Protein Rate
Salkım	81,7	397,9	23,9
Filiz 99	70,5	288,3	25,5
Kıtık 2003	87,5	427,2	26,0
LSD_C	ns	49,14	ns
LSD_A	ns	41,50	ns
LSD_{C*A}	19,20	68,50	ns

C:Cultivar A:Application C*A:interaction of cultivar and application * significant (*P<0,05*) ns:nonsignificant (*p>0,05*)

Harvest Index

It was determined that the interaction between cultivar and application was significantly affected on Harvest Index. The highest average value was obtained from Kıtık 2003 variety.

Seed Yield

It was determined that the interaction between cultivar and application was significantly affected on seed yield. According to the average results, the highest grain yield value was obtained from Kıtık 2003 variety. The increase in seed yield might be associated with high 100 seed weight. The results are in accordance with those obtained by El-Moursy 1998, Said 1998, Ahmed and El-Abagy 2007.

Seed Protein Rate

It was determined that the interaction between cultivar and application was nonsignificant affected on seed protein rate. According to mean values, Kıtık 2003 variety has the highest protein ratio.

RESULTS

As a result, while the application interaction was not found statistically significant in features such as plant height, number of main branches, number of pods per plant, number of pods per pod, pod height, grain protein ratio, the first pod, one hundred grains, the obtained index and grain yield were its interaction is important. Thanks to these results, it was revealed that the zinc fertilization application was planned on a multi-annual basis.

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POSSIBILITIES OF USING SOLAR PANELS IN SMALL RUMINANT BARNS

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ABSTRACT

Our production of red meat from animal proteins, which are of great importance in the nutrition of the people of our country, is not enough.

Due to the rapid increase of population, our consumption of red meat per capita decreases over the years. If the required support is given to sheep and goat breeding from the Ministry of Agriculture and Forestry, an increase in the number and productivity of animals over the years can be a significant solution to the problem of meeting the protein needs.

Environmental control of the active sheep folds of animals will increase their efficiency in sheep and goats. When the outside weather conditions are warm and windless, weather conditions inside the sheep folds can be achieved by mandatory ventilations. Failure to provide suitable weather conditions in the barn decreases the productivity of animals and the risk of getting some diseases.

In mandatory ventilation and cooling, electrical fans and fan-pads are needed. However, animal barns are required to be to rural areas according to the environmental laws and regulation, which means access to the electricity is difficult if not impossible. Because of that, solar panels can be utilized in animal barns to generate electricity to power fans and fan-pads.

The projected ventilation and cooling required for the floor area of 374 m^2 (11.0 * 34.0) and 300 heads sheep fold is suitable for the use of 4 aspirators and fan-pads. For this system, a 4.0 kWh solar panel system provides the necessary energy. It can be used in panels and increased excess energy is stored in batteries, which can be used in other appliances in the barn.

Keywords: *Green energy, sheep-goat fold, solar panel system, ventilation, cooling.*

INTRODUCTION

Animal breeding is a key business in the Turkish economy in terms of social lives and eating habits of people. The community will have a healthy diet if protein, calorie, fat and carb needs must be balanced and met. Furthermore, 40 % of the protein consumed must be from animals. However, only 29 % of the protein consumed in our country per capita is from animal products (Gündüz et al., 2006[1]). On the other hand, animal breeding is a key business operation because it supplies raw materials to the industry (food, wool woven fabrics, leather and stout leather etc.) and because of its contributions to employment. Organic wastes and residues of

animals might not be directly used as human food but might be used to the benefit of people in other ways.

Animal protein is one of the key elements in nutrition and red meat is a source of animal protein however our red meat production capacity cannot meet the demand. The per capita consumption of red meat gradually decreases in our country.

Other crucial problems in animal production are accessing to energy and covering the ever-increasing energy costs. Majority of the energy consumed around the world comes from non-renewable and fossil fuels. Fossil fuels can be found in specific places around the world and there are restrictions such as processing and transportation difficulties.

Fossil energy resources used all around the world lead to destructive environmental impacts such as carbon emission. Use of fossil energy resources increases release of greenhouse gases and this leads to an increase in global temperature. The increased global temperature causes unbalanced meteorological events such as drought, storm and flood (Anonymous, 2020a).

Rise of energy prices increases the cost of agricultural production and finished products tend to be more expensive. Use of environment friendly and green energy resources must be preferred instead of using fossil fuels that are subject to depletion of reserves and price increases. Green energy can be defined as an energy resource that causes very low or no CO_2 emission in the atmosphere. In case of green energy, renewable energy resources such as solar energy, wind, geothermal energy, hydraulic energy and waves (Anonymous, 2019).

Solar energy can be converted to electricity with photovoltaic systems and thermal energy with solar thermal systems (Yüksel and Yüksel-Türkboyları, 2017).

Importance of small ruminant breeding in Turkey

Turkey is an optimum place for small ruminant breeding because of factors such as natural resources, particularly grasslands, fit for sheep and goats and consumption habits of families in rural areas. Small ruminant breeding is generally preferred more than bovine breeding because handling a herd of small ruminants is easier and less workforce is needed (Amak, 2018). Recently, number of animals and production of animal products have increased significantly thanks to the support and incentives offered by the Ministry of Agriculture and Forestry. In 2002, the number of sheep in small ruminants' category was 25.2 million and this quantity increased up to 35.2 million in 2019. The increase in sheep quantity was slight in some years and high in other year however average increase in 18-year period was 2.21 %. If we review the number of goats in Turkey, we can see that the number of goats was 6.8 million in 2002 and 10.9 million in 2019. The increase in number of goats was slightly higher than sheep and recorded as 3.33 % (TUIK, Turkish Statistical Institute, 2020).

Despite the significant increase in livestock and production of animal products, per capita consumption of meat decreases in our country. 2018 consumption of meat in some countries was as follows; 101.3 kg in the USA (27.1 kg beef, 24.0 kg pig, 49.8 kg poultry and 0.4 kg sheep), 69.5 kg in the EU (10.8 kg beef, 32.3 kg pig, 24.5 kg poultry and 1.9 kg sheep) and this was 36.2 kg in Turkey (12.9 kg beef, 21.9 kg poultry and 1.4 kg sheep) (BESD-BİR, 2020). In 2015, the per capital consumption of meat was 37.4 kg in Turkey and this consumption decreased by 1.2 kg in three years. It is reported that small ruminant products will be very useful in closing this gap of animal products.

Ambient conditions in folds

Several factors are considered when constructing a fold for small ruminants. These are providing the optimum ambient conditions for animals, covering nutrition and animal care needs and protecting from negative external environment effects (Amak, 2018).

In our country, sheep breeding can be divided into two groups; conventional (extensive) and modern (intensive) housing. The conventional housing is commonly characterized with primitive fold structures. This type of housing is generally used by small family business in this country. Intensive housing offers high efficiency by sheltering sheep in ambient conditions and assuring animal welfare. This housing system is preferred by the commercial and large-capacity businesses recently established in our country (Koyuncu, 2005, Taşkın et al., 2015).

In intensive housing system, sheep folds offering optimum ambient conditions to sheep and goats are constructed and tunnel & greenhouse type sheep folds are preferred recently. These closed sheep folds are mainly preferred in very cold regions (Ünal and Yılmaz, 2009).

Number of animals housed in a sheep fold must be proportional to the floor area. Number of animals kept in the sheep fold section should not exceed the sheep fold capacity because increased microorganism population might cause a number of problems such as udder, foot and respiratory diseases. Hygiene in a sheep fold is very important for animal welfare and particularly for preventing mastitis (inflammation of udder tissue). Therefore, summer and winter air requirements in sheep folds must be met (Casamassima et al., 2001; Faerevik et al., 2005).

Ventilation is the key of assuring comfort in sheep folds and providing optimum ambient conditions. Ventilation keeps climate and chemical ambient conditions in the sheep fold at a good level for the animals. Air circulation balances climate ambient conditions, air temperature and humidity. Chemical ambient conditions preserve the desired levels of dust, harmful microorganisms and gases in the sheep fold (Yüksel and Şişman, 2015).

Natural and necessary (mechanical) ventilation systems are used in the barns. If the air circulation outside the sheep fold is not sufficient and the air temperature is high, natural ventilation cannot create the desired effect in the sheep folds. In this case, sufficient ventilation and cooling can only be provided with mechanical ventilation systems.

Sheep fold is a barn / animal shelter generally seen in the rural areas and the environmental protection law requires construction of a sheep fold minimum 1000 meters away from any residential area. Sometimes, this rule might lead to difficulty in accessing to electricity supply system.

In rural areas, green energy might be preferred and electricity might be generated and used with photovoltaic cells for using electricity if electricity network is not available, during a power cut or for reducing energy costs (Yüksel and Yüksel-Turkboyları, 2018).

Solar energy potential in Turkey

Turkey's solar energy potential is higher than most countries. Sunshine hours decrease from south to north in the country. This study focused on Tekirdağ province located in Marmara region; total solar radiation in this province is 1400-1450 kWh m^{-2} $year^{-1}$ (3.97 kWh m^{-2} day^{-1}) and this value is close to the average figures in Turkey (Anonymous, 2020b). According to the Turkey's Solar Energy Potential Atlas (GEPA), total sunshine hours in a year is 2741 hours

(average 7.5 hours / day) and total solar energy received in a year is 1.527 kWh m^{-2} $year^{-1}$ (average 4.18 kWh m^{-2} day^{-1} per day) (Anonymous, 2020c).
If we consider the solar radiation and sunshine hours in Tekirdağ, we can say that a solar panel system of sufficient size might be used to generate electricity for barns.

Material and Methods

The study was completed in Tekirdağ province which is located in the northwest of Turkey along Marmara sea coast. Tekirdağ is located in the European side of Turkey between 26˚41′-28˚10′ east longitudes and 40˚ 35′-41˚35′ north latitudes. The land structure in Tekirdağ is slightly rugged and the geological structure is young; it was formed during the fourth eon.Tekirdağ has semi-humid climate and summers as well as winters are windy (Anonymous, 2007).
Sheep folds are used as small ruminant houses and a ventilation project will be designed for assuring comfort and welfare of animals. Furthermore, fan-pad system project shall be designed for cooling the animals when the weather is hot. Ventilation and cooling requires mechanical ventilation system. The ventilation system shall be installed in a sheep fold with 300-animal capacity and the size of sheep fold was around 374 m^2 (11.0*34.0) (Karaman et al., 2012). For designing a more cost effective system project, the barns must be insulated. This might allow reducing ventilation and cooling capacities. Low-cost and low capacity fans, fan-pads and photovoltaic cells might be used in the project.

Photovoltaic cell and ventilation system design

A solar panel is a photovoltaic tool that has solar cells and that directly converts sunrays into electric current. Photovoltaic cells might be organic and inorganic based. Generally, inorganic based photovoltaic cells are more common because they have output around 15 to 20 % and they are highly efficient (Grätzel, 2009).
Inorganic solar cells are produced by semiconductor cells. There are several substances classified as semiconductor but the optimum and most common one for the solar cell (photovoltaic cell) is silicon. Silicon must be mixed with phosphor and aluminum so that it can be used as photovoltaic cell. This will give the cell one positive and one negative layer to generate electricity current. The surfaces of the produced photovoltaic cells can be rectangular, square and round. The surface areas are generally 100 cm^2 and the thickness is between 0.2 and 0.4 mm. To increase the power output, the photovoltaic cells might have parallel or serial connection. The panels convert sunrays to direct electricity current. The solar cells are installed on a surface to build the solar panel (Anonymous, 2020d).
This study discusses the alternative use of sun, one of the green energy resources, in small ruminant sheep folds. Methods of using electricity generated with renewable energy resources in ventilation and cooling systems of sheep folds were researched. The objective is to determine the sizes and capacities of the photovoltaic cells, fans, fan-pad (wet pad) and circulation pump of the ventilation and cooling system.

Solar panel and ventilation system design

The structure of photovoltaic cell system includes photovoltaic cells, battery group, battery charging regulator, inverter and auxiliary electronic circuits however this depends on the application.

A solar panel is a photovoltaic tool that has several cells and that directly converts sunrays into electric current. It generates direct current. The number of solar panels can be determined based on the energy needed.

The battery group can be defined as devices that store electric energy as chemical energy and, if needed, they deliver it as electric energy. The system generally has gel battery models (gelled electrolyte sealed lead acid) and includes fully closed and maintenance-free batteries. Battery charging regulator generally balances the energy generated in the solar panels. The charge regulator is used for preventing overcharge or discharge of the batteries. Depending on the battery condition, it interrupts the current coming from the photovoltaic cells or current of the system providing the load. High-efficiency (98 %) charge regulators working with MPPT (maximum power point tracking) technique must be preferred. This type of charge regulator charges the battery properly and this extends the battery life by 50 % and the system becomes more cost effective.

The inverter converts linear current coming from the solar panels to alternating current of 220 V 50 Hz. If the system will use alternating current or if current is to be provided to mains supply, the system must have an inverter (Köroğlu et al., 2010, Anonymous, 2020d). The solar panel system to be used for generating green energy and fan, wet pad (fan-pad) system that will ventilate and cool the folds are shown in Figure 1.

Figure 1. Components of ventilation and cooling system used in sheep folds, a type of animal house, with solar panel system

A number of different methods might be preferred for using water to cool barns. Water might be used in barns with irrigation methods, namely sprinkling or fogging systems. Water drops

coming from these systems fall on animals or barn floor. In time, falling water drops vaporize. The water's cooling effect takes away 598 calories (2500 J) from air and environment when a gram of water evaporates. The air in the barn will be loaded with water vapor. Vaporization required for water vaporization takes almost all of the latent heat from the perceptible heat. This reduces the air temperature in the barn. If mechanical ventilation is not used to support the process when cooling the barn with sprinkling and fogging systems, humidity of the air inside the barn might exceed the desired levels. Very high level of humidity is not good for animal health and development. However, in case of sheep breeds used for their wool, high level of humidity damages the natural texture of wool and gives a pale color. Therefore, the ideal relative humidity in the sheep fold is 55 to 65 % in average for the breeds kept for their wool (Ekmekyapar, 1991).

If sheep folds have cooling system with water, mechanical ventilation and fan-pad system, the humidity in the sheep fold will not be high. Fan-pads keep the temperature in barn between 6˚C and 16˚C (Abdalla and Narendran, 1991, Karaca et al., 2016).

Cooling systems in sheep barns

Sheep are sensitive to climate, environmental conditions, animal care and feeling conditions. Low and high temperatures, sudden temperature changes, high level of humidity and draft might cause particularly respiratory system diseases. Also, this has impact on the change of substance in an animal's metabolism and this effect slows down development of lambs and kids as well as decreasing the efficiency (Kaymakçı and Taşkın, 1995).

In order to prevent such negative effects, sheep folds must be cooled at a certain level during the hot seasons and temperature stress on the animals must be minimized as much as possible. This will allow sustaining healthy and productive production.

The optimum temperature levels in the sheep folds are generally accepted to be between 10˚C and 20˚C however there are several researchers accepting a wider range of values. Location of the research and measurements made in different seasons are the main factors that can explain the difference in temperature assuring optimum life comfort of sheep.

There are researches suggesting that the optimum temperature in sheep folds is between 10˚C and 13˚C (Anonymous, 1987) but there are also researchers suggesting a temperature range between 4˚C and 24˚C (Hahn, 1974), 8˚C and 17˚C (Mutaf and Sönmez, 1984), between 0˚C and 20˚C (Webster, 1994), between 10˚C to 15˚C (Olgun, 2011).

The optimum temperature limits in sheep folds vary between 10˚C and 20˚C (Olgun, 2011) but the optimum temperature might fall down to 7˚C and 8˚C in cold seasons (Mutaf and Sönmez, 1984). The critical high temperatures start after 22˚C and 24˚C (Hahn, 1974) and the folds must be cooled down after these temperatures (Figure 2).

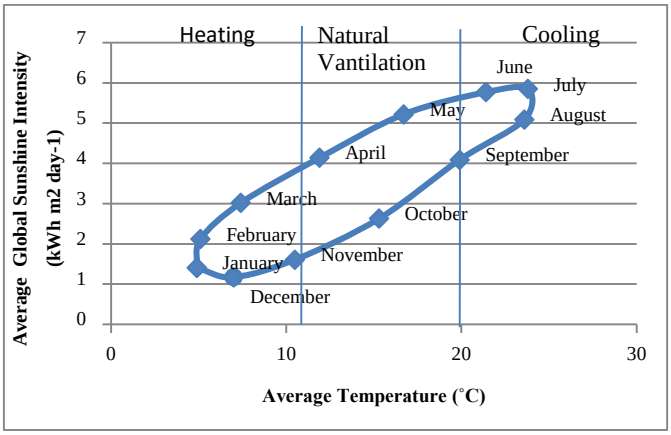

Figure 2. Air conditioning in sheep folds based on outdoor air temperature in Tekirdağ

Air conditioning in sheep folds is shown in Figure 2 based on the exterior weather conditions in Tekirdağ. In some months, the barn must be ventilated, heated and cooled for assuring comfort of animals kept in sheep barns. In December, January, February and March, the exterior air temperature falls below 7˚C or 8˚C; the temperature in the barn decreases and humidity density increases during these months and heating might be required. In April, May, September, October and November, the exterior temperatures will be between 7˚C&8˚C and between 22˚C&24˚C and therefore ventilation will not be needed. If the exterior air temperature exceeds 22˚C or 24˚C and the weather is not windy, mechanical ventilation must be supported with wet pads (Yüksel and Şişman, 2015). Cooling will be required in June, July and August.
It is reported that relative humidity in sheep folds, which is the other climate related ambient condition, should be between 50 to 60 %. According to Ekmekyapar (1991), it must be 55 to 65 % whereas Olgun (2011) notes that it should be between 50 and 60 %. Relative humidity in the barns increase significantly during the winter since exterior temperatures fall down and it might be reduced down to certain levels only through heating the barn.

Designing fan-pads to be installed in sheep folds

We must know the size of barn to design the fan-pad project. The sheep fold can house 300 animals; its width is 11.0 meters, length 34.0 meters, height 3.0 meters and the floor space is 374 m^2 (11.0*34.0) (Karaman et al., 2012).
1 m^2 of wet pad is required for each 25 m^2 of floor space in barns (Yüksel and Şişman, 2015). The following quantity of wet pad shall be enough for a sheep fold with a floor space of 374 m^2:

$$374/25 = 14.96 \sim 15\ m^2$$

In hot days, fan pad needs 30 to 40 liters per 1 m^2 of wet pad area (Bucklin et al., 1993). A circulation pump with a capacity of 0.2 kWh is required for transferring water to the top of fan pad in the sheep fold and for the water cycle back to the fan pad.
Ventilation in a barn might be calculated using different methods. This calculation might be based on internal volume of the barn or number of animals kept in the barn. Since the number of animals in barns might be less or more than the actual capacity, using number of animals as

the basis of calculating ventilation will be better. In summer, ventilation required for one kg sheep is 0.7 $m^3h^{-1}kg^{-1}$ (Olgun, 2011).Sheep and goats weight between 40 to 60 kg and the average is 55 to 60 kg (Olgun, 2011).

Sheep and goat weights start at 40 kilograms and might reach up to 90 to 100 kilograms depending on the sex and breed of animals (Tagem, 2009).

Number of animals to be kept in the sheep fold subject to the project is 300 and the average weight is 60 kg. Therefore the ventilation in the sheep fold is as follows per hour:

$$300 \times 60 \times 0.7 = 12600 m^3 h^{-1}$$

Fans to be used in the barn must be operated separately depending on the need of sheep and climate conditions. The diameter of fans to be used in the system must be 60 cm. If the diameter is larger, the fans will be noisy. The properties of fans with a diameter of 60 cm are as follows; capacity: 9500 m^3h^{-1}, power: 0.55 kWh, cycle: 1400 rpm (dd^{-1}) and single-phase 230 V (Anonymous, 2020e). Since the ventilation is equal to 12600 m^3h^{-1}, 2 fans are used for air change from inside to outside of barn.

The barn is longer than 25 to 30 meters and 2 more aspirators must be placed in the middle of the barn for so that the entire barn can be ventilated (Yüksel and Yüksel-Türkboyları, 2018).

Total 4 fans with a capacity of 0.55 kWh and one circulation pump with a capacity of 0.2 kWh are required for the ventilation and fan pad system project. Accordingly, the total energy requirement of the system is as follows:

$$0.55 \times 4 + 0.2 = 2.4\ kWh$$

Results and Discussion

Requirements of a ventilation and cooling system with solar panels were calculated for using green energy in sheep folds. In this system, the fan pad area is about 15 m^2; the ventilation is 12600 m^3h^{-1}; there are 4 fans and the energy requirement of the system is 2.4 kWh.

This project focuses on a closed sheep fold capable of housing 300 animals and the sheep fold needs a panel system to supply its energy demand of 2.4 kWh. However, some climate conditions might have negative impact on the solar panel system and this system might fail to supply the necessary energy. The adverse conditions, which prevent generation of sufficient energy, can be listed as dusty and dirty panel glasses, very hot or very cold weather, bent sunrays seen in the morning and evening, cloudy and rainy weather and system losses (Anonymous, 2020e). The calculated value was 2.4 kWh but a system having a capacity 25 to 30 % more than this value must be installed, namely 4 kWh, so that the solar panel system can function efficiently under such conditions. The system calculation must be based on the seasons receiving minimum sunrays.

When the system works with full capacity, excess energy will be stored in the battery to be used when sunrays do not reach the solar panels. Excess energy can be used in sheep folds for operating tools and instruments such as milking, cleaning and automatic feeding as well as using interior lighting.

Conclusion

Natural resources, particularly grasslands in Turkey are known to be optimum for breeding small ruminants, mainly sheep and goats. Furthermore, number of animals increase

significantly due to a number of elements such as consumption habits of families in rural areas. This might have a key role in closing the animal protein gap which is an alarming nutrition problem.

Recently, sheep breeding in our country has shifted from conventional methods to modern methods. Modern (intensive) housing offers optimum ambient conditions and methods assuring animal welfare and therefore achieves high efficiency.

Ventilation has a key role in assuring comfort and optimum ambient conditions in sheep folds. Ventilation improves climate and chemical ambient conditions inside a barn and keeps them at a good level for animals. Insufficient ventilation slows down efficiency and development of sheep and increases the risk of catching a disease. Natural and mechanical ventilation systems are used in sheep folds. If there is no air circulation outside the sheep fold and if the temperature is high, natural ventilation shall not create the desired effect in sheep folds. In this case, fans and fan pads will be used to sufficiently ventilate and cool the sheep folds. Fans and fan pads need electricity to function.

Barns must be constructed minimum 1000 meters away from any residential area and this rule might lead to difficulty in accessing to electricity supply system. Green energy might be used in rural areas not having electricity supply system and photovoltaic cells might be used to generate electricity.

Electricity generated with the solar panels can be used to supply the demand of barns as well as offering an uninterrupted energy source in case of power cuts and preventing any delays. Green energy reduces energy costs of a business and plays a part in reducing the production cost and product price.

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BIOCLIMATES OF THE LUMBARDH VALLEY OF PRIZREN, KOSOVO

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ABSTRACT

The climate of a region is the main determining factor in the distribution of vegetation in that region. The study of the average multi-year performance of the climate and its two main determining parameters, the regime of temperatures and precipitation, as well as their analysis through bioclimatic indicators, allows to judge the distribution and type of vegetation in that region. For proper explanation of the relationships between climate and vegetation, for the purpose of phyto-climatic characterization, based on thermal and pluviometry data, in this study are used the bioclimatic indicators proposed by Rivas Martinez, the pluvial-factor indicator of Lang, the drought indicator of De Martonn and the ombro-thermal diagrams of Walter and Leith, as the most suitable methods for this purpose. They are valuable indicators in orderto determine its climatic features in relation to vegetationfor the Mediterranean environment, where also the Prizren region in Kosovo is part of it too. Calculating the values of bioclimatic indicators and analyzing them enables us to compare between different types of bio climates and the bio climates between different areas (thermotypes and ombrotypes) and to evaluate the extent and type of vegetation for each bioclimatic type. Precisely, this study, located in the Lumbardh valley of Prizren in Kosovo, serves this purpose.
Keywords: *climate, bioclimatic indices, precipitation, temperature, Mediterranean vegetation*

INTRODUCTION

The study of climate and its impact on the distribution of vegetation and plant species, requires knowledge of the concept of climate and the study of its average multi-year progress, ie the climatic characterization of a given area. While to determine the impact it has on vegetation should be studied its climatic behavior and the relationships it creates with plants. This is achieved through the bioclimatic characterization of the area and the use of bioclimatic and phytoclimatic indicators, which highlight the link between climatic performance and plant growth. To determine the climate of a region should be recorded the performance of its main constituent meteorological parameters such as air temperature, precipitation, air humidity, wind speed, for a period of over 30 years (WMO - World Meteorological Organization). Climate is a dynamic system, the result of the interaction of its constituent elements and that determine the climatic course of different areas.In particular, the climate of the Mediterranean basin is represented by a great variability (Mitrakos, 1980), which is due to the interaction of different factors in this area. This climate variability is most evident at local levels where very different climate subtypes are created (Grove & Rackham 2001). Particularly in the mountainous areas are created significant climatic differences from the lowlands, influenced by the degree of continentally of the area. Warm Mediterranean currents often penetrate into the valleys of mountainous areas creating differentiated climatic subtypes (Krasniqi, 2017).These climatic subtypes in narrow areas have their impact on the distribution of vegetation. Climatic characterization of different ecological areas is with study interest for many disciplines but

especially for studies of biological nature, which highlight the relationships between climate and vegetation. Through them we can argue the geographical distribution of plants. In phytoclimatic studies, bioclimatic characterization, which is based on the relationships between biological parameters of plants and climatic parameters, is quite useful from a practical point of view.Unlike the climatic classification which are based only on meteorological parameters, which makes the classification only from the climatic point of view of different areas, the bioclimatic characterization is important because it points out the connection between vegetation and climatic factors. The different types of vegetation that we distinguish in an area, seen both in terms of space and time, or in relation to altimetry (altitude), are the result of changing climatic parameters, the identification of which in floristic and vegetation studies is a necessity. The distribution of plant communities is closely related to the type of climate (Blasi, 1996).The study of physical-geographical characteristics, altitude from level sea, distance from the sea and other factors are important in the study of climate as part of climate-forming factors. The study of the vegetation features of the areas under study is important as the vegetation itself is an influential factor in the formation of zonal climates and microclimates. This is due to the fact that: *"... vegetation regions are climatic regions"*(Giacobbe, 1948,1949) and *"... the comparable climatic conditions correspond to similar aspects of vegetation"*(Braun-Blanquet,1932).In different climatic zones are formed many bioclimatic types and subtypes which determine the distribution of plant species. The study and determination of bioclimatic types and subtypes of a region is made possible through the use of bioclimatic indicators. In many countries in the Mediterranean basin and in Europe, the Rivas- Martinez indicator (1995,1996,1999, 2004), are more appropriate for this purpose, giving a clearer picture of the relationship between climate and vegetation; pluviofactor indicator of Lang (1915); drought indicator of the De Martonn (1926); ohmothermic diagrams of Bagnouls e Gaussen (1957) modified by Walter e Lieth (1960); which are among the most used indicators and efficient for this purpose.Looking at the types of climate in relation to the distribution of vegetation, we can distinguish in the Mediterranean space bioclimatic types such as infra-Mediterranean, thermo-Mediterranean, eu-Mediterranean, super-Mediterranean, mountain-Mediterranean and oro-Mediterranean (Quézel, 1976).This bioclimatic classification takes into account the distribution of plant and vegetation species which have been studied early (Von Humboldt & Bonpland, 1807). The physico-chemical characteristics of the soil also play an important role in the distribution of vegetation.Therefore, when we study the floristic and vegetative aspects of an area or their dynamics, in fact we should study the pedo-climatic characteristics of the certain area. In the study area, which lies in the valley along the river Lumbardhi of Prizren, based on the physical-geographical position, altitude above the level sea and the influence of climatic, hydrological and terrestrial factors, there are four phytoclimatic belts with more or less clearly between them and which are: *(1) shrub and oak forest belt; 2) belt of deciduous forests (beeches); (3) belt of beech and coniferous forests (beech and fir) and, (4) belt of alpine pasture;* where in each belt we find a typical vegetation representative of the Mediterranean regions.These phytoclimatic belts, with a more or less clear difference between them, are an expression of the interaction of the vegetation of this area related with the conditions of the physical environment, is of the habitats and in particular of the altitude above sea level. In the interior of each belt we find a floristic wealth with defined features, represented by herbaceous, shrubby and woody plant species.

1) Shrub and oak forest belt. In this forest belt dominate the species:*Quercus petreae (Mat) Liebl., Fraxinus ornus L., Ostrya carpinifolia Scop., Quercus cerris L., Quercus frainetto Ten., Carpinus betulus L., Quercus pubescens Willd., Carpinus orientalis Miller., Robinia pseudoacacia L., Cornus mas L., Corylus avellana L., Pyrus pyraster Burgsd., Prunus spinosa L., Juniperus communis L., etc.*

2) Mesophilic belt of deciduous forests (beeches). In this forest belt dominate the species: *Fagus moesiaca (K.Maly)., Carpinus betulus L., Carpinus orientalis Miller., Quercus petreae (Mat) Liebl., Quercus pubescens Willd., Acer plantanoides L., Acer obtusatum Waldst.et, Kit., Acer campestre L., Prunus avium L., Populus tremula L., Viburnum lantana L.*
3) Belt of beech and coniferous forests (beech and fir). In this forest belt dominate the species:*Fagus moesiaca (K.Maly).,Pinus heldreichii Christ.,Pinus peuce Griseb., Abies alba Mill., Acer pseudoplantanus L., Acer plantanoides L., where are represented some shrubs such as: Juniperus nana Willd.,Vacinium myrtillus L., Robus idaeus L., Rosa pendulina L., Bruckenthalia spiculifolia (Spike Heath).*
4) Alpine pasture belt. This belt extends over the area of beech and conifers to the highest peaks. Represented by plant associations *Agrostis rupestris All., Alopecurus gerardii Vill., Alchemilla alpina L., Bromus racemosus L., Crocus scardicus Koš., Crocus velchensis Herb., Caltha palustris L., Dianthus scardicus Wedst., Festuca panciculata (L.),Schinz&Thell.,Gentiana verna L.,Gentiana punctata L., Geum montanum L.,Geum reptans L., Lilium albanicum Griseb., Nardus stricta L., Narthecium scardicum Koš., Potentilla dorfleri Wettst., Ranunculus crenatus Waldest.et.Kit., Ranunculus montanus Willd., Primula veris L., Veratrum album L., Salix reticulata L., Sesleria coerulans Friv., Viola elegantula Schott.*
In alpine pastures, in addition to alpine vegetation, many shrubs or bushes grow, among which: *Juniperus nana Willd.,*blueberry associations *Vaccinium myrtillus L.*

MATERIAL AND METHODS

The bioclimatic study was conducted in the Prizren region of Kosovo and analyzed the bioclimatic and phytodiversity of the Lumbardh valley of Prizren. This valley is characterized by a dense vegetation with a variety in its composition, which is differentiated in relation to the altitude and which is a consequence of climatic, hydrographic and pedological conditions. Bioclimatic analysis of this valley will enable the differentiation of its bioclimatic types and plant diversity for each bioclimatic type. Bioclimatic indicators were used to realize this study, among which those of Rivas- Martinez (1996, 1999, 2004), have found application in many countries in the Mediterranean basin and in Europe, as they give a clearer picture of the connection of climate with vegetation.The Rivas-Martinez method provides the opportunity to make a more detailed classification of phytoclimatic generations. Other indicators such as pluviofactor of Lang (1915) and drought indicator ofDe Martonn (1926) further serve this purpose. The calculation of indicators is done through formulas, while graphic methods (ombrothermal diagrams), record the progress of different climates and their impact on vegetation. The othermotherm diagrams of Bagnouls e Gaussen (1957) modified by Walter e Lieth (1960) are among the most widely used and efficient for this purpose. On the basis of temperature and precipitation databasesare constructed the ombrothermal diagrams, following the methodology of thermodograms (Walter e Lieth, 1960). For their realization are taken into account the average values of temperature and monthly precipitation, as the most significant meteorological factors, which are reflected in a Cartesian graph in which the temperature values are presented with a scale twice of the precipitation values (1°C = 2 mm). The overlap of the thermal and pluviometric curves (ombrothermal zone, shaded), which occurs when the amount of precipitation is less than twice of the temperature (P <2T), represents the climatic situation in relation to drought (which in this period of the year is considered aride or dry).Evidence of the climatic situation, through the use of this graphic indicator is important especially in areas with little rainfall, because it highlights the conditions of hydric stress for plants. For the bioclimatic inclusion of the study area, the data of the thermo-pluviometric station of Prizren (1948-1978) for a period of 30 years have been taken into account (Jarosllav *et al.*,1983). The performance of these data and their climatic variability has been compared with other

subsequent data for the Prizren station for the years 2017 and 2018 (IHMK, 2001-2018). Bioclimatic classification in the study area was done through the use of the above bioclimatic indicators, as the most appropriate methods for this purpose. For the bioclimatic interpretation of the study space, the network www.globalbioclimatics.org was consulted to determine its features and climatic behavior.

RESULTS AND DISCUSSION

Climatic characterization. Kosovo's climate is mostly continental, it is characterized by harsh and cold winters, with more rain and snow, while the summer season is hot. It is influenced by both Mediterranean and Alpine currents. The climate of the Prizren region is characterized by a somewhat modified climate, milder than what would be expected from its latitude. The climate is continental alpine, harsh climate, but also penetrates the Mediterranean Adriatic currents through the "*Drini i Bardhe*" canyon which have favored the adaptation and development of various plant forms which appear through its morphological diversity. Average annual temperatures in the Prizren area are around 11.9^{0}C; the coldest month is January and December with perennial minimum average temperatures going to -1.6^{0}C and 0.19^{0}C respectively and the hottest month is July with a maximum average temperature going up to 26.8^{0}C followed by August at 26.3^{0}C. This area is included in the Dukagjini Plain which is one of the hottest in Kosovo.There are about 229 days without frost. The average date of the first frost is November 11th, while the average date of the last frost is March 27th. The average value of precipitation in this area varies from 670 to 1200 mm/year. The largest amount of precipitation recorded is about 79 mm in October and the average annual amount is about 747 mm. The largest amount of precipitation falls during the autumn-winter period from October to April by about 455 mm (IHMK, 2001-2018).

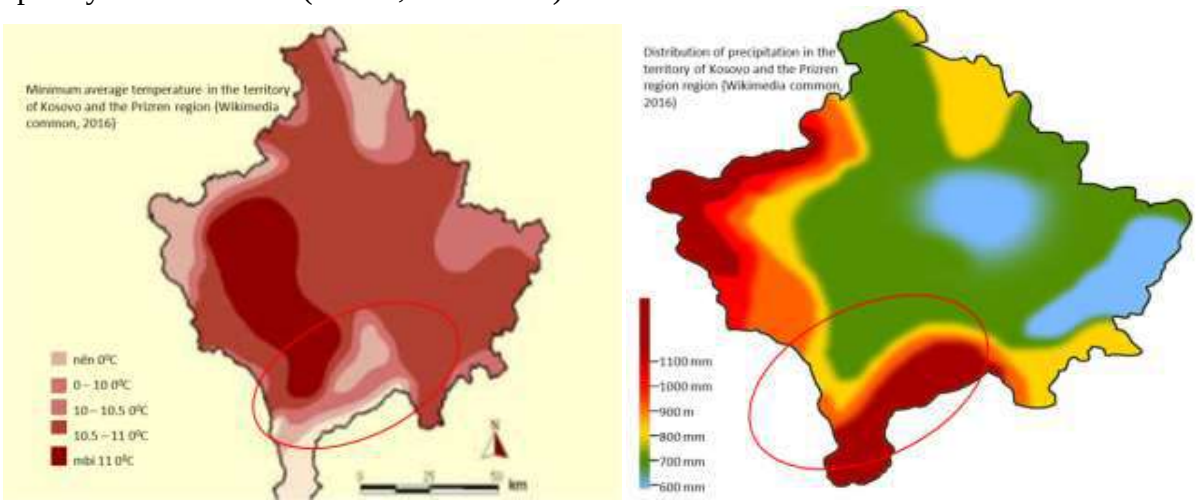

Figure 1. Temperatures and rainfall in Kosovo and the Prizren region

Average air humidity varies, depending on the seasons and climatic conditions. It reaches its highest level during the winter period (December-January) where it reaches the values 82–83%, while in the summer months, the air humidity decreases, reaching the lowest values during the month of August, by about 59%. The average value of relative air humidity is about 60-70%. There are 220 to 280 sunny days a year in this region. In Kosovo, on average there are about 2066 hours of sunshine (IHMK, 2013), this value varies according to the seasons of the year, terrain topography, altitude and degree of cloud cover. Solar radiation in the vegetation period goes 458 kcal/cm^2/day. The average value of wind speed is about 2.8 m/s. The wind ranges from 1 to 30 m/s where the wind directions from the south, southwest and south-east dominate and the lowest average wind speed occurs in August and September (IHMK, 2001-2018). This

climate has created the conditions for a very rich natural phytodiversity.For the bioclimatic characterization of the study area are taken the values of thermo-pluviometrical indicators of average temperature, maximum average temperature, minimum average temperature and the amount of precipitation at the Prizren station for a period of 30 years (1948-1978) (I.H., Jarosllav Çerni, 1983).

Table 1. Average temperature, maximum average, minimum average and amount of precipitation at Prizren station (1948-1978).

Indicators	January	February	March	April	May	June	July	August	September	October	November	December	Annual average
Average temperature	0.18	2.49	6.46	11.87	16.67	20.23	22.27	22.09	17.99	12.08	7.36	2.46	11.87
Maximum temperature	1.27	4.81	9.83	16.03	20.9	24.73	26.83	26.31	21.92	15.38	10.02	4.73	15.99
Minimum temperature	-1.63	0.17	3.09	7.7.1	12.44	15.73	17.71	17.87	14.06	8.78	4.70	0.19	7.75
The amount of precipitation	65	56	59	61	72	59	58	38	65	62	79	73	747

Bioclimatic characterization. For the bioclimatic characterization of the sudy area, the indicators were taken into analysis and calculated according to the method of Rivas-Martinez (1996,1999) such as the simple continentality indicator (Ic), the thermal index (It), the compensated thermal index (Itc), the indicator, annual ohmbrothermic (Io) and four-month summer ohmothermic indicator (Ios4).

Table 2. Bioclimatic thermotype horizons and related indicators (Rivas–Martinez, 1999).

Bioclimatic thermotype horizons and related indicators (Rivas–Martinez, 1999).			
Thermotype horizons	**Acronyms**	**It, Itc**	**Tp**
Infra-Mediterranean inferior	Lime	515-580	>2650
Infra-Mediterranean superior	Uime	450-515	2450-2650
Termo-Mediterranean inferior	Ltme	400-450	2300-2450
Termo-Mediterranean superior	Utme	350-400	2150-2300
Meso-Mediterranean inferior	Lmme	280-350	1825-2150
Meso-Mediterranean superior	Umme	210-280	1500-1825
Supra-Mediterranean inferior	Lsme	145-210	1200-1500
Supra-Mediterranean superior	Usme	80-145	900-1200
Oro-Mediterranean inferior	Lome	-	675-900
Oro-Mediterranean superior	Uome	-	450-675
Cryo-Mediterranean inferior	Lcme	-	150-450
Cryo-Mediterranean superior	Ucme	-	1-50
Mediterranean athermic	Mea	-	0
Infra-temperato inferiore	Lite	445-480	>2450
Infra-temperato superiore	Uite	410-445	2350-2450
Thermo-temperato inferiore	Ltte	355-410	2175-2350
Thermo-temperato superiore	Utte	300-355	2000-2175
Meso-themperato inferiore	Lmte	240-300	1700-2000
Meso-themperato superiore	Umte	180-240	1400-1700
Supra-themperato inferiore	Lste	100-180	1100-1400
Supra-themperato superiore	Uste	(20-100)	800-1100
Orotemperato inferiore	Lote	-	590-800
Oro-themperto superiore	Uote	-	380-590
Cryo-themperato inferiore	Lcte	-	80-380
Cryo-themperato superiore	Ucte	-	1-80
Athermic temperato	Tea	-	0

The definition of the thermotype and the low or high horizon is determined by the simple continentality indicator: *Ic = tw - tc*; where: *tw* - average temperature of the hottest month, *tc* - average temperature of the coldest month), where from the calculations it results that: *Ic* = 22.27 - 0.18 = 22.09. For values of this indicator >21 the climate is continental and in the range 21-28 it is considered semi-continental (Rivas-Martinez classification, 1995).

Since the value of this indicator is > 21 it is calculated the offset thermal index (Itc). Its calculation requires the calculation of the thermal index: *It = (T + M + m) x 10*; where: *T* - is the average annual temperature; *M* - maximum average temperature in the coldest month and *m* - minimum average temperature in the coldest month. *Itc = It + Ci*; where: *It* - the thermal index which *It = (T + M + m) × 10* and *Ci* has a compensation value equal to *Ci = 5 x (Ic - 18)*. *It = (11.87 + 1.27 + (-1.63)) x 10 = 115.1; Ci = 20.45; Itc = 115.1 + 20.45 = 135.55.* For values of the compensated thermal index *Itc = 135* the climate is considered mountain tempered (Rivas-Martinez, 1995, modified). For values of the *It* thermal index, included in the range 200 <It <70; according to the definitions of the Rivas-Martinez method (1999), where respectively the values are 115.1 and 104 the climate is considered *Supratemperato inferior*. Based on the values of the calculated indicators (It and Itc) the thermotype and the horizon are determined according to the classes defined by Rivas Martinez (1999).

To determine the ombrotype, calculate the annual ohmothermic indicator (Io). *Io = 10 x Pp / Tp*; where: *Pp* - the amount of monthly precipitation of months with average temperatures greater than 0^0C and *Tp* - the sum of average temperatures of the same months. *Io* = 10 x 747/2100.65 = 3.5. The relatively low value of *Io* highlights the lack of rainfall in the region making it relatively dry, especially in low-lying areas. Based on the value of Io the ombrotype is defined referring to the classes defined by Rivas-Martinez (1999).

Table 3. Horizons of bioclimatic ombrotypes and relevant indicators (Rivas-Matinez, 1999)

Horizons of bioclimatic ombrotypes and relevant indicators (Rivas-Matinez, 1999)		
Horizons of ombrotypes	**Acronyms**	**Io**
Ultra-periaride	Uha	<0.1
Inferior hyperaride	Lhar	0.1-0-2
Superior hyperaride	Uhar	0.2-0.3
Aride inferiore	Lari	0.3-0.6
Aride superiore	Uari	0.6-1.0
Semi-aride inferiore	Lsar	1.0-1.5
Semi-aride superiore	Usar	1.5-2.0
Dry inferire	Ldry	2.0-2.8
Dry superiore	Udry	2.8-3.6
Sub-umide inferiore	Lshu	3.6-4.8
Sub-umide superiore	Ushu	4.8-6.0
Umide inferiore	Lhum	6.0-9.0
Umide superiore	Uhum	9.0-12.0
Hiperumide inferiore	Lhhu	12.0-18.0
Superior hyperumide	Uhhu	12.0-24.0
Ultra-hiperumide	Uhh	>24.0

For annual precipitation values from 500-700 mm in tempered regions determines the subumido ombrotype, the inferior horizon (Blasi, 1994), with a tendency towards dry ombrotype. The four-month summer was also calculated also the ohmbrothermic index (Ios4). This indicator serves to distinguish the regions with Mediterranean climate from those with temperate climate. If its value is> 2 the region is tempered and if it is <2 the region has a Mediterranean climate.

$Ios4 = (P_{May} + P_{June} + P_{July} + P_{August}) / (T_{midMay} + T_{midJune} + T_{midJuly} + T_{midAugust})$.
Ios4 = (72 + 59 + 58 + 38) / (16.67 + 20.23 + 22.27 + 22.09) = 227 / 81.26 = 2.79.
The value of *Ios4 = 2.79* means that the climate of the region is temperate and that the amount of precipitation in the period considered is twice the sum of average temperatures (P> 2T).

Table 4. Bioclimatic indicators for Prizren station (Rivaz-Martinez,1999)

Bioclimatic indicators for Prizren station (Rivaz-Martinez,1999)									
tw	tc	Ic	Tp	It	Itc	Io	Ios4	Thermotypes	Ombrotypes
22.27	0.18	22.09	2100.65	115.1	135.55	3.55	2.79	Lste	Lshu

From the processing of thermometric and pluviometric data the studied area is determined: Macroclimate: *Temperato*; Variants: *Submediterranea*; Bioclimate: *Continental temperature*; Thermotype: *Inferiored supratemperato (Lste)*; *Ombrotype*: Superior dry (Udry) in high areas and Inferior subumide (Lshu) in low areas. Based on the obtained values of the indicators according to the Rivas-Martinez method (1999), the bioclimate is defined as *temperate* and the *thermotype supratemperato inferior (Lste)*. According to the definitions, this bioclimatic type (thermotype) includes the lower mountain part located at an altitude between 950-1000 m and 1350-1450 m. It is characterized by an average annual temperature of 9-11^{0}C, minimum average temperature below 0^{0}C in December, January and February; average annual rainfall between 1100-1300 mm and summer drought.
Heavy frosts are verified from period of November to March, accompanied by snowfall. The vegetative period is about 150-180 days. Forest vegetation is composed of semi-mesophilic and mesophilic deciduous plants (mainly beech) accompanied by hilly floristic elements and mountain species. Here are found plant species such as: *Fagus moesiaca (K.Maly).,Pinus heldreichii Christ.,Pinus peuce Griseb., Acer pseudoplantanus L.,Acer plantanoides L., Acer obtusatum Waldst.et.Kit., Populus tremula L.,* where are represented and some shrubs such as*: Juniperus nana Willd., Vacinium myrtillus L., Robus idaeus L., Rosa pendulina L., Bruckenthalia speciduifolia (Spike Heath).*
On the basis of interpolation of values are also classified other boundary zones based and on altitude based on the definitions given in the Rivas-Martinez method.
In the lower area (low and hilly) the limiting bioclimatic type is lower *Inferior mesotemperato (Lmte)*, which lies at an altitude of 300-450 m above sea level and is characterized by an average temperature of 13-15^{0}C, annual rainfall including between 750 and 850 mm, summer drought for about a month (during July), most pronounced in southern areas. The vegetative period varies between 210-240 days. In the higher areas lies the bioclimatic type of *Mesotemperato superior (Umte)* (high hills) between 450-1 000 m above sea level with an average annual temperature around 11-13^{0}C, precipitation between 850 and 1 100 mm, lack of summer drought period, with minimum winter temperatures in January and February, with frosts from November to March. The vegetative period is about 180-210 days. Here we find plant species such as: *Quercus petreae (Mat) Liebl., Fraxinus ornus (K. Maly)., Ostrya carpinifolia Scop., Quercus cerris L., Quercus frainetto Ten., Carpinus betulus L., Quercus pubescens Willd., Carpinus orientalis Miller., Robinia pseudoacacia L., Alnus incana L.(Moench)., Betula pendula Roth., Ulmus minor Miller., Salix alba L., Alnus glutinosa L.,Cornus sanguinea L.,Cornus mas L.,Corylus avellana L., Pyrus pyraster (L.)Burgsd.,Juniperus communis L.,Rosa canina L.,Eupotarium canabium L.,Sambucus ebulus L.,Juglans regia L.,Rubus ulmifolius Schot.*
In the upper part of the bioclimatic type of the study area is the bioclimatic type *Supertemperato superior(Uste)* (high mountains) located at an altitude of 1400-1450 and 1850-1900 m. It is

characterized by an average annual temperature of 7-9^{0}C and an average annual rainfall of 1300-1500 mm, without summer drought, with a minimum temperature below 0^{0}C during the months of December, January, February and March. Frosts are verified from October to April, with prolonged winter cold. In this area snow can cover the ground for weeks. The vegetative period is 120-150 days. Forest vegetation is composed of mesophilic deciduous (beech) and typical mountain species such as: *Fagus moesiaca (K.Maly)., Pinus heldreichii Christ., Pinus peuce Griseb., Acer pseudoplantanus L., Salix reticulata L.,where they are represented and some shrubs such as Juniperus nana Willd., Vacinium myrtillus L., Rododendron ferrugineum L., Robus idaeus L., Rosa pendulina L., Bruckenthalia speculifolia (Spike Heath).,Sambucus racemosa L.*

Above this belt is the bioclimatic type *Orotemperato (subalpino)* which is located at an altitude between 1850-1900 and 2300 m. It is characterized by an average annual temperature of 5-7^{0}C, rainfall included between 1300-1500 mm/year, minimum temperature below 0^{0}C for more than 5 months. In this area snow can cover the ground from December up to April. The vegetative period is 90-120 days. Forest vegetation is missing. It is represented by plant associations such as: *Agrostisrupestris All.,Alopecurus gerardii Vill., Achillea multifida DC.(Boiss.)., Barbarea longirostris Vel., Bromus racemosus L., Crocus scardicus Koš., Crocus velchensis Herb., Calthapalustris L., Dryas octoptala L., Dianthus scardicus Wedst., Festuca panciculata L., Festuca halleri Schinz&Thell., Gentianaverna L., Gentiana punctata L., Geummontanum L., Geumreptans L., Geumcoccineum Sibth.et Sm., Lilium albanicum Griseb., Narthecium scardicum Koš., Nardusstricta L., Primulaveris L., Potentilla dorfleri Wettst., Ranunculus montanus Willd., Ranunculus crenatus Waldest.et.Kit., Rumexalpinus L., Salix reticulata L.,Sesleria coerulans Friv., Sorbus aria L., Sedum alpestre Vill.,Troliuseuropaeus L.,Trifolium badium Schreb.,Viola elegantula Schott. In alpine pastures, in addition to alpine vegetation, many shrubs or bushes grow, among which: Juniperus nana Willd.*, blueberry associations *Vaccinium myrtillus L.*

In the upper area it is the bioclimatic type *Criotemperato (alpine)* which is located above 2300 m above sea level, where we find: *Alchemilla alpina L.,Asperulla doerfleri Wettst., Crocus scardicus Koš., Crocus velchensis Herb.,Campanula alpina L.,Carex paniceae L.,Carex leporine L.,Dianthus scardicus Wedst., Dianthus crenatus Griseb.,Draba korabensis Kum.et.Deg.,Empetrum nigrum L.,Galanthus nivalis L.,Gentiana verna L., Narthecium scardicum Koš., Nardus stricta L., Lilium albanicum Griseb., Ligusticum albanicum Jav., Linaria alpina L.,Ranunculus crenatus Walt.et. Kit.,Ranunculus incomparabilis Janka., Rumex alpinus L., Nardus stricta L., Juncus alpines Vill., Potentilla dorfleri Wettst., Poa annua L., Poa cenisia All., Salix herbaceae L., Scilla bifolia L.,Silene asterias Griseb.,Silene pusilla W.K.,Saxifraga scardica Gris.,Saxifraga glabella Bertol.,Scabiosa crenata Cyr., Trifolium norcium L.,Viola elegantula Schott., Viola gracilis L.*

From a previous floristic study, conducted in this area (Krasniqi S., 2017), it results that have been found ***735 plant species***, which are distributed in the bioclimatic type *Supratemperato inferior (Lste)*; in the bioclimatic type of *Mesotemperato inferior (Lmte)*; in the bioclimatic type *Supertemperato superior (Uste)*; in the bioclimatic type *Orotemperato (subalpino)* and in the bioclimatic type *Criotemperato (alpine)*.

The calculated bioclimatic indicators highlight the bioclimatic characteristics of the study area, but it should be noted that the bioclimatic study has a limited character, especially in terms of altitude, as the lack of meteorological stations and data available in narrow areas, does not create the possibility of a complete bioclimatic characterization of the space under consideration.

The comparison between different types of bioclimates (ombrotypes) in bioclimatic analysis is done by constructing ombrothermal diagrams according to the method of Walter and Lieth (1960). From the analysis of the graphic model and based on the classification of Walter and

Lieth (1960), it results that the bioclimate of the region it is of the *submediterrane type,* with drought periods included in the interval of 1 up to 2 months.

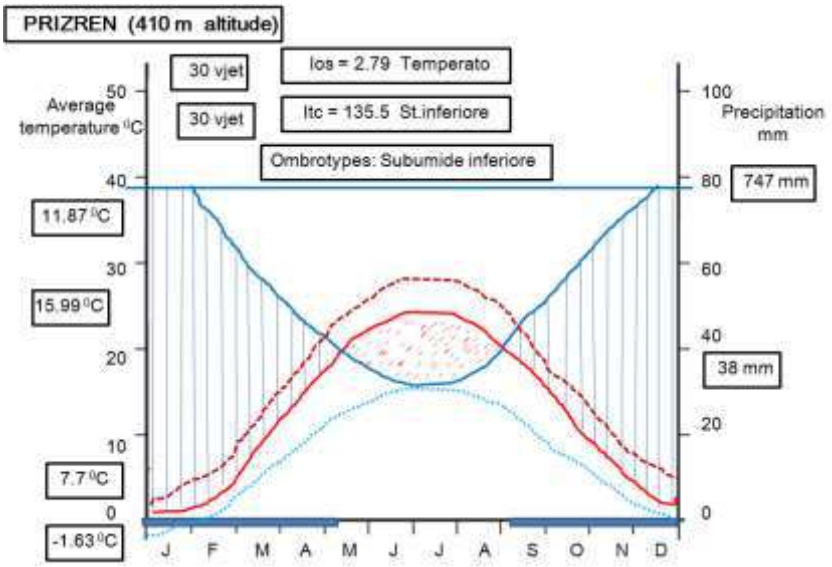

Figure 2. Bioclimate of the Prizren region (Walter e Lieth (1960)

To further explain of the relationships between climate and vegetation, for the purpose of phytoclimatic characterization itwas calculatedon the basis of thermal and pluviometric data, the pluvio-factor indicator of Lang (1915), which is valid for Mediterranean environments in relation to drought. This indicator relates the average annual rainfall P (mm) to the average annual temperature T (°C), giving a ratio called the *Lang pluviofactor* (PI). *PI = P / T*: where: *P* = average annual rainfall in (mm) and *T* = average annual temperature (°C). This indicator gives the humidity level of an area within certain defined temperature limits. *PI* = 747/11.87 = 62.93. Based on the definitions of Lang (1915), for this indicator this value included in the range 100 - 60 corresponds to a hot tempered climate. Based on Lang's definitions, in cases where P/T> 1 –the vegetation is of wood type.

The De Martonn (1926) indicator is used to further specification of the bioclimatic features on the study area. It is given by: *IA = P/(T + 10)*; where: *P* - average annual rainfall and *T* - average annual temperature. Specifically: *IA* = 747 / (11.87 + 10) = 34.1. Based on the definitions of De Martonn (1926), when I> 20 - the vegetation is of the forest type. Based on the classification done for the degrees of drought, the climate of the area can be defined as *subumide* (relatively humid), which is dominated by a vegetation of Mediterranean shrubs. This definition, based on the indicator of De Martonn (1926), is valid from the point of view of the assessment of vegetation and special floristic elements to determine their geographical distribution in this area. Regarding the above, we can say that this bioclimatic study in the area of the Lumbardh valley of Prizren gives us the opportunity to judge and explain the way of growth of plant species and their distribution in this area estimating them closely related with the performance of climatic parameters.

CONCLUSIONS

From the bioclimatic analysis of the studied area, based on the Rivas-Martinez method (1999), and the values of the indicators, it results that the macroclimate is *"temperate"*, of the *"sub-Mediterranean"* climatic variant; with *"continental tempered"* bioclimates; the thermotype *"supratemperato inferior (Lste)"* and the ombrotype *"dry superior (Udry)"* in the high areas and the *"inferior subumide (Lshu)"* in the lowlands. Based on the classification made for the

degree of drought, the climate of the area can be defined as *"subumide"* (relatively humid), which is dominated by a vegetation of Mediterranean shrubs. This bioclimatic study is valuable from the point of view of evaluating specific floristic and vegetation elements to determine the relationship between climate and plants and their geographical distribution in this area.

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BIOCHEMICAL AND HISTOPATHOLOGICAL MONITORING OF HEAVY METAL POLLUTION IN CATFISH (*SILURUS GLANIS*) TISSUES IN SAPANCA LAKE

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ABSTRACT

This study aims to determine the toxic effects of heavy metal pollution in Sapanca Lake on the catfish (Silurus glanis Linnaeus, 1758) by biochemical and histological methods. For this reason, fish samples were taken from the Kırkpınar location of the lake with the help of professional fishermen every month of 2015. Heavy metal (Cu, Fe, Zn, Pb, and Cd) analyzes in fish tissues (muscle, liver, gill) were determined with ICP-OES and compared with reference materials. In addition, catalase enzyme activity (CAT), total glutathione (GSH) and lipid peroxidation (MDA) levels, which are oxidative stress bioindicators in tissues, were measured by spectrophotometric methods. Histopathological findings in liver and gill tissue were determined by Hematoxylin & Eosin staining. As a result, the amount of Cu, Fe, and Cd in the fish were liver> gill> muscle and Zn amount was gill> liver> muscle. Generally, determined metal concentrations can be listed as Fe> Zn> Cu> Cd> Pb in muscle and Fe> Zn> Cu> Pb> Cd in liver and gill. According to the result of staining with H&E, the primary findings in the gills are the separation of the primary lamella and hyperplasia and irregularity in the secondary lamella. In the liver, necrotic conditions such as an obstruction in sinusoids, an increase in kupffer cells, and karyolysis in hepatocytes were observed. Enzymatic and non-enzymatic antioxidants are at different levels according to months, and these methods are useful in the biological monitoring of environmental pollution. The fact that the amount of heavy metal in fish tissues does not exceed the specified standards, however, the presence of biochemical and histopathological findings in fish tissues suggest that the lake is under the influence of not only heavy metals but also other sources of pollution.

Keyword: Heavy metal, pollution, catfish, oxidative stress, Sapanca Lake

INTRODUCTION

From yesterday to today, human beings have started to pollute their natural environment, to change it and to disrupt the balances in nature. In the century we live in, our natural environment is being destroyed by factors such as an unregulated and uncontrolled industry, rapid increase in population and excessive consumption. Due to the pollution in the air, water and soil, which are the basic elements of life, all living creatures, especially humans, are damaged and negatively affected (Kahvecioğlu et al., 2003; Özyürek, 2016). The pollution source does not only affect the area where it is located, the environmental problems that occur cause it to be adversely affected in other areas directly or indirectly. Heavy metal pollution that occurs in the

soil as a result of agricultural activities causes pollution of groundwater by rains and surface water pollution with runoff, and it can also cause water pollution in fuels containing heavy metals used for heating and energy. Water is one of the most important resources for all living things to continue their vital activities (Pérez-Coyotl et al., 2019). The extent to which the pollutant that causes water pollution participates in the ecosystem and to what extent it harms the ecosystem depends on the type, quantity, physical, chemical and biological structure and size of the pollution environment and the species and size of the affected organisms. Pollution is examined in three groups as physical, biological and chemical pollution according to pollutant types. Chemical pollution arising from the mixing of chemical substances that are released into the natural world, whether unintentionally or unintentionally, with industrial waste is chemical pollution.

As lakes from aquatic environments are calmer and open to human influence than streams, they feel the impact of human activities more. The latitudes of the lake environment, climatic characteristics and the sources it feeds affect the water quality. In the investigation of pollution in lakes, it is important to evaluate the biological, physical and chemical parameters of the environment with each other and their changes over time. Heavy metals are the most important inorganic factors that pollute water, especially in industrial wastes and some pesticides (Fırat and Kargın, 2010). Heavy metals are of great importance because they can remain in the environment where they are discharged for a long time, cause toxic effects on aquatic organisms and threaten human health as accumulators in the food chain. Sediments contaminated with heavy metals in aquatic ecosystems are a major stress source that threatens ecosystem health and constitute a major risk factor for aquatic organisms in contact with sediments. For this reason, aquatic organisms living in contaminated ecosystems and accumulating heavy metals in their tissues are used as biomonitors to determine the degree of pollution of their environment and the effects of contaminants. In recent years, studies on biomonitor species have increased in pollution research. Since Sapanca Lake is an important water source for the Marmara Region and contains many species, studies have been carried out on Sapanca Lake before. However, these studies usually only concern water quality and pollution. Unlike other studies, this study aims to determine the effects of water pollution on heavy metal accumulation in catfish tissues and biochemistry and histology of the tissues. In addition, it is of great importance both in determining the source of pollution and determining the effects of the fish living in Sapanca Lake, which is the source of drinking water, on human health.

MATERIAL AND METHOD

Study area, test organism and sample collection

The perimeter of the Sapanca Lake is 39 km long, 26 km of it is within the borders of Sakarya and 13 km of it is in the borders of Kocaeli (Figure 1). The long axis of the lake is in the east-west direction and the short axis in the south-north direction. The average depth of the lake is 31–33 m, but its maximum depth is 61 m. A Study conducted in Sapanca Lake in previous years indicate that there are 32 fish species in the lake, but a recent study show that this number has decreased to 22.

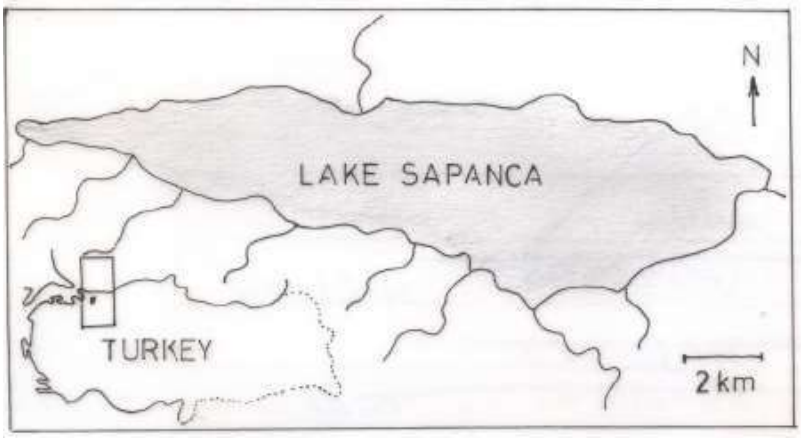

Figure 1. Study area, the localization of Sapanca Lake.

In this study, in which heavy metal pollution was investigated in Sapanca Lake, catfish (*Siluris glanis*) was chosen, because of they was hunted heavily due to its economic importance and the best represents the effects of environmental pollutants due to its feeding habits. The fish samples were collected from Kırkpınar location between January-December 2015. The necessary permissions were obtained from The Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Fisheries and Aquaculture, and Marmara University Animal Experiments Local Ethics Committee. With the help of a professional fisherman, fish samples were caught in the fishnet and brought to the laboratory, and water samples were also taken to the laboratory on ice, in brown bottles. In the laboratory, after measuring of fish's weight and height, muscle, liver and gill tissues were taken from fish samples.

Heavy metal analyses

Samples stored in the freezer were subjected to wet-burning with the nitric acid (HNO_3) in the Milestone Start D (Italy) microwave oven equipped with a temperature control program. Metal concentrations were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Spectro Arcos, Germany). High Purity- ICP- 200-7-5 brand ICP-OES multi-element standard was used as standard, DORM-3 was used as certified reference material for fish tissues, NW-KEJIM-02 Soft Lake Water was used as a reference material for Lake Water. Heavy metal analyzes in water samples taken in brown bottles were determined with ICP-OES by adding 65% HNO_3 at the rate of 10%.

Assessment of oxidative stress parameters

For the purpose of homogenization, the tissues taken from the freezer were thawed on ice. Tissues weighed for the purpose of homogenization of muscle, liver and gill samples were taken into eppendorf tubes with a 10% (w/v) cooled homogenate buffer and disintegrated in the homogenizer with the help of glass beads. Samples were preserved in ice at all stages of the studies. The homogenate was centrifuged for 20 minutes at 10000 rpm at 4°C. After the centrifuge, the supernatant part was taken and the pellet part was discarded.

a. LPO

The lipid peroxidation in the tissue samples were measured using the thiobarbituric acid reaction according to the method described by Ledwozyw. The absorbance was determined at 535 nm and its concentration was expressed as nmol MDA/g tissue.

b. CAT

The enzyme activity was measured following the decrease of absorbance at 240 nm due to hydrogen peroxide (H_2O_2) consumption. The activity was expressed as U/mg tissue.

c. GSH

GSH concentration was measured with an assay using the dithionitrobenzoic acid (DTNB) recycling method described by Beutler. GSH concentration was expressed as nmol GSH/g tissue.

d. Total Protein Content

Total protein was determined according to the method of Bradford. The intensity of the developed blue color was measured at 595 nm against the blank. Its concentration was expressed as µg/µL.

Histopathology analysis

The liver tissues were fixed in 10% neutral buffered formalin were dehydrated using a series of graded ethanol solutions (70–100%), cleared in xylene, embedded in paraffin and sectioned at 5 µm. The gill tissues were fixed again in Bouin's solution for 24 h for decalcification. Then the tissues were dehydrated and embedded in the paraffin wax and sectioned at 5 µm thickness and stained with Hematoxylin and Eosin (H&E) for standard histopathological evaluation. For each month, 5 secondary filaments from the inner section on 10 slides were analyzed. Slides were examined under the light microscopy.

Statistical analysis

Statistical analyzes were made using IBM SPSS Statistic 23 computer program. Study findings were expressed as mean ± standard error of mean (SE). Comparisons between the two groups are the parametric Student's t test and nonparametric Mann-Whitney U test in unequal variances, and comparisons between more than two groups are one-way ANOVA; in statistically significant results, Tukey's post hoc test was performed to compare the significant difference between groups following ANOVA. In all statistical comparisons, those with a significance level less than $p < 0.05$ were considered significant.

Result and Discussion

In this study, in which the effects of possible water pollution in Sapanca Lake, heavy metal accumulation in fish tissues and biochemistry and histology of tissues were investigated, catfish were not obtained except in May, August, September, October and November in the fishing, which was caught with special permission every month of 2015. Parametric findings of caught catfish; Cu, Fe, Zn, Pb and Cd amounts in muscle, liver and gill tissues; total protein, lipid

peroxidation, total glutathione levels and catalase enzyme activity; Histopathology of liver and gill tissues was examined.

Parametric findings of catfish caught from Sapanca Lake are given in Table 1. A total of 10 live catfish were caught during the hunting with special permission in every month of 2015. A total of 8 male individuals and 2 female individuals were caught.

Table 1. Parametric findings of catfish caught from Sapanca Lake.

Months	**n gender**	**Weight (±SE) (min.-max., g)**	**Lenght(±SE) (min.-max., cm)**	**Condition Factor (±SE) (min.-max.)**
May	2 (1♂+1♀)	1710,5±224,15[a] (1869-1552)	77,5 ± 5,5[a] (72-83)	0,371 ± 0,04[b] (0,326-0,415)
August	2 (2♂)	456,5 ± 56,5 (400-513)	42 ± 1 (41-43)	0,6128 ± 0,032 (0,58-0,64)
September	2 (1♂ + 1♀)	516 ± 47 (469-563)	42,5 ± 2,5 (40-45)	0,675 ± 0,057 (0,61-0,73)
October	2 (2♂)	350 ± 10,2[b] (248- 452)	37 ± 2[b] (35- 39)	0,67 ±0,091 (0,57- 0,76)
November	2 (2♂)	495,5 ± 72,5 (423- 568)	39,5 ± 0,5 (39- 40)	0,713 ± 0,087[a] (0,71- 0,88)

Cu, Fe, Zn, Pb and Cd quantities determined from the muscle, liver and gill tissues of catfish caught from Sapanca Lake and NRC Dorm-3 used as a reference, the maximum values that can be found in the metals determined by the FAO and Turkish Food Codex (TGK) are also given in Table 2. Accordingly, when comparing the concentrations of heavy metals determined from the tissues of catfish, Cu, Fe and Cd amounts can be listed as liver> gill> muscle, and the amount of Zn as gill> liver> muscle.

Generally determined metal concentrations can be listed as Fe> Zn> Cu> Cd> Pb in muscle tissue, and as Fe> Zn> Cu> Pb> Cd in liver and gill. All the data obtained remained well below the maximum values determined by FAO and TFC for fish.

Table 2. The results of heavy metals analyses of fish tissues.

MUSCLE					
Months	**Cu (µg kg^{-1})**	**Fe (µg kg^{-1})**	**Zn (µg kg^{-1})**	**Pb (µg kg^{-1})**	**Cd (µg kg^{-1})**
May	22,48±1,68^{a}	1182,8±58,6^{a}	456,16±24,6	*	*
August	2,03±0,65^{b}	341,95±31,2^{b}	405,23±74,5	*	*
September	25,93±5,4	614,23±10,6	368,6±19,7	*	*
October	2,41±0,53	366,5±22,4	330±14,3^{b}	*	*
November	13,46±2,4	463,45±5,8	547,98±9,45^{a}	*	0,37±0,09
LIVER					
Months	**Cu (µg kg^{-1})**	**Fe (µg kg^{-1})**	**Zn (µg kg^{-1})**	**Pb (µg kg^{-1})**	**Cd (µg kg^{-1})**
May	441,9±62,4	24675,3±102,8^{a}	1094,29±56,4	*	*
August	501,33±22,3^{a}	8235,6±58,6	1026,5±5,6	*	*
September	253,4±16,3	8022,7±214,3	2025,67±415,8^{a}	96,1±6,5	9,71±1,2^{a}
October	161,63±5,9	6291,4±47,8	879,3±61,5^{b}	*	0,94±0,1^{b}
November	125,96±14,7^{b}	2104±95,6^{b}	909,4±57,3	*	2,28±0,98
GILL					
Months	**Cu (µg kg^{-1})**	**Fe (µg kg^{-1})**	**Zn (µg kg^{-1})**	**Pb (µg kg^{-1})**	**Cd (µg kg^{-1})**
May	18,67±5,6^{b}	1626,9±5,7	813,75±68,3^{b}	*	*
August	68,91±4,8^{a}	1652,5±24,6	1279,7±102,4	*	1,85±0,7^{b}
September	38,57±8,5	3016,2±16,7^{a}	1504,8±63,2^{a}	15,34±1,4	2,96±0,6^{a}
October	46,36±11,7	1625,27±87,4^{b}	1462,6±55,8	*	2,48±0,52
November	22,74±4,8	1773,14±29,5	1215,6±108,9	25,69±7,4	2,95±0,42
NRC DORM-3	752,37±38,06	12252,1±750,6	1986,28±144,94	218,15±28,5	34,7±0,51
FAO (max.)	30.000	-	40.000	500	100
TFC (max.)	20.000	-	50.000	300	50

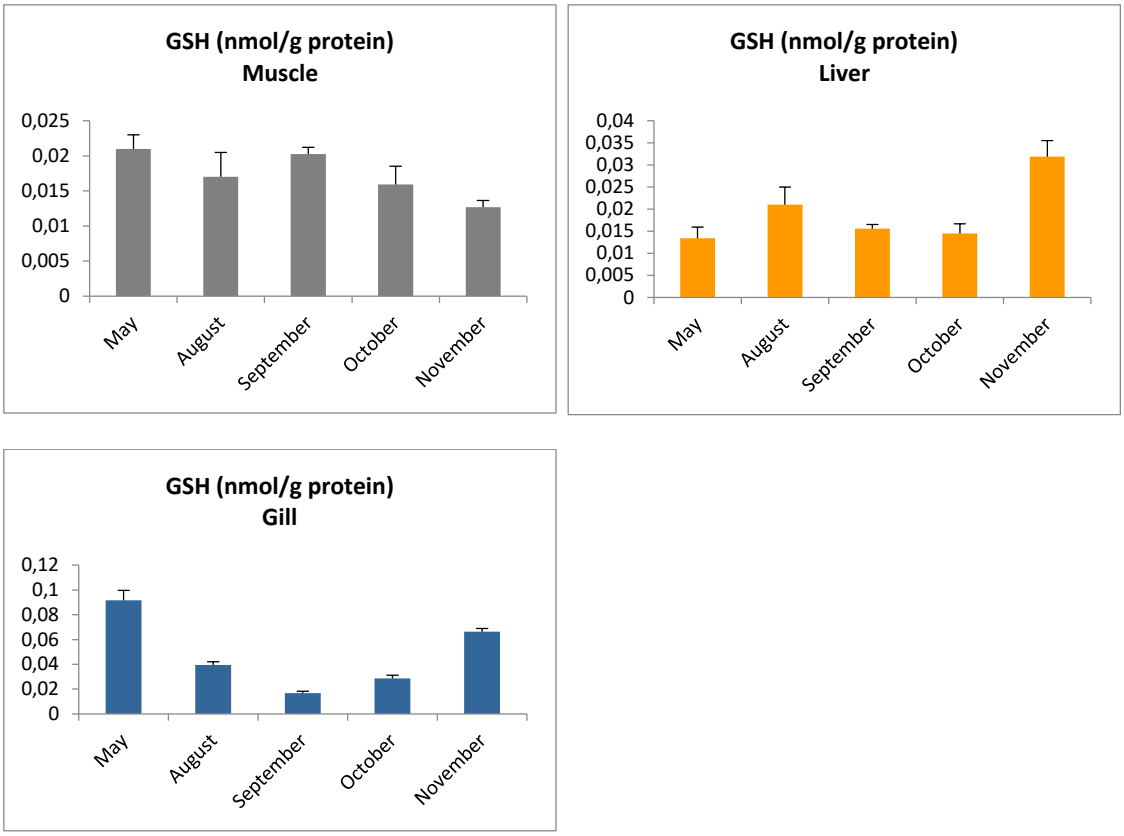

Figure 2. The results of total glutathione (GSH) levels of catfish tissues.

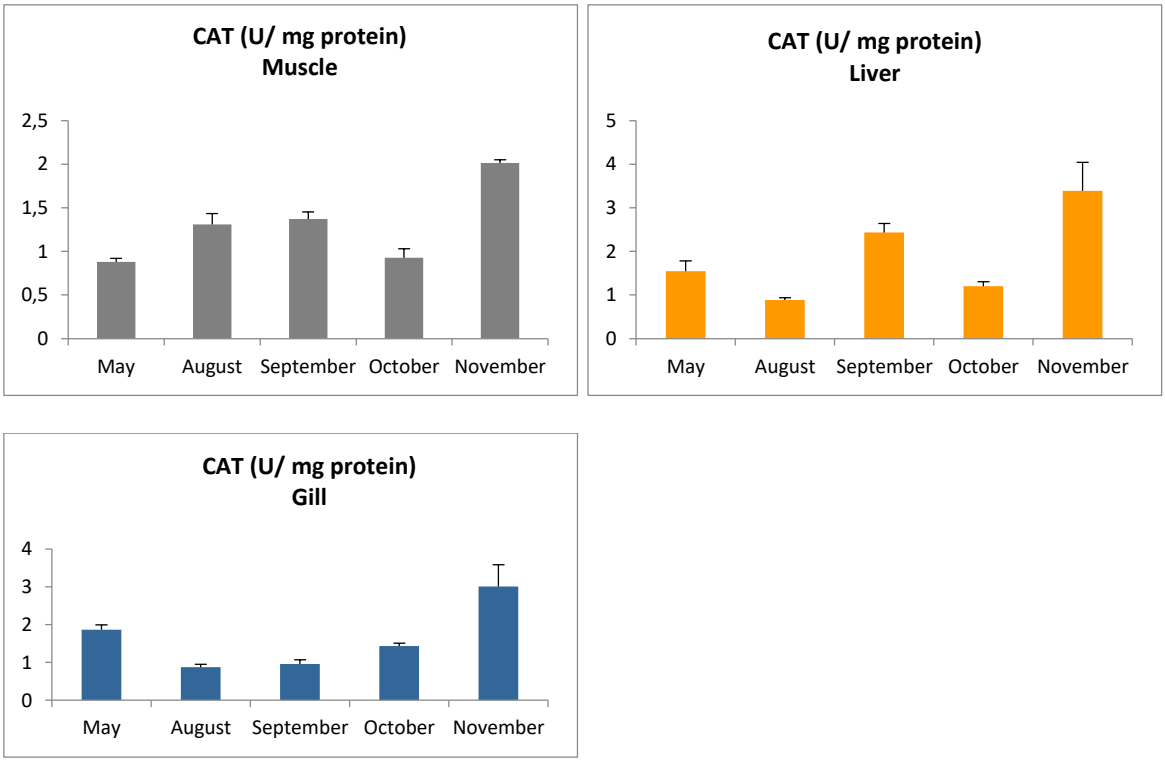

Figure 3. The results of catalase enzyme (CAT) activity levels of catfish tissues.

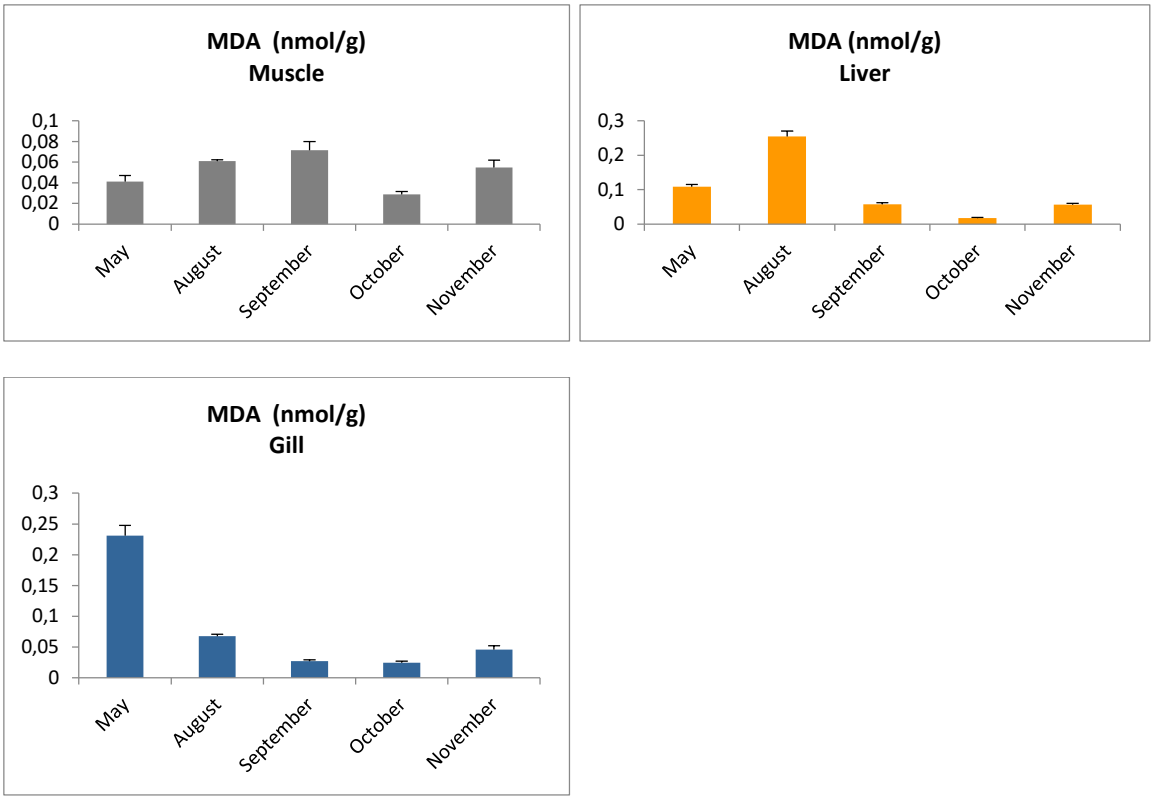

Figure 4. The results of lipid peroxidation (MDA) levels of catfish tissues.

Histological findings obtained by staining liver tissues of catfish caught from Sapanca Lake with Hematoxylin & Eosin are shown in Figure 5. Accordingly, there is no lobular arrangement in the liver parenchyma, in some examples regular polygonal shaped hepatocytes separated from each other by sinusoids formed clumps. However, there is bleeding (hemorrhage) in hepatocytes and sinusoids, which is evident with dark pink staining. Hepatopancreatic cells contain zymogen granules inside. In some samples, bile ducts and hepatic artery are prominent. Extensive fibrosis was seen in one sample. Kuppfer cells, a type of macrophage specialized in the liver, are evident in all samples. While the nucleus and nucleolus can be easily detected in some hepatocytes, some are distinctly hypertrophic.

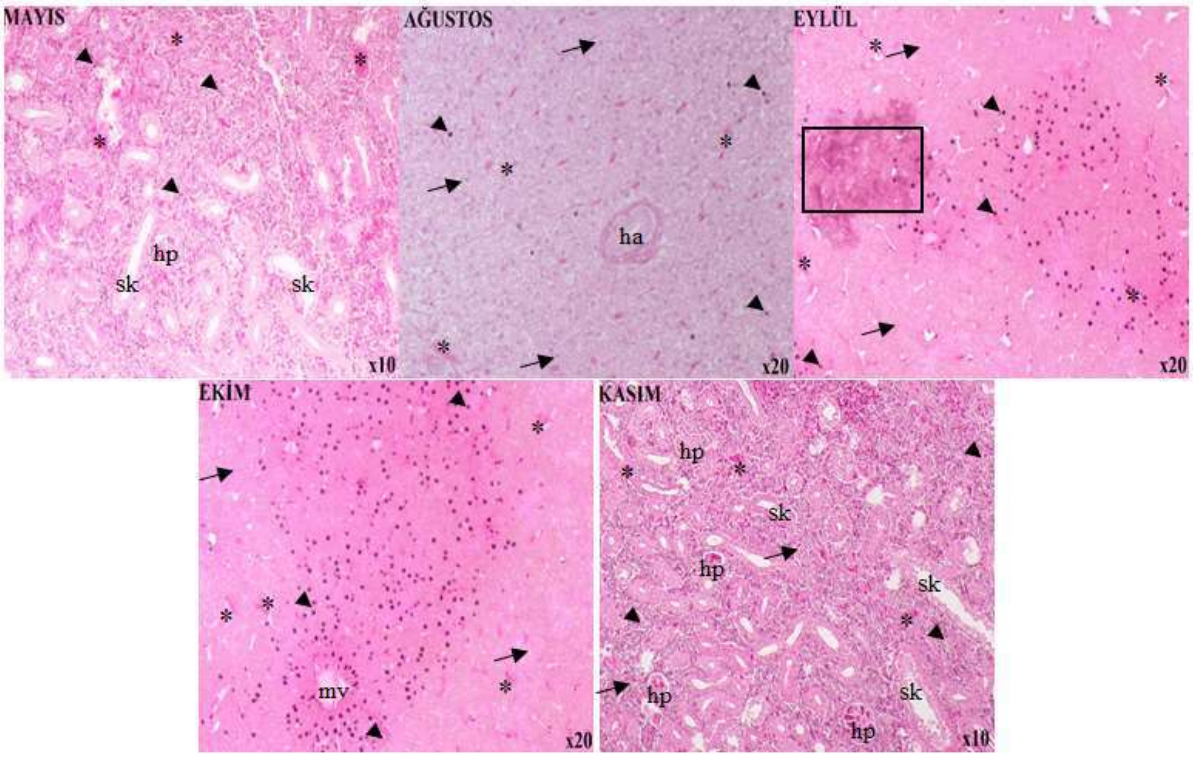

Figure 5. The histological findings obtained by staining liver tissues of catfish caught from Sapanca Lake with Hematoxylin & Eosin. In catfish liver tissue central vena (mv), hepatocytes (arrows), cupfer cells (arrowheads), hepatopancreas (hp), bile ducts (sk), hepatic artery (ha), fibrosis (rectangular), and blood supply in hepatocytes and sinusoids (asterisks)), H&E.

Histological findings obtained by staining the gill tissues of catfish caught from Sapanca Lake with Hematoxylin & Eosin are shown in Figure 6. Accordingly, separation in primary lamellae and hyperplasia and irregularity in secondary lamellae are the first striking findings. Desquamation (flaking, deformity) was observed in epithelial cells of secondary lamellae and hyperplasic lamellae. Although rare, vacuolization has been observed in the primary lamella. In addition, inter-lamellar mucus-like clumping has been encountered many times.

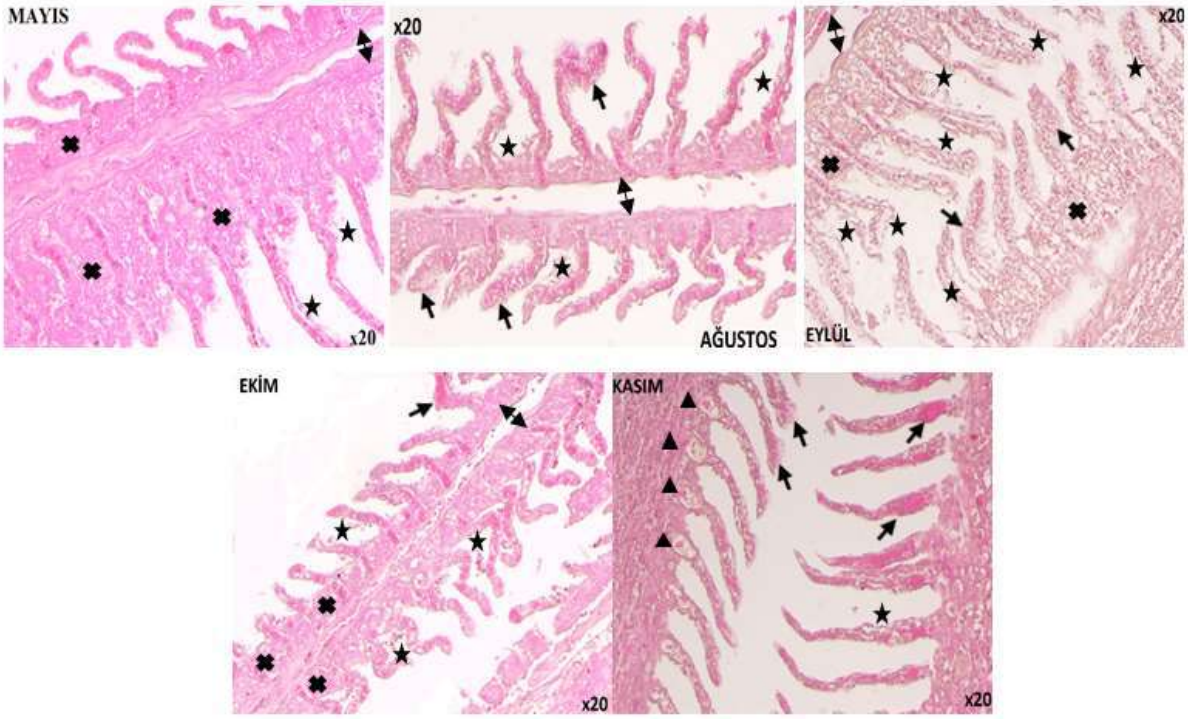

Figure 6. Histological findings obtained by staining the gill tissues of catfish caught from Sapanca Lake with Hematoxylin & Eosin. Hyperplasia in catfish gill tissue, secondary lamellae (arrows), separation of primary lamellae (bidirectional arrows), desquamation of secondary lamellae (asterisks), vacuolization of primary lamellae (triangle), as well as inter-lamellar mucus-like agglomeration (cross). H&E.

In conclusion, it is thought that this study will be of great importance in determining the effects of fish living in Sapanca Lake, which is a drinking water source, on human health. In general, the heavy metal load in the water and fish tissues does not exceed the specified standards, and the presence of biochemical and histopathological findings in the fish tissues suggests that the lake is under the influence of not only heavy metals but also other pollution sources. With this study, it has been shown that enzymatic and non-enzymatic antioxidants and histological analyzes are useful methods in biological monitoring of environmental pollution.

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INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF THE NON-LETHAL DOSE OF QUATERNIUM-15 ON ZEBRAFISH

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ABSTRACT

Natural or synthetic preservatives are added to products such as foods, medicines and personal care products to prevent microbial growth, unwanted chemical changes and accidental contamination during consumer use. 3- (3-Chloroallyl) -3-azonia-1,5,7-triazaadamantane (Quaternium-15), quaternary ammonium salt, which is used as a surfactant and preservative in many cosmetics and industrial substances, is also among these substances. Quaternium-15 is found in shampoos, conditioners, liquid soaps, shaving products, moisturizing creams and lotions, sunscreens, cleaners, disinfectants and laundry soaps, plastic gloves, metalworking fluids and cutting fluids, latex paints, glues and adhesives, food packaging, paper, cardboard and polyurethane resins. In order to determine the antimicrobial activity of the dose of quaternium-15, which has no negative effects on the vital activity of adult zebrafish (*Danio rerio*), gram (-): *Pseudomonas aeruginosa* ATCC 277853, *Escherichia coli* ATCC 25922, gram (+): *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Candida parapsilopsis* ATCC 22019 were used. As a control, Vancomycin 30µg for bacteria and Amphotericin-B 100U for yeast were prepared and disc diffusion method was applied. When the antimicrobial effect of the determined dose of Quaternium-15 was examined, it was determined that it was not effective in some bacterial species and fungi. It has been determined that it has a very weak effect compared to the antibiotic used as a control. Considering that Quaternium-15 is used as a protective additive, it is foreseen that it should be used at very high rates in order to provide effective protection in cosmetics and industrial products. It is known that quaternium-15 is a very strong formaldehyde releaser and therefore causes skin disease in humans.

Keywords: Antimicrobial activity, preservative, quaternium-15, bacteria, fungi.

INTRODUCTION

Due to the increasing human population and the rapid development of the industry, environmental pollutants are becoming more involved in nature and this situation is an urgent research subject in technical and scientific communities all over the world. Pollutants released to the market every day with hundreds of different formulations become threatening the

sustainability of environmental health and therefore the life of human beings. One of these pollutants is natural or synthetic preservative additives added to prevent microbial growth, unwanted chemical changes and accidental contamination of the consumer during use in products such as foods, medicines and personal care products. Preservative additives are specially designed to be resistant to metabolic degradation, and their high lipophilic properties are often seen as an essential requirement to maximize absorption by target organisms (Wielogórska et al., 2015). The number of preservatives allowed for cosmetic products in Europe is over 50 (Lv et al., 2015). 3- (3-Chloroallyl) -3-azonia-1,5,7-triazaadamantane (quaternium-15), which is used as a surfactant and preservative in many cosmetic and industrial substances, is also among these substances. Quaternium-15 found in shampoos, conditioners, liquid soaps, shaving products, moisturizing creams and lotions, sunscreens, cleaners, disinfectants and laundry soaps, plastic gloves, metalworking fluids and cutting fluids, latex polishes, glues and adhesives, food packaging, paper, cardboard and polyurethane resins (Siti et al., 2015). It is known that quaternium-15 is a very powerful formaldehyde releaser and therefore causes skin disorders in humans. It is also stated that formaldehydes are classified as carcinogens and have toxic effects on reproduction and development. Acceptable level of quaternium-15 in Europe is up to 0.2% (Toholka et al., 2014). However, 0.1% concentration (1000 ppm) quaternium-15 releases about 100 ppm formaldehyde, and this amount can cause dermatitis for a person with formaldehyde sensitivity. However, the degree of formaldehyde release of a product is affected by various factors such as the concentration of the preservative in the product, the percentage of water in the product, the rate and time of formaldehyde separation from the specific preservative. The irritant effects of the quaternium-15 substance draw attention in the literature (Marks et al., 1998; Maier et al., 2009; Odhav and Belsito, 2012; Shaughnessy et al., 2014).

Toxic agents are discharged into aquatic habitats in various ways. Pollutants that spread to the ecosystem are widespread, especially in water and sediments, and threaten other aquatic species (Hua et al., 2006; Yu et al., 2011; Zang et al., 2017; Marlatt et al., 2017). Especially recently, the number of studies that monitored changes in aquatic organisms due to environmental contamination has increased (Sancho et al., 2000; Lionetto et al., 2003; Miron et al., 2005; Moraes et al., 2007; Schirmer et al., 2013). As a result, the increasing environmental pollution in aquatic ecosystems seriously threatens the aquatic products that constitute very important protein resources, and this threat reaches human beings through the food chain. All chemical preservatives cause disruption of the ecological balance, damaging non-target organisms such as fish. Aquatic vertebrates such as fish are more exposed to various environmental pollutants emanating from the environment than other creatures. Fish constitute the vertebrate group with the most diversity, with 20,000 different species and being found in all aquatic niches (Schirmer et al., 2005). As a result, fish are considered the most suitable biomarker organisms to be used in studies of monitoring aquatic systems and determining environmental pollution (Loro et al., 2017). Because of these features, the effects of pollutants on fish make important contributions to understanding the root causes of degradation of aquatic habitats and their possible effects on human health (Caetano et al., 2017; Marlatt et al., 2017). Knowing that various biological systems are protected during evolution and the effects of these agents on fish provide information about their possible effects on humans. In addition, the economic importance of fish ensures that they are considered as important models in ecotoxicological studies (Caetano et al., 2017). For these reasons, fish are primarily preferred in controlled analyzes and modeling studies to determine aquatic ecosystem health. Due to its close homology to the human genome, zebrafish (*Danio rerio* Hamilton, 1822) is preferred in many studies. Small teleost species such as zebrafish are cheap and easily available creatures because they can easily adapt to laboratory environments (Luzio et al., 2013; Pereira et al.,

2013). In addition, completing the life cycle in a shorter time and being able to follow more generations is another reason for preference (Barbazuk et al., 2000; Paiono et al., 2017). Recent studies show that the use of zebrafish can provide the determination of the pollution level of the water and its toxicity value in detail (Hill et al., 2005; Coz et al., 2008; Pelayo et al., 2011; Zhang et al., 2017). The aim of this study is to determine the antimicrobial effect of the non-lethal dose of quaternium-15 used as a protective additive in cosmetic and industrial products in adult zebrafish.

MATERIAL AND METHODS

Fish Husbandory

The zebrafish used in the study were grown from the embryo in the laboratory. Female and male individuals of the broodstock fish were kept in separate aquariums. They were fed with protein-rich feeds for 15 days at 28 ºC, 14/10 hours in light / dark environment, chlorine-free tap water, and adapted to laboratory conditions. Later, 1 female and 1 male zebrafish were taken into the mating tank and live transparent embryos were collected in the morning and kept there until they came out of the chorion. The larvae emerging from the chorion were taken to 5 liter aquariums and fed with micron sized feeds. When the fish became mature individuals after about two months, they were used in experiments.

Q-15 exposure in zebrafish

For the determination of the LD50 ratio of Quaternium-15 in adult zebrafish kept under appropriate aeration and light / dark (14:10) conditions at the appropriate temperature range (24-28 ° C) in 25 liter glass aquariums, 0,5 mg/L; 1 mg/L; 5 mg/L; 10 mg/L ve 50 mg/L (n=10) doses application was made. Controls were made at the 24th, 48th, 72nd and 96th hours and the deaths were noted.

Antimicrobial analysis

Disk diffusion technique (Kirby-Bauer method) has been used in determining antimicrobial activity due to its low cost, less effort and easy application. In order to determine the antimicrobial activity of the dose (1mg / L) of Quaternium-15 applied to cells and fish, previously obtained from culture collections and available in our laboratory, gram (-): *Pseudomonas aeruginosa* ATCC 277853, *Escherichia coli* ATCC 25922, gram (+): *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 bacteria and *Candida parapsilopsis* ATCC 22019 standard strains were used. Disk diffusion method was used to determine the antimicrobial activity. 100 µL of the active microorganism suspension in sterile physiological water in a sterile cabinet, adjusted at 0.5 McFarland turbidity, under sterile conditions, and into the previously prepared solid medium (Mueller-Hinton agar (Sigma-Aldrich) for bacteria, Sabouroud dextrose agar for yeast (Sigma-Aldrich)) It was planted by the spread plate method. 15 µL of quaternium-15 was impregnated into empty sterile antibiotic discs. These discs were neatly placed on solid media on which standard strains were cultivated. As the control group, Vancomycin 30µg for bacteria and Amphotericin-B 100U for yeast were prepared in parallel. After these processes, the petri dishes containing bacteria were incubated at 37 ° C for 24 hours, and the dishes containing yeast at 35 ° C for 48 hours. Inhibition zones formed as a result of the incubation period were measured with the help of calipers and the zone diameters were recorded in millimeters (mm). All analyzes were carried out in three parallel (CLSI, 2009).

RESULTS AND DISCUSSION

In this study, the antimicrobial effect of the non-lethal dose of quaternium-15 used as a protective additive in cosmetic and industrial products in adult zebrafish was determined. For this purpose, Quaternium-15 was applied to zebrafish at increasing doses and times. In the first 24 hours, death was observed in all of the fish that received 10 and 50 mg / L doses. All of the fish that were dosed with 5 mg / L within 96 hours died. No deaths were observed in fish dosed with 0.5 and 1 mg / L. Based on these results, a dose of 1 mg / L Quaternium-15 was determined for use in antimicrobial analyzes.

Natural or synthetic preservative additives added to prevent microbial growth, unwanted chemical changes and accidental contamination during consumer use in products such as foods, pharmaceuticals and personal care products are specially designed to be resistant to metabolic degradation, and their high lipophilic properties are generally absorbed by target organisms. it is seen as a basic requirement to maximize (Wielogórska et al., 2015). When the antimicrobial effect of the determined dose of quaternium-15 was examined, it was determined that it was not effective on some bacterial species and fungi, and on the species it was effective, it had a rather weak effect compared to the antibiotic used as a control (Table 1). Considering that quaternium-15 is used as a protective additive, it is predicted that it should be used at very high rates in order to provide effective protection in cosmetic and industrial products. The number of preservatives allowed for cosmetic products in Europe is over 50 (Lv et al., 2015).

Table 1: Antimicrobial activity analysis of Quaternium-15

	Zone of inhibition (mm)	
Bacteria	**Q-15**	**Vankomisin**
Enterococcus faecalis ATCC 29212	-	16 ±0.4
Escherichia coli ATCC 25922	9,5 ±0.4	15 ±0.2
Pseudomonas aeruginosa ATCC 27853	-	18 ±0.3
Staphylococcus aureus ATTC 29213	9 ±0.6	18 ±0.3
Fungi		**Amfoterisin-B**
Candida parapsilopsis ATCC 22019	-	20 ±0.2

In conclusion, with this study, considering that even a very low dose of quaternium-15, which is used as a protective additive in cosmetic and industrial products and which is known to cause dermatological problems in humans, has a toxic effect in a short time on zebrafish, the human model organism, cosmetic and industrial It is thought that the dose in the products harms both the people who are the users of these products and all living things in the ecosystems of natural areas discharged with waste water. In addition, with this study, a new perspective is gained to identify and solve the problem of water pollution, which is not considered a major pollution factor yet, which is caused by the widespread use of protective additives, but can affect all humanity.

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EFFECT OF THE ENVIRONMENTAL CONDITION IN LEAF RUST AND RESPONSE OF BREAD WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS UNDER RAINFED CONDITION

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ABSTRACT

The prevalence level and development of leaf diseases depend on many factors, particularly climatic conditions. Leaf rust is the most widespread and imported diseases of wheat in the region. Temperature, rainfall, and humidity are some of the abiotic environmental factors that may influence bread wheat yield during the grain filling period in the Trakya region, Turkey. It was investigated the effect of the temperature, rainfall, and humidity during GS31-47, GS49-75, and GS77-90 into yield and leaf rust of the bread wheat cultivars. Temperature, rainfall, and humidity from shooting up to grain filling period were taken in the experimental area. This research was established with 25 genotypes in RCBD with 4 replications in Edirne, between 2005 and 2015 growing seasons. Cultivars Aldane, Selimiye, Pehlivan, and Gelibolu were selected from this experiment due to their various reaction to leaf rust. Grain yield, leaf rust, days of heading, plant height and relationship amongst traits were investigated based on cultivars. According to result there were significant differences for yield and other investigated traits. The highest average yield was 8636 kg ha^{-1} in 2007-2008, and in 2013-2014 with 8244 kg ha^{-1}. The lowest yield was determined in 2015-2016, where leaf rust infection was higher under rainfed environmental condition. Pehlivan and Gelibolu were susceptible cultivars. In this study it was investigated in order to determining correlation coefficient of the characters based on cultivars. It was found various relations among investigated parameters. There was a significant negative relation between grain yield and leaf rust in cv. Pehlivan (r= -0.644*) and Selimiye (r= -0.652*). Leaf rust negatively affected and decreased grain yield in cultivar Gelibolu (r= -0.437) and Aldane (r= -0.337). The rainfall during the GS31-47 growth stage negatively affected and increased infection of leaf rust in Selimiye and slightly affected other cultivars based on their development of growth period. Rainfall during GS31-47 growth stage contributed to leaf rust epidemic in susceptible cultivar Pehlivan. There was no any effect during GS77-90 between yield and leaf rust. There were various degrees of leaf rust infection and correlation between yield and leaf rust based on mean and maximum temperature during GS31 and GS90. The mean and maximum temperature during GS31-47 promoted leaf rust infection in Selimiye cultivar. Increasing in temperature during GS49-75 growth stage negatively affected leaf rust in Pehlivan cultivar.

Keywords: *Bread wheat, Cultivar, Climate effect, Yield, Leaf rust*

INTRODUCTION

Wheat leaf rust, caused by the obligate parasitic fungus Puccinia triticina Eriks (syn. P. recondita Roberg ex Desmaz. f. sp. tritici), is considered to be the most widely distributed wheat disease in the world (Wiese 1987; Gupta et al. 2006). Leaf rust caused by Puccinia triticina (syn. P. recondita Rob. Ex Desm. f. sp. tritici Eriks. And Henn) in particular, is one of the most

dangerous diseases in worldwide causing significant losses in grain yield (Long et al. 1994; Khan et al. 2013). Wheat plants are suffering from many destructive diseases. Rusts are the most important diseases of wheat because of their ability to move for a long distance and their ability to form new virulent races causing serious losses (Yahyaoui et al., 2004; Huerta-Espino et al., 2011). Susceptible wheat cultivars to leaf rust suffer from yield reductions between 5 to 60% (Smith, Lauren, 2008). Genetic resistance is the most reasonable and preferable technique to minimize yield losses due to leaf rust infection (Kolmer, 1996). Although, leaf rust disease is able within a short time to form new races that are capable of disabling the resistance of the newly produced commercial cultivars (Sayre et al. 1998 and Negm et al., 2013). The pathogen interferes with wheat plants at all developmental stages and serious yield losses of up to 62.7% have been reported in susceptible cultivars (Singh et al. 1998). Leaf rust caused by *Puccinia triticina* is one of the most important and widespread diseases of common wheat (*Triticum aestivum* L.) worldwide. It is adapted to a wide range of environments that occurs wherever wheat is grown and can cause significant yield and economic losses (Wamishe and Milus, 2004). The occurrence of severe and damaging epidemics of leaf rust lead to that many new wheat cultivars including high yielding were eliminated and discarded very shortly after their release and farmer's use in agriculture (Nazim el al. 1990; Negm, 2004). Early infection of leaf rust usually causes higher yield losses 60-70% depending on the susceptibility of the wheat varieties and the severity of epidemics (Appel et al., 2009). Host resistance is the most economical and safest method for controlling the disease (Mebrate et al., 2008). Leaf or brown rust of wheat caused by Puccinia recondita Rob. ex Desm. f.sp. tritici is a major disease of wheat worldwide. The fungus can infect with dew periods of 3 hours or less at temperatures of about 20°C, however, more infections occur with longer dew periods. At cooler temperatures, longer dew periods are required, for example at 10°C a 12-hour dew period is necessary. Few if any infections occur where dew period temperatures are above 32°C (Stubbs et al., 1986) or below 2°C (Roelfs et al., 1992). Severe epidemics and losses can occur when the flag leaf is infected before anthesis (Chester 1946). The disease can develop rapidly when free moisture is available and temperatures are near 20°C (Duveiller et al., 2012). Fungicides and different cultural practices can be used to reduce yield losses. However, the development of resistant cultivars is the most efficient, sustainable, and economic method to prevent leaf rust epidemics (Naz et al., 2008). Therefore, the success of a wheat breeding program depends on the regional adaptability of the cultivars improved and adaptability of such cultivars in the target environments determined by its tolerance to biotic and abiotic stresses (Altay, 2012).

Leaf rust is one of the main diseases affecting wheat yield production in the Trakya region, Turkey. Therefore, the objectives of the study aimed to determine and compare the efficiency of different levels of wheat cultivar resistance in decreasing losses in grain yield under various environmental conditions in rainfed conditions. In addition to some of the biotic stress factors and the relationship between these characters was investigated under rainfed conditions.

MATERIAL AND METHODS

Leaf rust (*Puccinia triticina*) is a vital disease that causes significant yield losses in wheat fields. The relation between yield related due to leaf rust disease and host resistance was studied in four bread wheat cultivars through the twelve successive growing seasons 2005-2006 and 2015-2016. The experiment was conducted in Trakya Agricultural Research Institute experimental area. Twenty-five winter wheat genotypes were examined under field conditions with randomized complete block design (RCBD) with four replications. Four cultivars which are Pehlivan, Gelibolu, Aldane, and Selimiye were selected from these experiments because of their various level of reaction to leaf rust disease. In the experiment, each plot was 6 meters

long and had 6 rows, spaced 0.17 meters apart. Using a plot drill performed sowing and 500 seeds per square meter were used. Sowings were performed by using a plot drill in October and in November, and nitrogen was applied three times, and for weed control chemical was used. Data on grain yield, leaf rust, days of heading, plant height, and relationship amongst these traits and rainfall, humidity, and temperature were investigated based on cultivars.

***Leaf rust disease severity*:** Leaf rust severity is recorded as a percentage, according to the modified Cobb scale (Prescott et al., 1986). This recording process relies upon visual observations at Z55 (One-half of ear emerged)

***Plant height*:** The height of ten randomly taken plants was measured at harvest maturity from the ground level to the tip of the tallest spike in centimeter and averaged.

***Days to 50% heading*:** The number of days from the date of 1 October up to the date when the tips of the spike first emerged from the main shoots on 50% of the plants in a plot.

The rainfall, temperature and mean humidity in April (GS31-47), May (GS49-75), and June (GS77-90) from shooting up to ripening period were taken from 2005 up to 2016 growing years in experimental field (Table 1). The Zadoks Decimal Code (GS) was used to describe plant growth stages of cereals. The described plant development stages are; April: GS31 (first node detectable) from up to GS47 (flag leaf sheath opening), May: GS49 (first awns visible) from up to GS75 (medium milk), June: GS77 (late milk) from up to GS90 (ripening). Also, regression graphs are used to predict the comparison of characters.

Table 1. Rainfall, mean humidity and temperature scored in GS31-47, GS49-75, and GS77-90

Year	Rainfall (mm)			Humidity (%)			Mean temperature (°C)			Max temperature (°C)		
	GS 31-47	GS 49-75	GS 77-90	GS 31-47	GS 49-75	GS 77-90	GS 31-47	GS 49-75	GS 77-90	GS 31-47	GS 49-75	GS 77-90
2005	17.6	55.4	15.1	62.2	67.2	61.9	13.4	18.6	21.5	28.7	30.2	34.4
2006	24.0	36.5	57.8	65.8	63.8	64.3	14.0	18.4	22.4	27.0	33.6	35.8
2007	17.0	122.0	8.2	59.2	67.4	56.1	12.5	19.6	24.8	25.9	32.1	42.6
2008	44.1	33.4	45.7	71.2	62.7	62.1	14.3	18.0	23.3	27.7	35.1	36.7
2009	15.8	27.7	25.9	68.8	66.1	62.5	12.3	19.1	22.6	25.9	32.1	36.4
2010	17.8	16.0	30.8	76.0	68.6	72.3	12.7	18.1	22.5	24.9	33.6	38.7
2011	47.4	12.4	32.4	78.9	74.9	68.6	10.5	17.4	21.9	24.1	31.8	34.4
2012	55.7	104.6	0.4	72.5	75.8	64.1	15.5	19.1	25.3	30.2	31.7	36.9
2013	51.0	11.0	26.6	67.5	66.7	70.1	20.3	20.8	23.3	32.0	32.9	36.2
2014	36.8	61.7	68.8	81.6	76.6	73.8	13.6	18.6	22.9	25.5	32.1	33.6
2015	44.4	45.2	31.0	70.9	68.4	68.2	13.1	20.4	22.5	25.7	33.3	35.3
2016	116.1	81.4	10.2	66.6	71.8	61.8	15.5	17.4	23.9	31.8	32.2	38.4
Mean	40.6	50.6	29.4	70.1	69.2	65.5	14.0	18.8	23.1	27.5	32.6	36.6

Four cultivars selected from regional yield trials, consist of 25 genotypes, evaluated under natural field condition and reaction types and rust levels scored using the Modified Cobb scale (Peterson et. al., 1948; Roelfs et al., 1992; Saari and Prescott, 1975; Stubbs et al., 1986; Prescott et al., 1986) in Edirne, Turkey. A uniform epidemic was achieved and susceptible genotypes were rated as 80-100S.

Statistical analysis

All data obtained were statically individually analyzed for each season. Analysis of variance over seasons was also determined. The correlation coefficient was also used to detect the relationship between yield and disease progresses. The statistical analyses of measurement data were performed by using statistics program and the differences among the means were compared with LSD at a 5% significant level. To evaluate significant differences between genotypes, the analysis of variance (ANOVA) was performed. The differences between genotype means of parameters were tested by the L.S.D test. Letter groupings were generated by using a 5% level of significance. Data were analyzed statistically for analysis of variance the method described by Gomez and Gomez (1984). The significance of differences among means was compared by using the L.S.D test (Kalaycı, 2005). The regression equations were calculated according to Finlay and Wilkinson (1963), and Eberhart and Russell (1969). Regression graphs were used to predict the adaptability of genotypes and the correlations between the quality parameters were determined by Pearson's correlation analysis.

RESULTS AND DISCUSSION

Rainfall, temperature, and humidity are mainly abiotic environmental factors that may influence bread wheat yield production during the growing cycle in the Trakya region, Turkey. Although the average annual rainfall is enough during the growing season (October-June) the distribution of this rainfall is not regular. So, this fluctuation of rainfall causes biotic and abiotic stress factors.

Table 2. Mean yield, leaf rust, days of heading and plant height of the cultivars Pehlivan and Gelibolu both of them susceptible to leaf rust

Year	Pehlivan				Gelibolu			
	GY	**LR**	**DH**	**PH**	**GY**	**LR**	**DH**	**PH**
2005	7660	80S	123	90	8110	60S	122	80
2006	7528	100S	120	105	7038	80S	120	95
2007	6617	80S	117	110	6810	60S	126	100
2008	8429	10MS	124	100	9032	10MS	122	95
2009	6047	70S	123	120	6273	20S	123	110
2010	7247	60S	118	105	6812	60S	128	100
2011	7133	60S	134	115	6212	40S	133	110
2012	7056	60S	123	90	7685	20S	120	80
2013	7439	40S	117	120	6767	60S	115	110
2014	8045	40S	110	115	8768	40S	109	115
2015	6399	80S	124	95	7934	60S	121	95
2016	3821	100S	105	105	5327	80S	105	100
Mean	6952		119.8	105.8	7231		120.3	99.2

GY: Grain yield (kg ha^{-1}), LR: Leaf rust, DH: Days of heading, PH: plant height (cm)

Leaf rust, caused by *Puccinia triticina* is important diseases of wheat worldwide. In the study bread wheat cultivars were chosen to show different levels of field resistance against leaf rust infection. Due to the changes in environmental conditions from one year to another, leaf rust epidemic was found to be more severe in its degree during the all growing season from 2005 to 2016 in susceptible cultivars Pehlivan and Gelibolu. Variation in disease severity was noticed

among genotypes and environment. Data in Table 2 and 3 revealed that rust incidence in 2006 and 2016 was higher than the other seasons.

Analysis of variance revealed highly significant difference among cultivar Pehlivan for grain yield. Mean grain yield for twelve years was 6952 kg ha^{-1}. The highest grain yield performed in 2007-2008 (8429 kg ha^{-1}) and the lowest in 2015-2016 (3821 kg ha^{-1}) (Table 2). Analysis of variance showed that highly significant difference among cultivar Gelibolu for grain yield. Mean grain yield for twelve years was 7231 kg ha^{-1}. The highest grain yield performed in 2007-2008 (9032 kg ha^{-1}) and the lowest in 2015-2016 (5327 kg ha^{-1}) (Table 2).

Table 3. Mean yield, leaf rust, days of heading and plant height of the cv Aldane and Selimiye

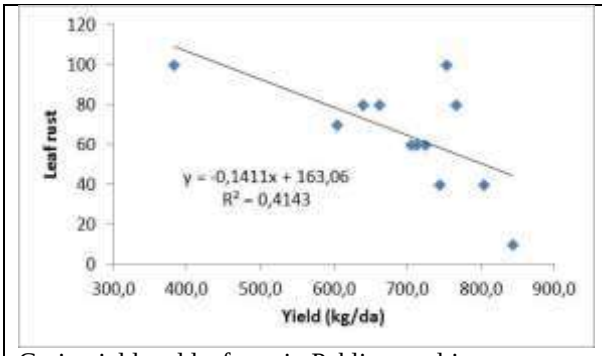
Year	Aldane				Selimiye			
	GY	**LR**	**DH**	**PH**	**GY**	**LR**	**DH**	**PH**
2005	6430	0	120	80	7860	0	120	90
2006	6351	0	117	100	7550	TR	118	100
2007	5787	0	112	100	7290	0	115	90
2008	8323	0	116	100	8760	0	114	100
2009	5353	0	120	120	6791	0	122	105
2010	5822	10MS	116	110	6993	20S	118	100
2011	6323	0	129	120	6970	0	136	100
2012	5904	0	121	95	7143	0	121	85
2013	7030	TR	116	115	7555	70S	115	115
2014	7601	TR	108	110	8560	10MS	109	110
2015	6583	TR	120	90	6625	60S	125	90
2016	4922	5R	98	100	3947	90S	105	95
Mean	6369		116.1	103.3	7170		118.2	98,3

GY: Grain yield (kg ha^{-1}), LR: Leaf rust, DH: Days of heading, PH: plant height (cm)

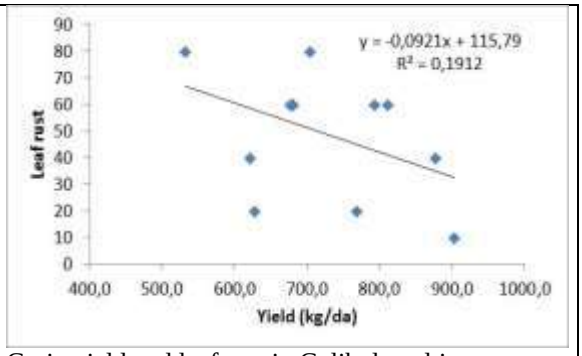
Grain yield was varied among the growing cycle from 2004-2005 and 2015-2016 in cv Aldane from 4922 kg ha^{-1} to 8323 kg ha^{-1} and, mean was 6369 kg ha^{-1}. Grain yield was varied among the growing cycle from 2004-2005 and 2015-2016 in cv Selimiye from 3947 kg ha^{-1} to 8760 kg ha^{-1} and, mean was 7170 kg ha^{-1} (Table 3). As a consequently, 2015-2016 had the lowest and 2007-2008 had the highest yielding crop cycles.

Data in Tables 2 and 3 indicated that all tested cultivars showed different final rust severity. Disease severity was high for cultivars Pehlivan (40-100S) and Gelibolu (20-80S). Whereas, the final rust severity was the lowest on wheat cultivars showing the low levels of susceptibility Selimiye (0-90S) and Aldane (0-10MS).

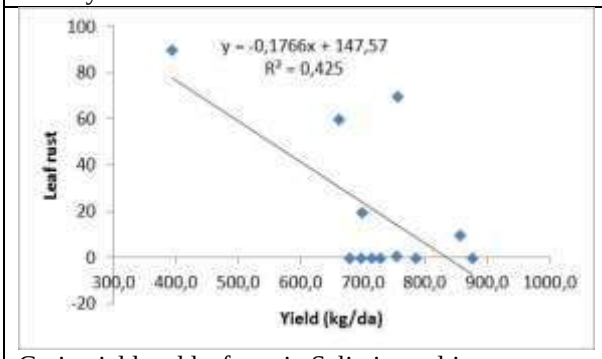
It is necessary to take into consideration yield performance because a high yielding line with high leaf rust tolerance will have a greater value for wheat breeders and producers. These lines are perfect parents for crossing with other slow-rusting lines that have better resistance to achieve high yield potential with a high level of resistance to leaf rust disease. In the study, four cultivars were used, cultivar Aldane has a high level of tolerance to leaf rust Selimiye is the medium tolerance, cvs Gelibolu, and Pehlivan very susceptible to leaf rust.



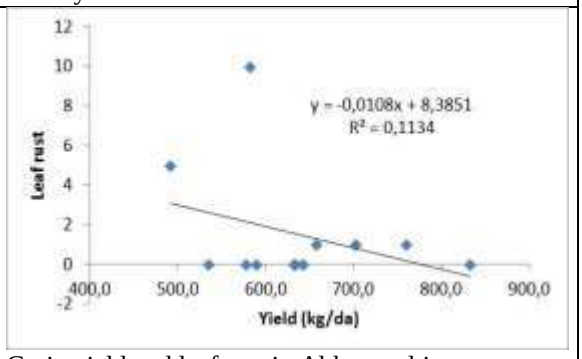
Grain yield and leaf rust in Pehlivan cultivar



Grain yield and leaf rust in Gelibolu cultivar

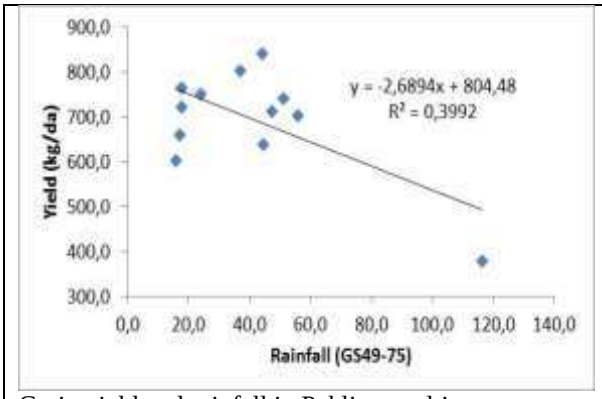


Grain yield and leaf rust in Selimiye cultivar

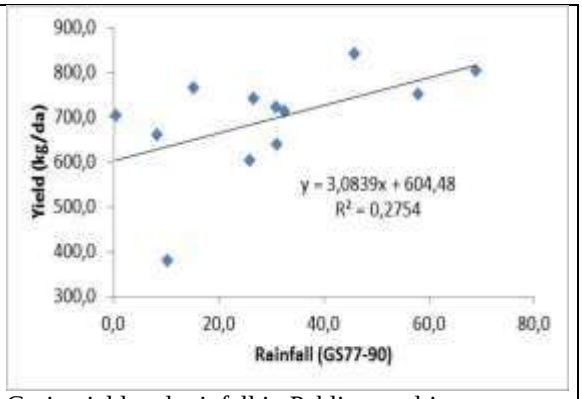


Grain yield and leaf rust in Aldane cultivar

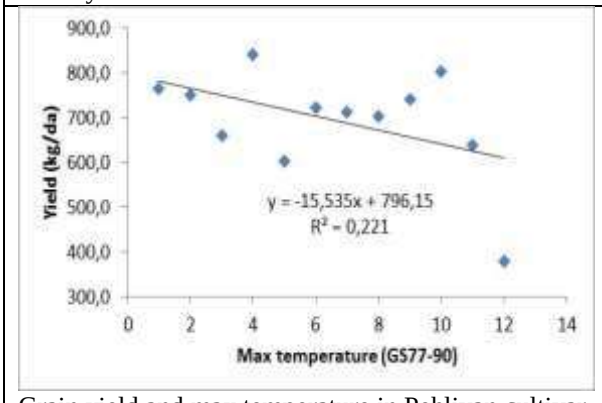
Figure 1. Relationship between grain yield and leaf rust infection in cultivars Pehlivan, Gelibolu, Selimiye and Aldane



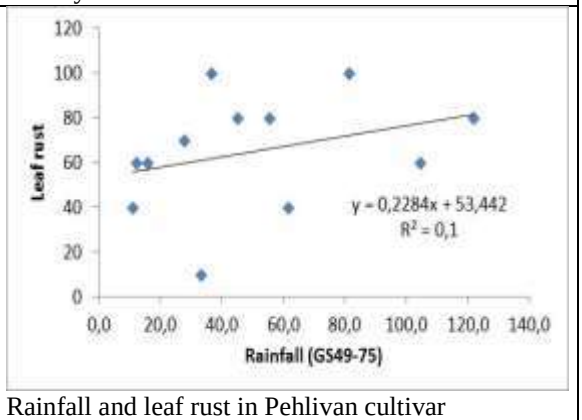
Grain yield and rainfall in Pehlivan cultivar



Grain yield and rainfall in Pehlivan cultivar



Grain yield and max temperature in Pehlivan cultivar



Rainfall and leaf rust in Pehlivan cultivar

Figure 2. Regression between yield and leaf rust infection and climate factors in susceptible Pehlivan cultivars

According to mean yield and other investigated traits there were significant differences among years. The highest average grain yield was 8636 kg ha^{-1} in 2007-2008, and in 2013-2014 with 8244 kg ha^{-1}, where leaf rust infection was very low under rainfed environmental conditions. The lowest yield was also determined in 2015-2016 crop cycles where leaf rust infection was higher under rainfed environmental conditions.

The rainfall, humidity, and temperature during GS31 up to GS90, from shooting, booting, heading, and grain filling period were investigated (Figure 1). The relationship between yield and leaf rust infection was investigated in Pehlivan, Gelibolu, Selimiye and Aldane cultivars based on their genotypic characters. The genotypes and environmental interactions varied by genotypic traits and climate conditions. As expected there was a negative relationship between grain yield and leaf rust in Pehlivan (R^2=0.414), Selimiye (R^2=0.425), and Gelibolu (R^2=0.191). A negative slight relation was found between grain yield and leaf rust in Aldane (R^2= 0.113) cultivar which has resistant to leaf rust and carries the Lr9 gene.

Table 4. Correlation coefficients among tested parameters in cultivars Pehlivan and Gelibolu

Parameters	Pehlivan				Gelibolu			
	GY	LR	DH	PH	GY	LR	DH	PH
LR	-0.644*				-0.437			
DH	0.422	-0.198			-0.021	-0.263		
PH	-0.066	-0.180	-0.143		-0.291	-0.020	-0.112	
Rainfall GS31-47	-0.632*	0.117	-0.432	-0.074	-0.353	0.188	-0.625*	0.021
Rainfall GS49-75	-0.307	0.316	-0.380	-0.377	0.043	0.054	-0.270	-0.410
Rainfall GS77-90	0.525	-0.325	-0.039	0.327	0.434	-0.035	-0.124	0.475
Humidity GS31-47	0.277	-0.488	0.144	0.208	0.221	-0.396	0.033	0.424
Humidity GS49-75	-0.166	-0.005	-0.117	0.018	-0.076	-0.117	-0.195	0.146
Humidity GS77-90	0.317	-0.351	-0.024	0.259	0.172	0.014	-0.093	0.440
Mean Temp. GS31-47	-0.012	-0.213	-0.427	0.059	0.001	0.158	-0.587*	-0.034
Mean Temp. GS49-75	0.123	-0.067	0.009	0.072	0.173	0.040	-0.058	0.014
Mean Temp. GS77-90	-0.259	-0.060	-0.357	-0.105	-0.075	-0.179	-0.270	-0.178
Max Temp. GS31-47	-0.306	0.046	-0.438	-0.191	-0.159	0.182	-0.631*	-0.312
Max Temp. GS49-75	0.228	-0.403	-0.003	0.082	0.215	-0.115	0.040	0.176
Max Temp. GS77-90	-0.372	0.215	-0.303	0.035	-0.382	0.189	0.126	-0.083

Note: Significance at *: P<0.05; **: P<0.01; GY: Grain yield (kg ha^{-1}), DH: Days of heading, PH: plant height (cm), LR: Leaf rust, GS: Growth stage

In this study, it was investigated in order to determine the correlation coefficient of the characters based on cultivars. It was found various relations among investigated parameters. There was a significant negative relation between grain yield and leaf rust in susceptible cultivars Pehlivan (r= -0.644*) and Selimiye (r= -0.652*). Leaf rust also negatively affected

and decreased grain yield in Gelibolu (r= -0.437) and Aldane (r= -0.337) cultivar. The rainfall during GS31-47 growth stage negatively affected and increased infection of leaf rust in Selimiye and slightly affected other cultivars based on their growth period. Rainfall during the GS31-47 growth stage contributed to the leaf rust epidemic in susceptible cultivar Pehlivan (r=0.316). There was no any effect during GS77-90 between yield and leaf rust. There were various degrees of leaf rust infection and correlation between yield and leaf rust based on mean and maximum temperature during GS31 and GS90. The mean and maximum temperature during GS31-47 promoted leaf rust infection in Selimiye cultivar. Increasing in temperature during GS49-75 growth stage has been negatively affected leaf rust in Pehlivan cultivar.

Table 5. Correlation coefficients among tested parameters in cultivars Selimiye and Aldane

Parameters	Selimiye				Aldane			
	GY	LR	DH	PH	GY	LR	DH	PH
LR	-0.652*				-0.337			
DH	0.132	-0.383			0.158	-0.374		
PH	0.268	0.162	-0.197		-0.044	0.136	0.109	
Rainfall GS31-47	-0.682*	0.715**	-0.359	-0.066	-0.194	0.171	-0.535	-0.034
Rainfall GS49-75	-0.199	-0.071	-0.373	-0.643*	-0.304	-0.188	-0.414	-0.490
Rainfall GS77-90	0.519	-0.189	-0.092	0.609*	0.635*	-0.065	-0.016	0.285
Humidity GS31-47	0.192	-0.093	0.225	0.361	0.328	0.234	0.187	0.484
Humidity GS49-75	-0.206	0.046	0.098	-0.102	-0.167	0.076	-0.053	0.161
Humidity GS77-90	0.199	0.200	0.118	0.555	0.330	0.381	0.125	0.400
Mean Temp. GS31-47	-0.033	0.569*	-0.495	0.344	0.184	-0.001	-0.335	-0.037
Mean Temp. GS49-75	0.206	0.247	0.027	0.094	0.127	-0.292	0.147	-0.099
Mean Temp. GS77-90	-0.188	0.102	-0.404	-0.309	-0.190	-0.057	-0.376	-0.073
Max Temp. GS31-47	-0.323	0.554*	-0.505	-0.006	-0.099	-0.033	-0.422	-0.270
Max Temp. GS49-75	0.191	0.119	-0.173	0.278	0.445	0.224	-0.090	0.185
Max Temp. GS77-90	-0.316	0.072	-0.316	-0.317	-0.446	0.309	-0.366	-0.025

Significance at *: P<0.05; **: P<0.01; GY: Grain yield (kg ha^{-1}), DH: Days of heading, PH: plant height (cm), LR: Leaf rust, GS: Growth stage.

There was also a slightly negative correlation between grain yield and maximum temperature at GS77-90 in four cultivars. Rainfall at GS49-75 growth stages promoted infection of leaf rust in susceptible cultivars Pehlivan and Gelibolu. Rainfall at GS31-47 growth phase had a negative correlation with GY but a positive correlation with rainfall at GS77-90 in all cultivars.

Wheat leaf rust is the most destructive and widespread disease in the Trakya region and susceptible cultivars leading to serious yield losses. There were differences among environmental conditions according to leaf rust (Puccinia recondita) infection. There is a low incidence of wheat leaf rust in the 2010-2011 and 2011-2012 cropping cycle because of suitable

environmental conditions, and disease severities in susceptible varieties Pehlivan reached 60S, in Gelibolu 40S, but there is no incidence in cultivar Selimiye and Aldane (Table 2 and 3).

CONCLUSION

According to the result, there were various levels of yield and leaf rust infection based on the degree of temperature, humidity, and rainfall during the various growth stages. The lowest yield was determined in environmental conditions where leaf rust infection was higher. The susceptible cultivar was various degrees affected by leaf rust. There were various relations among investigated parameters. There was a significant negative relation between grain yield and leaf rust in Pehlivan and Selimiye. Leaf rust also negatively affected and decreased grain yield in cultivar Gelibolu and Aldane. The rainfall during the GS31-47 growth stage negatively affected and increased infection of leaf rust in Selimiye and slightly affected other cultivars based on their growth period. Rainfall during the GS31-47 growth stage contributed to the leaf rust epidemic in susceptible cultivar Pehlivan. There was no any effect during GS77-90 between yield and leaf rust. There were various degrees of leaf rust infection and correlation between yield and leaf rust based on mean and maximum temperature during GS31 and GS90. The mean and maximum temperature during GS31-47 promoted leaf rust infection in Selimiye cultivar. Increasing in temperature during GS49-75 growth stage has been negatively affected leaf rust in Pehlivan cultivar.

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MORPHOMETRIC PARAMETERS OF THE SAGITTAL OTOLITHS OF 10 FISH SPECIES BELONGING TO SPARIDAE FAMILY FROM THE COASTAL AREAS OF ÇANAKKALE STRAIT, TURKEY

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ABSTRACT

This study aimed to compare the morphometric parameters between the right and left sagittal otoliths of ten species belonging to the Sparidae family, from the coastal areas of Çanakkale Strait, Turkey. A total of 600 specimens were collected monthly using beach seine (0–2 m) and beam trawl (5–20 m) from June 2013 to June 2014. The morphometric parameters such as length, width, area and perimeter of the sagittal otoliths were measured and compared between right and left otoliths. The relationships between otolith morphometric parameters (length, width, area and perimeter) and total length were also examined. These relationships can be useful for researchers who examining stomach contents of piscivorous predators.

Keywords: Otolith morhometry, Sparidae, Çanakkale

INTRODUCTION

Sparidae family are marine fishes, rarely found in brackish or freshwater, present in the Atlantic, Indian and Pacific oceans (Parenti, 2019). According to Nelson et al. (2016), Sparidae family comprises 148 species in 37 genera arranged in six subfamilies. However, this concept has recently been challenged by Parenti (2019) studies demonstrating that the family Sparidae representing 166 valid species in 39 genera and 260 synonyms. They have a wide distribution area in the world seas. They spread throughout the Mediterranean, in the East Atlantic, from the British Islands to Senegal, from North Carolina to Mexico in the West Atlantic, and from Venezuela to Argentina. The highest density is found in the Northeast Atlantic Ocean and the Mediterranean. This family, which has 22 species in the Mediterranean, is of great importance in the fisheries resources (Arculeo et al., 2003).

Otoliths are structures that are used in balance and / or hearing in all bony fishes and are hardened by the accumulation of calcium salts in the inner ear cavity (Campana, 1999). There are three pairs of otoliths called "lapillus", "asteriscus" and "sagitta" in the inner ear (Green et al., 2009, Tuset et al., 2008). These are natural data recorders that record information about growth and the environment at different temporal scales in their microstructure and chemistry (Campana, 1999, Kalish, 1989).

Scientists from past to present have examined the relationship between the size and weight of otolith and the growth of the fish. These structures record the periods when the fish grew and did not, and the changes caused by them. The length and age of the fish can be determined with otolith morphometric measurements. Knowing the relationships between otolith size and fish size provides important information to determine fish sizes from otoliths in stomach of predators (Aguilar-Perera and Quijano-Puerto, 2016, Battaglia et al., 2010, Granadeiro and Silva, 2000). Trout (1954) and Templemann and Squires (1956) were the first to demonstrate a positive relationship between otolith size and fish size of *Boreogadus saida* and *Melanogrammus aeglefinus*.

This study aimed to compare the morphometric parameters between the right and left sagittal otoliths of ten species belonging to the Sparidae family, from the coastal areas of Çanakkale Strait, Turkey. The relationships between otolith morphometric parameters (length, width, area and perimeter) and total length were also examined.

MATERIAL AND METHODS

The current study was conducted in the coastal areas of the Çanakkale Strait, Turkey between April 2019, and January 2020 (Figure 1). Fish samples were caught with a beach seine of 32 m in total length, with 15 m wing lengths. The net was constructed of 13 mm stretch mesh. The dimensions of the bag were 2 × 2 × 0.6 m, and the bag was constructed with 5 mm mesh. In the laboratory, the total length of fish was measured to the nearest 0.1 cm and weight to the nearest 0.1 g.

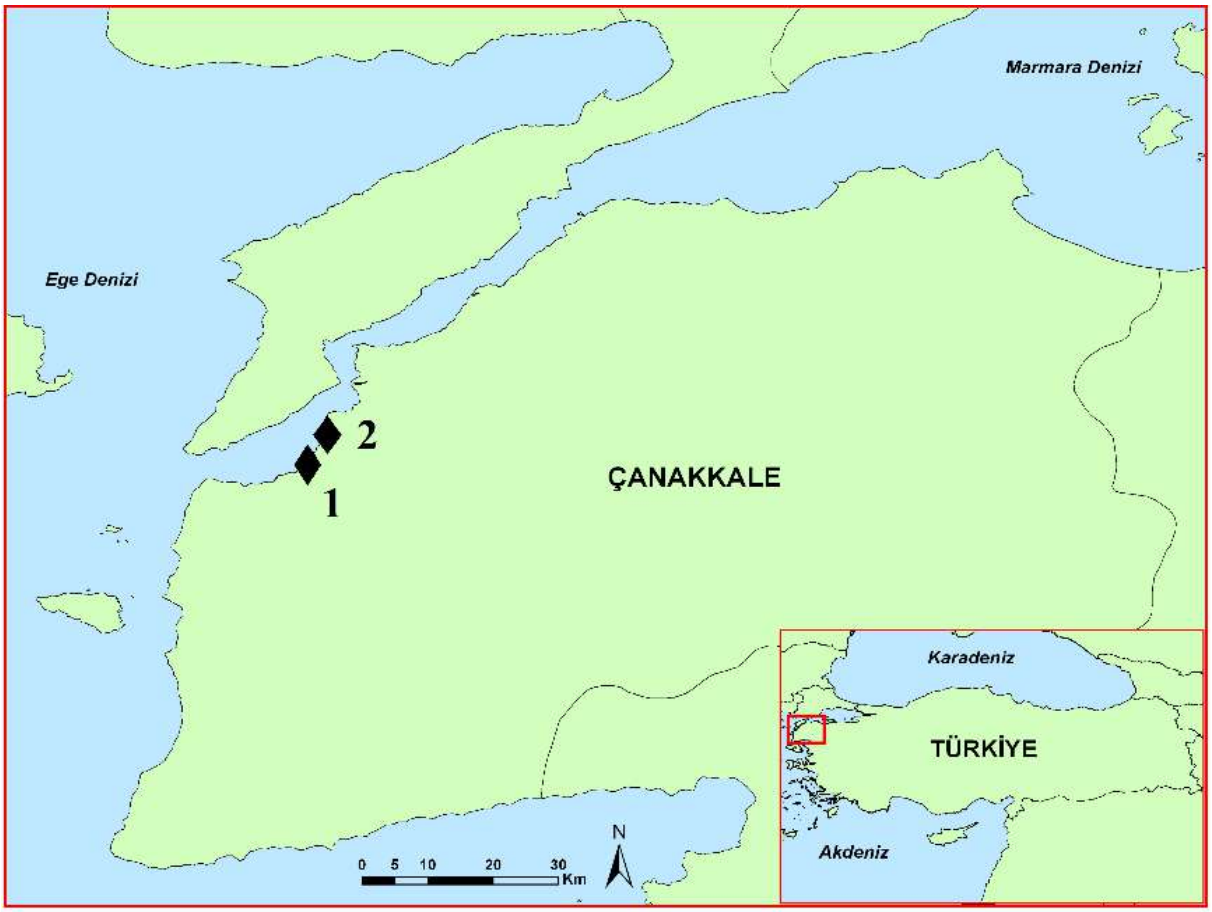

Figure 1. Sampling locations.

Sagittal otoliths were removed and cleaned with ethanol 70%. Left and right sagitta were dried and preserved in an eppendorf tube. Sagittal otoliths were placed on microscope slides for observation and photography as the sulcus acusticus upward. Otolith length (OL), width (OW), area (OA), and perimeter (OP) were measured to the nearest 0.001 mm below using Q Capture Imaging Software. Otolith length was defined as the longest axis between the anterior and the posterior otolith edge and OW as the distance from the dorsal to the ventral edge taken perpendicular to the length through the otolith focus. Paired t-tests were used to determine if there were different measurements between left and right otoliths.

The relationships between fish total length (TL) and otolith measurements (OL and OW) were described using the linear model while the exponential model was best fit for OA and OP measurements (Huxley, 1924, 1932).

RESULTS

A total of 600 individuals of 10 fish species belonging to Sparidae family were used in the analysis. The relationships between fish total length and otolith measurements are presented in Table 1 and 2. The coefficients of determination (R^2) ranged from 0.463 to 0.957 in all cases for the left otoliths. The coefficients of determination (R^2) ranged from 0.427 to 0.947 in all cases for the right otoliths.

The lowest correlation coefficient was found in relations between TL-OW (R2= 0.463) for *Pagellus acarne* while the highest correlation coefficient was calculated for the relations between TL-OA (R^2= 0.957) for *Lithognathus mormyrus*. Exponential model is the best model for OA and OP measurements while the linear model is the best model for OL and OW for most of the Sparidae species. The otolith area (OA) showed the highest correlation for predicting fish total length in all species. Regression coefficients of length otolith measurements to fish total length were larger than 0.9 in 11 cases and between 0.8 and 0.9 in 14 cases (Table 1). Regression coefficients of right otolith measurements to fish total length were larger than 0.9 in 17 cases and between 0.8 and 0.9 in 9 cases (Table 2).

The relationships of otolith length, width, area and perimeter with total length were determined, using left and right otolith values for all individuals. The best fit for the TL-OA relationship was recorded for left otoliths species of *L. mormyrus* (R^2= 0.957), and the second best R^2 (0.949) belongs to the left otoliths of the same species.

Table 1. Regression parameters of the relationships between Left otolith size and fish total length for 10 Sparidae species from the coastal areas of Çanakkale Strait, Turkey. Sample size (N) and coefficients of determination (R^2) given along with power equations

Species	**N**	**TL-OL**		**TL-OW**		**TL-OA**		**TL-OP**	
		Equation	***R²***	**Equation**	***R²***	**Equation**	***R²***	**Equation**	***R²***
Dentex dentex	22	y = 235.65x + 1386.7	0.786	y = 120.83x + 1136.7	0.744	$y = 210216x^{1.4543}$	0.890	$y = 2285.9x^{0.7}$	0.852
Diplodus annularis	132	y = 289.93x + 747.37	0.905	y = 186.47x + 481.75	0.835	$y = 219864x^{1.4542}$	0.915	$y = 1920.6x^{0.7385}$	0.910
Diplodus puntazzo	80	y = 412.51x + 244.32	0.866	y = 242.5x + 297.08	0.738	$y = 154376x^{1.6947}$	0.920	$y = 1474.2x^{0.9155}$	0.882
Diplodus vulgaris	64	y = 325.9x + 613.66	0.845	y = 185.03x + 467.66	0.804	y = 651142x - 953269	0.934	y = 933.65x + 1564.6	0.890
Lithognathus mormyrus	55	$y = 570.97x^{0.8587}$	0.917	$y = 435.98x^{0.7415}$	0.949	$y = 183398x^{1.5854}$	0.957	$y = 1680.7x^{0.8171}$	0.949
Pagellus acarne	95	y = 212.27x + 474.66	0.515	y = 144.22x + 347.51	0.463	$y = 126866x^{1.4516}$	0.663	$y = 1446.9x^{0.7307}$	0.638
Pagrus pagrus	13	y = 114.88x + 2230.3	0.762	y = 101.77x + 1341.5	0.868	$y = 1E+06x^{0.7447}$	0.892	$y = 3108x^{0.5476}$	0.829
Sarpa salpa	111	y = 286.78x + 690.33	0.735	y = 136.59x + 700.57	0.653	$y = 296131x^{1.2209}$	0.730	$y = 2190x^{0.6377}$	0.636
Sparus aurata	72	y = 306.38x + 470.89	0.905	y = 209.04x + 298.13	0.872	y = 810297x - 2E+06	0.940	y = 993.57x + 981.16	0.829
Spondyliosoma cantharus	32	y = 270.12x + 1273.6	0.760	y = 154.05x + 1016.4	0.728	$y = 377110x^{1.2871}$	0.884	$y = 2913x^{0.5964}$	0.742

Table 2. Regression parameters of the relationships between right otolith size and fish total length for 10 Sparidae species from the coastal areas of Çanakkale Strait, Turkey. Sample size (N) and coefficients of determination (R^2) given along with power equations

Species	**N**	**TL-OL**		**TL-OW**		**TL-OA**		**TL-OP**	
		Equation	***R²***	**Equation**	***R²***	**Equation**	***R²***	**Equation**	***R²***
Dentex dentex	22	$y = 235.02x + 1426.6$	0.733	$y = 140.31x + 903.28$	0.716	$y = 239676x^{1.3995}$	0.831	$y = 2402.6x^{0.6825}$	0.879
Diplodus annularis	13	$y = 288.49x + 748.25$	0.922	$y = 180.14x + 524.39$	0.850	$y = 216758x^{1.4682}$	0.928	$y = 1918x^{0.7384}$	0.910
Diplodus puntazzo	80	$y = 396.4x + 324.58$	0.853	$y = 230.73x + 376.93$	0.701	$y = 148763x^{1.7225}$	0.908	$y = 1486.2x^{0.9189}$	0.903
Diplodus vulgaris	64	$y = 365.47x + 430.46$	0.902	$y = 194.23x + 435.54$	0.823	$y = 651940x - 969961$	0.941	$y = 983.68x + 1329$	0.909
Lithognathus mormyrus	55	$y = 604.34x^{0.8337}$	0.937	$y = 447.8x^{0.7252}$	0.939	$y = 189384x^{1.5701}$	0.947	$y = 1739.9x^{0.797}$	0.939
Pagellus acarne	95	$y = 214.84x + 473.78$	0.470	$y = 133.17x + 402.74$	0.427	$y = 148261x^{1.3533}$	0.597	$y = 1476x^{0.7231}$	0.606
Pagrus pagrus	13	$y = 117.09x + 2177.3$	0.858	$y = 115.38x + 1238.1$	0.931	$y = 965378x^{0.7753}$	0.916	$y = 2971x^{0.5711}$	0.941
Sarpa salpa	11	$y = 258.97x + 861.35$	0.658	$y = 131.09x + 722.19$	0.664	$y = 333315x^{1.1534}$	0.712	$y = 2315.2x^{0.6026}$	0.668
Sparus aurata	72	$y = 315.01x + 438.29$	0.892	$y = 211.03x + 280.24$	0.879	$y = 819442x - 2E+06$	0.932	$y = 977.92x + 1026$	0.829
Spondyliosoma cantharus	32	$y = 248.11x + 1445$	0.729	$y = 163.95x + 933.43$	0.787	$y = 325509x^{1.3566}$	0.905	$y = 4065.3x^{0.4387}$	0.558

DISCUSSION

In this study the relationships between OL, OW, OA and OP with fish total length for 10 fish species belonging to Sparidae family were determined. These results provide a baseline reference for trophic studies for identification of prey and the estimation of its size.

Growth rates of fishes have significant effects on the otolith growth (Munk, 2012). One of the most effective methods for predicting fish size in the stomachs of the fish predators is the relationships between otolith morphometrics and fish size. In the present study, our results showed a percentage of explained deviance higher than 46% for all regressions.

The present study provides additional information by considering four otolith measurements (OL, OW, OA, and OP). The otolith area (OA) showed the highest correlation for predicting fish total length in all species. It is more reliable to calculate more than one equation. Little information is available on the otolith area and perimeter values of most of the fish species. However, in our study, otolith area (OA) was the best model for predicting fish total length in all species. Therefore, we suggest that the otolith area measurements should be made in the future studies.

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THE DETERMINATION OF YIELD POTENTIAL, IMPORTANT AGRONOMIC AND QUALITY CHARACTERISTICS OF ADVANCED PEANUT BREEDING LINES (F_8) BELONGING TO DIFFERENT CROSSING COMBINATIONS IN MAIN CROP GROWING CONDITION

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ABSTRACT

This study was conducted as a main crop in University of Cukurova, Faculty of Agriculture Field Crops Department in 2019. The objective of this study was to determine the yield potential, important agronomic and quality characteristics of advanced peanut breeding lines (F_8) in main crop growing condition. In this study, 10 advanced peanut breeding lines belonging to Halisbey x Flower-22 (HC-1, HC-2, HC-3, HC-4 lines), Halisbey x Wilson (HW-1, HW-4, HW-5 lines), Halisbey x NC-7 (HN-3, HN-4 lines) and Halisbey x Ha-Runner (HR-1 lines) crossing and two standard varieties (Halisbey and NC-7) were used as a plant material. The experimental design was a Randomized Complete Block with three replications. Pod number and pod weight per plant, 100-seed weight, shelling percentage, pod and kernel yield per hectare, oil and protein content values of lines were investigated. As a result; the pod number per plant of breeding lines varied between 27.5-38.8 pod plant^{-1}, pod weight was 62.3-87.6 g plant^{-1}, 100-seed weight 80.4-124.3 g, shelling percentage was 66.0-70.9%, pod yield was 6434-7979 kg ha^{-1} and kernel yield was varied between 4401-5451 kg ha^{-1}. The oil and protein content of breeding lines varied between %45.4-47.9 and % 25.0-27.2, respectively. This breeding program will continue by the selected lines at the future.

Keywords: *Peanut, Breeding lines, Agronomic characteristic, Pod yield*

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is the fifth most important oilseeds crop in the world with production of approximately 41.9 million tons per year (FAO, 2018). Peanut seeds contain 35-56% oil and 25-30% protein and 9.5-19.0% carbohydrate on a dry seed basis (Savage and Keenan, 1994; El Naim et al., 2011). In addition, peanuts are a rich source of other nutrients such as minerals (P, Ca, Mg, Mn and K) and vitamins (E, K and B groups) (Hassan and Ahmed, 2012; Gulluoglu et al., 2017). For these reasons, peanut seed is an important source of edible oil and protein for human nutrition. The annual peanut production is around 41.9 million tons and it contributes 7.3% of the total oil seeds production in the world in 2018 (FAO, 2018). About two-thirds of total peanut production is crushed for oil and the remaining one-third is used in confectionery products in the world (Dwivedi et al., 1993). Peanut production was 173.800 ton in Turkey in 2018 and all of them are used roasted peanut for the human nutrition (FAO, 2018). Peanut is a legume crop and it fixes about 150 kg/ha nitrogen to soil from the atmosphere by the *Rhizobium* bacteria living their roots (Arioglu, 2014).

Peanut production was done mainly in the Mediterranean, Aegean and South-east Anatolian regions. Peanut can be grown at different seasons such as main and double crop for the suitable climate in Turkey. Main and double crop peanut production accounts for 60% and 40% of the country's total production, respectively. Peanut production importantly was increased after 2010 years in Turkey.

Pod yield per unit area in peanut production varies depending on applied cultural practices, variety selection and environmental factors during the growing season. Variety selection is one of the most effective factors to obtain high yield and quality product in peanut farming. Most of the productions (95%) are used as a roasted peanut in Turkey. For this reason, large seeded Virginia type peanut varieties are growing by the farmers in the country. Virginia market type peanut varieties such as NC-7, Osmaniye 2005, Arıoglu-2003, Halisbey, Sultan, Polen555 and Aysehanım are growing in Turkey. Some researchers (Arıoğlu et al., 2001; Söğüt et al., 2002; Çalışkan and Arıoğlu, 2005; Aslan et al., 2005; Arıoğlu et al., 2013; Özgören and Arıoğlu, 2015; Arıoğlu et al., 2016; Güllüoğlu et al., 2017; Güllüoğlu et al., 2018; Aşık et al., 2018) reported that the Virginia type peanut varieties can be grown successfully in Turkey. High yield and quality, earliness, large seed, shelling percentage and seed coat color characteristics are the main breeding objective in peanut breeding in Turkey. For this reason, a new breeding program was initiated to breed suitable varieties for main crop in 2012 using different crossing combinations. The objective of this study was to determination of yield potential and important agronomic and quality characteristics of advanced peanut breeding lines (F8) belonging to different crossing combinations (Halisbey x Flower-22, Halisbey x Wilson, Halisbey x NC-7 and Halisbey x Ha-Runner) in main crop growing condition in Cukurova region (Adana-Turkey).

MATERIAL AND METHODS

Field experiments were conducted at Research Farm of Cukurova University (Southern Turkey, $36^{o}59^{l}$ N, $35^{o}18^{1}$ E and 23 m elevation) as a main crop in 2019. In this study, 10 advanced peanut breeding lines belonging to Halisbey x Flower-22 (HC-1, HC-2, HC-3, HC-4 lines), Halisbey x Wilson (HW-1, HW-4, HW-5 lines), Halisbey x NC-7 (HN-3, HN-4 lines) and Halisbey x Ha-Runner (HR-1 lines) crossing and two standard varieties (Halisbey and NC-7) were used as a plant material.

The soil texture was clay loam. The soil tests indicated that pH of 7.7 with high concentrations of K_2O (705 kg ha^{-1}) and low concentrations of P_2O_5 (28 kg ha^{-1}). In addition, the organic matter (1.4%) and nitrogen content of the soil were very low. The lime content was 22.1% in the soil.

This study was conducted in Adana province in Turkey and in this region, winters are mild and rainy, summers are dry and warm, which is a typical of a Mediterranean climate. The climate data such as temperature, precipitation and relative humidity during the 2019 growing period and long term (LT) average (1929-2019) in Adana was shown in Table 1.

Table 1. The climate conditions during the 2019 growing period and long term (LT) average (1929-2019)

Months	**Average temperature** (^{o}C)		**Precipitation** (mm)		**Relative humidity** (%)	
	2019	**L.T.**	**2019**	**L.T.**	**2019**	**L.T.**
April	17.0	17.5	61.4	51.1	67.0	60.1
May	24.1	21.7	2.6	47.1	57.6	63.2
June	27.1	25.6	13.8	20.5	68.7	70.2
July	28.4	28.2	28.0	6.2	68.8	67.5
August	29.6	28.7	0.0	5.5	68.0	68.5
September	27.3	26.1	0.0	17.6	62.1	65.4

The average monthly air temperature during the research period (April-September) was varied between 17.0 and 29.6 ^{o}C in 2019. The average air temperature was the higher during

the research period than long term average temperature. The total rainfall was 105.8 mm during the growing period in 2019. The average relative humidity was ranged from 57.6% to 68.8% in 2019. The differences between the years and long term for the climate data were not found very significant (Table 1).

The experimental design was a Randomized Complete Block with three replications. 300 kg ha^{-1} of Di-ammonium phosphate (54 kg ha/ha N, 138 kg ha/ha P_2O_5) fertilizer was applied and incorporated to soil before planting. Urea (46%N) at the rates of 400 kg ha^{-1} was applied two times; before first (beginning of flowering) and third (pod formation) irrigation (200 kg ha/ha + 200 kg ha/ha) in each years. The pre-sowing herbicide was applied to the soil and the plots were kept weed-free by hand weeding during the growing period. The seeds were sown at the first week of April with 0.7 m spacing between rows and 0.15 m spacing between plants in a row. Individual plots consisted of 4 rows 5.0 m long (14.0 m^2). All plots were irrigated with sprinkler irrigation approximately every 2 weeks, starting from the flowering stage. During the growing period, recommended pesticides and fungicides were applied to control insects and diseases. The remaining cultural practices were applied during the growing period. The plants were harvested by hand after sowing 150 days at the beginning of September in 2019.

Data collection and analysis: The data belonging to agronomic and quality characteristics such as pod number and pod weight per plant, shelling percentage, 100-seed weight, pod and kernel yield per hectare, oil and protein content were collected according to Arioglu et al. (2018)'s method. The collected data on different parameters were statistically analyzed to obtain the level of significance using JMP 8.1.0 package program with split plot design. The means differences were compared with the Least Significant Differences (LSD, 5%) Test.

3. RESULTS AND DISCUSSION

The average values belonging to pod number and pod weight per plant, 100 seed weight and shelling percentage of breeding lines has been presented in Table 2, pod yield and seed yield per hectare, oil and protein content of breeding lines has been presented in Table 3.

Pod number and pod weight per plant

It can be seen in Table 2, pod number and pod weight per plant of breeding lines varied between 27.5-38.8 pods/plant and 62.3-87.6 g/plant, respectively. The differences between the breeding lines for the pod number and pod weight per plant were statistically significant. The average pod number and pos weight of breeding lines were 34.0 pods/plant and 75.0 g/plant. The highest pod number per plant was obtained from HW-4 (38.8 pods/plant) and HC-3 (38.0 pods/plant) lines. The pod weight per plant was the highest in HC-1 (87.6 g/plant) and HW-4 (87.0 g/plant) breeding lines. Pod number and pod weight per plant of the lines was higher than NC-7 variety. Similar results were reported by some other researchers (Arıoğlu et al., 2001; Söğüt et al., 2002; Çalışkan and Arıoğlu, 2005; Aslan et al., 2005; Arıoğlu et al., 2013; Özgören and Arıoğlu, 2015; Arıoğlu et al., 2016; Güllüoğlu et al., 2017; Güllüoğlu et al., 2018 and Aşık et al., 2018).

100 Seed weight

The statistically significant differences were found among the breeding lines for the 100 seed weight. The 100 seed weight of the lines varied between 80.4-124.3 g. The average 100 seed weight was calculated as 114.2 g. The 100 seed weight was the lowest in HR-1 (80.4 g) breeding line (Table 2). This line belongs to Halisbey x Ha-Runner crossing combination. The runner type characteristic for the 100 seed weight was dominant in HR-1 breeding line. Because, Ha-Runner variety is a runner market type and its 100 seed weight was lower. The 100 seed weight was the highest in HN-4 (124.3 g) and HC-2 (123.4g) lines. These two lines were suitable for

toasting consumption due to high 100 seed weight. These results are in agreement with the findings of Arıoğlu et al. (2001), Söğüt et al. (2002), Çalışkan and Arıoğlu (2005), Özgören and Arıoğlu (2015), Arıoğlu et al. (2016) and Aşık et al. (2018).

Table 2. The average values belonging pod number and pod weight per plant, 100 seed weight shelling percentage of breeding lines and Standard varieties

Breeding lines	**Pod number** (No/plant)	**Pod weight** (g/plant)	**100 Seed weight** (g)	**Shelling percentage** (%)
HC-1	35.8 a	87.6 a	119.5 ab	67.6 cde
HC-2	36.4 a	83.7 ab	123.4 a	69.1 abc
HC-3	38.0 a	76.6 bcd	115.8 bc	66.9 de
HC-4	36.7 a	68.9 def	113.3 cd	68.2 bcd
HN-3	27.6 b	64.2 ef	117.6 bc	70.9 a
HN-4	28.0 b	66.0 ef	124.3 a	68.2 bcd
HW-1	35.2 a	80.7 abc	116.0 bc	68.5 bcd
HW-4	38.8 a	87.0 a	117.3 bc	68.1 cd
HW-5	35.8 a	72.7 cde	114.5 bc	66.0 e
HR-1	27.5 b	62.3 f	80.4 e	70.2 ab
HALİSBEY *	35.9 a	84.7 ab	115.4 bc	66.7 de
NC-7*	25.3 b	45.6 g	108.1 d	71.1 a
Average**	34.0	75.0	114.2	68.4
LSD(%5)	5.02	9.72	5.71	2.02

*Standard varieties; **Average of breeding lines

Shelling percentage

As it can be seen in Table 2, shelling percentage of the lines varied between 66.0-79.9%. The differences between the breeding lines were found statistically significant. Shelling percentage is an important quality characteristic in peanut. Shelling percentage has to higher than 70% in Virginia type varieties. The shelling percentage was found higher in NH-3 (70.9%) and HR-1 (70.2%) among the breeding lines and the others has lower than 70% shelling percentage. The shelling percentage of the lines was found lower than NC-7 variety, but higher than Halisbey except HW-5 (66.0%). Similar results were supported by findings by Arıoğlu et al. (2001), Söğüt et al. (2002), Çalışkan and Arıoğlu (2005), Özgören and Arıoğlu (2015), Arıoğlu et al. (2016) and Aşık et al. (2018).

Pod yield

The yield potential has to high for to obtain high yield in peanut farming. In this research, the differences between the breeding lines for the pod yield per hectare were statistically significant. Pod yield values of the breeding lines varied between 6534-7979 kg/ha. The average pod yield of the peanut lines was calculated as 6395 kg/ha. The pod yield was found higher in HC-1 (7979 kg/ha), HW-1 (7949 kg/ha), HC-4 (7701 kg/ha), HC-3 (7691 kg/da) and HW-4 (7494 kg/da) than the others lines (Table 3). Pod yield of these lines were higher than NC-7 and Halisbey varieties. These results for the pod yield are in agreement with the findings of some researchers (Arıoğlu et al., 2001; Söğüt et al., 2002; Çalışkan and Arıoğlu, 2005; Aslan et al., 2005; Arıoğlu et al., 2013; Özgören and Arıoğlu, 2015; Arıoğlu et al., 2016; Güllüoğlu et al., 2017; Güllüoğlu et al., 2018 and Aşık et al., 2018).

Table 3. The average values belonging pod yield and seed yield per hectare, oil and protein content of breeding lines and Standard varieties

Breeding lines	Pod yield (kg ha^{-1})	Seed yield (kg ha^{-1})	Oil content (%)	Protein content (%)
HC-1	7979 a	5387 a	46.6	25.9
HC-2	7342 ab	5078 ab	46.2	25.6
HC-3	7691 a	5139 ab	46.2	25.8
HC-4	7701 a	5256 a	45.4	25.3
HN-3	6615 c	4689 bc	45.7	27.2
HN-4	6662 c	4547 c	47.5	25.6
HW-1	7949 a	5451 a	47.7	26.8
HW-4	7494 a	5106 ab	47.9	25.4
HW-5	6676 bc	4401 c	46.9	25.1
HR-1	6534 c	4587 c	47.3	25.0
HALİSBEY *	6525 c	4357 c	47.4	25.7
NC-7*	4983 d	3546 d	45.2	27.3
Average**	7254	4957	46.7	25.3
LSD(%5)	676.3	494.0	N.S.	N.S.

*Standard varieties; **Average of breeding lines

Seed yield

Seed yield of the breeding lines ranged from 4401kg/ha to 5451 kg/ha. The differences between the lines for the seed yield were found statistically significant. The average seed yield of the lines was calculated as 4957 kg/ha. Seed yield per hectare of the lines were calculated as pod yield per hectare x shelling percentage. The highest seed yield were obtained from HW-1 (5451 kg/ha), HC-1 (5387 kg/ha) and HC-4 (5256 kg/ha) breeding lines (Table 3). The peanut farmers need the seed yield of the grown varieties has to high for the high income in peanut farming. The seed yield of the breeding lines was found higher than standard varieties (NC-7 and Halisbey). Similar findings have been reported on seed yield by Arıoğlu et al. (2016), Güllüoğlu et al. (2017), Güllüoğlu et al. (2018) and Aşık et al. (2018).

Oil and Protein content

As it can be seen in Table 3, the oil and protein content values of the breeding lines varied between 45.4-47.9% and 25.0-27.2%, respectively. The differences between the breeding lines for the oil and protein content were not statistically significant. The average oil and protein content of the breeding lines were 46.7% and 25.3%, respectively. The highest oil content was found in HW-4 (47.9%), HW-1 (47.7), HN-4 (47.5%) and HR-1 (47.3%) breeding lines. The protein content was the highest in HN-3 (27.2%) and HW-1 (26.8%) lines. Similar results were reported by some other researchers (Arioglu et al., 2016; Berk and Arioglu, 2019).

CONCLUSION

In this study (breeding program), 10 advanced peanut breeding lines belonging to Halisbey x Flower-22, Halisbey x Wilson, Halisbey x NC-7 and Halisbey x Ha-Runner crossing combinations were tested as a main crop in Cukurova region. HC-1, HC-3, HC-4, HW-1 and HW-4 breeding lines were found high yielding and good quality. This breeding program will continue at the future using these lines in different location. According to obtained results, one or two lines will be registered as a new peanut variety.

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HYDROCHEMICAL ASSESSMENT AND GROUNDWATER QUALITY USING STATISTICAL APPROACHES OF THE TERMINAL COMPLEX AQUIFER IN THE REGION OF OUED RIGH (ALGERIAN SAHARA)

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ABSTRACT

Oued Righ valley is part of the northeastern Algerian Sahara, it is characterized by an arid desert climate with very high temperatures in summer. The Terminal Complex aquifer waters's in Oued Righ evoke serious problems related to the physical and the chemical quality; Larger variations in conductivity are observed that probably due to multiple geochemical processes; these waters which are highly mineralized and concentrated of some elements shown the higher value in recommended potable waters norms of Algeria.In this work, several hydrochemical and statistical approaches are using to demonstrate the correlation between the chemical composition of water of the Terminal Complex and lithology.

Key words: *Oued Righ, Terminal Complex, hydrochemical , statistical.*

INTRODUCTION

Evaluation of groundwater quality is very important in arid regions like Oued Righ where increasing groundwater extraction.

The Terminal complex is known for its intensive agriculture activities which depend on groundwater exploitation. The purpose of this study was to assess the drinking groundwater to determine the hydrochemical processes governing the groundwater chemistry evaluation of groundwater quality in the region of Oued Righ.

Hydrochemical investigations, including geochemical analyses and multivariate statistics were conducted to assess the factors that influence groundwater geochemistry in complex Terminal, valley Oued Righ.

II. Localization of the study area

The valley Oued Righ is situated inone of the largest Southeastern Algerian deserts, bordered to the north by the Saharan Atlas, in the south by the wilayas Tamanrasset and Illizi, in the northwest by the province of Djelfa, in the West by the wilaya of Ghardaia and east by Tunisia

and Libya., it have coordinate of 32 ° 54 '39 ° 9' North in latitude, and 05 ° 50 'to 05 ° 75' East in longitude, with surface measure of 600.00 km^2 (, Fig.1)..

The Saharan climate of the region of Oued Righ is characterized by low rainfall levels, high temperatures, a significant evaporation and excessive solar radiation.

The agricultural domain which was once the main activity of the region [2] covers almost 16,000 hectares of palm groves. [8]

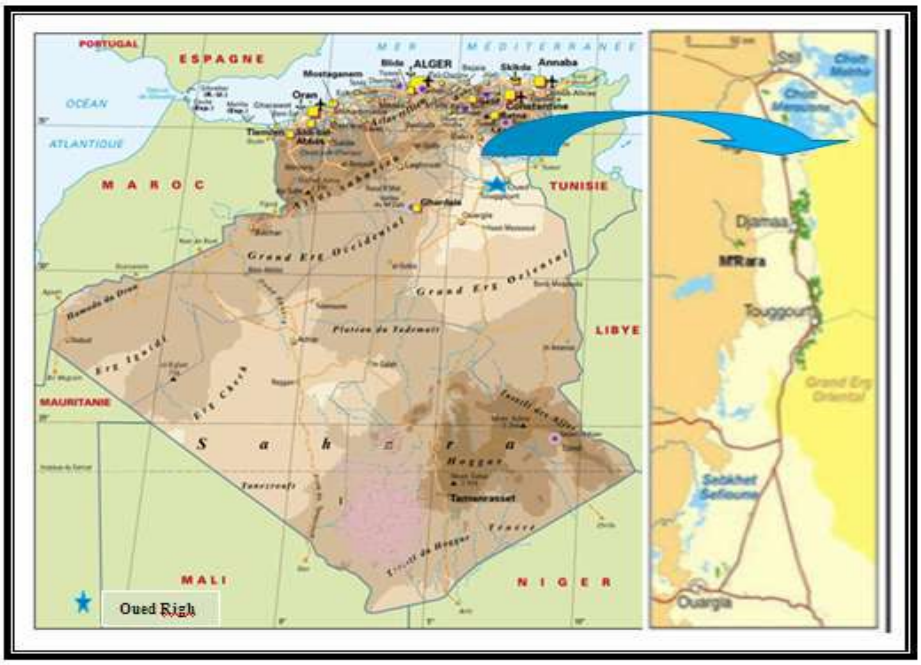

Figure1. Localization of the study area. [1]

III. Geology and hydrogeology of the region

The study area is formed by two structural units; Precambrian basement composed of igneous and metamorphic rocks, surmounted by thousands of meters of sedimentary rocks from Cambrian to Quaternary, devoid of significant tectonic deformations.

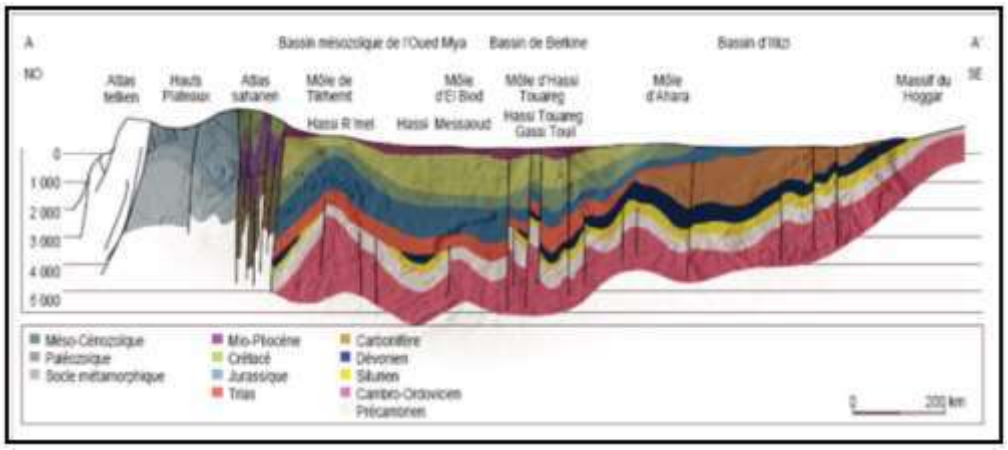

Figure 2. Geo-structural transversal cross (WEC, 2007).[10]

From a hydrogeological point of view, the Northern Sahara basin is composed of several heterogeneous formations very extensive, separated by impermeable formations, known as: the Intercalary Continental and the Terminal Complex. In the study area, three encountered layers are defined from bottom to top:

- The Continental Intercalary aquifer.
- The Terminal Complex aquifer which is the subject of our study.
- Phreatic aquifer.

These current levels of aquifers reflect the result of a pure drying up since the Holocene. [7]

In the region of the Oued Righ, three l are well differentiated in the terminal complex: The first and second layers are composed of Mio- Pliocene sands, then the third of inferior Eocene limestone (Fig.3). The continuity of the underground flow passing from one zone to another.[6]

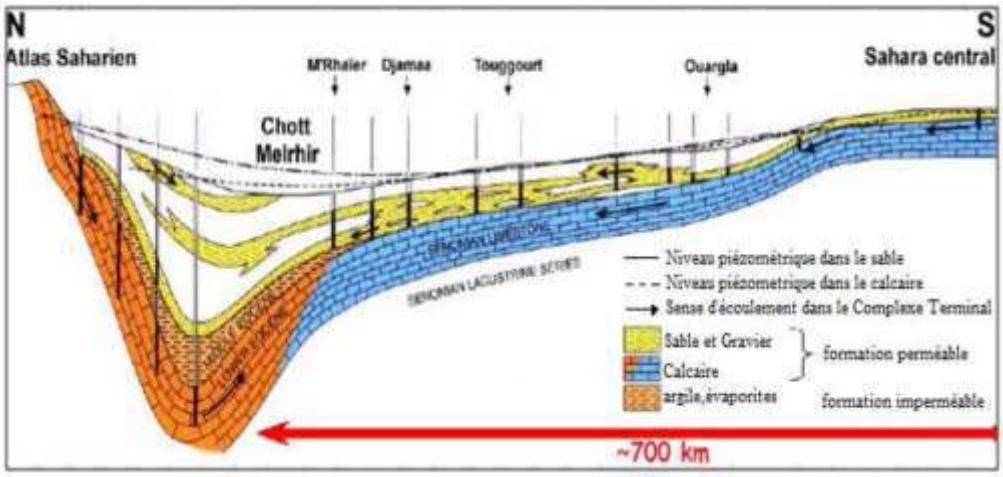

Figure 3. Hydrogeological transversal cutting of the Terminal Complex (UNESCO, 1972).[9]

MATERIAL AND METHODS

IV.1. Sampling

The water samples in this study are taken from 12 drilling waters's of the Terminal Complex in the Northern of Oued Righ Valley. The water samples were analyzed for physico-chemical parameters Temperature, pH, electrical conductivity and total hardness. Furthermore, the cations of $Na^{+,}$ K^{+}, Ca^{2++}, Mg^{++} and anions Cl^{-}, SO_4^{-}, HCO_3^{-} et NO_3^{-}.

RESULTS AND DISCUSSION

V.1.Hydrochemical classification

The research results plot on the Piper's diagrams show that the hydrochemical facies for the majority of the groundwater samples is sodium sulfated type with some minor hydrochemical facies of the sodium chloride types.

Stabler classification confirms the existence of two chemical facies: sodium chloride and sodium sulfate, with the predominance of sodium sulfate facies (75% of samples).

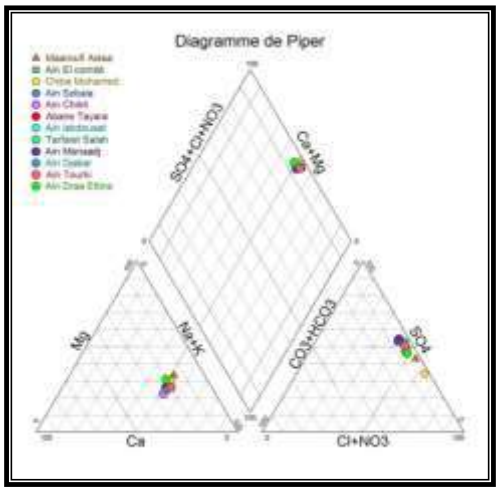

Figure. 4: Piper diagram waters of the Terminal Complex.

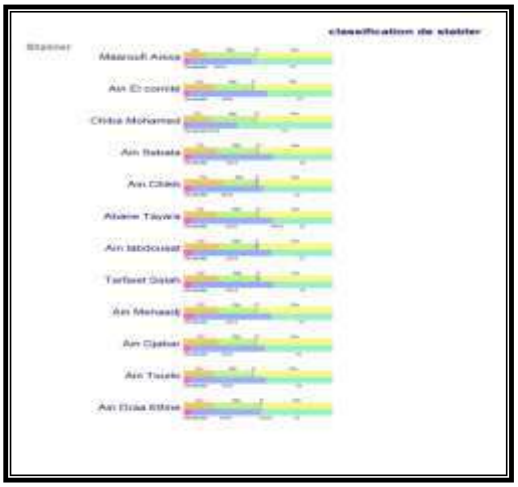

Figure.5: Graphic representations of chemical facies classification Stabler.

V.2. Study of correlations between parameters measured Terminal Complex water:

V.2.1. bivariate Correlation (simple)

the purpose of bivariate Correlation is to study the links that may exist between the physical and chemical parameters measured using the correlation matrix, The Table.2 show significant links between Cl^{-}, SO_4^{2-}, Mg^{2+} and Na^{+} with the conductivity (EC), the same as Mg^{2+} , Na^{+}, Cl^{-} and SO_4^{2-}. Indeed, these ions characterize studied waters facies, without significant remark of bicarbonates role's.

Table.1: Correlation matrix between the physico-chemical elements.

	CE	Ca^{2++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^-	Cl^-	SO_4^-	NO_3^-
CE	1								
Ca^{2++}	0.52	1							
Mg^{++}	**0.97**	0.37	1						
Na^{+}	**0.99**	0.4	**0.96**	1					
K^{+}	0.66	-0.24	0.72	0.75	1				
HCO_3^-	0.02	-0.81	0.14	0.16	0.73	1			
Cl^-	**0.98**	0.51	**0.95**	**0.94**	0.65	0.01	1		
SO_4^-	**0.87**	0.53	0.8	**0.88**	0.46	-0.06	0.74	1	
NO_3^-	-0.46	0.38	-0.52	-0.57	-0.85	-0.78	-0.43	-0.35	1

V.2.2. Multiple Correlation

V.2.2.1. Ascending hierarchical Classification (CAH)

In diagram of Figure.6, we note the presence of three groups, two of which have a liaison forming a single group linked in turn with the latter.

The detail of the groups is illustrated in the following table:

Table.2: distribution of water points using the method "CAH".

Group	Drillings	Conductivity (µS/Cm)	Area
I	F1, F2, F3	Entre 8100 et 8900	Djamaa
II	F4, F5, F6, F7, F8, F9	Entre 4300 et 4800	Sidi khlil et el-Mghair
III	F10, F11, F12	Entre 3700 et 3900	Oum Thiour

The affectation of each group of the study area shows a low mineralization in the North (OumThiour), it is medium in the center (Khlil and Sidi El-Meghair) and becomes strong in the South (Djamaa). The relationship of groups II and III can be explained by their similar ion content , while those in Group I are more important.

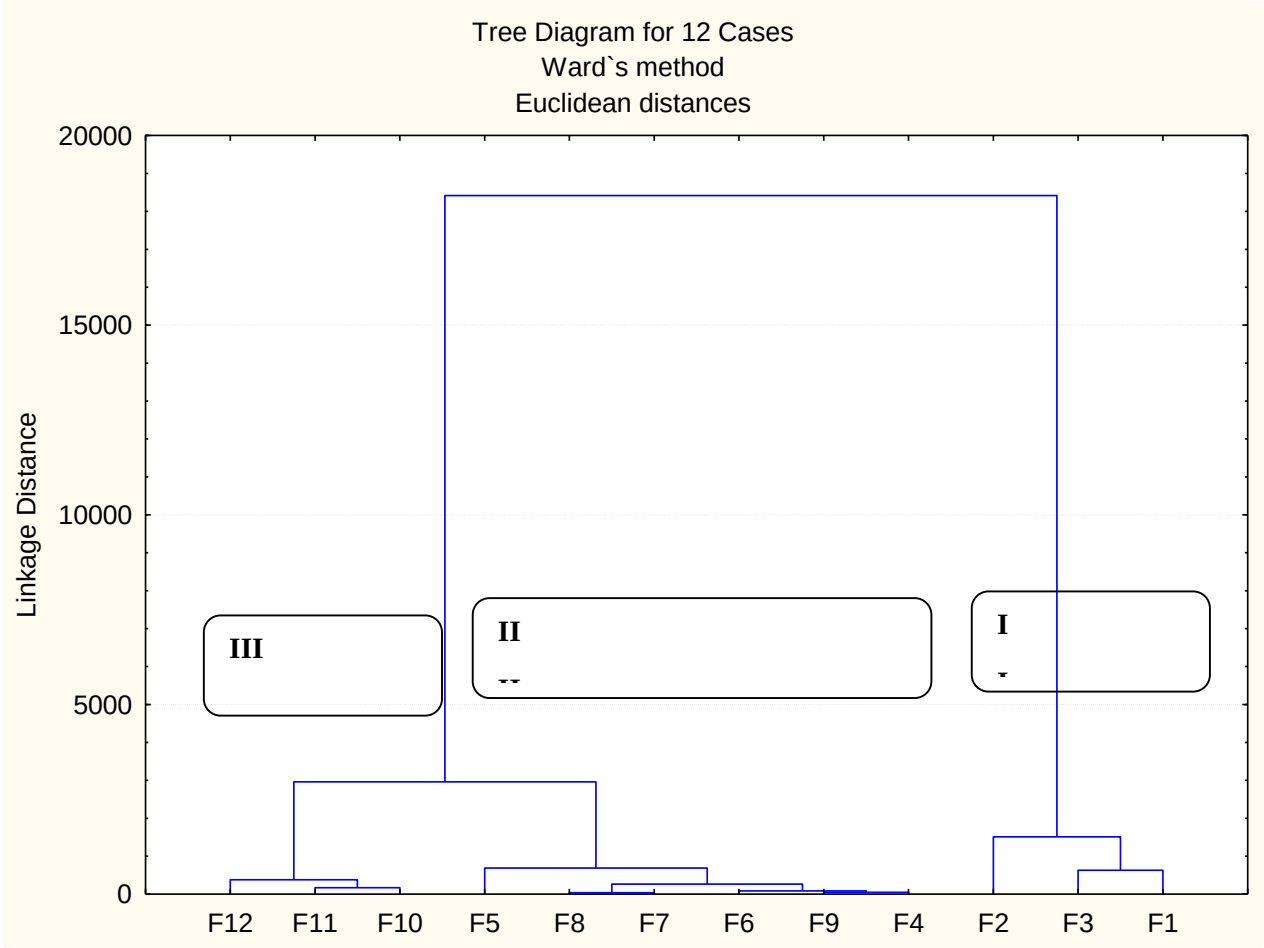

Figure.6: Ascending hierarchical classification of variables (C.A.H) by group of drilling.

Conclusion

The hydrochemical study allowed us to characterize the chemical quality of the water of Terminal Complex aquifer in the North region Oued Righ.

Oued Righ Water's Classification are devised into two types of chemical: sulfated sodium facies and sodium chloride.

These different facies are related to the contact rock water according to the geological nature of the area, since the interaction between water and the rock causes a dissolution resulting an enrichment of water to sulphate, chloride and sodium reveal excessive mineralization, expressed by very high values of electrical conductivity, increasing from North to South.

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IS THE EXPLOITABLE BIOMASS (B) VULNERABLE TO CHANGES OF NATURAL MORTALITY (M) BY AGE? CASE OF THE ROUND SARDINELLA

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ABSTRACT

A proper estimation of biomass is critical for environmental and decision making to conserve fish stocks. This work aims to explore the vulnerability of biomass estimation to the variability of natural mortality M by age. 2460 individuals of all sexes, sizes ranging from 7.25 to 21.75 cm, were studied. Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners in the central region of Algeria between Tenes and Bejaïa. Descriptive statistics were performed using FISAT II and VIT4Win. The results generated two different scenarios: first, the current total biomass (Bc) was estimated at 8686.6 tons for M = constant = 0.51 yr^{-1} ; secondly total biomass was estimated at Bc = 10636.4 tons for M = variable by age n, M_n, [M_1 = 1.130 ; M_2 = 0.791 ; M_3 = 0.721 ; M_4 = 0.606 ; M_5 = 0.545 yr^{-1}]. Evaluating Bc with constant M showed a decrease of 1914.19 tons compared to biomass assessment with Mn. Judging by these results, considering that mortality M varies with age has a great impact on biomass evaluation. Naturally, juvenile fish are exposed to larger predation mortality than an adult fish. This difference in predation mortality may be quite significant. Finally, we recommend adjusting the catch effort factor F_C = 1 to $F_{0.1}$ = 0.54 for M variable. This precautionary approach would permit long-term renewal of the stock of *S. aurita* in the central region of the Algerian coast (increasing sea biomass from 10632.91 to 14848.9 tons).

Keywords: *Sardinella aurita, Algeria, Biomass (B), Mortality (M), Stock assessment.*

INTRODUCTION

Fishing in Algeria constitutes an economic activity that provide not only a food source for the population but also thousands of jobs for the Algerians. Fishing has to be controlled and handled according to a sustainable approach to maintain this resource. Thus, fish stocks biomass needs to be constantly evaluated while making sure that the values obtained are the most representative of the reality. In literature many authors as Benamar (2011), Amponsah (2013) and many others, conducted fish stock biomass assessment with the assumption that natural mortality (M) is a constant value (M_{cte}) that isn't linked to fish age. However, it seems that fish mortality (M) varies with age according to Garrido (2016) and Quattrocchi and Maynou (2017), its value is the highest at the youngest age and diminishes with the aging of the fish This can be explained by juveniles, eggs and fish larvae being the most prone to predation and less resistant to environmental conditions than adult fish. This work aims to underline the impact of natural mortality values used when assessing fish stock biomass, in the case of *Sardinella aurita* (Valenciennes, 1847), a small pelagic fish of the family of clupeoids. To do so, we compared the biomass assessed while considering natural mortality as a constant (M_{cte}) with the biomass

calculated using natural mortality as a variable (M_n). This study was conducted in the central region of the Algerian coast between Tenes and Bejaïa.

MATERIAL AND METHODS

Monthly sampling was conducted from December 2018 to March 2020 from landings of seiners situated in the central region of the Algerian coast, 2460 *Sardinella aurita* individuals of all sexes were sampled and each eviscerated and weighed (W_{ev}) and measured from the extremity of the fish muzzle to the end of the caudal fin (TL). Sizes were arranged into length classes ranging from 7.25 to 21.75 cm, with a class width of 0.5 cm. The age-length key was then calculated using Bhattacharya's (1967) method following the recommendation of the DYNPOP working group of the CIESM (Abella *et al.*, 1995 ; Aldebert and Recasen, 1995 ; Alemany and Oliver, 1995 and Campana, 2001 in Bouaziz, 2007). The estimation of the age-length key using Bhattacharya's method was performed on the software FISAT II version 1.2.2 (Gayanilo and Sparre, 2005). We then used the software VONBIT_ALXL (2012) to assess the von Bertalanffy's (1938) growth parameters (L_∞, K and t_0), which is a fish growth model translating into the equation: $L_t = L_\infty(1 - e^{-K(t-to)})$.

We then used the growth parameters obtained to calculate the mortality of the fish stock (M_{cte}) using Djabali's *et al.*, (1993) natural mortality equation:

$$\mathrm{Log}_{10} M = -0{,}0278 - 0{,}1172\ \mathrm{Log}_{10} L_\infty + 0{,}5092\ \mathrm{Log}_{10} K$$

To calculate mortality for each age ($M_n, n = \text{age}$), we first calculated the K for each age using von Bertalanffy's model equation as follows:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$$
$$L_t = L_\infty - L_\infty e^{-K(t-t_0)}$$
$$(L_t - L_\infty) = -L_\infty e^{-K(t-t_0)}$$
$$\ln(L_\infty - L_t) = \ln\left(L_\infty e^{-K(t-t_0)}\right)$$
$$\ln(L_\infty - L_t) = \ln L_\infty + \ln e^{-K(t-t_0)}$$
$$\ln(L_\infty - L_t) - \ln L_\infty = -K(t - t_0)$$
$$K = \frac{\ln L_\infty - \ln(L_\infty - L_t)}{t - t_0}$$
$$K = \frac{-\ln\left(\frac{L_\infty - L_t}{L_\infty}\right)}{t - t_0}$$

We calculated L_∞ for each age ($L_{\infty n}$) using Taylor's (1962) in Bouaziz (2007) equation:

$$L_\infty = \frac{L_{max}}{0{,}95}$$

And then we used Djabali's *et al.*, (1993) natural mortality equation cited above to calculate mortality for each age (M_n) as the following:

$$\mathrm{Log}_{10} M_n = -0{,}0278 - 0{,}1172\ \mathrm{Log}_{10} L_{\infty n} + 0{,}5092\ \mathrm{Log}_{10} K_n$$

To calculate fishing mortality (F) we first calculated total instantaneous mortality rate (Z) using FISAT II, and then we calculated fishing mortality (F) using the following equation:

Knowing Z=F+M therefore F=Z-M

We also calculated the length weight relationship ($W_t = a\ Lt^{b}$) constants a and b.

After having calculated VIT4Win inputs, that said, mortality F, M_n, M_{cte} and the von Bertalanffy's (1938) growth parameters (L_∞, K and t_0), we ran the software VIT4win 1.3 (Lleonart and Salat, 2011) to assess current (Bc) biomass using M_n and M_{Cte}.

RESULTS AND DISCUSSION

The estimation of the average length by age resulted of the following:

Age (year)	Length (cm) ± Standard deviation	Population (NI)	Population %	Separation index (S.I.)
1	10.59 ± 0.960	889.75	36.20%	n.a
2	14.44 ± 1.290	1309.76	53.28%	2.280
3	17.25 ± 0.510	186.28	7.58%	2.130
4	19.11 ± 0.370	68.04	2.77%	2.110
5	22.75 ± 0.420	4.34	0.18%	2.290

Table 8. The average length by age for the population of *Sardinella aurita* sampled using FISAT II.

SI > 2 at all ages, therefore, the separation index is significant. This means that, the decomposition of size frequencies of our sample reflects representative results.

Bhattacharya's method performed on the software FISAT II 1.2.2 provided us with the decomposition of our sample into five (05) cohorts with a median length of 10.59, 14.44, 17.25, 19.11 and 22.75 cm for the ages 1, 2, 3, 4 and 5 respectively.

According to the table 1, age 1 and 2 are the most present in our sample, constituting 36.2% and 53.3% respectively. On the other hand, cohorts of age 3, 4 and 5 constitutes only around 10% of our sample, therefore it is likely that ages 1 and 2 are the most caught by seiners in the study zone.

The calculation of L_∞ and K (growth parameters of the whole sample):

By entering the age-length values to the VONBIT_ALXL (2012) we obtained the following growth parameters, L_∞ = 53.16 cm, K = 0.08 $year^{-1}$, t_0 = -1.838 year, which we consider as aberrant. This can be explained by L_∞ not being close to the value of L_{max} (the maximal length of the sample, which is 22.8 cm) as it should according to Pauly and Moreau (1997) and Bocar *et al.,* (2019), also K in this case has a low value that doesn't represent the growth speed of small pelagic fish which has a fast growth (Pauly et Moreau, 1997 and Bocar *et al.,* 2019), as for t_0 the value obtained doesn't make sense regarding this specie firstly because the later has a low longevity (05 years in this case), moreover, this value of t_0 seemed aberrant when compared to the values of t_0 obtained in other studies (Tsikliras et *al.,* 2005 ; Bouaziz, 2007 ; Apostolidis and Stergiou, 2014 ; Benamar, 2019), these results can be the effect of a non-regular growth of the fish caused by ecosystemic disturbances, this non-regular growth would then translate into an irregular growth curve (in other words a growth graphic with non-distinguishable curvature), which in turn distort the growth values obtained. To correct the growth parameters obtained we added the values age = 0 and TL = 0 to the age-length key, when using VONBIT_ALXL (2012), doing so, we considerately improved the curvature of the growth graphic, as a result, we obtained growth parameters that are largely more representative of the specie in reality, that are: L_∞ = 23.54 cm, a value close to L_{max} = 22.8 cm, K = 0.48 $year^{-1}$ a value representative of the fast growth of small pelagic fish, t_0 = -0.043 year a t_0 that concords with literature (Tsikliras et *al.,* 2005 ; Bouaziz, 2007 ; Apostolidis and Stergiou, 2014 ; Benamar, 2019).

The calculation of asymptotic length (L_∞) for each age ($L_{\infty n}$, asymptotic length for each age n) using Taylor (1962) in Bouaziz (2007) equation and K_n using von Bertalanffy's model equation provided us with the following results:

Table 9. Values of $L_{\infty n}$ (asymptotic length) and K_n (curvature parameter of the von Bertalanffy growth function) calculated for each age.

Age classes	$L_{\infty n}$ (cm)	K_n (year^{-1})
1	12.11	1.99
2	16.32	1.06
3	18.42	0.91
4	20.53	0.66
5	24.21	0.57

Results of the calculation of the mortality M_{cte} and M_n using Djabali's *et al.,* (1993) natural mortality equation with K_n and $L_{\infty n}$ results for each age (table 2):

$M_{cte} = 0.51$ year^{-1}

$M_1 = 1.13$, $M_2 = 0.79$, $M_3 = 0.72$, $M_4 = 0.61$, $M_5 = 0.54$

The results obtained show that mortality varies with age, it is the highest at age 1 and then diminishes with age (rapidly at first and then diminishes with a slower pace) which concords with Garrido (2016) and Quattrocchi and Maynou (2017).

Concerning length weight relationship ($W_t = a\,Lt^{b}$) constants a and b, we obtained the following:

a = 0.0045 and b = 3.123

The results of biomass assessment using VIT4Win 1.3:

Critical age and size of the virgin stock (mean size and age of the cohort having the highest biomass) with M_n were 2.20 year, 15.5 cm meanwhile with M_{cte} were 2.63 year, 17 cm. Opposingly mean age and size of the current stock didn't shift much (1.37 year, 11,21 cm for M_n and 1.43 year, 11.54 cm for M_{cte}). The same conclusion was drawn for the current stock critical age and length which were 1.63 year, 13 cm for M_n and 1.73 year, 13.5 cm for M_{cte}.

Concerning exploitable biomass, we used the virtual population analysis (VPA) feature in VIT4Win software to obtain the results presented in the table 3. Using natural mortality as a constant (M_{Cte}) the current biomass obtained was Bc = 86866.6 tons (biomass Bc = (Bc/R)*R, R is the number of recruits). On the other hand, when assessing biomass using mortality as a variable (M_n) we obtained Bc = 10636.4 tons, a value different from Bc calculated with M_{cte} by 1949.8 tons.

Table 10. Yield and biomass parameters obtained by the VIT4Win 1.3, for round sardinella, *Sardinella aurita*, of the central region of the Algerian coast.

Parameters for M_{Cte}					Parameters for M_n				
	Factor	Y/R (g)	B/R (g)	SSB/R (g)		Factor	Y/R (g)	B/R (g)	SSB/R (g)
F(0)	0	0	41.024	34.093	F(0)	0	0	20.929	15.967
F(0.1)	0.400	9.202	18.918	12.681	F(0.1)	0.540	5.176	10.152	5.657
F_{MSY}	0.700	9.808	13.205	7.394	F_{MSY}	1.200	5.664	6.521	2.485
F_C	1	9.585	10.092	4.675	F_C	1	5.640	7.272	3.103
Number of recruits: 860736484.88					Number of recruits: 1462652863.53				

Factor = Factor of effort, Y/R = Yield per recruit, B/R = Biomass per recruit, SSB/R = Spawning stock biomass per recruit, F(0) = Factor of non-fishing, F(0.1) = factor of fishing mortality rate at which the marginal yield-per-recruit is only 10 percent of the marginal yield per recruit on the unexploited stock, F_{MSY} = factor of fishing mortality rate corresponding to the maximum sustainable yield, F_c = factor of current effort fishing.

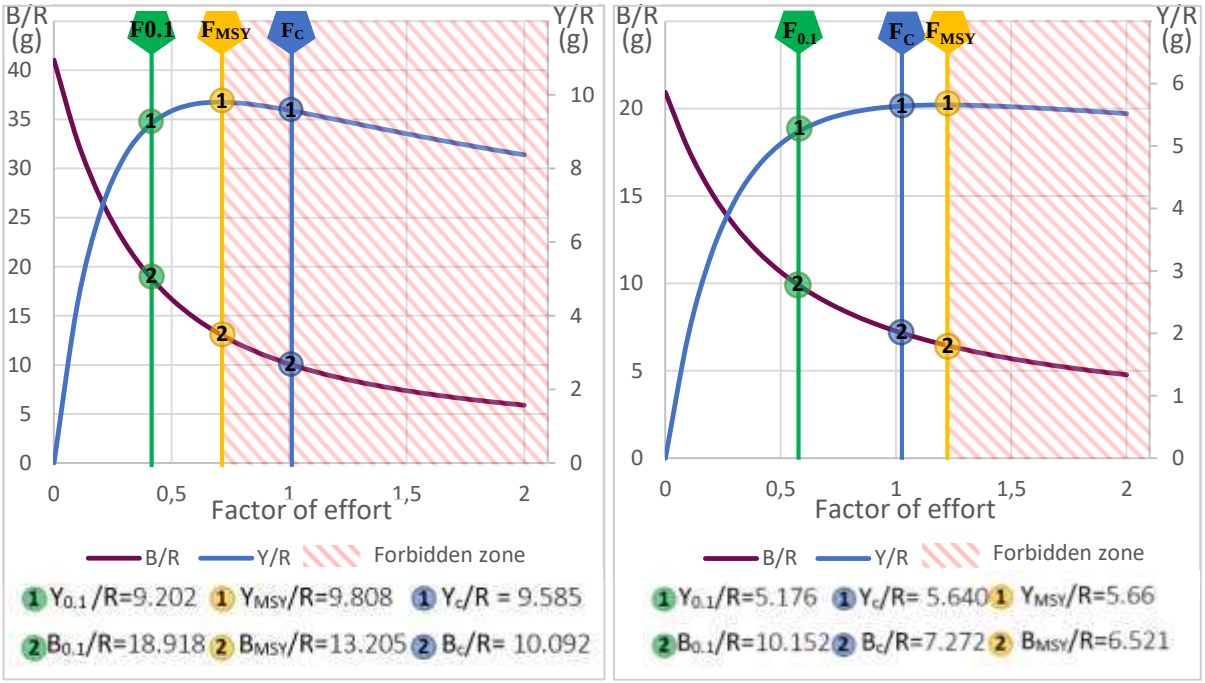

Figure 2. Yield per recruit (Y/R) and biomass per recruit (B/R) according to the effort factor with constant natural mortality (M_{cte}) (left) and with variable natural mortality (M_n) (right).

At the first glance on the graphics in figure 1 we can already see that the results differ according to the natural mortality used, we can also see that with M_{cte} the current factor effort (F_c) exceeds the maximum sustainable yield effort factor (F_{MSY}) and is positioned in the forbidden zone. On the other hand with M_n the current effort factor is positioned before the maximum sustainable yield and thus it is not in the forbidden zone, moreover, according to the values with M_{cte}, $F_{0.1}$ = 0.400, this value imply that to maximise a sustainable exploitation we would have to reduce fishing effort by 60% (to increase Bc to 16337.6 tons), that said, with M_n, $F_{0.1}$ = 0.54, so to have a sustainable exploitation we would need a reduction of 46% of fishing efforts (to increase the biomass to Bc = 14848.9 tons).
To investigate the occurrence of an overexploitation we used the Froese and Proelss (2012) criteria, and obtained the following, for constant natural mortality (M_{cte}) the results showed an overfished and overfishing state (dark grey), on the other hand, with variable natural mortality (M_n) the results showed a not overfished and not overfishing state (light grey).

CONCLUSIONS

According to the previous results biomass assessment is largely sensitive to mortality values. By comparing biomass assessment using M_{cte} with biomass obtained using M_n we obtained an estimated shift of the biomass value of 1949.8 tons, (Bc = 8686.6 tons with M_{cte} to Bc = 106364.4 tons with M_n). Additionally, results obtained led to different stock evaluations and even different suggestions. Consequently, while using M_{cte} the results pointed that the stock was in a state of overfishing and overfished, while when using M_n, the results showed a state of not overfished and not overfishing. Also, when using M_{cte} the results suggested a fishing effort reduction of 60%, while with M_n the results suggested a reduction of fishing effort of 46% (to increase biomass to Bc = 14848.9 tons). Since mortality values changes with age and

considering the impact of the natural mortality used on the biomass assessment, we therefore suggest the use of variable natural mortality when conducting similar procedures.

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PHYSIOLOGICAL AND BIOMETRICAL PARAMETERS OF ORGANICALLY GROWN LETTUCE (*L. SATIVA*)

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ABSTRACT

Global warming is the leading cause of the extreme meteorological events increase in the last three decades. Increase of annual average air temperature and temperature values during winter season and early spring has been recorded in comparison to the (1961-1990) referent period in Plovdiv region. Climate change impacts food security, crop growth, as well as their quality and nutrition value. Therefore, modern scientific research should be focused on growing produce using sustainable and organic methodology that also produces pure and delicious foods. The (type Batavia, variety Maritima) lettuce development was researched in a greenhouse environment in the experimental field of the Agricultural University Plovdiv. During the lettuce growth six different fertilizing variants were used: no fertilizer, one chemical, and four organic fertilizers. The effect of the different fertilizes was studied through specific physiological parameters and productivity. This paper analyzes changes in functional activity of the plant photosynthetic apparatus and productivity of variants with different fertilizers in an unheated greenhouse. The ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of PS II was determined to be more optimal in dark-adapted leaves for the organic fertilizer variants, compared to the no fertilizer variant. No significant difference was observed in the values of the minimal fluorescence Fo in reaction centers of PS II after the dark-adaptation of leaves using the different fertilizer variants. Higher values of chlorophyll content index (CCI) were documented for organic and chemical fertilizers compared to the no fertilizer variant.

Keywords: *photosynthetic activity, chlorophyll content index, lettuce, yield, greenhouse*.

INTRODUCTION

In recent years, with the intensification of extreme phenomena of meteorological origin, the advancement of agricultural science and the desire of people to produce qualitative and safe food with minimal risk to the environment, biological farming technologies have aroused scientific interest. Production without mineral fertilizers is important for the environment protection, for the balance and fertility of the soil, as well as for human health. The lettuce is a vegetable, intended only for fresh consumption, which requires good taste and purity of production. Vitamins A, B, C, D, and E can be found in the leaves of the species (Fogg, 1983). The vegetable is one of the main components from the dietary menu and the table in Bulgaria.

The resistance of the species to low temperatures and the duration of the period up to their typical leaf mass reached growth stage (Feller et al., 1995) make it preferred both for autumn-winter production in unheated facilities and for early spring cultivation.

Both the higher temperatures and the changes in the humidification conditions in the country (Marinova et al., 2018; Frantzova, 2014; **Alexandrov et al., 2004**), and the studied area (Georgieva et al., 2017), registered in the recent decades, affect the specific meteorological conditions, the growth and development of the different production **(**Popova et al., 2014**)** and types of lettuces. It is necessary to specify the varieties and the fertilization. Therefore, an experiment in polyethylene greenhouses with a type of lettuce was set (type Batavia, variety 'Maritima') with six different variants of biological fertilization, namely: no fertilization; fertilization by means of one chemical; and fertilization by four organic fertilizers.

There are studies on the interaction of different factors on the physiological status of plants (Shopova and Cholakov, 2014). The physiological condition of plants and effect of various stressful factors thereon have been studied using chlorophyll fluorescence properties by many researchers (Mathur et al, 2014; Kalaji et al.,2016). Chlorophyll fluorescence is a non-invasive measurement of photosystem II (PSII) activity and is a commonly used technique in plant physiology. The sensitivity of PSII activity to abiotic and biotic factors has made this a key technique not only for understanding the photosynthetic mechanisms but also as a broader indicator of how plants respond to environmental change (Murchie and Lawson, 2013). The fluorescence is emitted mainly from chlorophyll *a* of PSII and reflects the primary processes of photosynthesis by light absorption, distribution and transfer of excitation energy and photochemical reactions in PSII. Because of the functional relation of PSII with other components of the photosynthetic apparatus of the chlorophyll fluorescence, it is seen as a proxy for the state of the integral photosynthetic process and the plant organism as a whole (Roháček, 2002). Chlorophyll fluorescence, among others, has been satisfactorily used for monitoring leaf health status in lamb's lettuce (Ferrante and Maggiore, 2007) and storage potential of iceberg lettuce (Schofiled et al., 2005).

The CCM (Chlorophyll Content Meter) 200 plus is useful for improving nitrogen and fertilizer management, and is ideal for crop stress, leaf senescence, plant breeding, health determination, and other studies. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses (Opti-Sciences 2002; Richardson et al., 2002). The aim of the present study was to monitor the reaction of the leafy lettuce (Batavian variety 'Maritima') to six different fertilization variants by analyzing the temperature conditions and measuring the main parameters of productivity and photosynthetic activity.

MATERIAL AND METHODS

The experiment was conducted on the experimental field of the Agricultural University of Plovdiv in 2019-2020 in unheated greenhouses on alluvial meadow soil (Mollic fluvisol, FAO 2006).. The soil texture is sandy clay loam to clay loam. despite the small amount of total carbonates (2-3%), the soil reaction is slightly alkaline $pH_{(H2O)}$ – 7,7-8,0 (Valcheva et al 2015). The same authors found a high amount of exchange bases (Ca^{2+}+Mg^{2+} - 20-30meq/100g soil) in the composition of the soil sorption complex, and a low content of nitrogen, phosphorus and potassium. The importance of the organic matter of the soil for its fertility is indisputable. However, the nitrogen bound in the organic matter remains hidden in this indicator. The nitrogen in organic form, which is over 95% of total soil nitrogen is the basis of soil fertility. Organic nitrogen is the source that supports the plants throughout the growing season and

ensures an even supply of nitrogen to the plants. The active fraction of soil nitrogen varies with different soil types and depends on a number of factors - degree of cultivation, field history (previous crops in the crop rotation, fertilization system), biotic and abiotic soil characteristics and some environmental factors (mainly temperature and humidity). The lettuce plants (Batavian type, variety 'Maritima') were planted on 8th of November in polyethylene greenhouses in 4 rows according to the scheme 70+30+30+30/30cm with a profile of the soil surface a high level bed (100+60cm.) The experiment was based on the block method with four repetitions, using 28 plants per repetition, and a plot size of 3.36m^2. Organic seeds were provided for seedling production using container technology with 150-hole Styrofoam boards in the following combination – organic seeds - 80%, Perlite - 20%, Lumbricompost for bioproduction of seedlings (Kostadinov & Filipov, 2013). Several variants were tested: 1. NPK (mineral fertilization); 2. Control (non-fertilization); 3. Italpollina; 4. Arkobaleno; 5. LK (Lumbricompost); and 6. Ekoprop NX. The granular fertilizers were introduced as basic fertilization, with soil pre-transplantation at the following norms: N- 12.5kg/da, P_2O_5- 1.25kg/da, + K_2O – 4.75kg/da, Italpollina- 25 kg/da, Arkobaleno - 100 kg/da, and Lumbricompost - 400 l/da. The liquid bio fertilizer Ekoprop NX was applied by double treatment in a dose of 100g/da: before planting - in the 5th leaf seedling phase; and 10 days later on, after the adaptation to the soil. The remaining bio fertilizers are granulated and introduced into the soil before the last tillage and before planting the seedlings. Growing stages were note according BBCH, 2011 and Feller et al., 1995). The biometric measurements were taken three times at one-week intervals in stage-typical leaf mass reached.

Experimental setting: Meteorological observations from Plovdiv AU (Agricultural University) station (42 ° 14′N, 24 ° 75′E and 162 m above sea level) were used for the outdoor temperature analysis. The data were collected according to the recommendations of the WMO (World Meteorological Organization) and are also comparable with the climatic norm for the region. In greenhouse conditions, the air temperature was recorded by means of a weather station M**eteobot® Pro** (https://meteobot.com/). The active temperatures were calculated as the sum Σ^0C) =T-Tb, where T-Tb is taken 0 when T<Tb and T is taken as Tu when T>Tb (WMO, 2011).

Chlorophyll fluorescence imaging: The Chlorophyll fluorescence of the lettuce leaves was measured using a portable device PAR-FluorPen FP 110/D manufactured by Photon Systems Instruments Ltd., Czech Republic. The fluorescence measurement protocol uses short (30 μs) measuring flashes to measure zero level fluorescence (F_0) followed by a strong saturating flash [duration 0.8 s, intensity about 3000 μmol m−2 s−1] to measure the maximum fluorescence (*F*m). Three strong flashes of saturating light probed the effective quantum yield (Qy) of PSII during the actinic light exposure (Maxwell & Johnson, 2000; Nedbal et al., 2000). Light Meter for direct digital readouts of Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm, the span in which plants use energy during photosynthesis. PAR is measured as Photosynthetic Photon Flux Density (PPFD), which is indicated by units of quanta (photons) per unit time per unit surface area. The chlorophyll fluorescence transients were measured on the same day in the morning. The dates of measurement were 20/03/2020, 28/03/2020 and 04/04/2020 when the plants were in their typical leaf mass reached growth stage. The nine leaves from each variant were dark adapted for about 30 min by detachable leaf-clips prior each measurement. The numeric value of each parameter (Fv/Fm, F_o, PAR) was determined by integrating it over the measured leaf area. ***Physiological estimate of the***

chlorophyll content index (CCI)*:* The Chlorophyll content index of the leaves was measured using a portable apparatus CCM 200 plus Chlorophyll Content Meter manufactured by Opti-sciences, Inc., NH, USA. The physiological assessment was carried out *in vivo* on the field. The measurements were taken on three dates from a sample of leaves at their typical leaf mass reached growth stage. The dates of the measurements were 20/03/2020, 28/03/2020 and 04/04/2020; 20 leaf measurements in the central part of the leaves were taken for each variant (in each of the repetitions). ***Statistical evaluation of the results*****:** To prove differences in the mean values, a one-way ANOVA analysis of variance was performed within the variants of each variety, followed by LSD analysis by means of a Fisher's test. Mathematical processing and statistical analysis were made and visualized using Microsoft® Office products and StatGrafics® statistical software.

RESULTS AND DISCUSSION

Temperature conditions

Table 1. Temperature conditions November 2019-April 2020 and deviation from the reference period 1961-1990

		temperatures ° C				
Months	***Average***	***δ t***	***t max***	***date***	***t min***	***Date***
November	10,8	3,8	23,0	5	2,8	30
Desember	4,4	2,0	17,0	15	-6,5	6
January	3,5	3,2	17,0	28	**-10,5**	8
February	6,4	3,6	21,5	1	-7,0	09
March	8,9	2,1	23,8	13	-4,0	17
April	11,5	-0,7	**27,8**	18	-2,5	8

The observed climatic fluctuations in the study area during the last 3 decades undoubtedly influence the conditions of growth and development of lettuce in the open air and have an economic effect on plants grown in unheated greenhouses. Therefore, the authors believe that temperature, as a key element for the development and productivity of the lettuces is a mandatory element of the study. The average temperature by months and by days for the period of growth and development of the lettuce from planting at the moment of having its 5th true leaf to the stage Typical leaf mass reached - November, December 2019 and January, February, March and April 2020 – has been considered. For November, December, January, February and March the deviation from the climatic norm is positive - by 2.0˚C - December; 2.1˚C in March; 3.2˚C - January and, with the warmest months being November and February (Δ> 3.5 ˚C) degrees above the norm (Table 1). For the period November-April, the lowest reported air temperature was - 10.5˚C, and the highest 27.8˚C, both measured on the 8th day from the first ten days of the month (Table 1). April was cooler than usual (Δ = (- 0.7) ˚C), but the lettuces were harvested at the beginning of the month 04.04 and the low values did not significantly affect their growth and development. In summary, we can say that the period had a warmer winter and a cooler spring than the usual spring, compared to the reference period 1961-1990 and the period 1991-2019 (Fig.1).

As with any plant species, Batavia lettuce defines three cardinal temperature points - minimum, maximum and optimal value. According to some Bulgarian and foreign authors (Kartalov et al. 2007; Lorenz and Maynrad, 1988), the optimal temperature for lettuce development is 16.0-18.0˚C. Morgan (1999) proved that lettuce would produce better quality plants in the cooler conditions of winter and spring. Morgan (1999) also said the optimum temperatures of lettuce were 12 to 21˚C. Well-rooted plants in phenophase 7-9 leaves can withstand temperatures down to -6.0˚C (Cholakov, 1999). The range of development of the species is between 5.0 ˚C and 25.0˚C and below 5.0˚C; the growth slows down and stops (Cholakov, 1999). Other authors (Kristensen et al., 1987; Morgan, 1999) consider the temperature of 4.0˚C to be Tb (base). The temperature conditions have affected the growth and development of the studied plants. The greenhouse is made of polyethylene with a thickness of the cover foil of 0.20 mm. The outside temperature also determines the temperature inside and the excessive values are the reason for the higher values inside in the discussed period (Fig. 1). The lettuces were planted on November 8 in their growth stage: leaf development (Main shoot) - 5th true leaf unfolded with first harvest on March 21 in growth stage typical leaf mass reached. The results show that compared to the conditions outside, the average temperature in the greenhouse is higher by 2.5^{0}C (Fig. 2). From the whole period of 149 days, the days with temperatures below the biological minimum of 5.0˚C (Cholakov, 1999), noted in fig. 1 by a green dotted line, were reduced from 58 outside to 25 inside the greenhouse. The collected active amount for the vegetation period of 124 days was ΣAt^{0}C = 1267^{0}C. The effective temperature amount (ΣEft^{0}C) from the moment of planting to the last harvest of the lettuces in the polyethylene greenhouse was ΣEft^{0}C = 647^{0}C. For the period 21st of March 21- 4th of April 4 there were only two days with temperatures below the biological minimum and the collected active temperatures from the first to the last harvest were 137^{0}C while the value of the effective temperature >Tb was ΣEft^{0}C = 72.0 ^{0}C.

Figure 1. Temperature conditions November 2019-April 2020 and deviation from the period 1991-2019

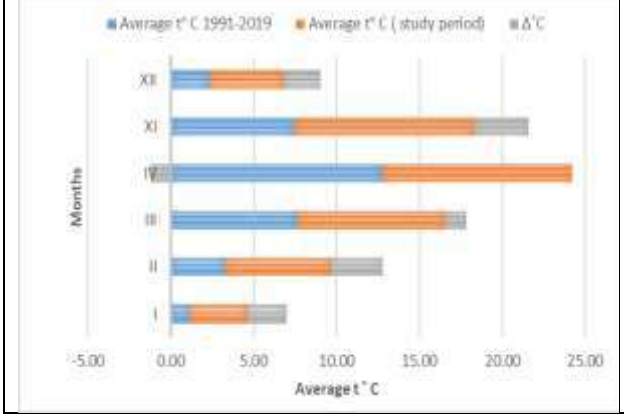
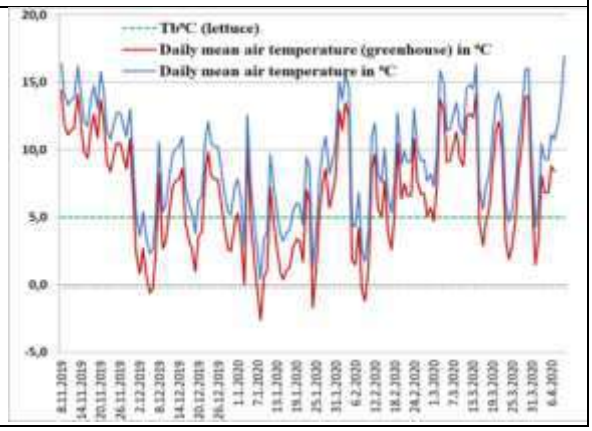


Figure 2. Average daily values of the air temperature outdoors and in an unheated greenhouse during the period of growth and development of the lettuce 2019-2020



It has already been mentioned that in unheated facilities the outside temperature determines the conditions of growth and development of the plants inside. Therefore, a comparison was made between the average temperature values outdoors and indoors (in the greenhouse). A linear relationship ***(y = 0.9421x + 2.8671; r^2=0.83, SS=1, 5; MAE=1.2)*** was obtained (Fig. 3). A model is proposed for calculating the average daily temperature inside the polyethylene greenhouse using the values, obtained from the meteorological site outside. The model was

applicable (Table 2) for the needs of various agrometeorological studies in conventional and organically grown vegetable crops in polyethylene greenhouses.

Figure 3. Plot of fitted model (StatGrafics®)

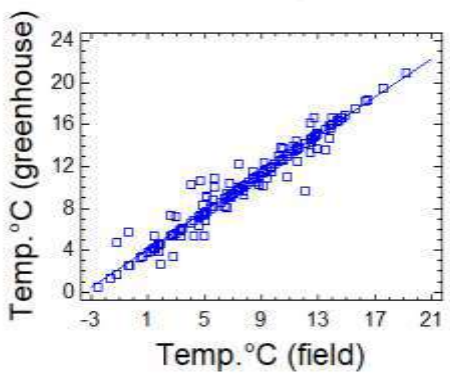


Table 2. Statistical analysis (StatGrafics®)

Dependent variable:

Parameter	Estimate	Standard Error	T Statistic	P-Value
CONSTANT	3.16	0.13	23.37	0.0000
x	0.91	0.015	60.11	0.0000

Analysis of Variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Model	2945.24	1	2945.24	3614.37	0.0000
Residual	140.972	173	0.81486		
Total (Corr.)	3086.21	174			

Analysis and Statistical evaluation of the fresh mass (g.)

The paper considers the biometric indicator of fresh weight as a key element of productivity. An analysis was made between the control and the tested variants (Fig. 4).

The unfertilized lettuce (the control) has an average weight of 589.18g – starting from 499 when the first measurement was made to 723 during the last one. After mineral fertilization, the weight of the plants varied from 538g to 721g, with an average weight of 629. The average weight of Italpollina was 620.4 with increase from 523.2 to 736.9; of Arkobaleno- 527.4 with the lowest value 472.3 and the highest - 563.9; LK had an average weight of 543.6, starting from 514.3 and reaching 589; Ekoprop - 628.3 with the lowest weight 535.7 and the highest 711.7. It is noteworthy that Italpollina has the best parameters, as in the last measurement it exceeds the results of all the other variants, including for the case of mineral fertilization. The biological variants Arkobaleno and LK have a minimal negative difference compared to the variant with mineral fertilization, while the variants Italpollina and Ekoprop exceed it by 31 g. and 39 g. respectively (Fig. 4).

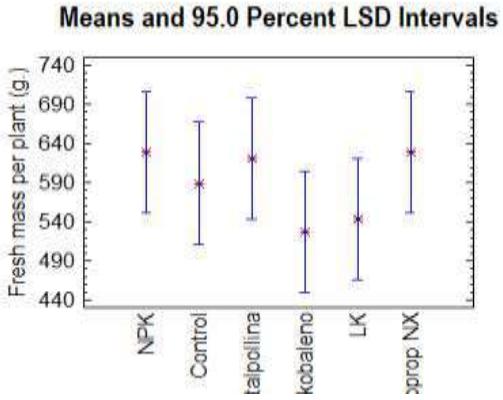


Figure 4. Fresh mass (g.) for the variants of lettuce Batavia variety 'Maritima'

The analysis of the data for this particular year does not show statistically significant differences between the control, the minerally fertilized plants and the ones, fertilized by organic fertilizers (Fig.4 and Tabl. 3). Ekoprop can be considered the best option, and at the last measurement on April 4, Italpollina exceeded all the other fertilization variants. The plants from the variants with organic fertilization have better taste qualities and no deviations from the typical color and shape of the species.

Table 3. ANOVA analysis Fresh mass (g.) of lettuce Batavia variety 'Maritima'

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	29933.9	5	5986.79	0.78596	0.57916	3.10588
Within Groups	91406	12	7617.17			
Total	121340	17				

Physiological parameters

The averaged value of the initial fluorescence (F_0) of the oxidized reaction centers of PSII is highest in case of biological fertilization with LK, and lowest in case of mineral fertilization (Table 4).

Table 4. Chlorophyll fluorescence parameters of the plant leaves for the lettuce Batavia, variety 'Maritima' in an unheated greenhouse

indices / **variants**	***Control***	***NPK***	***Italpollina***	***Arkobaleno***	***LK***	***Ekoprop NX***
Ft=Fo	4413	4668	4468	4761	4130	4103
Ft=Fo	3573	3730	3822	4578	4387	4165
Ft=Fo	4838	4032	4448	3699	4646	4467
mean value	**4275**	**4143** n.s	**4246** n.s.	**4346** n.s.	**4388** n.s.	**4245** n.s.
Qy=Fv/Fm	0,82	0,80	0,80	0,82	0,81	0,81
Qy=Fv/Fm	0,80	0,83	0,81	0,80	0,81	0,83
Qy=Fv/Fm	0,82	0,82	0,84	0,84	0,83	0,83
mean value	**0.814**	**0.816** n.s.	**0.817** n.s.	**0.821***	**0.819** n.s.	**0.823****
PAR	330,7	349,3	480,7	203,7	481,0	200.0
PAR	231,7	290,0	265,7	276,7	285,0	206,3
PAR	83,0	61,0	73,3	97,0	64,7	68,3
mean value	**215.1**	**233.4** n.s.	**273.2****	**192.4** n.s.	**276.9*****	**158.2*****

LSD	F0	Qy	PAR
p=0.05*	191.5	0.0059	33.4
p=0.01**	256.3	0.0079	44.7
p=0.001**	336.5	0.0104	58.7

n.s. no significance difference

With this indicator only no significance of the differences in the average values was established, which proves the weak influence of the fertilization method on it. Also, Zlatev & Kolev, 2012 and Chen et al., 2018 believe that the high temperature leads to an increase in F_0, in which case

the temperature conditions in the greenhouse do not lead to a negative change in the photosynthetic activity of the different variants. The highest value of F_0 for Arkobaleno on the first and second reporting dates can be observed, on average 17.0% higher than the control and significantly the highest value of F_0 of the control for the third reporting date. The average value of the Fv/Fm ratio or the quantum yield Qy of the different variants is close to the normal for healthy leaves - 0.83 (Demmig and Björkman, 1987). The lowest value was registered for the control variant, while the highest value together with a statistically significant difference were measured for the variants with the organic fertilizers Ekoprop and Arkobaleno (Table 4). The comparative characteristic made by dates of measurements shows the largest difference between the unfertilized variant and the variants with organic fertilizers on the third measurement date. With this important indicator of photosynthetic activity, there is a proven positive effect of the organic fertilizers, not only compared to the control variant, but also to the variant with mineral fertilization. On the other hand, Qy values for all variants in this study do not indicate the presence of stress in the plants during the period of reporting their physiological activity. The measured photosynthetically active radiation (PAR) is higher on the first two dates, which is associated with the higher daily temperatures and the increased solar radiation compared to the atmospheric conditions during the third reporting date. The almost double reduction of PAR does not lead to inhibition of the photosynthetic activity. The most optimal ratio between PAR and Qy was reported for Ekoprop and Arkobaleno (Table 4).

In parallel with the readings of some indicators of the chlorophyll fluorescence of the leaves, the chlorophyll index - Chlorophyll Content Index (CCI) was measured (Table 5). With the exception of the variant Arkobaleno, in all the other fertilization variants the CCI has a higher and statistically significant average value compared to the control variant. The leaves of the variants fertilized by organic fertilizers Lumbricompost and Ekoprop have the highest CCI. The lowest and highly variable CCI was reported for Arkobaleno, the low average value being mainly due to the 20.0% lower CCI compared to the control during the third measurement. The values of the chlorophyll index are in a positive correlation with the values of the quantum yield-Qy, which proves the inducing effect of the organic fertilizers on the photosynthetic activity of the plants. The highest average value of fresh mass of the plants, fertilized by Ekoprop, correlates positively with the reported good indicators of the chlorophyll fluorescence and the high chlorophyll content index.

Table 5. Chlorophyll content index (CCI) of the leaves for the lettuce Batavia variety ‘Maritima’ in an unheated greenhouse

date of estimate / variants	Control	NPK	Italpollina	Arkobaleno	LK	Ekoprop
20.3.2020	8.63	8.46 n.s.	8.84 n.s.	9.06**	8.86 n.s.	8.73 n.s.
28.3.2020	9.12	10.06***	10.35***	9.21n.s.	10.23***	11.12***
04.4.2020	7.35	7.66 n.s.	7.70 n.s.	5.90***	8.56***	7.58 n.s.
mean value	**8.37**	**8.72***	**8.96****	**8.06 n.s.**	**9.22*****	**9.14*****

LSD	**20.03.2020**	**27.03.2020**	**08.04.2020**	**Mean value**
p=0.05*	0.29	0.34	0.40	0.32
p=0.01*	0.39	0.45	0.53	0.47
p=0.001*	0.50	0.58	0.69	0.71

CONCLUSIONS

During the studied period (November 2019 - April 2020) the temperature conditions were determined by a warm winter and cool spring compared to the reference period 1961-1990 and the period 1991-2019. The collected effective temperature sum >Tb (ΣtE^0C) reached up to the typical leaf mass reached for the Batavian lettuce, variety 'Maritima', was 647^0C. The higher by 2.5°C temperature in the greenhouse reduced the days with temperatures below the biological minimum to the number of 25, compared to the number of 58 outside. The evaluation of the fresh mass of the rosette did not show statistically significant differences between unfertilized plants and plants, fertilized by either mineral or biological fertilizers. Ekoprop and Italpollina have the best parameters since Italpollina has the highest productivity of all the other variants for the last reporting date – 4th of April, 2020. Regarding the photosynthetic activity Qy, there is a proven positive effect of the organic fertilizers, not only compared to the control variant, but also compared to the variant with mineral fertilization. The most optimal ratio between PAR and Qy was reported for the variants Ekoprop and Arkobaleno. The leaves of the variants, fertilized with the organic fertilizers Lumbricompost and Ekoprop have the highest CCI.

ACKNOWLEDGEMENTS

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COMPARATIVE STUDY OF ELEVEN APRICOT CULTIVARS IN THE CONDITIONS OF COASTAL REGION OF ALBANIA.

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ABSTRACT

The study of eleven apricot cultivars was carried out in a collection planted in the Experimental Base of ATTC Vlore during the period 2017-2020, to evaluate, compare and list among apricot varieties, according to a common protocol for vegetative, pomological, horticultural and technological characteristics, in order to give assistance to farmers who grow apricots, that often fail to plant new cultivars suitable for the conditions of their farms. Antonio Errani, Bulida, San Castrese and Pellecchiella cultivars were planted in 2010, while Spring Blush, Luna, Bora, Magic Cot, Prima, Tsunami, Rubista cultivars were planted in 2014, having the same rootstock (Myrobalan 29C). The results of the study showed that these varieties have different habitats and capacity of growth. The cultivars Bora, Prima, San Castrese, and Spring Blush resulted in strong growth, while the cultivar Rubista resulted to have less potential of growth. Early flowering was recorded in Magic Cot and San Castrese, while the late flowering recorded the Rubista cultivar. Spring Blush, Magic Cot, Prima, Tsunami, Luna cultivars need pollinating plants, while others are self-pollinating. Fruit ripening period, compared to the standard (San Castrese), was recorded ; for Tsunami (-28), Spring Blush (-27), Luna, Magic Cot (-25), Prima (-23), Rubista (-19), Antonio Errani (-18), Bora (-15), Bulida (-12) and Pellecchiella (+15) which was the cultivar with latest ripening period. The comparison of fruit size pointed out as cultivars with largest fruit, Antonio Errani, Bora, Bulida and Magic Cot, while with smallest fruit Spring Blush, San Castrese and Rubista. The highest acidic content is recorded in San Castrese cultivar. The total yield has shown variability throughout the years and has confirmed the impact that environmental conditions have on apricot productivity. Bora and Pellecchiella cultivars resulted to have more cracked fruit compared to other varieties, during rainy years. Economic analysis showed that cultivars with earlier ripening are more effective than late ripening varieties. Obviously, an expanded study is required for spreading these varieties in whole regions of the country.

Keywords: apricots, cultivars, self- pollination, maturity, regionalization

INTRODUCTION

The climatic soil conditions of Albania and the diversity that characterizes it as a Mediterranean country, offer many opportunities for the cultivation of apricots, even in steep terrain, not very favorable for many other crops [9]. This has led that apricot is being cultivated throughout the country but without succeeding to dominate as the main culture. In 2018 the number of apricots in Albania reached 260,360 plants, 337 ha planted, with a production of 5131kv and an average yield of 23 kg / plant. The number of new plantings reaches 6673 roots, but in reduced quantities in blocks (3ha) (INSTAT). It is more spread in Korçe, Fier, Berat, Vlore and Peshkopi [8, 16].

Apricot is also known as a crop that has poor adaptability to environmental conditions 1, 17, 19]. In this vast area of cultivation with different zone and microzones, the total yield of apricots is not always favoured, there are many risks, mainly during the post-dormant period (winter dormancy) [1, 9]. Spring frosts that hit the plant during flowering when the buds are more sensitive, often accompanied by excess moisture, or conversely relatively high temperatures that inhibit microsporogenesis, can have serious economic consequences for apricot orchard [13, 14, 17]. Climatic factors of the environment can greatly affect the dormancy of flower buds, affecting the entity with the appearance of flower malformations [22, 24]. Also the limiting factors that prevent the extent of apricot cultivation are the climatic conditions related to accumulation of the frosts, a factor that has a significant impact on productivity [1, 19]. Selecting the most suitable variety for the terrain and climate in an ecological zone remains the main factor in improving successfully the yield, stability and profitability of apricot production [2, 4, 12, 20, 21]. First planted cultivars in Albania have local and foreign origin, which have been adapted to the conditions of the areas where they are positioned [9]. The increase of areas with this crop could not be tempted by the presence of new competing cultivars in the markets, of high quality, originating from Greece, Italy, France, Spain and the USA.The introduction of these new cultivars is a promising opportunity in the private and national agricultural economy, for the quantitative, qualitative and commercial improvement of apricots. But the experience of many countries and researchers has shown that apricot cultivars are rarely cosmopolitan [3, 11]. Even in Albania, most of the old cultivars have not been adapted outside their environment of origin [9], thus causing lack of manifestation of the best qualities for which they have been selected.

Rich germplasm with a variety of organoleptic and commercial qualities, size, color, taste and flavor of the fruit, with resistance to manipulation and refrigeration and which offer the opportunity to extend the harvest season until September, requires a period of time and testing before being analyzed for their inclusion in new planting schemes [23]. In apricots, the growing conditions enhances the influences of different natures and various biological, physiological and phenological intensiteties interrelated with the nature of growth and fruiting [1, 6, 10].

The behaviours of cultivars manifested in the flows of vegetative-productive growth, even if not completely identical with their features, are important not only for the selection of the cultivar, but also for the system of keeping the corolla and the method and time of pruning [5, 7].

To assist apricot growers, who often fail to plant new cultivars suitable for their farm conditions, has been realized a study of 11 new apricot cultivars, most of which were not previously tested, in a collection planted in The Experimental Base of ATTC Vlore, during the period 2017-2020, to evaluate, compare and list among them according to a common protocol for vegetative, pomological, horticultural and technological characteristics.

MATERIALS AND METHODS

The study of eleven apricot cultivars was carried out in a collection planted in the Experimental Base of ATTC Vlore, during the period 2017-2020. The cultivars Antonio Errani, Bulida, San Castrese and Pellecchiella, were planted in 2010, are also the cultivars that have been introduced earlier in Albania market, meanwhile the cultivars Spring Blush, Luna, Bora, Magic Cot, Prima,

Tsunami, Rubista were planted in 2014, representing new cultivars, without being tested before. They all have the same rootstock (Myrobalan 29C), planting distance (5x5 m) and are corolled according to the open vase system. For each cultivar were studied the following indicators:

7. Indicators of plant vegetative development, referred to the method used by Viti, R. and Guerriero, R. (2006) [25]. The annual increase in trunk diameter and corolla dimensions was measured to calculate the volumetric index from the ratio of height to corolla diameter. In the biennial branches emerging from the representative branches (selected according to the geographical coordinates since the first year), the elements of the branch insertion angle in relation to the vertical axis, the power of annual growth, the types and spread model of the sprigs are measured. All this measurments are carried out in order to determine the strength and habitat of the growth and fruiting of each cultivar.
8. The flowering period, considering the beginning when 10% of the flowers on the tree have blossomed, the full flowering when 70% of the flowers on the tree have blossomed, and the end when 70% of the petals have fallen.
9. The ripening period by observing the beginning and the end, comparing with the San Castrese cultivar which is considered as a reference cultivar.
10. Biometric indicators of fruit (dimensions Dx d, weight, color, form), other fruit indicators such; sugar content and total acidity, measured randomly in 30 fruits at maturity stage of each variety at ATTC Vlore bio-chemical laboratory.
11. Production for each tree calculating the production / ha for each cultivar according to years.
12. In this analysis was taken four plants for each cultivar, labeling since the winter pruning according to a randomized scheme, where each tree was been treated as a replication, while the representative branches were labeled and selected in N-S-E-W positions, preserving them for four years of experiment. The analysis of statistical indicators was carried out with the Comparisons for all pairs method using Tukey-Kramer HSD, for the error level 0.05.

RESULTS AND DISCUSSION

Referred to Table number 1, examined varieties are characterized from significative differences of vegetative growth indicators and growth habit. The differences are distinct within the same group regarding to indicators of growth habit, manifesting different growth habit for these varieties. Evidences on growth habit of each variety, ratio between different types of shoots and their position on trees are crucial in order to define correct pruning technique [7, 9]. In apricots, growth habit and fruiting performance are strongly interrelated 6, 25].

Table 1. Main indicators of vegetative growth and growth habit for all the compared cultivars. Every growth habit is classified according to the reference classes of each parameter, referred to Viti, R. and Guerriero, R. (2006).

Cultivar	Growth habit	Tree height (m.)	Tree canopy diameter (m)	Volumetric Index (h/l)	Branch insertion angle (°)
Antonio Errani	Regular	4.2 bc	4.1 abc	1.04 c	40.6 d
Bora	Upright	4.1 bcd	3.1 e	1.35 b	48.8 b
Bulida	Regular	4.6 a	4.1 ab	1.11 c	43.3 cd
Luna	Spur	3.8 de	3.3 de	1.15 c	50.1 b
Magic Cot	Upright	4.3 ab	3.2 de	1.35 b	48.6 b
Pellecchiella	Spur	3.9 cd	3.6 cd	1.10 c	49.3 b
Prima	Upright	4.2 bc	2.7 e	1.53 a	39.0 d
Rubista	Regular	3.1 f	2.8 e	1.11 c	42.5 cd
San Castrese	Open	3.4 ef	4.4 a	0.79 d	63.8 a
Spring Blush	Open	3.8 cde	4.5 a	0.84 d	67.2 a
Tsunami	Spur	4.1 bcd	3.8 bc	1.08 c	46.3 bc

The flowering stages of apricot cultivars are shown in Table 2. Magic Cot and San Castrese was the earliest cultivar to bloom, and Rubista was the latest. Full flowering period of the cultivars ranged between March 1 and March 20.

Table 2. Main characteristics of recently introduced apricot cultivars (average values of four years 2017-2020)

Cultivar	Flowering period	Autofertility*	Maturity period, compared to S. Castrese
Antonio Errani	2-10 March	SC	-18
Bora	4-16 March	SC	-15
Bulida	5-17 March	SC	-12
Luna	7-20 March	SI	-25
Magic Cot	27 February-8 March	SI	-25
Pellecchiella	8-17 March	SC	+15
Prima	5-15 March	SI	-23
Rubista	10-25 March	SC	-19
San Castrese	1-17 March	SC	
Spring Blush	2-12 March	SI	-27
Tsunami	3-15 March	SI	-28

* Autofertility : SC – auto-compatible, SI - auto-incompatible.

As turned out in the Table number 2, Spring Blush, Magic Cot, Prima, Tsunami, Luna cultivars need pollinating plants, while others are self-pollinating.

Fruit ripening period, compared to the standard (San Castrese), resulted to be (in number of days) for Tsunami (-28), Spring Blush (-27), Luna, Magic Cot (-25), Prima (-23), Rubista (-19), Antonio Errani (-18), Bora (-15), Bulida (-12) and Pellecchiella (+15) which was the variety with latest ripening period. Comparative assessment of ripening period, shows that most of the

varieties recorded early fruit ripening. Maturity time of these cultivars, creates a production conveyor in the market from the beginning of May until the last ten days of June.

As it's shown in Table number 3, there are some significative differences between traits of analyzed cultivars. The comparison of fruit size pointed out as varieties with largest fruit cultivars Antonio Errani, Bora, Bulida and Magic Cot, meanwhile with smallest fruit Spring Blush, San Castrese and Rubista. The highest malic acid content was measured for San Castrese variety.

Table 3. Some fruit characteristics of apricot cultivars (mean values of four years).

Cultivar	Fruit					
	Diameter (mm)	Weight (g)	Pulp /seed ratio	Soluble solids (°Brix)	Acidity (%)	SSC/TA*
Antonio Errani	51.8 a	73.9 a	22.8 bc	16.8 a	1.12 d	1.49 a
Bora	53.1 a	78.1 a	19.5 cd	16.5 ab	1.28 d	0.97 cd
Bulida	51.6 a	71.5 a	19.7 cd	12.5 de	1.7 b	0.97 cd
Luna	50.6 ab	58.9 bc	20.9 cd	10.2 e	1.75 abc	0.57 e
Magic Cot	51.9 a	72.3 a	27.1 ab	12.1 de	1.41 bcd	0.86 de
Pellecchiella	45.8 cd	53.3 c	18.7 cd	13.5 cd	1.27 d	1.06 bcd
Prima	47.8 bc	71.1 ab	21.3 cd	13.8 abcd	1.13 d	1.21 abcd
Rubista	41.4 ef	40.5 d	16.7 d	14.1 bcd	1.06 d	1.33 ab
San Castrese	43.4 de	46.5 cd	19.1 cd	11.8 de	2.1 a	0.56 e
Spring Blush	39.6 f	39.8 d	21.3 cd	11.6 de	1.32 cd	0.87 de
Tsunami	45.1 cde	51.4 cd	29.4 a	15.9 abc	1.05 d	1.23abc

*SSC/TA - Ratio of soluble solids (Brix) to titratable acidity.

SSC/TA ratio is a good indicator of fruit quality. As higher the ratio, the sweeter the fruit taste is perceived. Varieties such Antonio Errani and Rubista rezulted with the sweetest taste. .

Table number 4 shows average and cumulative values related to total production of analyzed apricot plants. Due to differences in planting period, varieties like Antonio Errani, Bulida, Pellecchiella and San Castrese are planted earlier and showed high total yield indicators. In terms of comparative assessment of yield/trunk section area, the most productive variety result Rubista, followed by Magic Cot and Bora.

Comparative survey of these apricot varieties throughout four years, clearly indicated significative differences in total productivity during different years (data not shown). The lability observed is related to fluctuating temperatures effects during and in the end of flowering period, especially occurred in 2018 and 2020 [15]. The last year resulted the year with the most damages in productivity because of drastic low temperatures in the end of the flowering.

fluctuating temperatures has affected the fruit cracking. Varieties Bora and Pellecchiella are recorded to have more cracked fruits compared to other varieties. Climate changes are frequently causing extreme climate events, as apricot is easily influenced, it would be beneficial that the most preferred commercial varieties to be in observation for long terms.

Economic analysis showed that cultivars with earlier ripening are more effective than later ripening varieties.

Table 4: Crosscut surface of stem 30 cm above ground and yield parameters of the apricot cultivars (average of four years)

Cultivar	Crosscut surface of stem 30 cm above ground	Yield (kg/tree)	Yield (t/ha)	Cumulative yield (kg/tree)	Cumulative yield (t/ha)	Yield/trunk section area (kg/cm2)
Antonio Errani	258.7 a	16.8 a	7.4 a	67.5 a	29.6 a	0.06 e
Bora	92.4 c	12.7 b	5.6 b	50.9 b	22.4 b	0.14 bc
Bulida	219.7 ab	17.1 a	7.4 a	68.1 d	29.9 a	0.08 cde
Luna	87.5 c	9.5 cde	4.2 cde	38.2 cde	16.8 cde	0.12 bcde
Magic Cot	85.1 c	12.5 bc	5.5 bc	50.1 sc	22.1 bc	0.16 b
Pellecchiella	209.2 b	16.4 a	7.2 a	65.7 a	28.9 a	0.08 cde
Prima	89.8 c	11.1 bcd	4.8 bcd	44.5 bcd	19.5 bed	0.13 bcd
Rubista	46.6 d	8.9 de	3.9 de	35.9 de	17.7 de	0.22 a
San Castrese	225.6 ab	18.7 a	8.2 a	75.1 a	33.1 a	0.08 de
Spring Blush	84.7 c	7.6 e	3.3 e	30.1 e	13.5 e	0.09 cde
Tsunami	79.3 cd	8.5 de	3.7 de	34.2 de	15.01 de	0.11 bcde

Other climatic phenomenon has affected apricot's productivity. Rainfalls associated with

CONCLUSIONS

Comparative study of eleven apricot varieties in coastal regions of Albania, proved potential of these varieties for cultivation in this region. Despite significative differences amongst them, regarding most of the indicators estimated, depend on investors to select the proper variety, based on their targets and market inclinations [23]. Evidences of this study provide enough data to determine the cultivation technology for these varieties. An expanded study is essential for spreading these varieties in whole regions of the country.

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THE DEPOLLUTION OF THE WHEY REJECTED BY THE CHEESE INDUSTRIES USING CHEMICAL, PHYSICAL AND MICROBIOLOGICAL METHODS

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ABSTRACT

In most developing countries, the cheese industry releases thousands of liters of whey a day into the wild. This whey is considered a waste. It has great biological value but it is also very polluting and harmful to the environment. The aim of this study is to evaluate several methods of depolluting whey on a laboratory scale and extrapolate the results on a large scale: the industrial scale. First, the physico-chemical characterization of the whey harvested from *GIPLAIT Tlemcen* (Algeria) was carried out. Then, different treatments were realized on our samples: physical, chemical and microbiological. The measurement of BOD5 (via Oxitop) of the different samples was made before and after treatment in order to evaluate the depollution performance. The results showed that the deproteinization and de-sugaring by microbiological culture had the highest depollution efficiency, a decrease of the BOD5 that reached 29%. These results can be obtained by ultrafiltration, nanofiltration, reverse osmosis and bioreactor systems on an industrial scale. As a conclusion, the depollution of the whey goes through its de-sugaring. The best and easiest applicable methods of depollution/valorization are the drying/atomization and the ultrafiltration/nanofiltration.

Key words: Whey, Depollution, BOD5

INTRODUCTION

The dairy industry, especially the cheese industry, occupies a large place in the food industry market. Large quantities of milk are used for cheese production, which result in the release of large quantities of whey after production. This release impacts heavily the environment due to the high organic matter of whey which is a perfect environment for many microorganisms. Thus, making it the food industries waste with the highest BOD and COD.

Whey generates significant organic pollution: 1 liter corresponds to around 85% of the daily pollution generated by an inhabitant (Laplanche, 2006). Despite this, whey has great biological value. In several countries, it is used to obtain several other products such as lactic acid, bio-ethanol, dietary proteins… etc. This recovery or valorization allows partial or total depollution

of the whey. Several studies have been discussed on this subject in Algeria, a country which neither values nor cleans up whey, such as Gana & al (2001) as well as Tebbouche (2012). Moreover, no action has been taken by the involved industries. In this study, authors assessed the performance of several methods for depolluting whey: physical, chemical and microbiological methods while focusing on what is achievable and applicable on a large scale and on the Algerian cheese industry (or the classic cheese industry).

MATERIALS AND METHODS

Physico-chemical characterization of the harvested whey

To begin with, we obtained the whey from the *GIPLAIT Tlemcen* cheese factory (in february 2020) that rejects around 3,500 liters of whey per 5,000 liters of milk used to produce Swiss cheese. The whey obtained was stored in plastic drums under a temperature between 0 °C and 10 °C. After the harvest, the physico-chemical characterization was carried out. The measured parameters, which are the most significant and important tools in our study are: Water, Dry extract, Protein content, Lactose content, Fat content and Acidity (pH).

- Water and **dry extract** (by *lyophilization* and *infrared drying*):

Lyophilization: The whey was frozen for 24 hours under a temperature of -80 °C via a ***Zhongke Meiling HL100/218/290*** freezer. After that, the frozen whey was placed in the ***Christ Alpha 12 LD+*** lyophilizer, the temperature and pressure were adjusted and lyophilization started. 24 hours later, the whey powder was recovered and the pre and post lyophilization weighing were compared.

Infrared drying: A 5g sample was placed on an aluminum display stand which was itself placed on a ***Ohaus MB25*** desiccator. Infrared drying occurred. Once finished, the displayed percentage which refer to the water content was noted.

-Protein content (by *spectrometry* /*Biuret method*):

We carried out increasing dilutions of our sample (1/8, 1/16 and 1/32) and added to the batch the standard solutions based on gelatin (1 g/ L, 5 g/L, 10 g/L) as well as the blank (distilled water) (all volumes are 1 ml). 1.2 ml of Biuret reagent were added to all the solutions and then were dried for 30 min at 30 ° C. After incubation, the optical density of all the solutions was measured, starting with the blank in order to calibrate the spectrometer (the wavelength was adjusted to 540 nm). The results obtained for the experimental curve were then compared to the standard curve and the protein content of our sample was extrapolated.

-Lactose content (by *titration* /*Fehling method*):

We started by centrifuging 20 ml of our sample at 3000 rpm for 5 minutes. 10 ml of supernatant were recovered and added to 30 ml of methanol and left for 10 minutes (liquid/liquid extraction). Centrifuge was carried out for 10 min at 3000 rpm and the supernatant that contains the sugars were recovered. Two titrations were carried out simultaneously, with the same titrating solution (2 ml Fehling liquor A + 2 ml Fehling liquor B). For the titrated solutions, we had on one side our sample V1 (supernatant obtained after liquid/liquid extraction and centrifugation) and on the other side a control solution V2 (glucose solution at a rate of 5 g/L C2). The sugar content was obtained by the following formula:

[C2 = ((C1xV1)/V2)x5x4]

-Fat content (by *acid-butyrometric assay* /*Gerber method*):

In a Gerber butyrometer: 10 ml of whey were added to 1 ml of isobutyl alcohol and 10 ml of NaOH (6.5%). The mixture was heated to 60-65 °C for 4-5 min and then centrifuged (1500 rpm) for 4-5 min. Reading by pointing the base of the butyrometer upwards.

-Acidity or **pH** (by *pH meter*).

Depollution tests

Several depollution methods have been carried out, each method retains or excludes a category of molecules from our sample.

The parameter measured was BOD5 or biochemical oxygen demand after 5 days of incubation, this parameter was chosen for its speed and ease of application as well as for the unnecessity of using nitrification inhibitors such as allyl thio- urea).

The treated solutions were named as follows: L.D.P.S (deproteinized whey by saline precipitation), L.D.P.T (deproteinized whey by thermoprecipitation), L.D.L.A (de-sugared whey by alcoholic solubilization), L.D.P.L.M (deproteinized and de-sugared whey by microbiological culture).

Two control solutions were added to the measurement batches: Lac N (untreated whey /in order to measure the depollution yield), Glucose control (glucose solution /in order to attest the validity of the microbiological strains used for the measurement).

The data conversion formula used is as follows: **[DBO5 = Readed value*Table factor*Dilution factor] (**this formula was designed according to the instructions presented in the manual directed by Tandia, 2007).

The BOD5 was mesured with an ***Oxitop*** device.

Figure 1 serves as a summary of the measurement protocol applied for the present study

RESULTS AND DISCUSSION

Physico-chemical characterization:

The results obtained (**Tab.1**) conform to the bibliographic references, however two parameters remain outside the batch:

The **lactose content***, which was 30% less than what was mentioned in the references, was due to the fact that the harvested whey was obtained by an acid coagulation process via lactic ferments, these same ferments had to consume lactose present in whey to produce lactic acid which lead to the coagulation of milk.

The **protein content****,which was 15% less than what was mentioned in the references, was due to the fact that the coagulation processes used to obtain the harvested whey were carried out at high temperatures thus leading to a low thermoprecipitation of the proteins found in the product of the milk coagulation (Swiss cheese).

Tab.1 Physico-chemical characteristics of the harvested whey.

	Harvested whey (%)	**Acid whey (%) (Morr & al. 1993 ; Linden & al. 1994)**
Water	**93,99**	**93,5**
Dry extract	**6,01**	**6,5**
Lactose	**3,1***	**4,71**
Proteins	**0,5****	**0,75**
Fats	**0,05**	**0,03**
Acidity (pH)	**4,57**	**4,6**

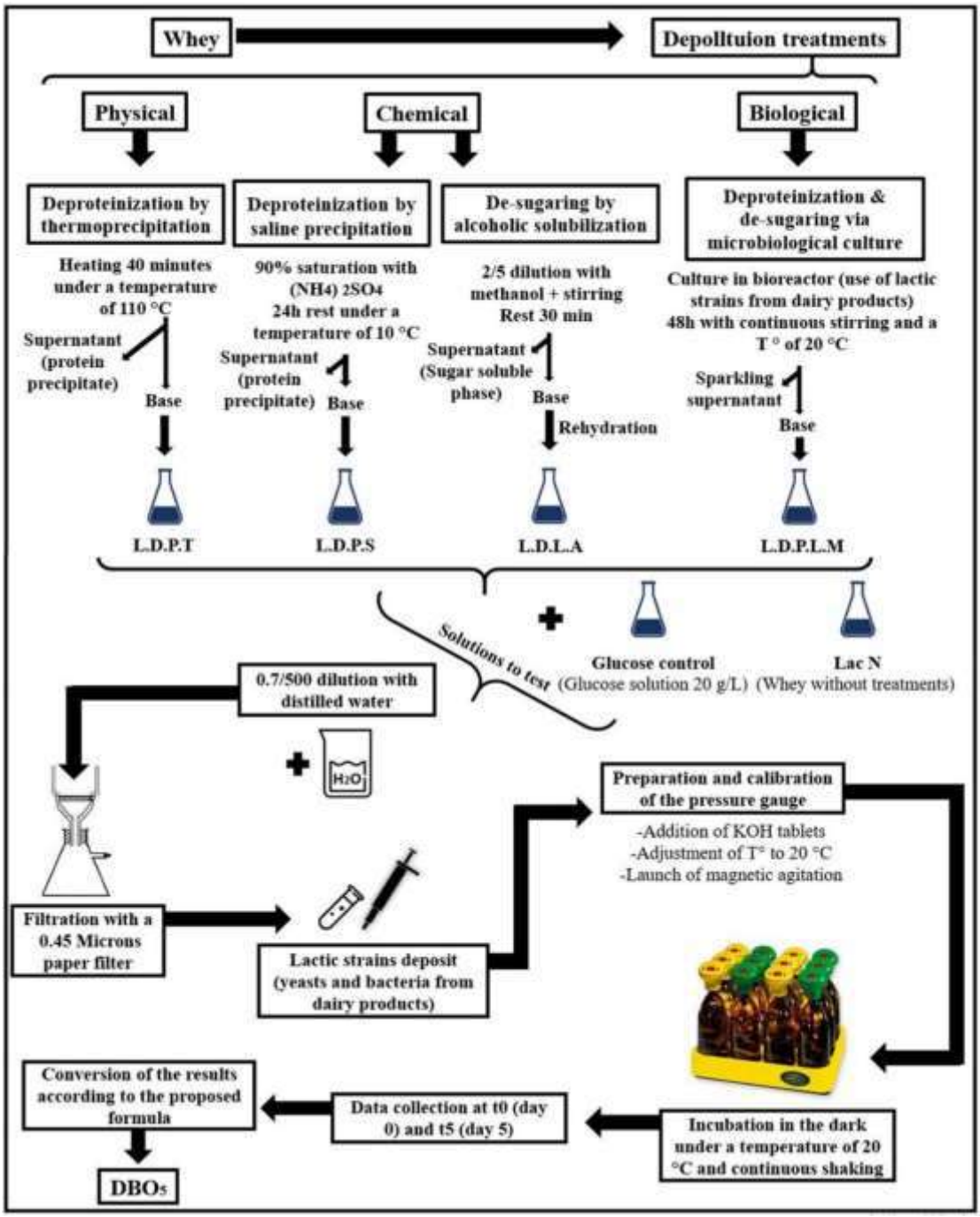

Fig.1 Summary of depollution protocols and their measurement.

Depollution tests

About the depollution tests, the results obtained are shown in Figure 2. The Glucose Control had a BOD5 of 28 751 mg O2/L which attests to the validity of the microbiological strains used for the measurement. Lac N had a BOD5 of 50 000 mg O2/L, a value that falls within the referential range proposed by Yorgun & al. 2008 (40 000 – 60 000 mg O2/L). The deproteinization by saline method L.D.P.S and by thermal method L.D.P.T had a depollution yield of 17% and 14% (BOD5 which reaches 41 428 and 42 857 mg O2/L) respectively. This small difference is due to the fact that the salt used for saline deproteinization was still present in the culture medium in small quantities and could have inhibited the growth of lactic strains in a rather weak manner. De-sugaring by alcoholic method L.D.L.A allowed a rise in BOD5 which reached 70 000 mg O2/L which represents a false positive explainable by two hypotheses:

-H1: The alcohol used for de-sugaring that remain present in the environment despite the dilution, had evaporated and interfered directly or indirectly with the measurement sensors integrated in the external caps of the opaque vials)

-H2: The alcohol present in the medium was so diluted that it could have been used as a source of carbon by microorganisms.

Deproteinization and de-sugaring by microbiological culture L.D.P.L.M had a depollution yield of 29% (BOD5 which nearly reached 35 000 mg O2/L) which represents the best depollution yield. This result can be explained by the fact that the microorganisms used during microbiological culture had to degrade a maximum of nutrients (Proteins and Lactose) thus leading to a depletion of the medium in organic molecules and therefore of its polluting effect. The L.D.P.L.M depollution yield can even reach 90% if carried out in better conditions as done by Gana.S & al., 2001 in their work on the DCO.

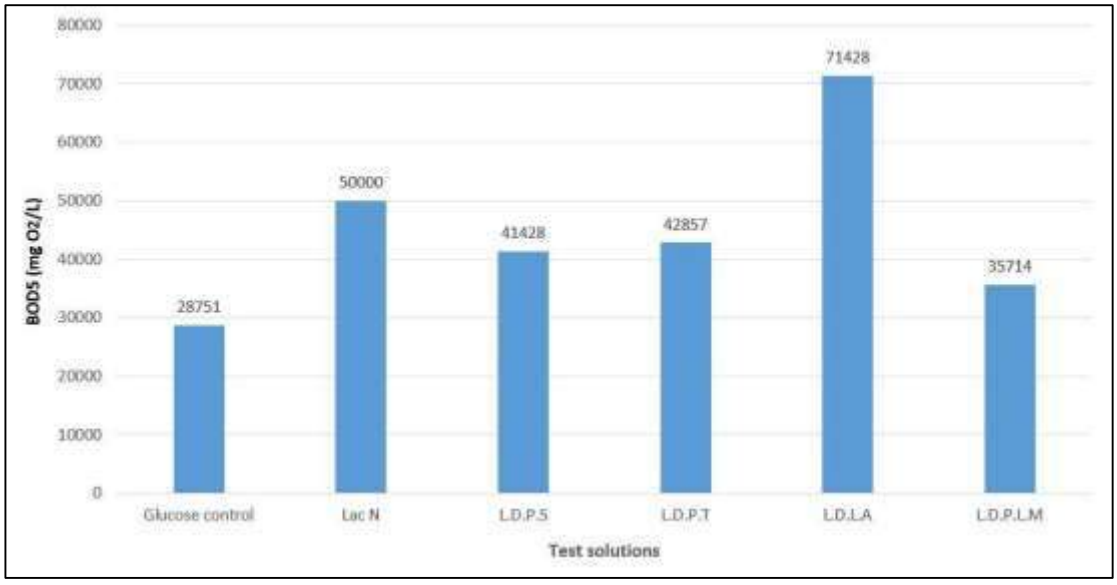

Fig.2 The variation in BOD5 according to the different treatments carried out on the harvested whey (as well as the controls).
*(**Glucose control** glucose solution at 20 g/L, **Lac N** whey without any treatment, **L.D.P.S** whey deproteinized by salts, **L.D.P.T** whey deproteinized by thermoprecipitation, **L.D.L.A** whey de-sugared by alcohol, **L.D.P.L.M** whey deproteinized and de-sugared by microorganisms).*

Extrapolation of the results

The extrapolation of the best results obtained to an industrial scale will be to opt for technologies such as ultrafiltration/nanofiltration or industrial bioreactors if we wish to totally depollute this waste and valorize it in an advanced way, or for drying/atomization in case we want to reduce the volume of the waste and recover it in its most practical form « powder » which is easily storable and salable (1 000 €/t in 2013, FranceAgriMer, 2013).

CONCLUSION

According to the tests carried out and the results obtained, deproteinization added to the desugaring of whey allowed a significant reduction of its BOD5: up tp 29%, thus enabling us to obtain a whey that can be rejected or reused as irrigation water without having harmful repercussions on the environment. There are several methods for depolluting whey: directly or through valorization processes. These methods are known and widely used in the West, and despite numerous researches and publications on the recovery and the polluting impact of whey in Algeria, industries that reject it, remain indifferent which is unfortunately, a common problem with several developing countries.

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COMPARISON OF NUTRIENT AND FATTY ACID CONTENTS OF MAIZE PRODUCED AS THE FIRST CROP AND THE SECOND CROP

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ABSTRACT

The present study was carried out to determine of nutrient and fatty acid contents of maize varieties grown as the first crop in Batman and the second crop in Mardin. In the research, four different types of maize were used. These maize varieties are PR (Pi), which is P1921 and P2088 as the first crop in Batman province, and PR32T83 and P0729 grown as the second crop in Mardin province between September and November, 2017-2018. It has been studied total 48 maize samples. In each one of Batman and Mardin province, 24 maize samples, from 3 fields for each variety, 6 fields in total and 4 samples from each field. For the maize varieties used in the study, it was determined that crude ash (CA) ranged between percent 0.13-1.22, organic matter (OM) 85.82-89.61, crude protein (CP) 5.60-6.89, crude oil (CO) 0.97-1.69, crude cellulose (CC) 2.55-2.96, free-nitrogen substance 75.73-79.50, acid detergent fiber (ADF) 2.69-3.03, neutral detergent fiber (NDF) 8.58-13.08, acid detergent lignin (ADL) 3.00-303, starch ratio 64.22-64.95, sugar 1.20-1.21, metabolic energy (ME) ruminant (Mcal /kg) 2.86-2.90, poultry (kcal/kg) 3126.96-3194.61, palmitic acid 3.50-3.68, oleic acid 8.16-8.63, linoleic acid 16.00-16.98, omega 6 fatty acid 16.00-16.98 and saturated fatty acids 32.27-34.17 %. The difference between the first crop and the second crop CA, OM, CP, CO, CC, NDF, ME poultry, palmitic acid, oleic acid, linoleic acid, omega 6 and saturated fatty acid levels were statistically significant ($p<0.05$ and 0.001). In general, it was determined that maize cultivars grown as the first crop were higher in terms of nutrient content and maize cultivars grown as the second crop were higher in terms of fatty acid content. Considering the importance of maize in animal nutrition, it can be recommended to grow PRT83 in Mardin province and P2088 in Batman province.

Keywords: Nutrient, Fatty acid, The fist crop, The second crop, Grain maize

INTRODUCTİON

It is estimated that 60% of the maize produced in the world is used as animal feed, 20% of maize as human food (direct consumption), 10% as processed food and 10% as other consumption and seed (Özcan, 2009). Since mazie contains unsaturated fatty acids, it causes soft body fat in beef cattle. Although it is suitable for use in the rations of beef cattle, excessive use in dairy cows is not suitable because it softens the butter consistency. Storing maize in crushed or powdered form for a long time will cause the oil in its content to become bitter. Maize should be given limited quantities to breeding herd. Otherwise, it causes fatten and negatively affects fertility. When maize is used in poultry diet, it should be supplemented with the amino acids lysine, tryptophan and methionine (Ergün ve ark., 2016). Kutlu (2009) reported

that maize can be used up to 60% in poultry diets and its use should be given attention, as its excessive use may cause body fat softening. Therefore, in this study, it was aimed to determine the nutrient and fat content of the PR two maize varieties grown as the first crop in Batman and as the second crop in Mardin, due to their importance in animal nutrition, especially in poultry feeding.

MATERİALS AND METHODS

Four different maize varieties were used, namely PR (Pioneer) two maize varieties grown as the first crop in Batman province (P1921 and P2088) and PR two corn varieties grown as the second crop in Mardin (PR32T83 and P0729). The study was conducted between September and November 2017-2018. The research was carried out in Mardin and Batman provinces. A total of 48 maize samples were analyzed, 24 corn samples from 3 fields (6 fields in total and 4 samples from each field) for each variety in Batman province, 24 corn samples from 3 fields for each variety in Mardin (6 fields in total and 4 samples from each field). The samples were grinded in the Van Yüzüncü Yıl University Faculty of Agriculture, Department of Animal Science and prepared for analysis.

In Grain maize; Crude ash, Organic matter, Crude protein, Crude oil, Crude fiber, ADF (Acid detergent fiber), NDF (Neutral detergent fiber) and Nitrogen-free extract According to the Weende analysis method and the method reported by Kutlu (2008), starch, sugar and fatty acids in maize and were made in NIR (Near Infrared) Spectroscopy device. Metabolic energy (ME) was calculated according to Alderman (1985) for ruminant animals and Carpenter and Clegs (1956) for poultry. The SAS (2015) package program was used while statistically evaluating the data obtained in the experiment. While t test is used when comparing first and second year products; Duncan multiple comparison test was used when comparing all varieties (Bek & Efe, 1988).

RESULTS AND DİSCUSSİON

Nutrient analysis values of first and second crop maize varieties are given in Table 1**.**

Table 1: Nutritional analysis values of first and second crop maize varieties

Maize Varieties	**1. Crop**		**2. Crop**	
	P1921	**P2088**	**P0729**	**PRT83**
Crude Ash (%)	1.03a	1.22a	0.13c	1.11b
Mean	1.13a		0.62b	
Organic Matter (%)	86.90c	89.61a	85.82d	88.84b
Mean	88.26a		87.33b	
Crude Protein (%)	6.53b	6.89a	6.16c	5.60d
Mean	6.70a		5.88b	
Crude Oil (%)	1.69a	1.45b	0.97d	1.23c
Mean	1.57a		1.10b	
Crude Fiber (%)	2.61b	2.65b	2.96a	2.55b
Mean	2.63b		2.75a	
Nitrogen-Free Extract (%)	76.21b	79.34a	75.73b	79.50a
Mean	77.71		77.61	
ADF (%)	2.83bc	2.96ab	2.69c	3.03a
Mean	2.89		2.86	

NDF (%)	9.63bc	13.08a	8.58c	10.39b
Mean	11.33a		9.48b	
ADL (%)	3.03	3.01	3.02	3.00
Mean	3.02		3.01	
Starch (%)	64.22b	64.95a	64.83a	64.83a
Mean	64.56		64.83	
Sugar (%)	1.20	1.21	1.20	1.20
Mean	1.20		1.20	
Poultry (kcal/kg)	3194.1a	3178.22a	3126.96b	3126.97b
Mean	3186.77a		3126.96b	
Ruminant (Mcal/kg)	2.90a	2.88b	2.90a	2.86c
Mean	2.89		2.88	
C16:0 (Palmitic Acid)	3.60a	3.50b	3.68a	3.61a
Mean	3.55b		3.65a	
C16:1 (Palmitoleic Acid)	0.10	0.10	0.10	0.10
Mean	0.10		0.10	
C18:0 (Stearic Acid)	0.60	0.60	0.60	0.60
Mean	0.60		0.60	
C18:1 (Oleic Acid)	8.40b	8.16c	8.63a	8.40d
Mean	8.29b		8.52a	
C18:2 (Linoleik Asit)	16.48b	16.00c	16.98a	16.55b
Mean	16.25b		16.77a	
C18:3 (Linolenik Asit)	0.30	0.30	0.30	0.30
Mean	0.30		0.30	
Omega 3	0.30	0.30	0.30	0.30
Mean	0.30		0.30	
Omega 6	16.48b	16.00c	16.98a	16.48b
Mean	16.25b		16.73a	
Saturated Fatty Acid	33.25b	32.27c	34.17a	33.33b
Mean	32.78b		33.75a	

*: The difference between the group average shown with different letters on the same line is statistically significant. (P<0.05).

For the crude ash values of first and second crop maize varieties, the differences between varieties and averages were found significant (P <0.05). As seen in Table 1, the highest crude ash ratio was found in P2088 maize variety with 1.22%, while the lowest crude ash ratio was found in P0729 maize variety with 0.13%. Biro et al. (2009) 1.41-1.66%, Dumral (2015) 1.07-1.16% and Sabancı (2016) 1.09-1.32% crude ash values were similar to the values we found in our study. Macro and micro minerals are found in crude ash. Therefore, grain maize with high crude ash content means higher macro and micronutrient content. If the feed samples were not grain maize but powder corn samples or mixeed feed samples taken from outside, it would be recommended to make an analysis of ash insoluble in 10% HCl acid to be able to say this clearly.

For the first and second crop maize varieties, the differences between the varieties and the averages in terms of organic matter values were found to be significant (P <0.05). The highest organic matter ratio was found in P2088 maize variety with 89.61%, while the lowest organic matter ratio was found in P0729 maize variety with 85.82% (Table 1). Denek and Deniz (2004) 94.32%, Ceresnakova et al. (2006) 98.57%, Öz düven et al. (2009) 92.18-94.82% and

Simko et al. (2011) 98.5% organic matter values were higher than the values we found in our study. Organic matter is the remaining portion of the feed after the crude ash portion is removed from the dry matter. Maize with high content of organic matter contains more nutrients than maize with low content. Because all nutrients (crude protein, crude oil, crude fiber and nitrogen-free extract) are found in organic matter.

For crude protein values of first and second crop maize varieties, differences between varieties and averages were found significant ($P < 0.05$). The highest crude protein ratio was found in P2088 maize variety with 6.89%, while the lowest crude protein ratio was found in PRT83 maize variety with 5.60% (Table 1). Dumral (2015) 6.1-7.9%, Sabancı (2016) 6.18-7.84% and Keskin et al. (2018) 5.69-8.14% crude protein values were in agreement with the values we found in our study. Crude protein is an important parameter in terms of digestion and feed value, as it contains all components containing nitrogen. The digestion degree of maize with high protein content is higher than that of corn with low protein level.

For the crude oil values of first and second crop maize varieties, the differences between varieties and averages were found significant ($P < 0.05$). The highest crude oil ratio was found in P1921 maize variety with 1.69%, while the lowest crude oil content was found in P0729 maize variety with 0.97% (Table 1). Kılınç (2016) 3.33-4.00%, Sabancı (2016) 2.71-3.24% and Kahraman et al. (2017) 3.07-4.17% crude oil values were lower than the results we found in our study. This situation can be attributed to the difference of maize varieties used, as well as climate and soil characteristics. The quality of maize with high crude oil content is higher than the quality of maize with low crude oil content. Maize with high oil content is important not only for animal feeding, but also for other industries, especially in the oil industry.

For the crude fiber values of first and second crop maize varieties, the differences between varieties and averages were found significant ($P < 0.05$). The highest crude fiber ratio was found in P0729 maize variety with 2.96%, while the lowest crude fiber ratio was found in PRT83 maize variety with 2.55% (Table 1). Lucchin et al. (2003) 1.88-2.36%, Güngör et al. (2007) 1.89-3.14% and Oner and Aykutlu (2019) 2.14-3.79% crude fiber values showed similarity with the values we found in our study. The degree of digestion of crude fiber in ruminants and poultry is different. In general, cellulose is well digested by ruminants but not by poultry. Cellulose content in maize provides feeling to fullness ruminant animals.

While the differences in nitrogen-free extract values of the first and second crop maize varieties were significant ($P<0.05$), it was determined that the difference between the averages was not significant ($P>0.05$). The highest nitrogen-free extract ratio was found in the PRT83 maize variety with 79.50%, while the lowest nitrogen-free extract was found in the P0729 maize variety with 75.73% (Table 1). Güngör et al. (2007) 75.62-78.71%, Biro et al. (2009) 81.75-82.99% and Simko et al. (2011) values of 82.5% were in agreement with the values we found in our study. The nitrogen-free extrtact is the part that forms the sugar and starch part of the feed. These differences seen in the nitrogen-free extract values of maize; can be attributed to the differences seen in the maize variety, the region where it is grown and the soil structure. Maize is important not only for animal nutrition but also for its use in starch industry.

ADF values of first and second crop maize varieties were found to be significant ($P <0.05$), but the difference between the averages was not significant ($P> 0.05$). While the highest ADF rate was found in PRT83 with 3.03%, the lowest ADF rate was found in P0729 maize variety with 2.69% (Table 1). Newman et al. (2016) 1.82-3.14% and Keskin et al. (2018) had

found as 2.72-4.72% ADF rate of maize, and the values they found were similar to the values we found in our study. ADF is the part of feed that consists of cellulose and lignin. Since cellulose is difficult to digest, the digestibility and energy level of maize with low ADF rate is higher than maize with high ADF rate.

NDF values of the first and second crop maize varieties, and the differences between both varieties and averages were found to be significant (P <0.05). While the highest NDF ratio was found in P2088 maize variety with 13.08%, the lowest NDF ratio was found in P0729 maize variety with 8.58% (Table 1). Zilic et al. (2011) 11.02% -14.72 and Newman et al. (2016) found 7.02-10.14% and the values that they found are in accordance with the values that we found in our study. NDF is the part consisting of cellulose, lignin and hemi cellulose. NDF provides information about the digestion of maize. High cellulose content of maize affects the NDF ratio. There is a direct proportion between the NDF ratio and the volume of maize. The volume of maize with a high NDF ratio is greater than the volume of maize with a low NDF ratio.

ADL values of first and second crop maize varieties were determined to be insignificant between both varieties and averages (P> 0.05). The highest ADL ratio was found in P1921 maize variety with 3.03%, while the lowest ADL ratio was found in PRT83 maize variety with 3.0% (Table 1). Özdüven et al. (2009) 2.64-4.44% and Reddy et al. (2013) values found as 2.8-5.3%, showed similarity with the values we found in our study. These differences seen in the study can be attributed to the variety of maize, climate and soil structure.

For the starch values of first and second crop maize varieties; While the difference between the varieties was found to be significant (P <0.05), it was found that there was insignificant difference between the means (P> 0.05). The highest starch rate was found in P2088 maize variety with 64.95%, while the lowest starch rate was found in P1921 maize variety with 64.22% (Table 1). Ali et al. (2010) 58.33-67%, Dumral (2015) 60.8-64.2% and Kılınç (2016) 64.28-65.57% were similar to the values we found in our study. These differences seen in the study can be attributed to the variety of maize, climate and soil structure.

It was determined that the difference in sugar content of first and second crop maize varieties, between both varieties and averages was not significant (P> 0.05). The highest sugar rate was found in maize variety with 1.21% P2088 (Table 1). Saleem et al. (2008) 0.65-1.93% and Ramchandran et al. (2016) 0.50-1.32% values showed a close relationship with the values we found in our study. These differences seen in the study can be attributed to the variety of maize, climate and soil structure.

In terms of ruminant (Mcal/kg) ME (Metabolic Energy) values of first and second crop maize varieties, the differences between maize varieties were found to be significant. (P <0.05). For ruminants, it was determined that the difference between the mean ME values of first and second crop maize was not significant (P> 0.05).
When a comparison is made in terms of ME values for poultry; It was determined that there is a significant difference between the groups in terms of the average values of first and second crop maize and first and second crop maize varieties (P <0.05). The highest ME value for poultry was found in P1921 maize variety with 3194.61 (Kcal/kg), while the lowest ME value for poultry was found in P0729 maize variety with 3126.96 (Kcal/kg). The highest ME value for ruminant was found in maize varieties P1921 and P0729 with 2.90 (Mcal/kg), while the lowest ME value for ruminant was found in PRT83 maize variety with 2.86 (Mcal/kg) (Table 1). In this study, the results of grain maize on metabolic energy values for both poultry and

ruminant animals was lower than values stated as Güngör et al. (2007), 3333-3462, Kalkan and Sade (2009) 3696-3771 kcal/kg, 12.1-14.5, Rodrigues et al. (2014) 13.6-15.4 and Keskin et al. (2018) 3.22-3.27. These differences between studies are due to the different nutrient content of maize varieties. Maize is an energy-rich feed. The reason why the energy value is different in ruminants and poultry is due to the difference in the contents of the nutrients used in the ME level calculation and the formula used in the calculation.

In the first and second crop maize, for C16:0, C18:1, C18:2, Omega 6 and saturated fatty acids values, differences between both varieties and averages were found to be significant ($P < 0.05$). For first and second crop maize C16:1 C18:0, C18:3 and Omega 3 values, differences between both varieties and averages were not found significant ($P > 0.05$). The highest C16:0 value was found in P0729 maize variety with 3.68, while the lowest (C16: 0) value was found in P2088 with 3.50. The highest C18:1 value was found in P0729 maize variety with 8.63, while the lowest value was found in P2088 with 8.16. The highest C18:2 value was found in P0729 maize variety with 16.98, while the lowest C18:2 value was found in P2088 with 16.00. The highest Omega 6 value was found in P0729 maize variety with 16.98, while the lowest Omega 6 value was found in P2088 corn variety with 16.00. The highest saturated fatty acids value was found in P0729 maize variety with 34.17, while the lowest saturated fatty acids value was found in P2088 with 32.27 (Table 1). When the results obtained in general are examined, (C16:0) palmitic acid, (C18:1) oleic acid, (C18:2) linoleic acid, Omega 6 and saturated fatty acid values in grain maize were lower than the results reported by Goffman and Böhme (2001), Saleem and et al. (2008), Saoussem et al. (2009), Ali et al. (2010), Della et al. (2010), Öner and Aykutlu (2019). The low values that we have found are thought to be due to the growing period, climatic conditions, agricultural practices and the corn variety used. Fatty acids have a positive effect on the growth, development and yield of livestock. Fatty acids in feeds are important in terms of closing the energy gap.

CONCLUSİON

In the study, the highest rate of organic matter, crude ash, crude protein, crude fiber, nitrogen-free extract, ADF and NDF was found in P2088 corn variety grown as the first crop in Batman; The highest crude fat, ADL, metabolic energy levels and sugar ratio was found in P1921 variety, which was grown as the first crop in Batman. The highest fatty acid levels (C16:0, C18:1, C18:2, Omega 6 and saturated fatty acids) were found in P0729 maize variety grown as the second crop in Mardin. In general, the nutrient content and yield-based parameters of the maize varieties grown in Batman were higher than the maize varieties grown as the second crop in Mardin. These differences can be attributed to the variety of maize grown, the period it was grown, environmental factors, climate and field practices.

Most of the maize grown as the first crop in Batman is processed in factories in Mardin province and turned into corn flour and starch or added to mixed feed. It is thought that the increase in nutrient content of maize, which has the highest energy content among grains, can both increase the quality of the products and reduce the cost of mixed feed by reducing the amount of expensive feeds to be added to the ration. So, this will contribute greatly to the country's economy. For this reason, suitable varieties for regions and territories should be determined and recommended to farmers. In this respect, new studies are needed to determine the yield, nutrient and fatty acid levels of different varieties of first and second crop maize in different regions.

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THE BEHAVIOUR OF DIFFERENT HYBRIDS OF SUNFLOWER IN THE CLIMATIC CONDITIONS OF THE YEARS 2019 AND 2020 IN SOUTH-EAST OF ROMANIA – DOBROGEA

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ABSTRACT

The experimental field was placed in Amzacea, Constanta County within the climatic conditions of years 2019 and 2020(Dobrogea area), had the largest weight regarding the surface cultivated in Romania with sunflower crop between 10-12%. The most drought area in Romania is Dobrogea (average 1961-1990: 464 mm. rainfall). Climatic change in recent years has accentuated this tendency. The number of hybrids taken into account was nineteen in 2019 and twenty in 2020. Of all tested hybrids elevens of them have been monitorized in both years (Genesis, Janis, Terramis, Loris, Odessa, Diamantis, Onestar, Eiffel, Aromatic, P64LE99, P64LE25). Genesis has been planted in two periods of the time. When the planting was delayed the yield was decreased between 1000 - 300 kg/ha. The aim of this study was i) the behaviour of the hybrids in the unbeliveble dry conditions, ii) to see the yield and the behaviour of sunflower hybrids to the attack of main pathogens - *Sclerotinia sclerotiorum*, *Phomposis helianthi, Orobanche cumana,* iii) how the planting date influence de yield, iiii) the importance of the pesticides used.

Keywords: sunflower, technological improvement, pest behaviour, yield, drought

INTRODUCTION

Constanta County (Dobrogea area), had the largest weight regarding the surface cultivated in Romania with sunflower crop (10,48%) from arable land in 2019, and 23,8% from Constanta area arable land (General Direction of Agriculture and Development, Constanta, 2019).
Nowadays there is a wide offer for sunflower hybrids which means without a screening of them is hard to decide which are the most suitable for every region. It should exist experimental fields not only for sunflower but for other important crops related to a specific region. The hybrids must be from different seed companies eliminating any suspicions. In Dobrogea such experiments were made over the years (Jinga et al., 2016; Manole et al., 2018, 2018b, 2019) which provided results for yield in very dry conditions, behavior to the attack of the main pathogens and quality indices.
The aim of this study was i) the behaviour of the hybrids in the unbeliveble dry conditions, ii) to see the yield and the behaviour of sunflower hybrids to the attack of main pathogens – *Orobanche cumana, Sclerotinia sclerotiorum, Phomposis helianthi, Alternaria helianthi* iii) how the planting date influence de yield, iiii) the importance of the pesticides used.

MATERIALS AND METHODS

The experimental plots were organised in 2019 and 2020 in the field of SC SPORT AGRA SRL Amzacea, Constanta County (South-East of Romania) (Figure 1 - 2). The number of hybrids taken into account was nineteen in 2019 and twenty in 2020. The soil is a cambic chernoziom with a deeper profile than other chernozioms, a blackish-brown soil of 40-50 cm thickness, medium texture (Demeter, 2009). The content of nutrients was: mobile P index -72; N index -4; K index -200; humus -3.11%; neutral pH -7.2. The area of each plot was 560 m^2 and 672 m^2 in 2020. The preceding crop was winter wheat for each year. Planting date was March 20th in 2019 and March 12th in 2020. Depth of planting in 2019 5-6 cm., and in 2020 7-8 cm. in considerations of moisture of the soil.

Figure 1. Experimental field of SC SPORT AGRA SRL Amzacea, Constanta County – 2019

Figure 2. Experimental field of SC SPORT AGRA SRL Amzacea, Constanta County – 2020

In 2019 the seeds have been treated against (i) *Botrytis* and *Sclerotinia* phytopathogens using Maxim 025 FS (fludioxonil 25g/l) at 0.6 l/100 kg, (ii) *Plasmopara helianthi* using Apron XL (metalaxil 339 g/l) at 3 l/t, (iii) *Agriotes* spp., *Tanymecus dilaticollis Gyll.* using Cruiser 350 FS (350 g/l tiametoxam) at 10 l/t, here having the most infested area. Looking to the year 2020 the seeds were'nt been treated with neonicotinoids.

Two fungicides were used in vegetative season, to control the pathogens: Mirage 45EC (procloraz 45%) - 1 l/ha 8-10 leaves, and Pictor (200g/l dimoxistrobin + 200g/l boscalid) - 0.5 l/ha before flowering.

To control weeds, the herbicides used were: glyphosate, autumn application, in a dose of 2 l/ha, Frontier Forte (dimetenamid-P) in a dose of 1.4 l/ha, Racer 25EC (fluorocloridon) in a dose of 2 l/ha, mixed up before emergency and Pulsar Plus (25g/l imazamox) in a dose of 2 l/ha (used only for the imazamox rezistant hybrids), at 6-8 leaves. Sulfonylurea was been applied for the hybrids resistent to the herbicides tribenurom methyl 30g./ha.

The soil was fertilized using complex fertilizers(18.46.0 + 20 SO_3) 200 kg/ha and nitrogen in vegetation two trips using 150kg/ha plus 100 kg/ha. Foliar fertilizers were performed using two complex fertilizers: 12.60.0 - 2 kg/ha and 145 SO_3, 5 MgO, 100 B, 2 Cu, 25 Fe, 50 Mn, 0.5 Mo, 20 Zn - 2 kg/ha.

Figure 3. Planting.

Figure 4. Temperature of the soil in the moment of planting.

Phytosanitary assessments of plants were performed on July 11th in 2019 and July 17th in 2020 over the main pathogens: *Phomopsis helianthi* Munt.-Cvet. et al., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Alternaria helianthi* (Hansf.) Tubaki & Nishihara and the parasite *Orobanche cumana* Wallr.. The degree of attack (DA%) was calculated using formula F x I/100 (F - frequency of the attacked plants I - intensity of plants attack).

Figure 5. *Orobanche cumana*, Onestar.

Technological sheet includes data about number of plants/m^2 after emergence, flowering and harvesting date and the yield at 9% moisture kg/ha.

Rainfall during 2019 and 2020 in Amzacea, reveal that, 2020 was the driest year at the time has been with 133 mm. rainfall during the growing season compared with 2019 when the rainfall sum was 178,5 mm. (Table 1).

Table 1. Rainfall during 2019 and 2020 growing season of sunflower (Amzacea, Constanta)

	Month								
	Jan.	Feb.	March	Apr	May	June	July	Aug.	
Days	The growing season 2019: Rainfall (mm) for 10-day periods								Sum
1-10	10	0	10	19	0	10	12	7	68
11-20	26	8	0	1	6	4	22	0	67
21-31	0	0	6	15,5	12	0	10	0	43,5
Sum	36	8	16	35,5	18	14	44	7	178,5
Days	The growing season 2020: Rainfall (mm) for 10-day periods								Sum
1-10	0	20	0	0	18	4	29	2	73
11-20	0	0	0	4	0	10	0	0	14
21-31	2	8	16	6	14	0	0	0	46
Sum	2	28	16	10	32	14	29	2	133
Days	Average 1961-1990: monthly values of rainfall (mm)								Sum
1-31	27.7	24.0	29.1	31.8	37.7	47.1	38.9	37.4	464.0

Figure 6. The height of the hybrids.

Figure 7. The height of the hybrids.

RESULTS AND DISCUSSIONS

The very dry conditions of the year 2020 has affected the height of the hybrids between 70 cm. (FD15E27, Genesis) to 105 cm.(P64LE25, P64LE99).

The diseases can affect the yield and hybrids presented a DA greater or less due to their resistance linked with the climatic conditions. From all tested hybrids, ten of them were monitorized in both years (Genesis, Janis, Terramis, Loris, Odessa, Diamantis, Onestar, Eiffel, Aromatic, P64LE99, P64LE25).

Table 2. Phytosanitary status (DA%) – July 11 2019

Hybrid	Pathogen			Parasite
	Sclerotinia sclerotiorum	*Phomopsis helianthi*	*Alternaria helianthi*	*Orobanche cumana*
SY Diamantis CL	0	15	14	4
SY Odessa CLP	0,2	14	24	0
SY Katana CLP	0	24	5	0
SY Onestar CLP	0,2	27	14	2,8
SY Neostar CLP	0	22,5	20,0	4,5
RGT Eiffell CL	0	14	24	0
RGT Tivolli CLP	0	30	33,25	3,75
Bellona	0	24,5	36,0	0,2
RGT Cllayton CL	0	6,0	20,0	0,2
Aurimi	0	14,0	28,0	12
ES Terramis CL	0	14,0	31,5	3,5
Loris CLP	0	10,0	10,5	2,5
ES Janis CL	0	5,0	21,0	0,9
ES Genesis CL 2	0	21,0	21,0	3,5
ES Genesis CL 1	0	5,0	20,0	1,5
ES Aromatic SU	0	1,0	3,0	7
P64LE99	0	5,3	17,5	0
P64LE25	0	10,5	10,0	0
Centrosol	0	12,5	7,0	15
Rubisol	12	7,5	12,0	15

In 2020, the attack of *Sclerotinia sclerotiorum* all of twenty hybrids were'nt been affected, in comparation with 2019 were three of the nineteen hybrids have been affected. *Phomopsis helianthi* and *Orobanche cumana* had a lower DA average than in 2019. ES Genesis CL 2 and SY NX82214(Onestar) CLP had a great DA average for pathogens and parasite combined (8,12% - 7%) (Tabel 3).

Table 3. Phytosanitary status (DA%) – July 17 2020

Hybrid	Pathogen			Parasite
	Sclerotinia sclerotiorum	*Phomopsis helianthi*	*Alternaria helianthi*	*Orobanche cumana*
ES Genesis CL	0	8	10	1
ES Genesis CL 2	0	10	20	2,5
ES Janis CL	0	5	12	0
ES Anthemis CLP	0	10	8	0
ES Terramis CL	0	12	15	0
Loris CLP	0	8	10	0
Coloris CL	0	10	6	0
SY Odessa CLP	0	8	0	0
SY Diamantis CL	0	11	0	0
SY NX82212 (Nexus) CLP	0	12	8	0
SY NX82214 (Onestar) CLP	0	20	8	2
RGT Absollute CL	0	12	14	0
RGT Eiffell CL	0	16	10	0
FD15CL44	0	10	8	2,5
ES Aromatic SU	0	0	2	3
SY NX81220 SU	0	15,5	12	0
P65LE99	0	6	10	0
P64LE25	0	2	8	0
P64LE137	0	8	10,5	0
FD15E27	0	10	7	0
FD18E41	0	8	12	0,2

All the hybrids tested had over 6 plants/ m^2 after emergence which means a good an uniform emergence. The average yield of the tested hybrids was 3678 kg/ha in 2019 exceeding of the county average yield of 2505 kg/ha(General Direction of Agriculture and Development, Constanta) and 1513 kg/ha in 2020.

The best hybrid from thouse twenty hybrids wich have been tested in the experimental field was FD15E27 with 1914 kg/ha, belongs to National Agricultural Research and Development Institute Fundulea, Romania, followed by P64LE25 with 1779 kg/ha.

Except Genesis 2 all the hybrids had over 6 plants/ m^2 after emergence. Flowering date was different due their genetic hybrids. Considering the hybrids cultivated in both years, all of them had a yield greater in 2019 then 2020 becouse this year 2020 was unforgettable looking to the very dry conditions. In 2020, when Genesis was planted with a delay of 22 days the yield has decreased with almost 303 kg/ha (Tabel 5). Same results were recorded in literature showed a higher duration for seed maturity increases yield in sunflower crop (Jonhson and Jellum, 1972; Ahmed et al., 2015; Demir, 2019).

Table 4. Technological sheet for sunflower - 2019

Hybrid	No. of plants/m^2 after emergence	Flowering date	Harvesting date	Yield at 9% moisture (kg/ha)
SY Diamantis CL	7	June 24	August 21	4525
SY Odessa CLP	6	June 22	August 21	4379
SY Katana CLP	7	June 22	August 21	4165
SY Onestar CLP	6.5	June 20	August 21	4248
SY Neostar CLP	6	June 20	August 21	4128
RGT Eiffell CL	6	July 1	August 21	3660
RGT Tivolli CLP	6	June 23	August 21	3245
Bellona	6	June 23	August 21	3814
RGT Cllayton CL	6.5	June 25	August 21	3518
Aurimi	6	July 1	August 21	3799
ES Terramis CL	7	June 20	August 21	3869
Loris CLP	6.5	June 23	August 21	3339
ES Janis CL	6	June 20	August 21	3655
ES Genesis CL 2	5.5	June 28	August 21	3474
ES Genesis CL 1	6	June 18	August 21	4512
ES Aromatic SU	6	June 23	August 21	2534
P64LE99	6	June 25	August 21	3254
P64LE25	6.5	June 25	August 21	3543
Centrosol	6	June 20	August 21	2763
Rubisol	6	June 20	August 21	2944

Table 5. Technological sheet for sunflower - 2020

Hybrid	# of plants/m^2 after emergence	Flowering date	Harvesting date	Yield at 9% moisture (kg/ha)
ES Genesis CL	6,5	June 21	August 11	1593
ES Genesis CL 2	5,5	July 8	August 18	1290
ES Janis CL	6,5	June 25	August 11	1428
ES Anthemis CLP	6,5	June 25	August 11	1774
ES Terramis CL	6,5	June 28	August 11	1555
Loris CLP	6,5	June 29	August 18	1420
Coloris CL	6,5	July 2	August 18	1514
SY Odessa CLP	6	June 25	August 18	1357
SY Diamantis CL	6,5	June 27	August 18	1415
SY NX82212 (Nexus) CLP	6,5	June 25	August 11	1345
SY NX82214 (Onestar) CLP	6	June 25	August 11	1227
RGT Absollute CL	6,5	July 2	August 11	1565
RGT Eiffell CL	6,5	July 2	August 18	1341
FD15CL44	6,5	June 29	August 18	1343
ES Aromatic SU	6,5	June 26	August 11	1617
SY NX81220 SU	6,5	June 20	August 11	1432
P65LE99	6,5	June 29	August 18	1537
P64LE25	6,5	June 29	August 18	1779
P64LE137	6,5	June 29	August 11	1745
FD15E27	6,5	July 2	August 18	1914
FD18E41	6,5	July 2	August 18	1375

Figure 8. Harvesting day 2020.

Figure 9. Harvesting day 2020.

CONCLUSIONS

In 2019, Onestar was the most susceptible hybrid to *Phomopsis helianthi*, Bellona to *Alternaria helianthi* and Neostar to *Orobanche Cumana*, while in 2019 Tivolli had the highest average of DA. *Sclerotinia sclerotiorum* no any chance for attack and *Alternaria helianthi* had a lower attack in 2020 than in 2019 and *Phomopsis helianthi*, *Orobanche cumana* had a lower attack in 2020 than in 2019.

Considering the hybrids cultivated in both years, all of them had a lower yield in 2020 then 2019 due to climatic conditions. In both years when the planting was delayed the yield was decreased with over 1000 - 303 kg/ha.

Taking into consideration the special climatic conditions, for plants cultivation, in Dobrogea area – South-Eastern Romania, it must be established the best cultivars, adapted to this situation. Testing sunflower hybrids which already are commercialized in the seed market, or some new hybrids, it could be recomanded the best ones, suitable for this area.

The year 2019 and 2020 were very different regarding the climatic conditions, the cultivated plants being high influenced.

Testing around 20 sunflower hybrids each year, some of them in both years, we found that for all hybrids the seed yield was considerable lower in 2020, comparing with 2019, due to very special conditions in 2020.
All important pathogens had not conditions for developing in 2020, so, their attack was significantly lower in this year. The same situation was for de attack of the parasite *Orobanche cumana*.

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INVESTIGATION OF THE EFFECTS OF *DAM* AND *SEQA* GENES ON BIOFILM FORMATION IN SALMONELLA WITH PLASMID COMPLEMENTATION TEST

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ABSTRACT

In this study, the effects of the genes that encoded DNA adenine methyltransferase (*dam*) enzyme and replication origin sites sequestration regulator protein (*seqA*) on biofilm formation in identified the different *Salmonella* serovarieties isolated from Turkey, were investigated by plasmid (pBAD24) complementation test with a promoter that inducible expression of genes of interest in the presence of arabinose was used. For this purpose, chromosomal *dam* and *seqA* genes have been deleted primarily by red recombinase enzyme system based mutation studies in the examined serovarieties. When the biofilm formation properties of the obtained *dam* and *seqA* deletion mutants were examined, it was determined that the ability of biofilm formation in these mutants decreased statistically significantly compared to wild type strains ($p < 0,05$). In the second step to test the accuracy of the data obtained, the *dam* and *seqA* genes were cloned behind the promoter found in the pBAD24 vector and induced in the presence of arabinose.
After the cloned recombinant vectors were transformed into *dam* and *seqA* deletion mutants, arabinose was added to the growth media to activate transcription of *dam* and *seqA* genes.
All of the *Salmonella* serovarieties complemented with the recombinant pBAD24 plasmid and mutant genes were either produced with similar to wild type strains or higher ($p < 0,05$) biofilm levels. These findings brought certainty to the findings obtained from mutation studies. Because of the common misleading in defining the phenotypic effects of mutations is the mixing of the polar effects of the mutations with their direct effects. Therefore, these data prove that in *Salmonella*, the main function of replication time is to regulate the dam and seqA genes, which are the regulation of pathogenicity-related properties, as well as in the biofilm regulation.
Keywords: *Salmonella, dam, seqA*, biofilm, plasmid complementation

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INTRODUCTION

Dam (DNA-adenine methyltransferase) and *seqA* (sequestration A) proteins were found to participate in the regulation of many genes with different functions in *Salmonella.* In this bacteria, mutant strains formed in terms of the dam and *seqA* genes; it was found that especially the attachment to biotic and abiotic surfaces and host cell occupation functions decreased significantly compared to natural strains (1, 2, 3, 4). On the other hand; In some bacteria, such as *S.* Typhimurium, *E. coli* and *Acinetobacter baumannii*, biofilm formation has been shown to be under the control of epigenetic arrangements (5). However, only a few studies are explaining the effect of dam methylation on biofilm formation in *Salmonella* (5,6). Aya Castaneda et al. (5) found that DNA methylation in *S.* Enteritidis increased the expression of effective factors such as cellulose and curli fimbria by changing the expression of *csgD*. Based on these findings, the researchers suggested that dam methylation triggered the formation of biofilms in *S.*

Enteritidis. However, as a result of comparative analysis between mutant strain and wild type, it was concluded that the *seqA* gene and therefore the *seqA* protein is not effective in biofilm formation (6). Badie et al. as a result of comparative genomic analysis carried out with pathogenic and non-pathogenic *Salmonella* isolates; found altered dam enzyme levels, the virulence-related gene expression, such as bacterial virulence gene expression, motility and flagella synthesis properties at *Salmonella* Typhimurium 14028 higher level than LT2 (7). These findings are important in terms of determining that dam methylation is associated with effective factors in biofilm formation in *Salmonella*. On the other hand, some researchers have identified that *dam* and/or *seqA* mutations lead to changes in the composition of membrane phospholipids in *S.* Typhimurium (6,8). In both *dam* and *seqA* mutants, DNA-adenine methyltransferase and *seqA* proteins encoded by relevant genes can perform these activities by modifying the activity of RNA polymerase enzyme or transcription factors in promoter regions. *SeqA* and *dam* proteins have been found to affect membrane fluidity, flagella and exopolysaccharide synthesis, which are important in biofilm formation, as well as the ability to regulate fatty acid biosynthesis in *S.* Typhimurium (6). These studies are important to point out that the *dam* and *seqA* genes in *Salmonella* contribute indirectly to biofilm formation.

In this study, direct effects of dam and seqA genes on biofilm formation in *Salmonella* serovaryets were determined using mutation and plasmid completion tests.

MATERIAL AND METHODS

Construction of *dam* and *seqA* Genes Mutants in Target *Salmonella* Strains

In *Salmonella* serovarieties, *dam* and *seqA* gene deletions on chromosomal DNA were created using the single-stage lambda (λ) Red recombination method and *dam* and *seqA* mutants were obtained in selective media (9). In the first phase of this study; Template pKD3 plasmids were developed for the target *dam* and *seqA* genes containing 50 base pairs of homologous arms at the right and left ends of the chloramphenicol gene cassette it contains. While performing this process, specific primers to the *dam* and *seqA* genes were used. These primers designed by us are specific to the chloramphenicol gene cassette, 50 base *dam* and *seqA* genes, on 20 base pKD3 plasmids. Consequently, pKD3 plasmids containing the *dam* and *seqA* genes in the right and left ends of the chloramphenicol gene cassette were designed with the Touchdown polymerase chain reaction (Touchdown PCR) performed using the pKD3 plasmid as a DNA template. After this stage, *dam* and *seqA* genes into target cells where deletion mutants will be generated; In the first step, the pKD3 plasmid containing the chloramphenicol cassette and target gene homologous arms, in the second step, the λ red recombinase expression vector plasmid pKD46 was transferred by the transformation. GeneJet Plasmid Miniprep K0503 (Fermentas) kit was used in all of the plasmid DNA isolations by following the manufacturer's procedure. In the transformation experiments, electrocompetent target cells were used. Whether homologous region recombination occurred in selected transformants was determined by PCR studies conducted using chloramphenicol gene cassette primers on chromosomal DNA samples isolated from transformants.

Control the Transcriptional Expression Levels of *dam* and *seqA* Genes by Cloning into the pBAD24 Vector have Arabinose-İnducible Promoter

These genes were cloned in front of the inducible arabinose pBAD24 promoter to control the expression levels of the *dam* and *seqA* genes, which we aimed to determine its effect on biofilm production. Primer pairs to be used in the amplification of dam and seqA gene were designed by adding SalI and PstI restriction endonuclease recognition series. The dam and seqA genes PZR products were purified with the High Pure PCR Product Purification kit (Roche

Chem. Co., USA). Circular pure pBAD24 vector and purified gene products were cut with SalI and PstI enzymes to form sticky ends. After purification of the linear pBAD24 vector and gene products obtained by restriction enzyme cutting, the formation of phosphodiester bonds between the sticky ends was achieved using the DNA ligase enzyme. Recombinant plasmids obtained as a result of the addition of *dam* and *seqA* genes were transferred to competent cells in the CelljecT Uno electroporator device (Thermo, USA) using electroporation cuvettes with a 2 mm wide sample chamber (Thermo, USA). Since the pBAD24 vector used in the study was resistant to chloramphenicol (Chl, 20 µg / mL), LB agar containing Chl was used as the selective medium.

Identification of biofilm formation on polystyrene surfaces

The method proposed by Woodward et al. (2000) was used to determine the amount of biofilm produced by the wild type *Salmonella* strains and their mutants with impaired *dam* and *seqA* genes on the polystyrene surface. In this method, the tested bacteria were inoculated at a rate of 1% in 5mL LB^{-NaCl} medium and activated for 18 hours under shaking at 37 ° C. Subsequently, bacterial cultures were adjusted to OD_{595} = 0,2 and 30 µL of these dilutions were transferred to 96-well microtitration plate wells containing 100 µL LB^{-NaCl}. Plates were incubated for 24, 48, 72 and 96 hours at 20 °C (10). At the end of the incubation process, the wells were washed 3 times with saline and planktonic cells were removed in this way. Fixation of the biofilm structure formed by incubation for 10 minutes by adding 130 µL of 95% (v/v) methanol to the wells dried at room temperature. At the end of the incubation, methanol was removed and these media were allowed to dry. Then, 130 µL of 1% crystal violet was added to the wells for the purpose of staining the biofilm structure and incubated for 30 minutes. At the end of this period, the wells were washed 3 times with distilled water and then 130 µL of 33% (v/v) glacial acetic acid was added to dissolve the dye attached to the biofilm structure and kept at room temperature for 45 minutes (11). This experiment was carried out in 4 parallel and 2 repetitions. Dye density was measured with the Elisa reader in OD_{595}, and biofilm production quantities were determined by subtracting the values obtained from the wells containing only LB^{-NaCl} used as control from the average of the obtained values. The results were evaluated as "non-productive" ($OD \leq ODc$), "weak"($ODc < OD \leq 2xODc$), "medium" ($2xODc < OD \leq 4xODc$), and "strong" ($4xODc < OD$) in terms of the level of biofilm produced according to "cut off" (limit, threshold; ODc) values (10, 11).

RESULTS

Obtaining mutants with *dam* and *seqA* deletions

Dam and *seqA* deletion mutants were obtained by homologous region recombination by transforming the mutagenic pKD3 plasmids produced by the Touchdown PCR method into the wild type control *Salmonella* (14028) strain using primers containing the specific regions of the *dam* and *seqA* homologous arms and the chloramphenicol gene cassette.

After this step, chloramphenicol gene cassette with added primers *dam* and *seqA* was transformed into all strains used in the experiment, and the transformants (*seqA* and *dam* genes chloramphenicol gene cassette mutants impaired by homologous region recombination) were selected in the medium containing chloramphenicol. As described above, the mutants of the *dam* and *seqA* gene degraded for all strains were produced in nutrient media containing chloramphenicol, mutant codes were given and stock cultures were prepared (Table 1). Stock cultures were kept at -80° C.

Table 1. dam and seqA gene degraded mutants of the strains used in the study

Strain Code	***dam* Mutant**	***seqA* Mutant**
Salmonella Group CI (DMC 2)	DMC2::*dam*	DMC1::*seqA*
Salmonella Typhimurium (DMC4)	DMC4::*dam*	DMC4::*seqA*
Salmonella Virchow (DMC 11)	DMC11::*dam*	DMC11::*seqA*
Salmonella Enteritidis (DMC 22)	DMC22::*dam*	DMC22::*seqA*
Salmonella Montevideo (DMC 89)	DMC89::*dam*	DMC89::*seqA*
Salmonella Typhimurium (Kontrol suş, 14028)	14028::*dam*	14028::*seqA*

Obtaining the pBAD24 recombinant vector containing *dam* and *seqA* genes

Recombinant pBAD24 vectors containing *dam* and *seqA* genes were transformed into target cells by electroporation, and transformants were identified in LB agar media containing chloramphenicol (20 µg / mL) due to the resistance encoded by the pBAD24 vector. The final controls of the selected transformants were performed by re-isolating the recombinant pBAD24 vector from these strains and detecting the presence of the insert (*dam* or *seqA* gene) on the isolated recombinant pBAD24 vectors by PCR application (Figure 1).

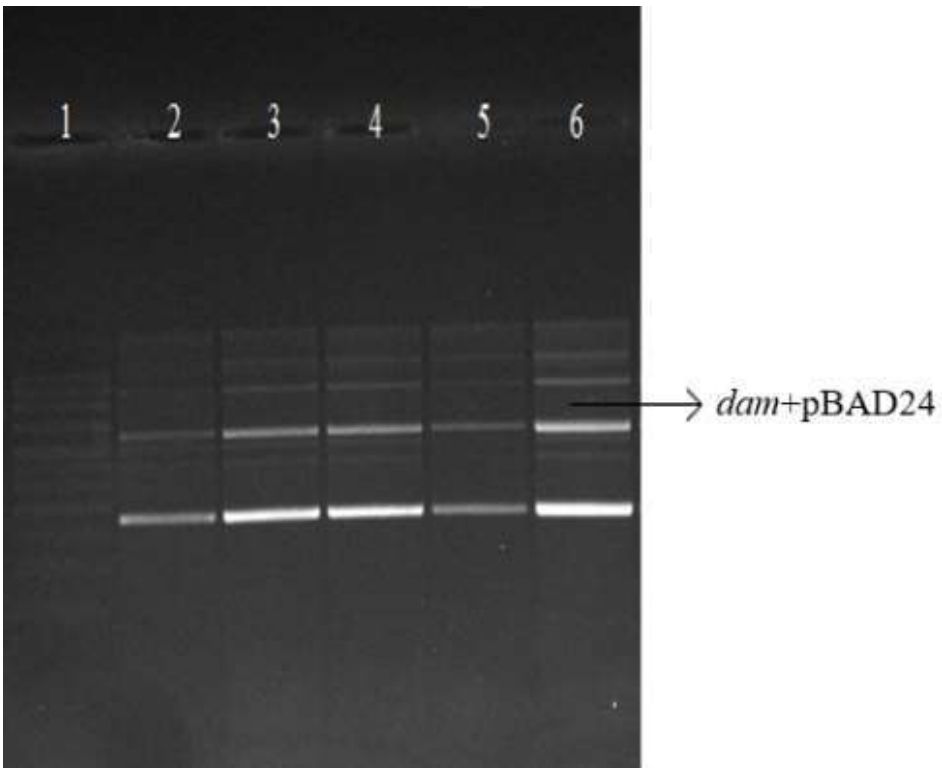

Figure 1. Plasmid content of STyphimurium 14028 colonies selected from *dam* + pBAD24 transformants**.**
1:1 kb DNA Marker, Fermentas; 2: plasmid vector pBAD24; 2-6: Plasmid DNA of transformants containing dam + pBAD24

Biofilm production levels in natural strains, mutants and recombinants

When the biofilm-forming properties of natural strains, their *dam* and *seqA* mutants and the recombinants formed by cloning the *dam* and *seqA* genes behind the arabinose promoter of said mutants were examined on polystyrene surfaces; while all-natural strains were strong biofilm producers, they were either transformed into weak biofilm producers in *dam* and *seqA* mutants, or lost their ability to produce biofilms, and turned into strong biofilm producers as in natural strains as a result of gene complement studies performed with the pBAD24 vector. In recombinants, 0.01% arabinose was used for induction of arabinose promoter (Table 2).

Table 2. Biofilm production levels in natural strains, mutants and gene complemented recombinants

Strain	Biofilm Production		
	24 h	**48 h**	**72 h**
S. Typhimurium 14028 (WT)	0,56	3,28	3,45
Δ*dam*	0,24	1,75	2,68
Δ*seqA*	-	-	0,38
pBAD*dam*+(Δ*dam*)	0,65	3,52	3,48
pBAD*seqA*+*(ΔseqA)*	0,58	3,60	3,74
S. Wirchow DMC11 (WT)	1,65	1,70	1,72
Δ*dam*	-	0,10	0,10
Δ*seqA*	0,10	0,10	0,10
pBAD*dam*+ (Δ*dam*)	1,72	1,68	1,74
pBAD*seqA*+(Δ*seqA*)	1,69	1,75	1,70
S. Group C1 DMC1 (WT)	0,23	1,24	1,50
Δ*dam*	0,12	-	0,05
Δ*seqA*	0,15	-	0,05
pBAD*dam*+ (Δ*dam*)	0,29	1,22	1,62
pBAD*seqA*+(Δ*seqA*)	0,33	1,28	1,47
S. Enteritidis DMC22 (WT)	0,21	1,35	1,76
Δ*dam*	-	-	0,10
Δ*seqA*	0,01	-	0,10
pBAD*dam*+(Δ*dam*)	0,20	1,44	1,87
pBAD*seqA*+(Δ*seqA*)	0,20	1,36	1,81
S. Montevideo DMC89 (WT)	2,45	2,55	2,50
Δ*dam*	0.08	0,15	0,10
Δ*seqA*	-	0,08	-
pBAD*dam*+(Δ*dam*)	2,86	2,89	2,60
pBAD*seqA*+(Δ*seqA*)	2,98	2,75	2,54
S. Typhimurium DMC4 (WT)	0,23	2,43	3,68
Δ*dam*	0,12	0,42	0,25
Δ*seqA*	0,15	0,30	0,67
pBAD*dam*+(Δ*dam*)	0,22	2.96	3,87
pBAD*seqA*+(Δ*seqA*)	0,26	2.98	3,71

DISCUSSION

Today, detailing the genetic regulation of biofilms, preventing the formation of biofilm structures or eradication is the basic step of their studies (12,13). However, in these studies, tests limited to mutation analyzes often lead to false or seriously misleading results (14,15,16). On the other hand, recombinant plasmid completion studies in which mutant genes are expressed with an inducible promoter are the tests that give the most accurate results in defining the effects of mutations. In this way, it is possible to eliminate the misleading results caused by the polar effects of mutations. The data obtained in this study, therefore, unambiguously describes the direct relationship of dam and seqA genes with biofilm formation in different *Salmonella* serovarieties.

CONCLUSIONS

Defining that *dam* and *seqA* genes are directly related to biofilm formation in different *Salmonella* serovarieties is a critical finding in combating biofilm structures in serovarieties where this study is conducted. Because, it is possible to minimize the biofilm structures by using food-grade inhibitors of proteins encoded by the genes in question, without negatively affecting consumer health.

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GREEK NATIVE FOREST FRUIT TREES AND SHRUBS: COLLECTION, DOCUMENTATION AND *EX SITU* CONSERVATION FOR SUSTAINABLE UTILIZATION

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ABSTRACT

Forest trees and shrubs fruits have a high nutritional value and therefore assure a present and future growing market potential. Although many such speciesare native in Greece, so far there has not been any documentation or any effort to commercially propagate and cultivate them. The Eco Variety research project focussed on 8 different fruit tree/shrub species:*Amelanchier ovalis*, *Cornus mas*, *Prunus spinosa*, *Rhuscoriaria*,*Rosa canina*, *Rubusidaeus*, *Sambucus nigra* and *Vaccinium myrtillus*. The project aims are to document these species in their habitat, collect several samples from different sites, evaluate their fruit characteristics, begin asexual propagation trials, develop propagation protocols and ultimatelyto apply this knowledge to pilot crop cultivations. The results are expected to set the basis for future sustainable utilization of these genetic resources through commercialization and breeding programs.

Keywords: native plants, small fruits, neglected and underutilized crops, cuttings, rooting

INTRODUCTION

Wild fruits and berries make up a specific category of fruits with well-known value for human health as they are sources of primary and secondary metabolites, which provide excellent antioxidant activity for humans (Cosmulescuet al., 2017). Small fruit trees and shrubs belong to plant families with several members that are both commercially cultivated and naturally occurring across Greece. Particularly for Greece, in addition to the nutritional value, the native forest fruit trees and shrub species are associated with significant natural and cultural heritage that has been neglected over the years and has not been properly utilised. Additionally, native plant species present innate high adaptability to domestic environmental conditions which may enhance fruit quality (Kozlowski et al., 1991; Milla et al., 2005; Gacheru et al., 2017).

In this framework, this study presents the national Greek research project 'Eco-Variety' (T1EDK-05434) which is being implemented across four large areas of high ecological importance of northern Greece. The project focuses on two plant groups: Group A - wild-growing Greek native forest fruit trees and shrubs with potential to become minor crops (which are presented herein), and Group B - local varieties of fruit trees that are traditionally cultivated or are neglected/abandoned. For all these, the project's aims are their survey through targeted collection and documentation (taxonomic identification, accession numbering, DNA barcoding and species-specific geographical and ecological information) as well as their *in-situ* and *ex-situ* conservation in order to facilitate their pilot propagation, evaluation and sustainable utilization. To date, no documented propagation material of Greek origin exists regarding these focal plants, thus limiting their possible use either as new crops, or as valuable phytogenetic resources for future plant breeding.

MATERIALS AND METHODS

Selected focal wild-growing species

The wild-growing native forest trees and shrubs species selected (Group A) are: *Amelanchier ovalis*, *Prunus spinosa*, *Rosa canina* and *Rubus idaeus* (all in Rosaceae family), *Cornus mas* (Cornaceae), *Rhus coriaria* (Anacardiaceae), *Sambucus nigra* (Caprifoliaceae) and *Vaccinium myrtillus* (Ericaceae). All wild focal species of Group A develop nutritious fruits (food for human consumption) with beneficial properties or can be used as spices (*R. coriaria*) or for infusion-decoctions and are associated with significant natural and cultural heritage in Greece and elsewhere.

Selection of study areas

The field research was conducted from October 2018 until October 2019, including a complete plant growth cycle. The work included multi-day visits to selectedsites, demarcation via remote sensing, species tracking, identification and description, collection of samples and collection of additional information from local authorities.The research was focused on fourlarge mountainous and ecologically important areas of Northern Greece (Table 1), and the fieldwork was divided between the different partners of the project. Sistada partner undertook five geographical areas of Eastern Macedonia and Thrace, Central and Western Macedonia (Rodopi mountain range, Almopia mountain range, Mt. Pieria, Mt. Grammos, Mt. Vitsi and Prespes lakes), HAO Demeter undertook Central and Western Macedonia (Almopia mountain range, Mt. Vermio, Mt. Paiko, Mt. Kroussia, Mt. Lakmos and Mt. Pieria) and the University of Ioannina focused on the mountain ranges of the Epirus area. In the context of the project, literature review was conducted in the first place, aiming at the organization and realization of the field expeditions in the focal areas that were set for the collection of propagation material and fruit samples. Additionally, species-specific information was sought from the online site of the botanical museum of Lund, Sweden (http://herbarium.emg.umu.se/index.html), and from the botanical museum of Berlin, Germany (http://ww2.bgbm.org/herbarium). These sources were particularly useful since they included specific location and habitat details as well as geographical coordinates for the target plants. According to the literature review results, the aim set for the project partners was to collectively undertake field research work for each of the eight selected taxa (Group A) in at least three different sites of at least three distinct geographical areas (see Table 1).

Table 1. Focal wild-growing plants with potential to become minor crops that were prioritised by the Eco-Variety project and number of collection sites per Greek prefecture as implemented by the project partners during the period of 10/2018 – 11/2019

Species	Area	Number of collection sites
Rhus coriaria	Pella Prefecture	7
	Imathia Prefecture	2
	Ioannina Prefecture	5
Rubus idaeus	Kastoria Prefecture (Mt Grammos)	4
	Grevena Prefecture	2
	Pella Prefecture	4
	Kozani Prefecture	3
	Kilkis Prefecture	3
	Trikala Prefecture	1
Vaccinium myrtillus	Pella Prefecture	9
	Florina Prefecture	1
	Kozani Prefecture	4
	Ioannina Prefecture	1
	Kilkis Prefecture	1
Cornus mas	Pella Prefecture	3
	Ioannina Prefecture	12
	Kilkis Prefecture	3
	Kozani Prefectures and Mt Pieria	2
	Kastoria Prefecture (Mt Grammos)	1
	of Drama Prefecture (Mt Rodopi)	1
	Preveza Prefecture	1
	Xanthi Prefecture	2
Amelanchier ovalis	Trikala Prefecture	2
	Kilkis Prefecture (Mt Paiko)	1
	Florina Prefecture	3
	Pella Prefectures	2
Rosa canina	Grevena Prefecture	1
	Kilkis Prefecture	4
	Ioannina Prefecture	2
	Pella Prefecture	4
	Imathia Prefecture (Mt Vermio)	1
	Preveza Prefecture	1
	Trikala Prefecture	1
Sambucus nigra	Preveza Prefecture	1
	Ioannina Prefecture	5
	Pella Prefecture	3
	Kilkis Prefecture (Mt Paiko)	3
	Imathia Prefecture (Mt Vermio)	1
	Trikala Prefecture	1
Prunus spinosa	Preveza Prefecture	1
	Ioannina Prefecture	2
	Pella Prefecture	3
	Kilkis Prefecture (Mt Paiko)	1

Imathia Prefecture (Mt Vermio)	1
Trikala Prefecture	1
Florina Prefecture	3

Documentation of collected samples

Each of the eight selected species was effectively documented in at least three different sites of at least three distinct geographical areas, thus resulting in a total of 70 surveyed sites from which sufficient plant material was collected (at least 20 cuttings and 10 g of fruits per taxon population from each site). The collection protocol included collection of suitable propagation material (cuttings) and/or live plants, leaf samples for DNA analysis, original soil samples, fruit samples and photographic documentation, all taken *in-situ*. The collection of plant material was conducted with the aid of a special collection permit issued yearly by the Greek Ministry of Environment and Energy. Additionally, botanic, geographical and ecological data were recorded for each site and for each focal species (codes and accession numbers, habitat characteristics, geographical coordinates, altitude, vegetative and fruit morphological traits). All this valuable information serve documentation purposes ('passport info') and is incorporated in the project's database (https://ecovariety.gr/).

Propagation trials

Consequently, propagation trials for each population of each species have been conducted in the laboratory of the Institute of Plant Breeding & Genetic Resources of HAO Demeter, using the collected material. The objective of the trials was the development of a successful propagation protocol prior to *ex situ* pilot cultivation trials. The collected material was handled on the day of its arrival. The cuttings were prepared, were treated with rooting hormone and were set for rooting in 3 perlite:1 peat substrate under mist (70 – 80 % of Relative Humidity). The rooting hormone used was Indole-3-butyric acid (IBA) in a range of concentrations from 1000 ppm to 10000 ppm dissolved in 50% ethanol and it was applied through a quick dip (5-7 sec) of the cutting's incision.

Statistical analysis of the data

In order to evaluate the effect of the hormonal treatments applied to the cuttings, the results of the rooting trials were analysed through ANOVA using the Tukey HSD for mean comparison ($P<0.05$) provided that the data satisfied the assumptions of the ANOVA. Otherwise, non-parametric tests were used as appropriate. The statistical software used was IBM-SPSS 20.

RESULTS

The number of population samples that were collected for the eight eligible taxa of the project were 124 in total. Collected from four designated areas of the project were 23 population samples of *C. mas*, 20 samples of *R. idaeus*,15 samples of *P. spinosa*, and 9 population samples of *A. ovalis*. Similarly, collected from three designated areas of the project were 16 population samples of *V. myrtillus*, 14 samples of *R. canina*, 14 samples of *S. nigra*, and 12 population samples of *R. coriaria*. The highest number of population samples were those of *C. mas* (23) due to great variability of fruits detected *in-situ*, whereas the lowest number was those of *A. ovalis* (9) due to the unforeseen rarity of this taxon in the wild habitats (Table 2). As far as leaf samples are concerned, 115 composite samples were collected in total, each including leaves from 20 individuals. Most of the samples came from *C. mas* (21) and *R. idaeus* (20) whereas the lowest number of samples came from *A. ovalis* (7) and *R. coriaria* (11) (Table 2). Soil samples proved to be more difficult to be collected due to the ruggy-rocky and inhospitable terrain of many surveyed areas. As a result, 93 soil samples were collected in total, most of

them being from habitats of *C. mas* populations (18), *R. idaeus* (18), *V. myrtillus* (15), *S. nigra* (11) and *R. coriaria* (10). Comparatively fewer soil samples were collected for *P. spinosa* (9), *R. canina* (6) and *A. ovalis* (6) (Table 2). Regarding fruit samples, already 45 collections have been made and much more are going to be implemented during the 2020 fruiting season (autumn).

During the propagation trials, 108 population samples were successfully propagated via cuttings (at least 20 cuttings per population) with rooting patterns ranging from 17.5% to 100%, which resulted in the production of 1,128 new plants raised *ex-situ* from seven focal species (Table 3). *S. nigra, C. mas, R. idaeus* and *R. canina* have shown high rooting rates of cuttings (>75% - 100%) which is commercially acceptable; *P. spinosa* showed intermediate rooting rates (45.65%), whereas *R. coriaria* and *A. ovalis* presented lower rates and further research is undertaken (Table 3). All produced plants have presented good adaptability at the *ex-situ* environment of the Balkan Botanic Garden of Kroussia in Thermi, Thessaloniki (sea level), where currently they are being cultivated successfully in pilot cultivation trials.

Table 2. Number of documented population sampling per focal taxon (alphabetically) of the Eco-Variety project (cuttings from different populations, leaf samples of 20 individuals, soil samples and fruit samples) collected *in-situ* from the designated focal areas of the project (Table 1), as stemmed from the field work of all partners of the project for the period of 10/2018 – 11/2019

Focal species	Population samples	Focal areas	Leaf samples	Soil samples	Fruit samples
Amelanchier ovalis	**9**	3	7	6	2
Cornus mas	**23**	4	21	18	9
Prunus spinosa	**15**	4	14	9	11
Rhus coriaria	**12**	3	11	10	4
Rosa canina	**14**	3	14	6	6
Rubus idaeus	**20**	4	20	18	5
Sambucus nigra	**15**	3	14	11	3
Vaccinium myrtillus	**16**	3	14	15	5
TOTAL	**124**	**8**	**115**	**93**	**45**

Table 3. Number of propagated clones (cuttings' groups from distant populations) for each focal species of the project Eco-Variety with corresponding rooting capacity and number of produced plants to date

Species	Propagated clones	Rooting (%) (in progress)	Produced plant individuals
Cornus mas	23	85,0	217
Rosa canina	14	75,7	172
Rubus idaeus	20	100	229
Sambucus nigra	15	100	454
Prunus spinosa	15	45,6	25
Rhus coriaria	12	18,5	17
Amelanchier ovalis	9	17,5	14

DISCUSSION

None of the targeted wild fruit species can be considered as ubiquitous (commonplace), at least in Greece. Some of them, like *C. mas* and *P. spinosa,* are more common, while others, like *A. ovalis* and *V. myrtillus,* are fairly uncommon and are rather difficult to encounter in the wild. Consequently, although the literature sources were theoretically a reliable indication for the existence of the eight focal taxa in the wild habitats of Greece (which determined the actual on-site visits), in practice, the focal species were not always easily or successfully located. Moreover, even when the target species were successfully located, ripe fruit samples were not always available during the on-site visits (in many cases fruits were absent or unripen, even in other subsequent efforts).

Concerning the propagation trials, the results showed that *S. nigra, C. mas, R. canina* and *R. idaeus* responded well to the application of the selected rooting hormone (IBA) and the tested treatments presented higher rooting rates compared to the control ($P<0.05$). On the other hand, *P. spinosa, R. coriaria* and *A.ovalis* presented comparatively lower rooting rates. However, the produced plants of all focal species showed high survival rates and good adaptability to the human-made *ex-situ* environment. The external application of auxin proved to be essential for high rooting rates, whereas the quick dip application method coupled with the least amount of time from excision of cuttings till quick dip (Blythe et al., 2007) also seemed to affect the rooting patterns detected. In addition, our results and observations suggest that the physiological condition and the developmental stage of the mother plants might have also affected the observed rooting of cuttings, as expected (see Da Costa et al., 2013).

The above work has resulted in the documentation of valuable Greek native population samples of wild-growing focal fruit species with potential to become minor crops which provides the basis for their sustainable utilisation. In addition, species-specific asexual propagation protocols have been developed in order to pave the way for their commercialisation and future breeding programmes. The work of the Eco-Variety project is in full development through pilot cultivation trials and targeted phytochemical analysis of the selected focal taxa in order to further document their respective potentials as new minor crops.

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DROUGHT TOLERANCE OF TWO BULGARIAN WINTER COMMON WHEAT CULTIVARS

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ABSTRACT

Drought tolerance of two winter common wheat cultivars (*Triticum aestivum L.*) was compared under controlled, green house and field conditions. The plants in the field were grown according to the standard technology accepted in IPGR, Sadovo. In a climatic chamber plants were developed at 20°C/18°C day/night temperature, the light intensity of 300 μmol $m^{-2}s^{-1}$, 16/8h light/dark photoperiod and 55% air humidity until 3rd leaf emerged. Measurements of photochemical activity of PSII and thermal energy dissipation after 3 and 7 days of dehydration of wheat plants in a climatic chamber showed higher drought resistance of cultivar Guinness compared to cultivar Nikky. While the ratio Fv/Fm was not significantly affected after 7 days of dehydration of cv. Guinness it decreased by 15% in Nikky, which was accompanied by 35% enhancement in thermal energy dissipation (1-Fv′/Fm′). In addition, the values of 1-Fv′/Fm′ remained high in cv. Nikky after 4 days of rehydration of dehydrated plants. Electron-transport rate was more sensitive to drought compared to Fv/Fm and it could be used as a stress marker. The higher drought sensitivity of cv. Nikky was also confirmed by experiments in the greenhouse and in the field. The reduction in dry weight in Guinness was slightly lower than in Nikky. The chlorophyll content and the ratio of chlorophyll content to leaf area were higher in the flag leaves of field-grown plants from Guinness compared to Nikky during the waxy maturity phase and especially after a period of drought and dry winds.

Keywords: *Drought tolerance, winter common wheat cultivars, photochemical activity of PSII, thermal energy dissipation*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the important staple food crops, providing more than one-fifth of the total calories and proteins for the global population (Comastri et al., 2018). In major wheat-growing areas of the world, its productivity is adversely affected by various abiotic stresses among which drought is the major abiotic stress causing serious damage (Saradadevi et al., 2017). Drought stress (commonly known as drought) can be defined as the absence of adequate moisture necessary for a plant to grow normally and complete its life cycle. The lack of adequate moisture leading to drought stress is a common occurrence in rain fed areas, drought about by infrequent rains and poor irrigation (Carlos et a.,2008; Ali and Saad, 2020). Drought is the crucial environmental stress factor, which severely affects plant development and advancement of plant, reduces plant yield as well as the functions of the plant, to a greater extent compared to some other natural factors (Shao et al. 2009). The grain yield performance in cereal crops is a complex physiological and biochemical process essentially associated with the accumulation and remobilization process of assimilates (Biswal and Kohli, 2013). In Bulgaria there are two main breeding centers for winter common wheat - IRGR - Sadovo and DAI G. Toshevo. IRGR "K. Malkov" in the town of Sadovo is located in the zone of

transitional-continental climate, typical for Central Southern Bulgaria. The summer is hot and dry, with moderate to very dry weather prevailing. There is usually drought ~~is~~ in autumn or spring, but in any case it is most typical during the period of filling of the grain. The period of the wheat grain filling for this region is naturally accompanied by high temperatures, low atmospheric humidity and dry winds. Due to these specific conditions, IPGR has the opportunity to conduct breeding on the basis of drought resistance. (Bojadjieva,1996). The Balkans are a region that has been strongly affected by rising temperatures, changes in rainfall distribution and the increasing frequency of extreme weather conditions mainly droughts and cold. Bulgarian agriculture develops under different agrometeorological conditions. The country's climate is characterized by a lack of atmospheric and soil moisture. During the summer months, it is rather dry in many parts of the country. Recently, the region has been experiencing trends of warming and drought with increasing duration and intensity. (Ivanova et al. 2020). Sowing dates should be shifted to adapt crops to rising temperatures. The change in the date of sowing will allow the crops to develop in a period with a temperature closer to the most favorable one, optimizing the duration of cultivation, especially the period of the grainfilling (Ivanova et al. 2020) The identification of genotypes with different degrees and mechanisms of action tolerance to drought involves different methods-from physiological to genetic markers (Aliyev, 2012; Khavarinejad and Karimov, 2012)

The aim of the study was to compare drought tolerance of two winter common wheat cultivars Guinness and Nikky under controlled, greenhouse and field conditions by measuring different growth and physiological parameters.

MATERIAL AND METHODS

Plant material

The experiments were performed by two common wheat cultivars Guinness and Nikky. The Guinness cultivar was created in 2004 through the method of physical mutagenesis, by irradiating seeds of the Katya variety with gamma rays Co60 in a dose of 50 Gy. Early cultivar, heading 3-4 days before standard Sadovo1. The plant height is 100 - 105 cm. The leaves are erect and narrow, score 1 according to Feekes. Its grain is relatively small with a 1000 grains weight of 40 g, but it is well executed and leveled. It is characterized by extremely high drought resistance and adaptability. It has a very high yield potential based on high and balanced productive tillering. Its average yield in last years is almost the same as the Gaia-1 variety and is about 740 kg/da. Cultivars Nikky was created through the method of inter-varietal hybridization of the cultivars Sadovo super x Pobeda and impact of F1 seeds with 1mM-NaN3 (sodium azide). Early variety, heading 2-3 days before Sadovo 1. It is characterized by a low but strong stem of 75-80 cm. It is resistant to lodging. The leaves are broad and planophytic, score 9 according to Feekes. Its grain is big with a 1000 grains weight of 49-50 g. It has good cold resistance, equal to the level of variety 301 and medium drought resistance.

Experiment under controlled conditions

Plants were grown on soil in a climatic chamber (Fytoscope FS-RI-1600, Photon System Instruments, Czech Republic) at 20°C/18°C day/night temperature, the light intensity of 300 μmol m^{-2} s^{-1}, 16/8 h light/dark photoperiod and 55% air humidity until 3rd leaf emerged. The measurements of maximum quantum efficiency of PSII (Fv/Fm), electron-transport rate (ETR) and thermal energy dissipation (1-Fv′/Fm′) were performed on the second leaf stage after 3 and 7 days of dehydration and following 4 days of rehydration of the dehydrated plants. Control plants were regularly watered. The same soil as in greenhouse experiment was used. Pot experiment with plants of two cultuvars of common winter wheat subjected to controlled drought. In a glass greenhouse, 50 medium-sized seeds of each variety were sown in two pots containing 1 kg of dry soil - 25 seeds per pot. The seeds germinated after seven days and the seedlings were grown under optimal soil moisture conditions until a second leaf was reached.

The plants in one pot were then dried in a controlled manner for 7 days, and the plants in the other pot were left in normal hydration for the control variant.The two variants were comparatively evaluated taking into account indirect physiological indicators. During the controlled drought, data on the relative amount of chlorophyll and depression in the leaf temperature were reported in dynamics, respectively at the beginning of the drought, on the third day and at the end of the drought (7th day). At the end of the drought in laboratory conditions, the relative water content (RWC), fresh mass and absolutely dry mass of the leaves were reported.

Field experiments

For the cultivars Nikky and Guinness were reported data on water exchange, morphometry and relative amount of chlorophyll on flag leaves of randomly selected plants from the experimental plot with dimensions of 10 m^2. Physiological evaluation was performed twice in dynamics every 10 days after the plants reached the grain filling phase. Basic parameters such as yield, 1000 grains weight and test weight were also monitored. Data on water exchange, morphometry and relative amount of chlorophyll on flag leaves of randomly selected plants from the experimental plot were reported. The data were recorded in dynamics every 10 days in the grain filling phase.

Morpho-Physiological analyses

The chlorophyll content index (CCI) of the leaves was measured with a portable apparatus Chlorophyll Content Meter-CCM 200 Plus manufactured by Opti-Sciences, Inc., NH, USA Measurements were made three times for the variants of the vascular experiment and twice on the flag leaves of the plants from the plots of the field experiment. For each variant of the pot and field experiment, 15 leaves (n = 15) from different plants were reported.

Canopy temperature depression (CTD) -surface temperature reading of plants for each variant and variety was reported with an infrared thermometer, and at the same time with a digital thermometer the atmospheric temperature in the glass greenhouse was read. From the values of the two temperatures, CTD was calculated according to the following formula CTD = T0 air - T0 leaf, proposed by Blum et al. (1982). 10 measurements were made (n = 10) for each variant of the vascular experiment.

The relative water content (RWC, %) is determined as percentage, according to the formula RWC (%) = (FW-DW)/(TW-DM) x 100 (Turner, 1981).

Chlorophyll fluorescence measurements - Chlorophyll *a* fluorescence induction was measured with a portable fluorometer *PAM-2500* (*Heinz Walz GmbH*, Effeltrich, Germany) after dehydration and recovery of plants in a climatic chamber. The leaves were dark-adapted for 30 min and photosynthetically active radiation of about 300 μmol m^{-2} s^{-1} was used for the measurements. The maximum quantum efficiency of PSII photochemistry was calculated as F_v/F_m immediately after the predarkening period. The relative proportion of energy absorbed and dissipated as heat in the PSII antennae (referred as thermal energy dissipation in the antenna) was estimated by 1 − (Fv'/Fm') (Demmig-Adams et al. 1996).

Morphometric characteristics of leaves- Leaf area was calculated by the formula of Chanda et al. (2002) and Berova et al. (2004): A=k*l*b, where: k-coefficient (0.65); l-length of the leaf along the central vain;

b-maximum leaf width. It was measured 10 leaves (n=10) for every variants of field experiment.

Fresh weight (FW) and dry weight (DW) of the leaves are measured using a precision electronic analytical balance Kern EW 220-3NM. Dry weight of leaves is determined by drying the leaves at 104ºC for 1 hour or until reaching a constant mass in three consecutive measurements (Beadle, 1993). 10 leaves (n=10) were measured for every variant of pot and field experiments.

Results are presented as mean ± arithmetic mean error (SE). To prove differences in the mean values, a one-way one-way ANOVA analysis of variance was performed within the variants of each variety, followed by LSD analysis by Fisher's test.

RESULTS

Experiments under controlled conditions

The changes in maximum quantum efficiency of PSII and thermal energy dissipation were measured after 3 and 7 days of dehydration of wheat plants and following 4 days of recovery. The results showed that RWC of leaves of control plants from both cultivars did not change significantly during the experiment (data not shown). In addition, RWC was not affected after 3 days of drought, but it significantly decreased after 7 days of water stress and it was stronger in cultivar Nikky. While the RWC of pants from cultivar Guinness completely recovered following 4 days of rehydration, it was still lower than control in cultivar Nikky. The results of changes in the maximum quantum efficiency of PSII, estimated by the ratio Fv/Fm, during dehydration of wheat plants confirmed the higher drought tolerance of cultivar Guinness. While the value of Fv/Fm was not affected by 7 days of drought of this cultivar, it decreased by 15% in cultivar Nikky and it was even 35% lower than the control after 4 days of recovery (Figure 1). Similarly to RWC and Fv/Fv, the electron transport rate through PSII of well-hydrated control plants was not affected during the experiment. As usual, the fluorescence parameters, measured in light-adapted state like ETR showed higher sensitivity to different stress factors compared to the parameters, measured in dark-adapted state (Fv/Fm). A decline in the efficiency of electron transport was observed after 7 days of dehydration of wheat plants from cultivar Guinness (Figure 2). However, 7 days of drought of cultivar Nikky resulted in a significant inhibition of ETR (by 75%) and it was not completely recovered after 4 days of rehydration. This reduction in ETR was accompanied by a corresponding increase in the proportion of thermal energy dissipation in the antenna (1− Fv'/Fm'), protecting the PSII reaction center from over excitation (Fig. 3).

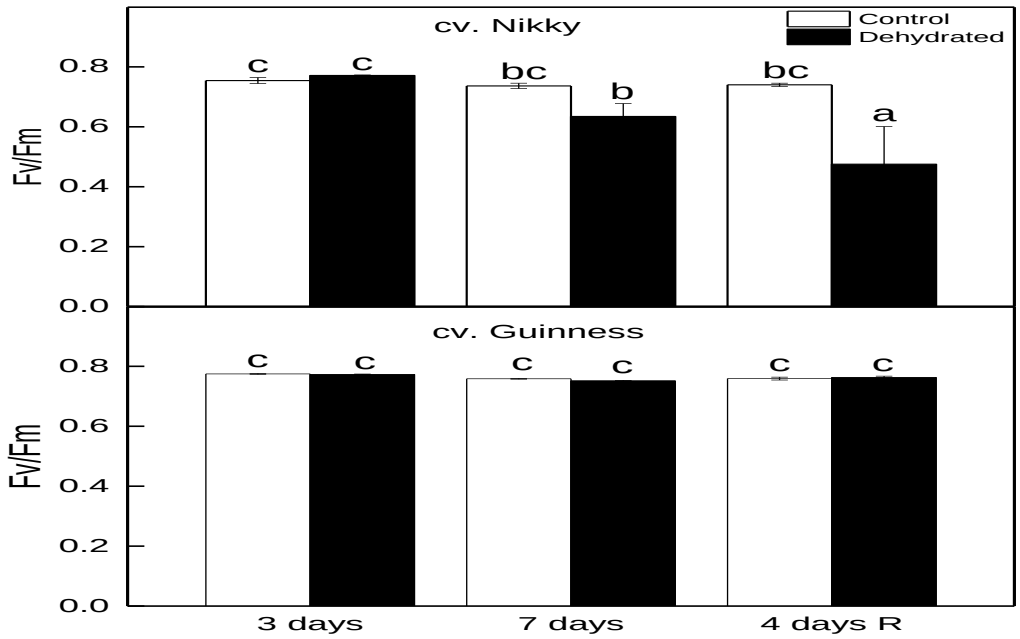

Figure 1. Effect of dehydration of wheat plants from cultivars Nikky and Guinness on maximum quantum efficiency of PSII, estimated by the ratio Fv/Fm. The same letters within a graph indicate no significant differences assessed by Fisher's LSD test (P≤0.05) after performing ANOVA.

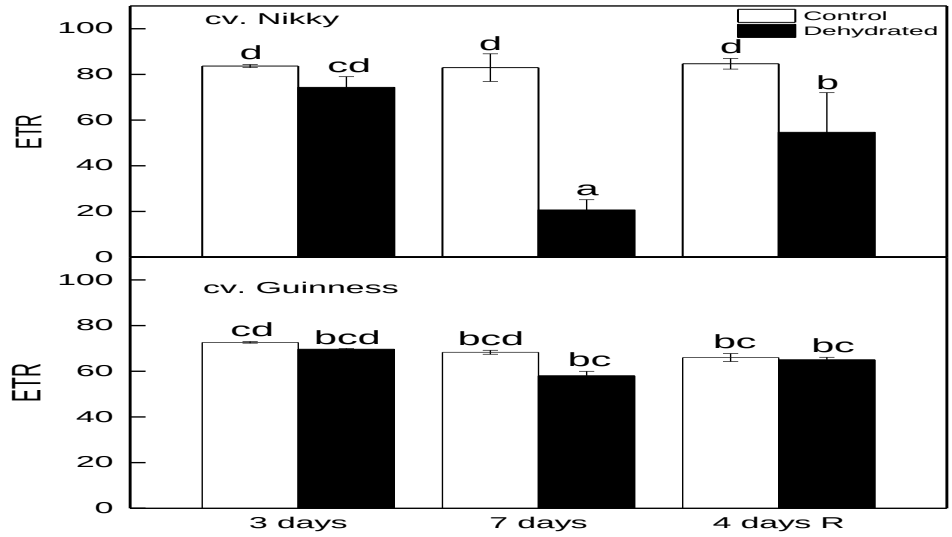

Figure 2. Changes in electron-transport rate (ETR) as a result of dehydration of wheat plants from cultivars Nikky and Guinness. The same letters within a graph indicate no significant differences assessed by Fisher's LSD test (P≤0.05) after performing ANOVA.

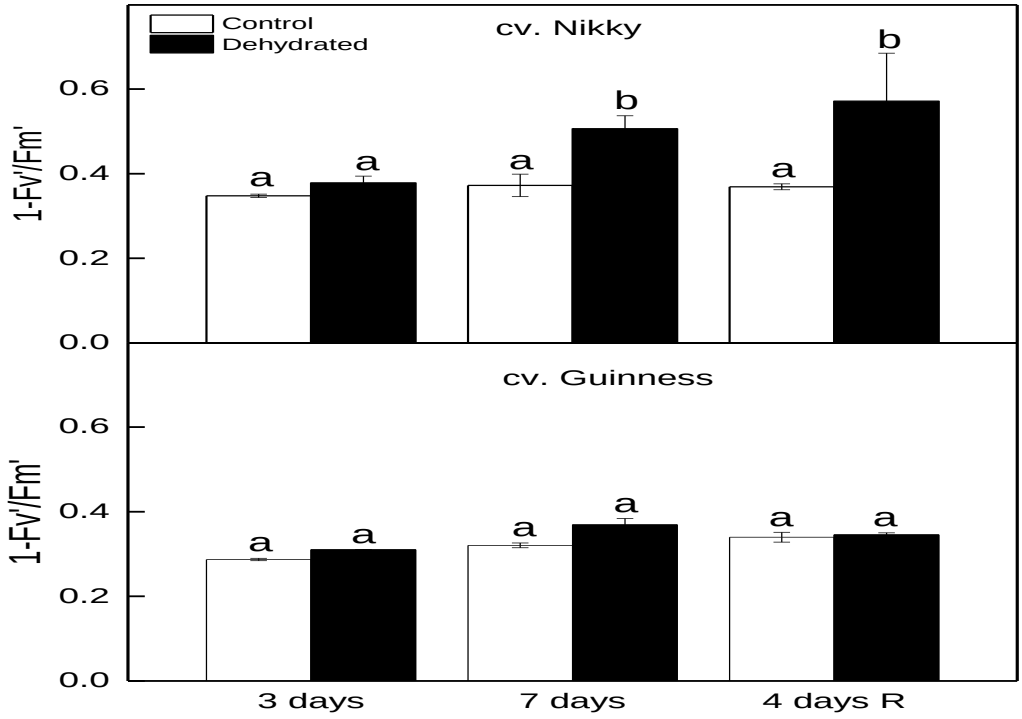

Figure 3. Drought-induced changes in the proportion of thermal energy dissipation in the antenna (1 − Fv'/Fm'), measured in cultivars Nikky and Guinness. The same letters within a graph indicate no significant differences assessed by Fisher's LSD test (P≤0.05) after performing ANOVA.

Pot experiments

Water exchange and morphometry-In both cultivars, as a result of drought, there is a significant reduction in the values of the studied indicators compared to the controls. (Table 1). The reduction values have low differences in RWC and fresh weight (FW) between Guinness and Niki. As an indicator of dry weight, the value of the reduction in the Guinness cultivar is by 6% lower, which is an indication of a more tolerant reaction compared to Nikky (Table 1). The result for the relative amount of chlorophyll (CCI) shows that in the dried plants in dynamics the chlorophyll decreases more intensively in the cultivar Nikky and at the end of the drought it reaches a value of 36.0% lower than the beginning of stress. For the Guinness cultivar this decrease is significantly lower by 5.5%, but the ratio by dates of reporting between the data of the drought compared to the controls is similar for the two varieties. These correlations are due to the increasing CCI value of the Guinness cultivar control as opposed to the lack of increase in the Nikky variety controls (Table 2).

The reported value of leaf canopy temperature depression (CTD) indicates a tolerant drought response at the beginning and middle of the applied stress for Niki, expressed by a lower difference between CTD of controls and drought and a more tolerant Guinness response on the 7th. day (Table 2).

Table 1. Morphometric characteristics of leaves of controlled irrigated and unirrigated plant in conditions of pot experiment

Cultivars/ variants of irrigated	FW mg±SE	DW mg±SE	RWC %±SE
Guinness irrigated	289.0±32.3[a]	54.0±6.0 [a]	85.7±4.3 [a]
Guinness-unirrigated	107.5 ±12.3[b]	41.5±5.2 [b]	39.9±8.6 [b]
% of control	37.2	76.9	46.5
Nikky-irrigated	347.3±35.3 [a]	74.0±8.1 [a]	88.4±3.9 [a]
Nikky-unirrigated	127.0 ±14.7 [b]	52.5±7.2 [b]	39.2±7.2 [b]
% of control	36.6	70.9	44.3

SE- standard error; The same letters indicate no significant differences between two variants assessed by Fisher's LSD test (P≤0.05) after performing ANOVA.

Table 2 Chlorophyll Content Index and Canopy Temperature Depression of leaves of controlled irrigated and unirrigated plant in conditions of pot experiment

Cultivars/ duration of dry stress	Chlorophyll content index-CCI			Canopy temperature depression-CTD		
	Irrigated ±SE	Unirrigated ±SE	% of control	Irrigated ^{0}C±SE	Unirrigated ^{0}C±SE	Difference ^{0}C
Guinness-0 days	1.73±0.18 [a]	1.64±0.21 [a]	94.8	0.99±0.15 [a]	0.09±0.18 [a]	0.90
Guinness-3 days	1.93±0.19 [a]	1.62±0.20 [b]	83.9	2.25±0.20 [a]	-3.03±0.22 [b]	5.28
Guinness-7 days	2.25±0.25 [a]	1.55±0.16 [b]	68.9	3.38±0.24 [a]	-5.13±0.32 [b]	8.51
Nikky-0 days	2.12±0.31 [a]	2.20±0.22 [a]	103.8	1.20±0.12 [a]	0.97±0.09 [a]	0.23
Nikky-3 days	2.00±0.36 [a]	1.62±0.21 [b]	81.0	2.88±0.21 [a]	-1.60±0.15 [b]	4.48
Nikky-7 days	2.15±0.22 [a]	1.41±0.13 [b]	65.6	3.94±0.35 [a]	-5.92±0.31 [b]	9.86

SE- standard error; The same letters indicate no significant differences between two variants assessed by Fisher's LSD test (P≤0.05) after performing ANOVA.

Field experiment

From the first measurement made during the wax maturity phase, a higher fresh and dry mass, leaf surface and relative hydration of Nikky flag leaves were reported. On the other hand, the relative chlorophyll content as well as the ratio between CCI and the leaf surface of the flag leaves is higher in Guinness (Table 3). In the second morphophysiological measurement of the flag leaves, there was a reduction in the values of all indicators in both varieties, except for a slight increase in the leaf area (LA) of the Niki variety. The decrease in the value of most of the studied Guinness parameters is much lower compared to Nikky. For instance, regarding the cultivar Guinness, the reduction of CCI and LA, as well as the ratio between them reaches a maximum of 10% compared to the first reading. An exception is the indicator of the amount of water related to the dry weight of flag leaves, where the values for both cultivars are close (Table 3). On table 4 is presented main biometric parameters of common winter wheat. For the Guinness cultivar was estimated a higher yield.

Table 3. Morphometric and physiological parameters of flag leaves of plant common winter wheat in conditions of field experiment- first date 21.05.2019 and second date 31.05.2020

Cultivars/estimate date	FW g±SE	DW g±SE	g WC/g DW ±SE	CCI индекс ±SE	LA cm^2 ±SE	CCI/LA
Guinness-21.05.2019	0.952±0.078[a]	0,354±0.027[a]	1.69±0.13 [a]	37.0±2.80 [a]	21.79±2.20[a]	1.69±0.10[a]
Guinness-31.05.2019	0.448±0.074[b]	0,217±0.035[b]	1.08±0.11 [b]	33.2±2.82 [b]	20.02±1.96[b]	1.65±0.12[a]
% of first date	47.1	61.3	63.9	89.7	91.9	97.6
Nikky-21.05.2019	1.521±0.193[a]	0.546±0.062[a]	1.78±0.10 [a]	33.3±2.83 [a]	29.95±3.27[a]	1.11±0.10[a]
Nikky-31.05.2019	0.282±0.034[b]	0.135±0.020[b]	1.12±0.11[b]	26.1±3.50 [b]	31.22±2.64[a]	0.74±0.12b
% of first date	18.5	24.7	62.9	78.4	104.2	66.7

S.E. standard error; The same letters indicate no significant differences between two dates assessed by Fisher's LSD test (P≤0.05) after performing ANOVA.

Table 4 Main biometric parameters of common winter wheat

Variety	Yield , kg/da	1000 grains weight, g	Test weight, kg/ha
Guinness	560	34	81,3
Nikky	510	39,6	78,4

DISCUSSION

A parallel study of the drought resistance potential of Guinness and Niki varieties helped to identify relationships between plants at a young age and in the final stages of their development. The drought tolerance of young wheat plants from cultivars Nikky and Guinness was compared by changes in photochemical activity of PSII. Chlorophyll fluorescence measurements have been widely used as a reliable and non-invasive method to determine the plants response to environmental stress conditions. Our results showed higher drought tolerance of cultivar Guinness compared to Nikky. RWC of leaves decreased by about 10% more in Nikky after seven days of desiccation. It was found that ETR was much more sensitive to drought compared to Fv/Fm and it could be used as a stress marker for screening the drought sensitivity of different wheat cultivars. While the values of ETR slightly decreased as a result of dehydration of plants from Guinness, the ETR was significantly inhibited in cultivar Nikky and not completely recovered after rehydration. This strong inhibition of ETR was accompanied by increased dissipation of excess excitation energy, which cannot be used for photochemistry, thus protecting the photosynthetic apparatus from overexcitation and photoinhibition. The weaker growth of Guinness leaves of control plants compared to Niki at an young age correlated with the reported lower fresh and dry mass, as well as the leaf surface of the flag leaves of adult plants on the first date of data collection from field experiment, when they have not fully manifested their exposure to drought conditions (Table 1 and Table 3). This is due to the fact that the amount of precipitation from late April to the second half of May is only 16.2 l / m^2, but most days there was morning dew and lower average daily temperatures. Also, the higher chlorophyll content and CTD in the controls and the initial stages of drought prove the strong start of Niki under normal hydration, but on the other hand there is a more tolerant Guinness reaction expressed by slower reduction of mass and chlorophyll after prolonged the adverse effects of drought. Similar data for the Guinness and Niki cultivars were reported by Chipilski, 2016. According to the analytical stress indicator CTD, neither of the studied varieties has roved to have any advantage, but still the lower difference in the 6th day of drought for variant

Guinness correlates with the higher RWC value of the leaves compared to the Niki variety. The results obtained in the second reading, which took place after a period of drought and high average daily temperatures, are very indicative of the drought tolerance of both varieties are.

Cultivar Guinness shows a more tolerant reaction expressed mainly in the relative amount of chlorophyll and its ratio to the leaf surface, as well as less expressed loss of fresh and dry leaf mass. Results reported for both cultivars correlate with the results obtained at the final stage of controlled drought of young plants.

The grain yield is an indicator for winter common wheat that is influenced by the interaction of the variety of environmental conditions. In this regard, the development of a comprehensive and targeted breeding program for creating high-yielding and well adapted to environmental conditions wheat varieties with grain with the necessary quality indicators for the respective field of use, will support Bulgarian agriculture and create conditions for its sustainable development (Reynolds et al., 2011, Graybosch and Peterson, 2010). For the period of our study the main parameters of plant height, mass of 1000 grains, hectolitre mass and grain yield were observed (Table 4). In the Guinness cultivar, for which a more tolerant reaction was found compared to the Niki cultivar after a period of drought in the field of field experiment, a higher yield was reported, similar data are published by Chipilski, 2016. The values of the following indicators-1000 grains weight and test weight, retain the same differences between varieties as the reported characteristics by the authors.

CONCLUSIONS

Measurements of photochemical activity of PSII and thermal energy dissipation after 3 and 7 days of dehydration showed higher drought resistance of cultivar Guinness compared to cultivar Nikky. ETR was the most drought sensitive parameter, which could be used as a stress marker for screening the drought tolerance of wheat cultivars. The Guinness cultivar showed a more tolerant reaction than the Niki variety after a period of drought in a field experience. This reaction was expressed by a higher value of the relative amount of chlorophyll and the calculated ratio between CCI and the leaf surface of their flag leaves, as well as a less pronounced loss of fresh and dry leaf mass. The higher chlorophyll content and CTD in the controls and initial stages of drought prove Niki's strong start at normal hydration, but on the other hand the more tolerant Guinness reaction is expressed by a slower reduction of mass and chlorophyll after prolonged adverse effects of drought. The Chlorophyll content index in field experience after a period of drought and in dried leaves of young plants grown in vascular experience is higher in the cultivar Guinness.

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DETERMINATION OF SAMPLE SIZE ON DIFFERENT PEARSON CORRELATION COEFFICIENT BY POWER ANALYSIS

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ABSTRACT

The purpose of this study was to determine the minimum sample size in order for the correlation coefficients with different power of the test (70% - 95%) and correlation coefficients (0.3 -0.99) to be significant. The material of the study consisted of random numbers generated from multivariate normal distribution with parameters $X \sim N(\mu, \sum)$. When the population correlation coefficient (ρ) was 0, the correlation coefficient (r) calculated from the sample was checked for significance with the t test. When this situation was tested in 10000 simulations, the required number of observations in each 5% power increases between 70% and 95% were determined. It was observed that similar or very close n values were obtained by controlling this situation with different power analysis programs. In the significance control of the correlation coefficient, r test statistics were taken between 0.3 and 0.99 and the number of experimental units required for 95% power varied between 138 and 5. The required sample size varied between 112 and 4 at 90% power value, and when the power value decreased to 70%, the sample size varied between 67 and 4. When the correlation coefficients were 0.90 and above, the required sample size did not change much when power was between 70% and 85%. In the correlation coefficient (r) statistics, which is widely used in scientific studies, our purpose was to provide the researchers the number of sample sizes required to reach sufficient power values. In this way, excess money expenditure and financial loss in scientific studies can be prevented and the opportunity to find financing more easily can be provided.

Keywords: Pearson Correlation, Power analysis, Sample size, Monte Carlo simulation

INTRODUCTION

In scientific world, reaserchers always wonder about relationship between the variables. Although the determination of the relationship between two variables is known as the correlation coefficient, the data types of the examined variables should be known. As the data forms change, the calculation of the correlation coefficient and the names also change. Pearson's correlation coefficient is a measure of the degree of linear relationship between two continuous variables obtained from the same experimental units (Snedecor and Cochran, 1937; Düzgüneş et al., 1987;Choi et al., 2010; Sheskin, 2011; Mendeş, 2012). The correlation coefficient is expressed by the letter r and takes values between -1 and +1 (Moore and McCabe, 1997; Navidi, 2006). When the relationship between two variables is complete, the correlation coefficient takes a value of 1 and the sign of the correlation coefficient (-, +) determines the direction of the relationship (Düzgüneş, 1963; Kocabaş et al., 2013). In other words, if the other variable increases as the first variable increases, then the coefficient is positive, and on the contrary, there is a negative (decreasing) relationship while one variable is increasing and the other variable decerasing (Kocabaş and Kesici 1998; Kocaçalışkan and Bingöl 2017). When the Pearson correlation coefficient is -1 or +1 this shows that there is a perfect linear relationship (Düzgüneş et al., 1984; Öztuna et al., 2008). When correlation coefficient is zero (0) this indicates that there is no linear relationship between the variables, however this does not mean

that there is no relation between the variables (Çil, 2008). In other words, the relationship between the two variables may not be linear, but there may be a quadratic, cubic, logarithmic and curvilinear (nonlinear) relationship (Figure).

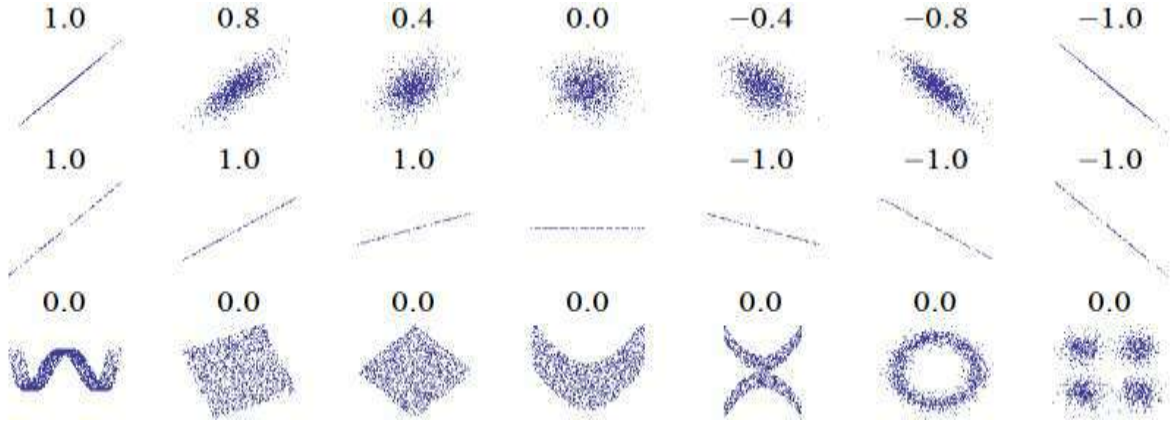

Figure 1. Representation of various correlation coefficient distributions for x and y variable pairs (Anonymous, 2007)

In order for the Pearson correlation coefficient to be applied, it must meet some criteria. These criteria are; variables must to be continuous, show normal distribution, sample volumes should be more than 10 and there should be no outliers in the data set (Messerges et al., 1999; Clavier et al., 2000; Coron et al., 2000; Akkar et al., 2000; Brier et al., 2004; Mendeş, 2012). Of the criteria, the most important criteria is to know what the required sample size should be at the determined power level (MacCallum et al,. 1996). The large sample size depends on the low type I error level and the high power of test (Işık, 2006). In other words, it is known that the power of the test affects the sample size, and the determination of this situation is obtained with the help of power analysis. In a study, the power of the test is an analysis that determines how much power the researcher wants to have and how many experimental units are needed in the sample (Ellis, 2010). Knowing the power of the test in a study helps to interpret the results accurately and reliably, while not knowing the power of the test may cause some misinterpretations. Thus, results of research conducted in this way may also give false information for the population (Murphy and Myors 2004). Knowing the number of experimental units in the sample required for a research will help to plan future studies (Hallahan and Rosenthal, 1996).

In the correlation coefficient (r) statistics, which is widely used in scientific studies, our purpose was to provide the researchers the number of sample sizes required to reach sufficient power values. In this way, excess money expenditure and financial loss in scientific studies can be prevented and the opportunity to find financing more easily can be provided. In this way, determining the sample sizes in advance will provide the opportunity to find project financing more easily by preventing waste and financial loss in scientific studies. The purpose of this study was to determine the minimum sample size in order for the correlation coefficients with different power of the test (70% - 95%) and correlation coefficients (0.3 -0.99) to be significant

MATERIALS AND METHODS

If the correlation coefficient between two variables is calculated from the population, then this is considered as a parameter and denoted by ρ. The material of the study was produced from the random numbers from the multivariate normal distribution with parameters $X \sim N(\mu, \sum)$

with the correlation coefficient of the population (ρ) being 0. The correlation coefficients of the samples drawn from the population were between 0.30 and 0.99 and significance was checked with the t test. When this situation was tested in 10000 simulations, the required number of observations in each 5% power increases between 70% and 95% were determined. Calculation of the correlation coefficients of the population and the sample are given in Equation 1 and Equation 2.

$$\rho_{xy} = \frac{\Sigma(x_i-\mu_x)(y_i-\mu_y)}{\sqrt{\Sigma(x_i-\mu_x)^2\,\Sigma(y_i-\mu_y)^2}}$$

(1)

$$r_{xy} = \sum d_x\, d_y \Big/ \sqrt{\sum d_x^2\ \sum d_y^2}$$

(2)

Calculation of the significance testing of the correlation coefficient was done by Equation 3. The t statistic calculated by Equation 3 was compared with the n-2 degree of freedom t table statistics, and the results were empirically determined and the control was done (it was assumed that ρ = 0).

$$t = \frac{r-\rho}{\sqrt{\frac{1-r^2}{n-2}}}$$

(3)

RESULTS AND DISCUSSION

The required sample sizes at specific power values in the commonly used Pearson correlation coefficient (r) statistics are presented in Table 1.

Table 1. Calculated sample sizes in the significance testing of the Pearson's correlation coefficient of different power

Test statistics	ρ	1-β	r=0.3	r=0.4	r=0.5	r=0.6	r=0.7	r=0.8	r=0.9	r=0.95	r=0.99
r	0	0.70	67	37	23	15	11	8	6	5	4
		0.75	75	41	26	17	12	8	6	5	4
		0.80	84	46	29	19	13	9	6	5	4
		0.85	96	53	32	21	15	10	7	5	4
		0.90	112	61	37	24	17	11	8	6	4
		0.95	138	75	46	30	20	13	9	7	5

Although there are quite different perspectives in the interpretation of the correlation coefficients, they are generally classified in 2 different ways.. The correlation coefficients are classified as weak, medium and high when they are between 0.10-0.29, 0.30-0.49 and 0.50-1.00, respectively (Cohen 1988). Based on this, it was determined that in moderate relationship (0.40), the number of sample size, for power values between 70% and 95%, were 37, 41, 46, 53, 61 and 75, respectively. In high relationship (0.50), the number of sample size, for power values between 70% and 95%, were 23, 26, 29, 32, 37 and 46, respectively. Çakır and Güneş stated that if r is <0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8 or >0.8 then there is a very weak, weak, moderate, high and very high relationship, respectively. In this context, when power was 80%,

sample size for a weak correlation (0.30), a moderate correlation (0.50), a high correlation (0.70), and a very high correlation (0.99), were 84, 29, 13 and 4, respectively.

When the results are examined in general, as the r test statistics increases in the significance control of the correlation coefficient, the sample size decreases. As the power increases for each r test statistics, the sample size increases. The correlation coefficient is a good effect size scale although most people do not know it is an effect size index (Ellis, 2010). The increase in the correlation coefficients and the decrease in the sample size is a proof that the correlation coefficient is an effect size measure (Table 1). This supports the results of our study.

Keskin (2012) determined the minimum sample size for the correlation coefficient for different power and correlation coefficients in his study. He found that when the correlation coefficient value was 0.60 and the power was 0.85, the sample size was 21, whereas when the correlation coefficient value was 0.50 and the power was 0.95, the sample size was 46. Similar results were found in our study.

CONCLUSION

It is important to know the sample size at the beginning of studies and however this is generally ignored by researchers. With the development of today's computer technology, there are statistical package programs that calculate the sample size with the help of power analysis. The use of package programs requires some basic statistical knowledge. In this study, by using the relevant tables provided, researchers can do a hypothesis testing for the correlation coefficient by determining the sample size with a power between 70% and 95% without using any statistical package program.

The sample sizes determined in the study can be used in the negative correlation, although the direction of the correlation coefficients is taken as positive. In addition, the hypothesis test used in the significance testing of the correlation coefficient is two-sided, and the sample size can be calculated easily by using one-sided hypothesis control. For example, sample size of the two-sided test with 90% power is equal to the sample size of one-sided test with 95% power. In this way, an easy way to calculate sample size is provided to the researchers.

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STUDY OF MATHEMATICAL MODELS WITH TWO, THREE AND FOUR CONSTANT OF RHEOLOGICAL PROPERTIES AGAINST TEMPERATURE, FOR ALBANIAN RED WINES

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ABSTRACT

In the first phase, several physicochemical and sensory properties of red wines available in Albanian market were investigated, which can be used as a way of characterizing the wine quality. The density, total and volatile acidity, alcohol content, reduced sugars, free and total SO_2 and pH following the analytical methods described in Albanian standard were determined. The limit values of physicochemical properties in red wines comply with the national law of the Albanian Food Law and EU Food legislation. The results indicated that all the samples possess good quality of sensory properties. In the second phase, in order to describe the temperature dependence of the dynamic viscosity of commercial red wines, was performed the experimental data fitting to mathematical models with two, three and four constant. The mean absolute percentage error, which indicates the deviance of the observed values from the theoretical ones, was calculated. Firstly, it was experimentally seen that the dynamic viscosity decreases with the temperature increment. Secondly, the mathematical model chosed to represent this behavior was highly effective with mean absolute percentage errors below 10%.

Keywords: R*ed wine, Quality, Mathematical model*

INTRODUCTION

The European Union represented around 45 % of the world's total area under vines, 3.2 million hectares, hence is the world-leading producer of wine. It accounts for 45% of world wine-growing areas, 65% of production, 60% of global consumption and 70% of exports. The most cultivated main red varieties were Cabernet Sauvignon, Garnacha tinta, Merlot, Cabernet franc and Montepulciano (FAOSTAT, 2018). The Republic of Albania is located in the south-eastern region of Europe, southwest of the Balkan Peninsula, along the Adriatic and the Ionian Sea. It is positioned between these geographical coordinates: 39 38' (Konispol) and 42 39' (Vermosh) south-north, 19 16' (Sazan Island) and 21 40' (Vernik village, Korca) west-east. The climate of the Albanian littoral lowlands is typically Mediterranean. Annual precipitation rates range from 930 to 2200 mm; the number of frosty days is 5−30 every year, maximum temperatures are 42.2−43.9°C and minimum ones range from - 3.5 to -7.2°C (Cataudella S., Crosetti D., Massa F.**,** 2015). Thanks to this position, Albania is under the influence of a mild Mediterranean climate, quite suitable for the cultivation of vineyards. In the climatic zoning of Albania, factors such as: latitude, altitude, temperature, air humidity, solar radiation and other local factors have been taken into account. Albania produced an estimated 17,500 tons of wine. According to these results, the wine is a very important product and a detailed description of its chemical and physical properties is essential. Knowledge of the physical and chemical properties of wine, especially density and viscosity data, are essential for the design and evaluation of industrial

processing equipment. Therefore, knowing the rheological properties of wines are very important (Trávníček P., Burg P., Krakowiak-Bal A., Junga P., Vítěz T., Ziemiańczyk U., 2016). In previous studies, we have determined the physicochemical properties and studied mathematical models of rheological properties of Merlot red wine, produced in Tirana and Korca. Since, Merlot wine is produced from the Merlot variety, one of the most widespread varieties in our country (Tirana, Lushnja, Fier, Berat, Permet, Vlora, Shkodra etc), we thought of expanding the study areas, including Fier and Permet. The wines were taken directly from the wineries. Hence, the aim of this work is to provide detailed description of the rheological properties of selected Albanian red wines and their mathematical evaluation applying the two, three and multi constant equations. Merlot is one of the world's most widely planted grape variety, a dark blue-colored wine grape variety that is used as both a blending grape and for varietal wines. The name Merlot is thought to be a diminutive of merle, the French name for the blackbird, probably a reference to the color of the grape. Merlot grapes are identified by their loose bunches of large berries. Merlot grapes with a thinner skin and fewer tannins per unit volume tend to have a higher sugar content and lower malic acid (Robinson J., 2013; Robinson J. ed, 2015).

Material and Methods

Red wines available in Albanian market were characterized for physicochemical and rheological properties produced in different regions of Albania (Tirana, Fier, Korca, Permet). Following red wines were purchased: No 1. Merlot from Tirana; No 2 Merlot from Fier; No 3. Merlot from Permet; No 4 Merlot from Korca.

We started wine analysis with the physicochemical parameters for each wine: Density, pH, total acidity, volatile acids, content of the alcohol, reduced sugars, free SO_2, total SO_2, and polyphenol index following the analytical methods described in Albanian Standard (General Directorate of Standardization Albania book., 2018). All measurements were performed in three repetitions. The determination of the density, the pycnometric method was used. The pH of red wines was obtained with a PHS-3CW microprocessor pH Meter. For the determination of total acidity, an analytical method was used (SSH 1446-3:1987). Free acidity was determined according to SSH 1446-4:1987. Content of alcohol in wine was determined by SSH 1446-1:1987 method. Free and total SO_2 were determined by titration of the standard solution of iodine (SSH 1446-7:1987 and SSH 1446-6:1987). Concentration of reduced sugars was determined with Fehling method (SSH 1446-2:1987. Dynamic viscosity and temperature of red wine samples were measured using the Falling Ball Viscosimeter, temperature range -20^o to 150^{o}C, viscosity range 0.5 mPas to 10^5 mPas, reproducibly <0.5%. Dynamic viscosity was experimentally determined as a function of temperature from 10 to 40^{0}C. Relation for calculation of kinematic viscosity is demonstrated by ratio of absolute or dynamic demonstrated by ratio of absolute or dynamic viscosity and density at the same temperature (Kumbár et al., 2015). Office Excel 2016 software was used to carry out the effect of temperature on dynamic viscosity. Then, the variation of the dynamic viscosity of red wines with the temperature is analyzed applying different mathematical models, with two, three and four constants. Equation 1 include two constant equation, the Duhne model.

$$Ln\mu = A + \frac{B}{T} \qquad (1)$$

Where μ is the dynamic viscosity in mPa.s and T is the absolute temperature Kelvin. Multi-constant formula known as Andrade (three constant) and Clements (four constant) models that are represented in the following equations (Abramovic H. *et al*; 1998; Clements L. *et al*; 1992, Clements C. *et al*., 2006):

$$Ln\mu = A + \frac{B}{T} + \frac{C}{T^2} \qquad Ln\mu = A + \frac{B}{T} + \frac{C}{T^2} + \frac{D}{T^3} \qquad (2)$$

Where μ is the dynamic viscosity in mPa.s, T is the temperature in Kelvin. A, B and C are constants. The mean absolute percentage error (MAPE), which indicates the deviance of the observed values from the calculated, was calculated

Results and Disccusion

The physicochemical parameters of red wines can be influenced by the viticulture and enological practices and can be used as a way of characterizing the wine quality. The values of physicochemical parameters in red wines produced in different regions of Albania were in accordance to the national law of the Albanian Food Law and EU Food legislation. Density of red wines ranges from 0.997 to 0.992 g/cm^3 (No.1 0.992 g/cm^3, No.2 0.994 g/cm^3, No.3 0.993 g/cm^3, No.4 0.997 g/cm^3). pH levels in wine normally range from 3 to 4, according to experimental results the pH values ranges within standard range (No.1 3.89, No.2 4.11, No.3 3.78, No.4 3.34). The acids are important in maintaining pH low enough to inhibit the growth of many undesirable bacteria, thus giving advantage to wine yeasts (Joshi V., Rao B. S., Reddy R. S., 2013; Ribéreau-Gayon P., and Traduction A., 2003). The total acidity in different samples ranges from 4.27 to 5.32 g/L tartaric acid (No.1 4.62 g/l, No.2 4.82 g/l, No.3 4.75 g/l, No.4 4.27 g/l). Volatile acidity is expressed in terms of g/L acetic acidity (No.1 0.63 g/l, No.2 0.79 g/l, No.3 0.72 g/l, No.4 0.70 g/l). The amount of acetic acid in wine, which at too high of levels can lead to an unpleasant, vinegar taste. Alcohol content was maximum in No 3, while No 1 has recorded minimum content (No.1 11.14%, No.2 11.46%, No.3 12.32%, No.4 11.18%). Content of alcohol in wine is affected by many factors, such as ripeness of the grapes at the time of harvest, grape processing technology, and fermentation technology (Kaltzin W., 2012). Wines ranging from 10 - 13% are usually produced when less-sweet grapes are used to make wine. Sugars are converted into alcohol and carbon dioxide in the process of fermentation. Reducing sugar content of wine was maximum in No 3 (3.62 g/L), while No 1 has recorded minimum content (2.15 g/L) ((No.1 2.15 g/l, No.2 2.97 g/l, No.3 3.62 g/l, No.4 3.73 g/l). A decrease in the reducing sugar content happened due to conversion of sugars amount into various by products like aldehydes, acetals, esters, tartaric acid and malic acids (Joslyn M.A., and Amerine M.A., 1964). According to the grams per liter residual sugar in a wine, can determined the product type: dry, medium dry, medium sweet and sweet. In accordance with Europe wine regulations is not higher than 4 g/L, so red wine can be classified into the category of dry wines.

Sulfur dioxide content determination is important in the winemaking process as it aids in preventing microbial growth and the oxidation of wine (Santos M. C., Nunes C., Saraiva J. A., Coimbra M. A., 2012). In must and wine sulphur dioxide are in equilibrium between different forms viz. total SO_2, free SO_2 and molecular SO_2 (Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2006). The total sulphur dioxide ranging from 113 to 160 mg/L, hence none of the evaluated samples exceeded the maximum allowable limit, 160 mg x L^{-1} (No.1 113 mg/l, No.2 160 mg/l, No.3 120 mg/l, No.4 137 mg/l). The free dioxide ranging from 16 to 26.4 mg/l indicated that values were under 30 mg/L (No.1 16 mg/l, No.2 26.4 mg/l, No.3 16.7 mg/l, No.4 18.5 mg/l). The experimental data of red wines, for density and dynamic viscosity versus temperature range 10 – 40^{o}C in Figure 1 (a, b) are presented, respectively. From figure 1 (a), it

is observed that the density decreases with increasing of temperature. Based on the studied of physic-chemical parameters, the higher density values correspond to the wine samples with the higher reducing sugar content. The dynamic viscosities of red wines were decreased with increased of temperature (Košmerl T., Abramovič H., and Klofutar C., 2000). As both, density and viscosity are highly temperature sensitive; it is possible to find out the dependence between them. The R^2 for both dependence obtained was greater than 0.95. In our case, studying the experimental values set in the graphs below we noticed that red wine No.1 with a lower reducing sugar content had a significantly lower viscosity (Rao, M. A., Kenny, J. F. and Nelson, R. R., 1977). The temperature effect was stronger in samples with higher density and higher reducing sugar content (Havlíček, M., Severa, L., Křivánek, I., 2007). The kinematic viscosity can be calculated from the dynamic viscosity and the density, is presented in Figure 1 (c).

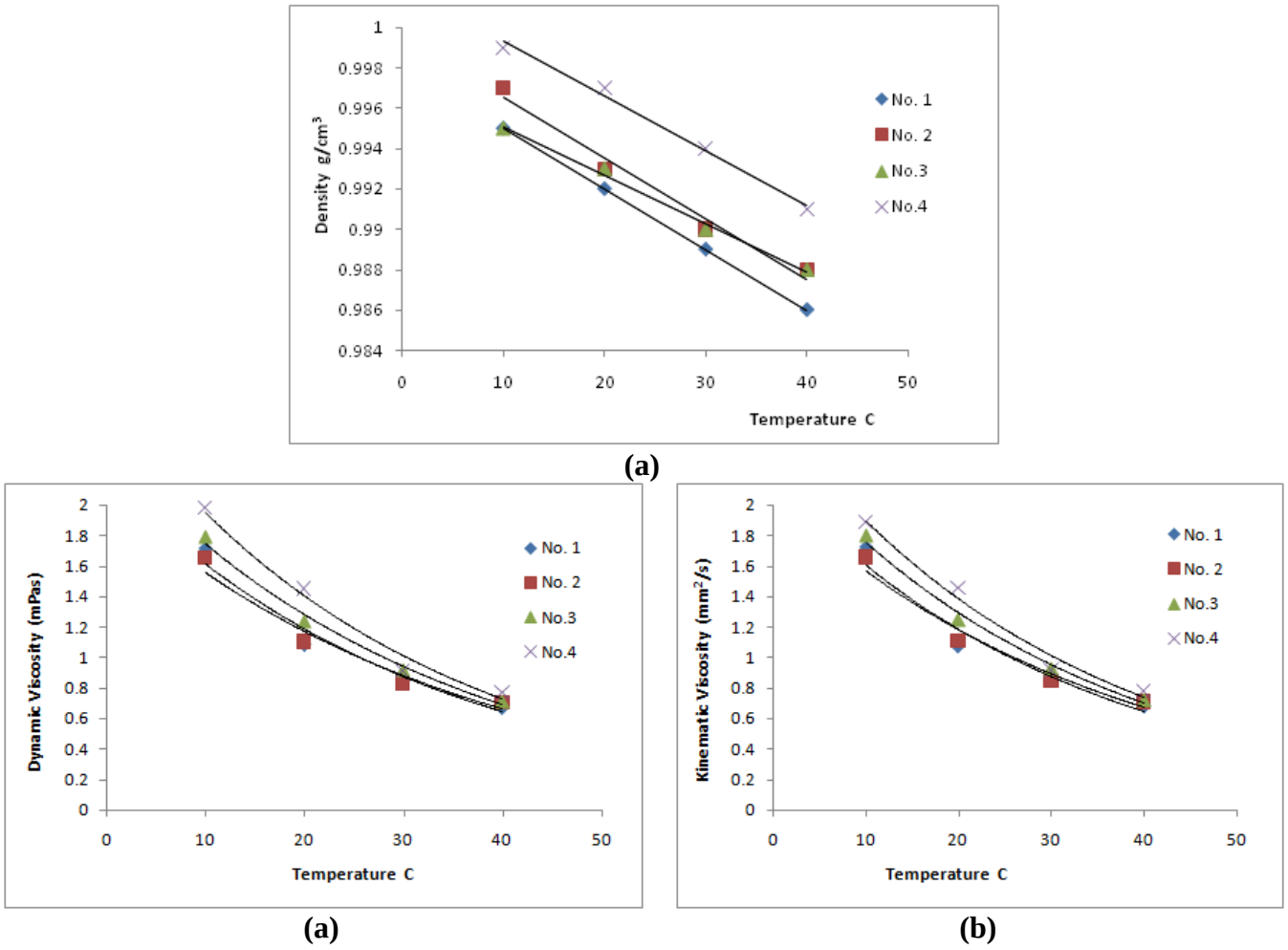
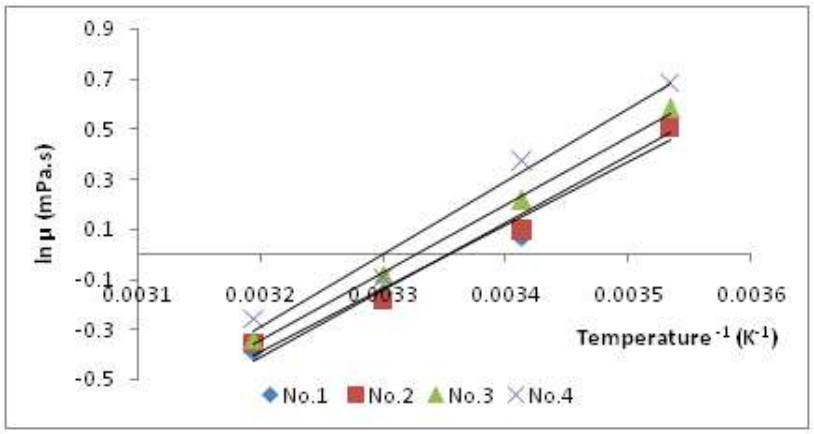
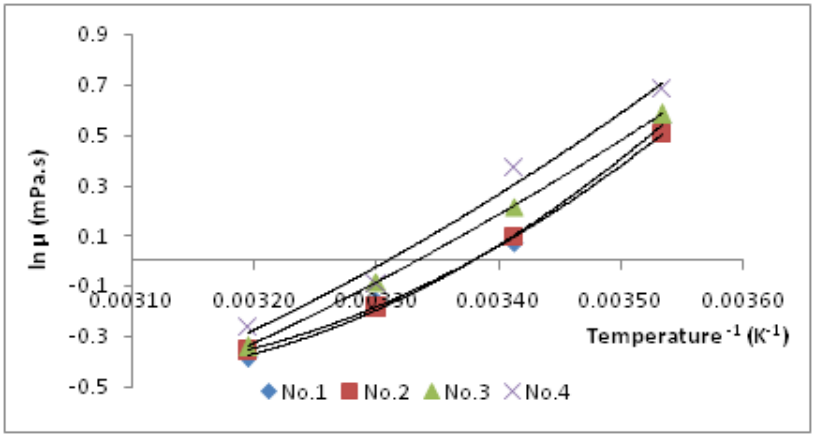


Figure 1. Effect of temperature on density, dynamic and kinematic viscosities for red wines

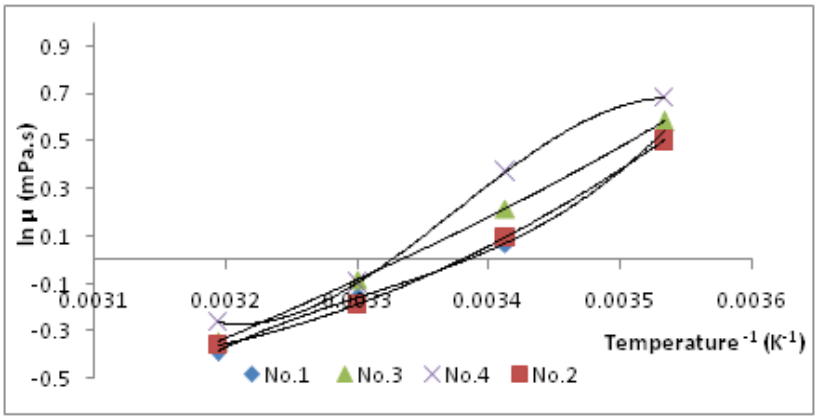
Dynamic viscosity of red wines decreases non-linearly with increasing temperature. Fitting of the experimental data in some theoretical models was performed in order to describe the temperature dependence of the dynamic viscosity of red wine. The experimental data of red wines, for dynamic viscosity fitting by different mathematical models are presented in Figure 2 (a-c), by using Equation 1 and 2 respectively. Equation 1 includes the Duhne model (two constant equations). Equation 2 includes Andrade and Clements models (three and four constant equations).



(a)



(b)



(c)

Figure 2. Effect of temperature on (a) Duhne, (b) Andrade and (c) Clements models of red wines

The values of the estimated constants, correlation coefficients and mean absolute percentage errors are presented in Table 1. The correlation coefficients for Duhne model were under 0.99, hence according to the experimental data three and four constant equations are more suitable to describe the dependence of dynamic viscosity from temperature of red wines samples. However, comparisons of the calculated data indicate that the temperature-dependence of viscosity for the red wines samples was best described by the Clements model. The R^2 values for red wines were very above 0.99, indicating experimental data fell on straight lines. Although, the mean absolute percentage errors determined for first model (No.2, No.3), second model (No. 2,3,4) and third model all the wines were below 10%, the best results are presented by the third model under 1%, which means that the viscosity values obtained were very stable.

Table 1: Two, three, multi-constant values, R^2, MAPE of models studied

Duhne Model	**A**	**B**			**R^2**	**MAPE**
No.1	-9.06	2702.37			0.97	29.62
No. 2	-8.52	2540.31			0.98	8.71
No. 3	-9.07	2726.28			0.98	7.25
No. 4	-9.67	2928.79			0.98	11.11
Andrade Model	**A**	**B**	**C**		**R^2**	**MAPE**
No.1	38.65	-25697.65	4219930		0.99	14.74
No. 2	35.14	-23446.5	3861353.88		0.99	5.24
No. 3	5.84	-6148.33	1318669.6		0.99	4.55
No. 4	10.5	-9075.34	1783682.13		0.99	6.11
Clements Model	**A**	**B**	**C**	**D**	**R^2**	**MAPE**
No.1	-916.61	827590.84	-249650141.60	25157719778.62	1	0.84
No. 2	75.82	-59782.07	14671899.90	-1071290859.80	1	0.45
No. 3	-84.52	74565.81	-22695370.79	2379715319.91	1	0.13
No. 4	2068.7	-1847556.56	548767928.8	-54204405877	1	0.28

Conclusions

The article is focused in physicochemical parameters determination and on influence of temperature on rheological parameters of red wine made in Albania. The density as well as the viscosity of wines are dependent on temperature and decreased non-linearly with increasing temperature from 10 °C to 40 °C. The influence of temperature on the dynamic viscosity of Albanian red wines, particularly those of higher reducing sugar content, was very high. The temperature dependence of dynamic viscosity for red wine samples was best described by Clements model. The value of correlation coefficient and mean absolute percentage error indicates that the models fit satisfactorily to experimental data.

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STUDY OF THE STRUCTURAL ELEMENTS OF PRODUCTIVITY AND DETERMINATION OF CORRELATIONS BETWEEN THEM IN TRITICOSECALE GENOTYPES

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ABSTRACT

During the period 2017-2019 in the experimental field of IPGR "K. Malkov"- Sadovo the structural elements of productivity of 35 triticosecale genotypes were studied. Biometric indicators: plant height, length of central spike, number of spikelets per central spike, number of grains per central spike, grain weight per central spike, harvest index, productive tillering per m^2, weight of 1000 grains and hectoliter mass were evaluated. Five triticosecale genotypes exceed the standard by the grain weight per central spike. Two breeding lines show over 500 productive tillers per m^2. The number BGR30814 is characterized by the highest number of grains per central spike and twenty-six genotypes exceed the standard in terms of the grain weight per central spike. The variation in productivity indicators is assessed as weak to strong. There is weak variation by the indicators hectoliter mass and weight of 1000 grains. The most variable indicators are the number and weight of grains per central spike. The strongest proven correlation is observed between indicators length of central spike and number of spikelets per central spike. The greatest influence, as a source of variation, is exerted by the genotype on the traits length of central spike and plant height. The growing conditions show a primary influence on the indicators number of spikelets per central spike and hectoliter mass.

Keywords: Triticosecale, elements of productivity, correlations, coefficient of variation, source of variation, genotype, environment

INTRODUCTION

Triticosecale (*x Triticosecale Wittm.*) is the newest cereal crop that was created by artificial crossbreeding of wheat and rye (Yankov et al., 2002). Characteristics of triticosecale are its great adaptability to different climatic conditions and high resistance to diseases (Motzo et al., 2001). The harvested grain is used for fodder, and from the flour of wheat and triticosecale in a ratio of 75% to 20%, there is bread, which has a high protein and lysine content. Advanced varieties of triticosecale, compared to other cereals, have the highest productive potential (Kirchev, 2019; Kirchev et al., 2019). It was found that compared to wheat and rye, triticosecale realists higher grain yield and higher crude protein content (Kolev & Ivanova, 2004; Kirchev et al., 2005, Dogan et al., 2011). In recent years, according to the Ministry of Agriculture and Forestry in Bulgaria, a decrease in the harvested areas has been reported, as in 2017 18,660 ha were harvested, in 2018 – 15,171 ha and in 2019 – 13,890 ha. There is also a decrease in the

total yield by 16.1% for the period 2017-2019, with a reported average yield of 303 kg/da (*https://www.mzh.government.bg*).

Global triticosecale breeding programs focus on improving economically important traits such as grain and biomass yield, grain quality, disease and pest resistance. Genetic diversity in triticosecale is relatively low and needs to be expanded (Mergoum et. al., 2019). It is necessary to work to enrich the existing germplasm by creating new genetic material to support the breeding process.

The aim of the present research work is to study the structural elements of productivity in triticosecale genotypes to create new highly productive lines and varieties.

Material and Methods

During the period 2017-2019 in the experimental field of IPGR "K. Malkov"- Sadovo the structural elements of productivity of 35 triticosecale genotypes were studied, and the Rakita variety was used as a standard. The technology adopted in IPGR Sadovo was used for growing of triticosecale, with a length of the experimental plot – 7 m^2, with a row spacing – 20 cm and a inside row spacing – 5 cm. Biometric measurements were performed on 10 randomized plants of each genotype in 3 replicates. The following productivity indicators were evaluated: plant height (cm), length of central spike (cm), number of spikelets per central spike, number of grains per central spike, grain weight per central spike (g), harvest index, productive tillering per m^2 (number), weight of 1000 grains, hectolitre mass (kg/hl). The degree of variation of each of the indicators of productivity is determined by calculating a coefficient of variation. It is accepted to consider the variation as weak if the coefficient of variation is up to 10%, on average – when it is greater than 10% and less than 20%, strong – when it is over 20% (Dimova & Marinkov, 1999). By applying a correlation analysis, the strength and direction of the relationship between the studied traits were established. When the correlation coefficient (r) is up to 0.33, the correlation is considered weak, from 0.34 to 0.66 - medium, and in the range 0.67-0.99 is strong. It can be positive or negative (Genchev et al., 1975; Dimova & Marinkov, 1999). The mathematical processing of the results was performed using the statistical processing programs SPSS 19 and Microsoft Excel 10 for Windows.

Results and discussion

The data obtained from the structural elements of productivity in the studied triticosecale genotypes are presented in Tables 1.1, 1.2 and 1.3. The height of the plants is a trait of the variety, but it also varies related to the growing conditions. The height of the stem affects the yield through the resistance to lodging and the harvest index, and it is extremely important for the crop breeding that the created varieties have a non-sloping stem. For the plant height indicator (Table 1.1) the values of the studied materials are in the range from 87.4 cm (BGR31374) to 131.8 cm (BGR26792). Fifteen genotypes with proven differences compared to the Rakita standard, and in twenty breeding materials the measured height of the plant is over 100 cm. According to (Kurkiev, 2007) as a result of reducing the plant height it is possible not only to preserve but also to significantly increase the yield and quality of grain.

The spike, as a symbol of yield, has always been a major part of triticosecale morphology, subject to crop breeding influence. The spike size is not only a morphological feature, but it is also one of the factors for increased photosynthesis. In our study, the length of the central spike (Table 1.1) had an average minimum of 7.8 cm (Sofia 3) and a maximum reaching 12.4 cm (BGR30814). In six triticosecale genotypes, the trait was exceeded compared to the standard. At nineteen genotypes, the length of the central spike is over 10.0 cm.

The number of spikelets per central spike is a factor that determines the density of the spike. The numbers BGR30814 (36.0), BGR30815 (35.9) and BGR30078 (32.5) are characterized by

the largest number of spikelets per central spike (Table 1.1). Thirty-one triticosecale genotypes fall below the level of the Rakita standard. The number of spikelets per central spike of sixteen triticosecale genotypes is less than 25.

The increasing the number of grains per central spike is directly related to the increasing in yield. In general, varieties with more grains have a higher breeding value (Tsenov and Tsenova, 2004). The number of grains per central spike (Table 1.2) varies from 31.3 (Sofia 3) to 72.4 (BGR30814). There are six genotypes with proven differences from the standard, and only one of them (BGR30814) is mathematically proven to exceed the Rakita variety. At twenty genotypes the reported number of grains per central spike is in the range of 50-60.

The grain weight of a plant is determined by a number of authors (McMaster et al., 1987; Fufa et al., 2005; Leilah & Al-Khateeb, 2005) as one of the most important breeding traits in the selection of breeding materials. The results obtained for the grain weight per central spike (Table 1.2) show that the highest values of the indicator were reported for the following breeding materials: BGR30816 (3.42 g), Belitsa (3.09 g) and BGR39005 (3.08 g). The last by this indicator are numbers BGR30813 (1.57 g) and variety Sofia 3 (1.20 g). There are ten triticosecale genotypes above the standard level, and only in one its difference with the Rakita variety is mathematically provided.

The harvest index is one of the main factors determining the productive potential of the variety. Although the stem of triticosecale is shorter than that of rye, its higher height is a prerequisite for a lower harvest index than wheat. This necessitates looking for ways to reduce it in order to increase the harvest index. The ratio of grain yield to harvest index during the years of survey ranged from 0.346 (BGR28728) to 0.533 (BGR39039). There are thirty genotypes with a proven difference from the standard. A higher value of the harvest index compared to the standard was reported only for BGR39039 (Table 1.2).

Tillering as a biological feature of cereals is determined by the hereditary qualities of the varieties, but it is also strongly influenced by the growing conditions. It has been established that when sowing is in the optimal time, a large part of the tillers are formed in the autumn, but when the sowing is late, the tillering takes place exclusively during the winter-spring period (Kasimov, 1976). In addition, the formation of the tillers is important for the realization of grain production by turning them into productive ones. The ratio between the numbers of formed tillers per unit area to the number of maximally formed tillers determines the productive tillering (Kirchev, 2005). In our study with the largest productive tillering per m^2 (Table 1.3) are characterized variety KT 81 (519.1) and breeding line BGR30815 (507.9 pcs.). Higher productive tillering than the standard was observed in fifteen triticosecale genotypes.

Various physical indicators are used to classify the grain, the most important of which are the weight of 1000 grains (absolute mass) and hectolitre (volume) mass. The weight of 1000 grains is one of the most significant indirect indicators, characterizing the grain size, its grinding quality and its quality as sowing material (Yanchev & Ivanov, 2012; Ivanov, 2019). For the trait weight of 1000 grains, the data shows that twenty-five breeding materials exceeded the standard Rakita (Table 1.3).

With the highest value of the trait is variety KT 81 (48.1 g). The lowest value of the indicator shows BGR30812 (33.5 g) and BGR26802 (33.8 g). The largest percentage (51.4%) of the total number of tested genotypes weight per 1000 grains is in the range from 35.0 to 40.0 g. Hectolitre mass is an important physical indicator of the quality of the grain. The higher the hectoliter mass, the higher the yield of flour. The low hectolitre mass of triticosecale grain is one of the main reasons for its poor application as a food crop (Kolev & Ivanova, 2004). Since the wheat grain has a hectoliter mass less than 72 kg/hl, it is unsuitable for bread production, therefore, triticosecale grain processed as flour increases its baking properties and should be mixed with wheat flour (Atanasova, 1985; Tsenov & Tsenova, 2004). The hectoliter mass (Table 1.3) of the studied breeding materials is in the range from 51.4 kg/hl (4047 TH 1) to 76.2

(Sofia 3) kg/hl. There are three triticosecale genotypes above the standard level. In twenty genotypes the reported hectolitre mass is over 65.0 kg/hl. Thirty breeding materials have proven differences from the standard.

Table 1.1. Results from biometric measurements of the structural elements of productivity of triticosecale genotypes (2017-2019)

N	Genotype/ Variety	Plant height, cm			Length of central spike, cm			Number of spikelets per central spike		
		$\bar{x}$	± D	Pr.	$\bar{x}$	± D	Pr.	$\bar{x}$	± D	Pr.
1	26787	112.1	+1.6	n.s.	9.3	-2.8	- - -	24.0	-8.4	- - -
2	26791	100.8	-9.8	-	10.4	-1.7	- -	25.5	-6.9	- - -
3	26792	131.8	+21.2	+ + +	9.7	-2.4	- - -	27.4	-4.9	- -
4	26797, KC 20	94.1	-16.4	---	9.1	-3.0	- - -	21.8	-10.6	- - -
5	26802	118.7	+8.1	n.s.	12.5	+0.4	n.s.	31.3	-1.0	n.s
6	28728, 9-25	116.2	+5.7	n.s.	9.9	-2.3	- - -	21.6	-10.8	- - -
7	28729, Trit.32/6	104.1	-6.4	n.s.	9.1	-3.1	- - -	23.7	-8.7	- - -
8	28731, Grado	91.7	-18.9	- - -	9.1	-3.1	- - -	21.6	-10.8	- - -
9	28733, KT 81	113.9	+3.3	n.s.	11.9	-0.2	n.s.	26.3	-6.0	- - -
10	30052, 130 TM 3-1	105.2	-5.3	n.s.	12.4	+0.3	n.s.	31.3	-1.1	n.s
11	30058, 4047 TH 1	109.2	-1.3	n.s.	10.9	-1.2	- -	31.6	-0.8	n.s
12	30071	101.7	-8.9	n.s.	9.2	-2.9	- - -	27.4	-5.0	- -
13	30078	133.8	+23.2	+ + +	13.1	+1.0	n.s.	32.5	+0.1	n.s
14	30812	110.9	+0.3	n.s.	11.6	-0.5	n.s.	30.7	-1.6	n.s
15	30813	89.8	-20.8	- - -	8.8	-3.3	- - -	23.4	-9.0	- - -
16	30814	112.3	+1.8	n.s.	13.4	+1.3	- -	36.0	+3.7	-
17	30815	114.9	+4.3	n.s.	12.7	+0.6	n.s.	35.9	+3.6	-
18	30816	104.9	-5.7	n.s.	11.0	-1.2	n.s.	25.7	-6.7	- - -
19	31355, Oak Treiwel	113.7	+3.1	n.s.	10.4	-1.7	- -	22.9	-9.5	- - -
20	31357, KS 60	105.1	-5.4	n.s.	9.9	-2.3	- - -	25.2	-7.1	- - -
21	31362, Coorong	91.2	-19.3	- - -	9.7	-2.4	- - -	22.3	-10.1	- - -
22	31370, Gama	97.1	-13.4	- -	12.3	+0.2	n.s.	25.2	-7.1	- - -
23	31371, Vronti	90.4	-20.1	- - -	8.4	-3.7	- - -	20.6	-11.7	- - -
24	31373	94.9	-15.7	- -	9.7	-2.5	- - -	22.3	-10.1	- - -
25	31374	87.4	-23.1	- - -	9.2	-2.9	- - -	21.6	-10.8	- - -
26	33827, Sofia 3	101.6	-9.0	n.s.	7.8	-4.3	- - -	21.6	-10.8	- - -
27	34816, Vihren	103.3	-7.2	n.s.	9.7	-2.5	- - -	23.5	-8.9	- - -
28	34817, Persenk	94.4	-16.1	- - -	10.4	-1.7	- -	23.9	-8.4	- - -
29	34818, Zaryad	104.0	-6.6	n.s.	10.9	-1.2	n.s.	28.0	-4.3	- -
30	34825, Belitsa	133.6	+23.0	+ + +	12.1	0.0	n.s.	31.3	-1.1	n.s
31	39005	112.8	+2.2	n.s.	11.3	-0.8	n.s.	25.9	-6.5	- - -
32	39039	97.4	-13.1	- -	11.0	-1.1	n.s.	25.8	-6.6	- - -
33	39046	93.7	-16.9	- - -	9.9	-2.3	- - -	22.1	-10.3	- - -
34	Rozhen	104.0	-6.6	n.s.	11.3	-0.9	n.s.	24.6	-7.7	- - -
35	34824, Rakita- st.	110.6			12.1			32.4		
$\bar{x}$		105.7			10.6			26.2		
GD 5.0%		9.3			1.2			3.3		
GD 1.0%		12.2			1.6			4.3		
GD 0.1%		15,7			2.1			5.5		

+ -,+ + - -,+ + + - - -, proven at GD 5.0%, GD 1.0% и GD 0.1%; n.s. – unproven

Table 1.2. Results from biometric measurements of the structural elements of productivity of triticosecale genotypes (2017-2019)

N	Genotype/ Variety	Number of grains per central spike			Grain weight per central spike, g			Harvest index		
		$\bar{x}$	± D	Pr.	$\bar{x}$	± D	Pr.	$\bar{x}$	± D	Pr.
1	26787	37.2	-19.0	- -	1.78	-0.66	n.s.	0.437	-0.091	- - -
2	26791	51.5	-4.7	n.s.	2.44	-0.01	n.s.	0.493	-0.035	- - -
3	26792	44.3	-12.0	n.s.	1.60	-0.84	n.s.	0.362	-0.166	- - -
4	26797, KC 20	50.1	-6.1	n.s.	1.84	-0.61	n.s.	0.402	-0.126	- - -
5	26802	53.6	-2.6	n.s.	1.93	-0.52	n.s.	0.426	-0.102	- - -
6	28728, 9-25	39.8	-16.4	-	1.77	-0.67	n.s.	0.346	-0.182	- - -
7	28729, Trit.32/6	58.6	+2.4	n.s.	2.24	-0.20	n.s.	0.455	-0.073	- - -
8	28731, Grado	45.7	-10.5	n.s.	2.07	-0.37	n.s.	0.493	-0.035	- - -
9	28733, KT 81	56.9	+0.7	n.s.	2.75	+0.30	n.s.	0.478	-0.050	- - -
10	30052, 130 TM 3-1	51.0	-5.2	n.s.	2.29	-0.16	n.s.	0.439	-0.089	- - -
11	30058, 4047 TH 1	54.4	-1.9	n.s.	2.76	+0.31	n.s.	0.479	-0.050	- - -
12	30071	45.4	-10.8	n.s.	2.06	-0.39	n.s.	0.499	-0.029	- - -
13	30078	55.4	-0.9	n.s.	2.21	-0.23	n.s.	0.431	-0.098	- - -
14	30812	53.5	-2.7	n.s.	2.22	-0.23	n.s.	0.513	-0.015	- -
15	30813	38.0	-18.3	- -	1.57	-0.88	- -	0.470	-0.058	- - -
16	30814	72.4	+16.2	+	2.78	+0.33	n.s.	0.506	-0.022	- - -
17	30815	64.4	+8.1	n.s.	2.76	+0.31	n.s.	0.489	-0.039	- - -
18	30816	61.0	+4.8	n.s.	3.42	+0.97	+ +	0.489	-0.039	- - -
19	31355, Oak Treiwel	40.6	-15.7	-	1.84	-0.60	n.s.	0.460	-0.068	- - -
20	31357, KS 60	58.9	+2.7	n.s.	2.31	-0.14	n.s.	0.498	-0.030	- - -
21	31362, Coorong	58.0	+1.7	n.s.	2.20	-0.25	n.s.	0.526	-0.002	n.s.
22	31370, Gama	60.4	+4.2	n.s.	2.61	+0.16	n.s.	0.494	-0.035	- - -
23	31371, Vronti	45.4	-10.8	n.s.	1.77	-0.68	n.s.	0.502	-0.026	- -
24	31373	52.0	-4.3	n.s.	2.06	-0.39	n.s.	0.473	-0.055	- - -
25	31374	51.9	-4.3	n.s.	1.78	-0.67	n.s.	0.512	-0.017	-
26	33827, Sofia 3	31.3	-24.9	- - -	1.20	-1.25	- - -	0.404	-0.124	- - -
27	34816, Vihren	49.6	-6.6	n.s.	2.31	-0.13	n.s.	0.509	-0.019	- -
28	34817, Persenk	43.9	-12.4	n.s.	1.95	-0.50	n.s.	0.476	-0.053	- - -
29	34818, Zaryad	61.0	+4.7	n.s.	2.68	+0.23	n.s.	0.466	-0.062	- - -
30	34825, Belitsa	57.0	+0.7	n.s.	3.09	+0.64	n.s.	0.487	-0.041	- - -
31	39005	60.4	+4.2	n.s.	3.08	+0.63	n.s.	0.516	-0.012	n.s.
32	39039	50.8	-5.4	n.s.	2.29	-0.16	n.s.	0.533	+0.005	n.s.
33	39046	56.7	+0.4	n.s.	2.36	-0.09	n.s.	0.526	-0.002	n.s.
34	Rozhen	53.5	-2.7	n.s.	2.52	+0.07	n.s.	0.464	-0.064	- - -
35	34824, Rakita- st.	56.2			2.45			0.528		
$\bar{x}$		52.0			2.26			0.474		
GD 5.0%		13.5			0.70			0.016		
GD 1.0%		17.8			0.92			0.021		
GD 0.1%		22.9			1.18			0.027		

+ -,+ + - -,+ + + - - -, proven at GD 5.0%, GD 1.0% и GD 0.1%; n.s. – unproven

Table 1.3. Results from biometric measurements of the structural elements of productivity of triticosecale genotypes (2017-2019)

N	Genotype/ Variety	Productive tillering per m^2			Weight of 1000 grains, g			Hectoliter mass, kg/hl		
		$\bar{x}$	± D	Pr.	$\bar{x}$	± D	Pr.	$\bar{x}$	± D	Pr.
1	26787	419.1	+2.1	n.s.	44.0	+6.0	+ + +	65.3	-3.3	- - -
2	26791	435.6	+18.6	n.s.	41.5	+3.4	+ + +	64.4	-4.1	- - -
3	26792	497.0	+80.0	+ + +	33.9	-4.2	- - -	65.1	-3.5	-- -
4	26797, KC 20	454.0	+37.0	+ +	34.8	-3.2	- - -	62.9	-5.7	- - -
5	26802	480.7	+63.7	+ + +	33.8	-4.2	- - -	66.0	-2.5	- - -
6	28728, 9-25	441.7	+24.7	+	43.7	+5.7	+ + +	63.8	-4.8	- - -
7	28729, Trit.32/6	397.8	-19.2	n.s.	39.1	+1.1	+	65.2	-3.3	- - -
8	28731, Grado	473.4	+56.4	+ + +	39.2	+1.2	+	64.3	-4.2	- - -
9	28733, KT 81	519.1	+102.1	+++	48.1	+10.0	+ + +	67.5	-1.1	n.s
10	30052, 130 TM 3-1	371.4	-45.6	- -	40.1	+2.1	+ + +	66.7	-1.8	---
11	30058, 4047 TH 1	397.2	-19.8	n.s.	40.6	+2.6	+ + +	56.4	-12.1	- - -
12	30071	408.7	-8.3	n.s.	43.8	+5.8	+ + +	67.6	-0.9	n.s
13	30078	426.2	+9.2	n.s.	37.5	-0.5	n.s	65.7	-2.9	---
14	30812	397.4	-19.6	n.s.	33.5	-4.5	- - -	66.4	-2.1	- - -
15	30813	461.2	+44.2	+ + +	39.5	+1.4	+	64.6	-3.9	- - -
16	30814	416.6	-0.4	n.s.	37.1	-1.0	n.s	68.6	+0.1	n.s
17	30815	507.9	+90.9	+ + +	36.3	-1.7	-	65.7	-2.9	- - -
18	30816	247.1	-169.9	- - -	44.6	+6.6	+ + +	67.0	-1.5	- -
19	31355, Oak Treiwel	420.3	+3.3	n.s.	42.2	+4.2	+ + +	65.6	-3.0	- - -
20	31357, KS 60	392.2	-24.8	-	40.2	+2.2	+ + +	66.7	-1.8	- -
21	31362, Coorong	375.7	-41.3	- - -	35.6	-2.5	+ + +	61.9	-6.7	- - -
22	31370, Gama	462.9	+45.9	- - -	38.2	+0.2	n.s	70.2	1.7	- -
23	31371, Vronti	431.3	+14.3	n.s.	39.3	+1.3	+	65.0	-3.6	- - -
24	31373	401.1	-15.9	n.s.	38.3	+0.2	n.s	64.3	-4.2	- - -
25	31374	518.6	+101.6	+ + +	38.3	+0.2	n.s	60.7	-7.8	- - -
26	33827, Sofia 3	378.4	-38.6	- - -	36.7	-1.3	+	76.2	+7.7	- - -
27	34816, Vihren	361.8	-55.2	- - -	40.0	+2.0	n.s	66.4	-2.1	- - -
28	34817, Persenk	314.6	-102.4	- - -	38.4	+0.4	n.s	65.0	-3.5	- - -
29	34818, Zaryad	326.0	-91.0	- - -	38.7	+0.6	n.s	63.2	-5.4	- - -
30	34825, Belitsa	329.9	-87.1	- - -	44.8	+6.7	+ + +	65.1	-3.4	- - -
31	39005	406.6	-10.4	n.s.	46.6	+8.6	+ + +	65.1	-3.4	- - -
32	39039	377.0	-40.0	- - -	39.0	+0.9	n.s	64.0	-4.6	- - -
33	39046	350.7	-66.3	- - -	40.3	+2.3	+ + +	68.3	-0.2	n.s
34	Rozhen	406.8	-10.2	n.s.	44.9	+6.9	+ + +	63.3	-5.2	- - -
35	34824, Rakita- st.	417.0			38.0			68.5		
$\bar{x}$		412.1			39.7			65.5		
GD 5.0%		22.5			1.1			1.1		
GD 1.0%		29.7			1.5			1.4		
GD 0.1%		38.2			1.9			1.8		

+ -,+ + - -,+ + + - - -, proven at GD 5.0%, GD 1.0% и GD 0.1%; n.s. – unproven

To determine the degree of variation of each of the performance indicators, a coefficient of variation was calculated based on average values for the study period. Table 2 presents the standard deviation (Std. Dev.) and the coefficient of variation (CV) for the different performance indicators. According to the coefficient of variation, the variation of the studied indicators is from weak to strong. The variation of the indicators hectolitre mass (CV = 6.4%) and weight of 1000 grains (CV = 9.6%) is weak. On average, the variation in the harvest index (CV = 11.2%), plant height (CV = 13.0%), productive tillering (CV = 15.3%), length of the

central spike (CV = 15.4%) and number of spikelets per central spike (CV = 18.0%). The most variable are the number (CV = 20.2%) and the weight of grains per the central spike (CV = 25.9%).

Table 2. Variation of the elements of productivity

Elements of productivity	Number of genotypes	Mean	Min	Max	Std. Dev.	CV,%
Plant height	35	109.8	73.0	136.3	14.3	13.0
Length of central spike	35	10.6	8.0	13.9	1.6	15.4
Number of spikelets per central spike	35	25.3	19.0	35.7	4.5	18.0
Number of grains per central spike	35	47.8	33.7	73.7	9.7	20.2
Grain weight per central spike	35	1.92	1.17	3.13	0.5	25.9
Harvest index	35	0.504	0.416	0.594	0.1	11.2
Productive tillering per m^2	35	415.9	304.3	560.0	63.5	15.3
Weight of 1000 grains	35	34.5	28.5	41.3	3.3	9.6
Hectoliter mass	35	60.2	51.6	70.0	3.9	6.4

To determine the relationships between the individual elements of productivity, correlation coefficients were calculated based on the average values of the individual productivity indicators. The calculated correlation coefficients (Table 3) show that the strongest positive and proven correlation is observed between the number of spikelets per central spike with the length of the central spike (r = 0.831**). The correlation between the grain weight per central spike with the number of grains per central spike (r = 0.806 **) and the length of the central spike (r = 0.634 **) is strong and proven. An average positive relationship was found between the grain weight per central spike with the number of spikelets per central spike (r = 0.490 *) and the harvest index (r = 0.481 *). The trait grain weight per central spike was negatively affected by the productive tillering (r = -0.359 *). The relationship between the harvest index and the plant height has a proven negative effect (r = -0.402 *). Similar correlations between the elements of productivity have been found by other scientists. In his study, Kirchev (2019) points out that the length of the spike leads to a proven increase of the number of spikelets per spike, the number of grains per spike and the grain weight per spike. The author also reports the negative impact of productive tillering on grain weight (Kirchev, 2019). The positive influence of the harvest index on the grain weight per central spike and the number of grains per central spike has been established by our previous study (Dimitrov, 2018).

Table 2. Correlations between studied traits

	Plant height	Length of central spike	Number of spikelets per central spike	Number of grains per central spike	Grain weight per central spike	Harvest index	Productive tillering per m	Weight of 1000 grains	Hectoliter mass
Plant height	1								
Length of central spike	0.554**	1							
Number of spikelets per central spike	0.621**	0.831**	1						
Number of grains per central spike	0.150	0.662**	0.553**	1					
Grain weight per central spike	0.247	0.634**	0.490**	0.806**	1				
Harvest index	-0.402*	0.161	0.098	0.481**	0.481**	1			
Productive tillering per m^2	0.072	0.01	0.034	-0.110	-0.359*	-0.226	1		
Weight of 1000 grains	0.100	0.007	-0.172	-0.026	0.423*	0.119	-0.196	1	
Hectoliter mass	0.093	0.059	0.047	-0.095	-0.111	-0.043	-0.098	-0.041	1

* proven at significance level α=0.05 ** proven at significance level α=0.01

Conclusions

Five triticosecale genotypes exceed the standard by grain weight per central spike. Two breeding lines show over 500 productive tillers per m^2. The number BGR30814 is characterized by the highest number of grains per central spike and twenty-six genotypes exceed the standard in terms of the grain weight per central spike. The variation in productivity indicators is assessed as weak to strong. There is weak variation by the indicators hectoliter mass and weight of 1000 grains. The most variable indicators are the number and weight of grains per central spike. The strongest proven correlation is observed between indicators length of central spike and number of spikelets per central spike. The greatest influence, as a source of variation, is exerted by the genotype on the traits length of central spike and plant height. The growing conditions show a primary influence on the indicators number of spikelets per central spike and hectoliter mass.

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THE INFLUENCE OF TWO STARTER CULTURES ON THE COLOR AND SENSOR PROPERTIES OF *MACEDONIAN TRADITIONAL SAUSAGE*

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ABSTRACT

Sausages belong to the widest range of meat products available in a wide variety of species and with various commercial names. The aim of this paper is to monitor the influence of two starter cultures on the instrumental values for color and sensory properties of industrially produced *Macedonian traditional sausage*. The research covered three variants: Variant 1: Control variant (conventionally produced Macedonian traditional sausages using nitrite salt and powdered acerola); Variant 2: Macedonian traditional sausages where the basic formulation was enriched by addition of starter culture CS-300 (*Staphylococcus carnosus ssp. utilis* + *Staphylococcus carnosus*) in combination with Swiss chard powder and powdered acerola; Variant 3: Macedonian traditional sausages where the basic formulation was enriched by addition of starter cultures CS-300 (*Staphylococcus carnosus ssp. utilis* + *Staphylococcus carnosus*) and BLC-78 (*Pediococcus acidilactici* + *Staphylococcus carnosus*) in combination with Swiss chard powder and powdered acerola. The lightness of the color (*L**) continuously decreases in the control variant, resulting in a loss of color in the specified time interval. This phenomenon is not observed in the samples from variant 2 and variant 3. From the aspect of retaining the values for redness (*a**) and the yellowness (*b**), better effect showed the starter culture CS-300. Thus, the samples of this variant showed statistically significant ($p<0.05$) better values for color saturation (C). On the other hand, according to the sensory analysis, the sausages from the variant 3 have statistically significant ($p<0.05$) higher grades for weighted average (4.52) and percent of maximum possible quality (90.40%) at the 30th day of production. Even that the sausages from the variant 3 have higher grades from the sensory analysis, starter culture CS-300 is recommended, while better stability of the color is achieved during the storage period, as well as a good quality. At the same time a safe product is obtained where the use of nitrite salt is completely eliminated.

Keywords: *Macedonian traditional sausages, starter cultures.*

INTRODUCTION

The processing of meat in meat products records its first beginnings in the Phoenicians, and hence it extends all over the world. According to numerous written data, the first meat preparations have been started with the salting of the meat, and later, other means of conservation began to be applied (Hammes and Hertel, 1998; Čavlek, 2001).

The various properties of sausages, including their overall quality, are conditioned, above all, by the type and quality of the meat, but also by the added additives, spices, preservatives, and the applied technological procedures in the production (Feiner, 2006; Honikel, 2008; Leroy et al., 2006).

One of the most types of sausages that are produced in the Republic of Macedonia is the traditional *Vevcanski* sausage, whose formulation is 1400 years old. Traditional food is considered as a legacy that is transmitted by generations, and consumers expect specific sensory properties and high quality food. At the same time, traditional food must be safe from the microbiological and chemical-physical aspect, without uncontrolled processing and without the presence of preservatives.

The color science (colorimetry) has been developed because of the need for an objective evaluation of color characteristics, which can not be achieved solely with human perception of color, that is, because of the need for color to be quantified and expressed in numerical values. The color of the objects does not depend only on the characteristics of the subject itself, but also depends on the light that illuminates the subject, as well as the condition in which the observer is located, because the weary eye has a reduced sensitivity to the color (Đurišić et al. 2007).

There are many color definitions, and according to SRPS ISO standard (SRPS EN ISO 5492: 2012), color is a feeling caused by the stimulation of the retina of light beams at different wavelengths. MacDougall (2002) defines color as a combination of visually understood information contained in the light reflected by the sample.

In sausages and other meat products, the desired color is achieved by adding nitrites and other chemicals, and in a natural way it is obtained by adding appropriate starter cultures (Janssens et al., 2012; Maksimović et al., 2015).

The quality properties of food are a set of characteristics that are acceptable to consumers. They are divided into external factors (size, shape, color, consistency, odor, taste, texture) and internal factors (chemical, physical and microbiological) (Ruiz Pérez-Cacho et al., 2005). The way of food production has a great influence on sensory characteristics. It is known that the typical taste and aroma of sausages produced with the addition of starter cultures, as the main sensory characteristics, is due to the activity of microorganisms and metabolic processes of decomposition of carbohydrates, proteins and lipids from meat, in combination with various spices (Pleadin et al ., 2013).

From the aspect of full realization of this potential, research on the influence of starter cultures and their metabolites on the quality of foodstuffs is of great importance, and in order to become their obligatory part of the regular, industrial production processes (Bhat et al., 2012; Casaburi et al., 2007). The use of starter cultures in the food industry is a substitute for many chemical additives (including additives containing natural components), which contributes to the creation of new and attractive products containing less chemical preservatives (Arihara, 2006; Demeyer et al., 2000).

The aim of this paper is to monitor the influence of two starter cultures on instrumental values for color and its stability on the cross section of industrial produced *Macedonian traditional sausage*, as well as its sensory properties.

Material and methods

As a material for work was used *Macedonian traditional sausage* produced in industrial conditions in Skopje. As a basis for the production of this product was taken the traditional formulation of *Vevcanski* sausage, modified for industrial use. Pork meat (I category) and dorsal bacon were used in the ratio 75:25%. Water was added in an amount of 150 g/kg mixture. Then additives, spices and starter cultures were added to the mixture. The following starter cultures were used: CS-300 *(Staphylococcus carnosus ssp. utilis*) and BLC-78 (*Pediococcus acidilactici + Staphylococcus carnosus*).
The research in this paper covered three variants:

- *Variant 1*: Control variant (conventionally produced *Macedonian traditional sausages* using nitrite salt and powdered acerola);
- *Variant 2*: *Macedonian traditional sausages* where the basic formulation was enriched by the addition of starter culture CS-300 in combination with powder Swiss chard (as a substitute for nitrite salt) and powdered acerola;
- *Variant 3*: *Macedonian traditional sausages* where the basic formulation was enriched by the addition of starter cultures CS-300 and BLC-78 in combination with powder Swiss chard (as a substitute for nitrite salt) and powdered acerola.

The meat and bacon were ground to pieces of 8 mm. Then all spices and starter cultures were added, according to the determined formulation. The aim of adding Swiss chard powder is to provide a natural source of nitrates that the added starter cultures will convert into nitrites with which it is expected to achieve better results compared to the control variant where nitrite salt is added during production, and the only source nitrates is the leek that is part of the basic formulation. In this way, not only nitrite salt is completely excluded from use, thus eliminating its adverse effects on the health of consumers, but also improvements in the quality of sausages have been achieved. The meat, together with the added spices and starter cultures, was mechanically mixed in a stirrer. Then, the mixture was left to stand for 48 hours in a refrigerator at a temperature of 1-3 °C.

After leaving the mixture, it was accessed to machine filling of the sausages, where during the filling of the mixture of each variant, detailed washing and cleaning of the filler was carried out. Sausages were then thermally treated according to a program that was created according to the needs and modification of the basic formulation.

For measuring the instrumental parameter color, randomly selected three *Macedonian traditional sausages* from each variant separately were taken, and the measurement was done on the surface of the samples. This parameter is determined on the finished product on the 4^{th} and 30^{th} day of production.

For determining this parameter, the colorimeter Dr Lange, spectro color, was used. Before each series of measurements, the instrument was calibrated using a white calibration plate CR-A43, according to the standard procedure of the production instructions. The color characteristics are expressed according to CIE *L* a* b** (CIE, 1976), which is based on three coordinates that define the color of the samples: *L** (lightness), *a** (redness (+*a**) or green (-*a**)) and *b** (yellowness (+*b**) or blue (-*b**)). The measured values *L* a* b** were read directly from the colorimeter, and on the basis of these three values, the following color parameters are calculated using the appropriate mathematical relations:

Total color change (ΔE):
The total color change (ΔE) is calculated in relation to the standard sample, which determines the influence of a factor (in this study the influence of starter cultures) on the characteristics and color quality.

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}$$

where: L_0^*, a_0^* и b_0^* - parameters of the standard (the control variant 1 was taken as the reference value in this study);
L^*, a^* и b^* - sample parameters (variants 2 and 3)

Color Saturation (C):*
Color Saturation (C*) is a measure of the degree of color purity. In the center of the coordinate system is 0 and increases with the distance of the color from the center to the peripheral parts. It is calculated on the basis of parameters a^* and b^*

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Hue angle (h):
The hue angle (h) is calculated on the basis of the parameters a^* and b^*, and determines the value of the angle under which the corresponding color is located (point A, B, C), counting with respect to the + a^* axis of the coordinate system .

$$h = \tan^{-1}(b^*/a^*)$$

Sensory analysis
The examination of the sensory characteristics is performed on the 30th day of production. Assessment of the sensory properties of the prepared product was performed by comparison-scoring (Radovanović and Popov-Rajlić, 2001). Sensory optimal features were: color, smell, taste, consistency, cross-sectional appearance, external cross-section. The assessment is performed on a scale of 0 to 5, where each grade represents a certain level of quality: grade 0 indicates a product with visible mechanical or microbiological damage, grade 1 indicates altered and atypical color or some other property of the product (unacceptable product), grade 2 indicates that the product has certain, significantly noticeable defects in quality, grade 3 indicates partially noticeable defects in quality, grade 4 indicates insignificant deviations in terms of color or some other property, and grade 5 indicates that the product has exceptional, characteristic sensory properties, optimal color , ie optimal overall quality. For each of the above characteristics, the coefficient of importance has been determined: external appearance - 1, the apparent average - 4, consistency - 3, color - 3, odor - 4, and taste - 5. The grade for each property is multiplied by the coefficient of importance. Values are collected and divided by the coefficients (20). The obtained value represents the weighted average value, i.e. the weighted value - general grade of the quality of the examined sausage. In addition, the percentage of the maximum possible quality is calculated, which represents the re-weighted average value (WAV) compared to the maximum value (5):

$$WAV / 5 \cdot 100$$

All sensor parameters are rated on fresh sausage samples from each variant separately.

Presented data are statistically processed with ANOVA, post hoc Tukey's test (p=0.05) in SPSS package.

Results

The results of the instrumentally measured values for the color of the sausage cross section, *L**, *a** and *b**, as well as the values for ΔE, C and h are shown in Table 1 and Table 2.

Table 1: Average values of instrumental color analysis on the cross section of sausages

Sample	n	4th day of production			30th day of production		
		*L**	*a**	*b**	*L**	*a**	*b**
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Variant 1 (control)	5	57,089[a] ± 1,79	22,848[a] ± 3,14	86,976[a] ± 1,87	52,911[a] ± 1,97	11,584[a] ± 0,87	25,455[a] ± 1,08
Variant 2 (CS-300)	5	50,518[a] ± 1,19	26,336[a] ± 2,09	83,951[ab] ± 1,87	55,274[a] ± 2,26	15,865[a] ± 1,65	24,344[a] ± 1,57
Variant 3 (CS-300 + BLC-78)	5	49,570[a] ± 4,52	23,950[a] ± 2,05	83,861[b] ± 1,87	56,163[a] ± 1,62	13,444[a] ± 1,69	25,546[a] ± 3,28

[a, b, c] – the values for *L**, *a**, *b** per days marked with different letters have a statistically significant difference between the examined variants ($p<0.05$)

Table 2. Average values of instrumental color analysis on the cross section of sausages

Sample	n	4th day of production			30th day of production		
		ΔE	C	h	ΔE	C	h
			$\bar{x}$ ± SD	$\bar{x}$ ± SD		$\bar{x}$ ± SD	$\bar{x}$ ± SD
Variant 1 (control)	5	Reference	89,93[a] ± 0,01	75,28[a] ± 0,01	Reference	27,97[a] ± 0,01	65,53[a] ± 0,01
Variant 2 (CS-300)	5	8,03	87,98[b] ± 0,01	72,58[b] ± 0,01	2,98	29,47[b] ± 0,01	56,90[b] ± 0,01
Variant 3 (CS-300 + BLC-78)	5	8,21	87,21[c] ± 0,01	74,06[c] ± 0,01	3,75	28,87[c] ± 0,01	62,24[c] ± 0,01

[a, b, c] – the values for Δ*E**, *C**, *h** per days marked with different letters have a statistically significant difference between the examined variants ($p<0.05$)

Lightness values (L)*

According to the data from Table 1, can be noted that with the highest value for *L**, i.e. with the brightest color on the 4th day of production, are characterized the sausages from the control variant (57.089), while on the 30th day of production, i.e. in the course of storage, a decrease in this value is noted up to 52.911. On the 4th day of the production, the sausages of variant 3 are characterized by the lowest value for the color light, which is 49.570, but already on the 30th day of the production, this variant shows an increase in value (56.163), indicating the fact during storage, the number and activity of the present microflora is reduced, but the sausages do not receive dark color, but it becomes brighter and does not lose the attractiveness, that is approaches the color of the sausages from the control variant.

A similar trend to increase the *L** value is also determined in the samples of variant 2, where from 50.518 on 4th day of production, the value reaches up to 55.274 on 30th day of the production. Nevertheless, there are no statistically significant differences between the examined variants.

Values for redness (a*)

From the data in Table 1, can be seen that the highest value for redness was measured in the sausages of variant 2 (26.336) on 4th day of the production. During storage, the number of microorganisms decreases, thereby reducing their activity. As a result, sausages have a tendency to decrease the intensity of red. However, on the 30th day of production, the most intense red color (15.865) was measured in the sausages of variant 2. In the sausages of variant 3, where two starter cultures were applied, the value for the intensity of redness, from 23.950 on the 4th day of production is reduced to 13.444 on the 30th day of production. The lowest average value for *a** (17,642) on the 4th day of production was measured in the sausages from the control variant, which slightly decreased to 30th day (11.584), which is also the lowest value compared to the other variants. There are no statistically significant differences between the examined variants ($p>0.05$).

Value for yellowness (b*)

Regarding the yellowness, the highest value on the 4th day of production was measured in sausages from variant 1 (86.976), which during the storage, on the 30th day is reduced to 25.455. Similar values were also measured in the sausages of variant 2, where from 83.951.855 on the 4th day of production, *b** is reduced to 24.344 on the 30th day of production. According to the obtained values, on the 4th day of production, statistically significant difference ($p<0.05$) is found in variant 3 compared to the control variant. On the 30th day of production, there is no statistically significant differences between the examined variants ($p>0.05$).

Total color change values (∆E)

In order to determine the total color change of the cross section of the sausages from varieties 2 and 3 where starter cultures are applied, the control variant (variant 1) is taken as a reference trial because it is produced according to the traditional recipe of *Vevcanski* sausage using nitrite salt, modified for industrial applications. According to the obtained calculations, on 4th day of the production, a greater total color change is observed in the samples of variant 3 (8.21), compared with the samples of variant 2 (8.03). On 30th day of the production, a greater color change is observed in variant 3 (3.75) compared to variant 2 (2.98). Based on these data, it can be seen that during the storage, in the sausages of variant 3, the activity and the presence of starter cultures and in general the present microflora decreases, which directly affects the decrease in the intensity of the color, i.e. the approximation to the color of the control variant, where this and other quality properties are achieved as a result of the added nitrite salt. On the other hand, sausages of variant 2 indicate greater color consistency during storage.

Color saturation values (C*)

According to the data shown in Table 2, sausages of variant 1 (89.93) on 4th day of the production are characterized with the highest color saturation, and this value decreases to 27.97 on the 30th day of production. Sausages of variant 2 on 4th day of the production show a slightly lower color saturation value (87.98) compared to the sausages of variant 3, but, on the contrary, on the 30th day of production, the sausages of variant 2 have the highest a color saturation value of 29.47. The lowest values for this parameter on the 30th day of production are characterized in the sausages from the control variant. According to the statistical analysis data, it can be noticed that on day 4th and 30th day of the production there is a statistically significant difference ($p<0.05$) in variants 2 and 3 compared to the control variant, as well as between variant 2 and variant 3.

Hue angle values (h)

On 4th day of the production, the highest value for the hue angle (*h*) on the sausage surface was calculated for the samples of variant 1 (75.28), as well as on the 30th day of the production this variant has the highest value (65.53). Sausages of variant 2, on 4th day of production, have the lowest value for this parameter (72.58), which increases to 56.90 on 30th day of production. The sausages from the variant 3, on the 30th day of the production are characterized with the middle value for this parameter (62.24). A statistically significant difference ($p<0.05$) on day 4th and 30th day of the production was noted in variants 2 and 3 compared to the control variant, as well as between variant 2 and variant 3.

Table 3: Average values from sensory analysis of the sausages on the 30th day of production

Sensory properties	n	KV	Variant 1 (control)		Variant 2 (CS-300)		Variant 3 (CS-300 + BLC-78)	
			O	K $\bar{x} \pm SD$	O	K $\bar{x} \pm SD$	O	K $\bar{x} \pm SD$
Color	20	3	4,38	$13,14^{a} \pm 0,03$	4,27	$12,81^{b} \pm 0,04$	4,72	$14,16^{c} \pm 0,01$
Smell	20	4	3,80	$15,20^{a} \pm 0,01$	4,35	$17,40^{b} \pm 0,04$	4,26	$17,04^{c} \pm 0,03$
Taste	20	5	3,79	$18,95^{a} \pm 0,01$	4,37	$21,85^{b} \pm 0,04$	4,55	$22,75^{c} \pm 0,03$
Consistency	20	3	4,00	$12,00^{a} \pm 0,07$	4,05	$12,15^{b} \pm 0,04$	4,35	$13,05^{c} \pm 0,07$
Appearance of cross section	20	4	4,41	$17,64^{a} \pm 0,03$	4,33	$17,32^{b} \pm 0,04$	4,67	$18,68^{c} \pm 0,04$
Outside look	20	1	4,82	$4,82^{a} \pm 0,01$	4,51	$4,51^{b} \pm 0,04$	4,76	$4,76^{a} \pm 0,04$
Total KV PSV % of maximum possible quality		20						
PSV				$4,09^{a} \pm 0,03$		$4,30^{b} \pm 0,01$		$4,52^{c} \pm 0,09$
% of maximum possible quality				$81,80^{a} \pm 0,71$		$86,00^{b} \pm 0,61$		$90,40^{c} \pm 1,71$

KI - coefficient of importance; O - average grade; K - corrected assessment; PSV - weighted average

^{a,b,c} - values marked with different letters have a statistically significant difference between the examined variants ($p<0.05$)

According to the results from sensory analysis (Table 3), can be seen that for the most of the tested features, the sausages from the variant 3 have the highest grades (color – 14.16; taste – 22.75; consistency – 13.05 and appearance of cross section – 18.68). The sausages from the variant 2, have the highest grades for the feature smell (17.40). The percent of maximum possible quality has the highest value for the sausages from the variant 3 (90.40%). There is statistically significant differences ($p<0.05$) in the variant 2 and 3 compared to the control variant, as well as between the variant 2 and variant 3 for the PSV value and for the percent of maximum possible quality.

Discussion

In the studies of Elías and Carrascosa (2010) for determining the color of the Portuguese traditional sausage *Paio do Alentejo,* the following color parameters have been obtained: *L** (43.1), *a** (16.4) and *b** (13.2). According to this recipe, the color is achieved as a result of the added nitrates in the form of $NaNO_3$ (0.039%) and KNO_3 (0.008%), and nitrites in the form of KNO_2 (0.0076%). Compared with the values of the control variant of the *Macedonian traditional sausage*, the Portuguese sausages show a lower color intensity for all three parameters.

In their study, Stojanova et al. (2017) examined the influence of some starter cultures on the instrumental values for color, on the surface of the Macedonian traditional sausages. They concluded that from the aspect of retaining the values for redness (*a**) and the yellowness (*b**), a better effect showed a starter culture CS-300 (*Staphylococcus carnosus spp. utilis* and *Staphylococcus carnosus*). Thus, the samples of this variant showed better values for total color change (ΔE) and color saturation (C).

Škaljac (2014) points out that sausages produced without the addition of starter cultures, in traditional conditions, were darker at the end of the production process ($p<0.05$) compared to sausages in which starter cultures were applied ($p<0.05$), where a higher pH value was also noted. The research conducted with the addition of starter cultures in the production of *Macedonian traditional sausage*, completely coincides with the literature data from the aspect of lightness. Namely, in sausages from both variants in which starter cultures are added, color illumination is observed during storage, compared with the control variant, where the value for the lightness decreases, i.e. the sausages have a darker color.

According to El Adab et al. (2014), the development of color in fermented sausages is largely conditioned by the duration of ripening of sausages rather than by added starter cultures. The authors point out that *L*, a** and *b** values are usually reduced during the maturation process in the event that starter cultures are not added.

Operta et al. (2012) investigated the sensory characteristics of Bosnian sausage produced from fresh, chilled beef (type A) and frozen beef (type B), adding starter cultures *Lactobacillus, Pediococcus, Staphylococcus* and *Candida.* They found that both types of sausages have similar sensory properties. There were statistically significant differences in the mean for the cross-sectional appearance. Type B sausages had a better consistency (7.55) compared to type A sausages (5.76), lighter color (6.13 / 7.79) and whiter adipose tissue color (1.73 / 4.30).

According to Toldrá (2002), the innate taste of sausages as well as the yellowish color of adipose tissue occurs as a result of oxidation of unsaturated fatty acids.

In the research of Operta et al. (2007), Bosnian sausage produced in industrial conditions, by tasters, is generally rated as "undesirable".

According to the results of sensory analysis of perennial domestic and wild boar sausages obtained by Kos et al. (2015), concluded that home-made pork sausages have a more intense smell, greater spiciness, as well as greater consistency and better overall quality.

According to Petrović et al. (2007), the highest sensory rating for the overall appearance, had the sausages produced in the first season that were smoked and dried in traditional conditions. The lowest sensory rating for the overall quality was given to the sausages that were smoked and dried in industrial conditions.

Conclusions

Based on the presented data for the instrumental measurement of the color of the cross sections of *Macedonian traditional sausage* from all three variants, on the 4^{th} and the 30^{th} day of production, as well as the sensory analysis on the 30^{th} day of the production, can be concluded that in all samples there is a tendency of reduction of the color values over the course of storage.

During the storage period, in the sausages of variant 3, the activity and the presence of starter cultures and in general the present microflora decreases, which directly affects the decrease in the intensity of the color, i.e. the approximation to the color of the control variant, where this and other quality properties are achieved as a result of the added nitrite salt. On the other hand, sausages of variant 2 indicate greater color consistency during storage.

Even that the sausages from the variant 3 have better values for some of the color parameters, as well as better grades for most of the tested sensory properties, they have lower color stability compared to the sausages from variant 2. In this way, the sausages from variant 2 are characterized with the middle, optimal values for the most of the analyzed parameters.

Generally, it can be concluded that the starter culture CS-300 (*Staphylococcus carnosus spp. utilis* and *Staphylococcus carnosus*) is recommended for the industrial production of sausages.

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BIODEGRADABLE ANTIBACTERIAL FOOD PACKAGE

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ABSTRACT

Plastic is one of the most commonly used materials for food packaging. Plastics are generally produced using oil derivative materials, which is both high in cost and takes too long to recycle. Nonetheless, plastic packages are also one of the most important reasons underlying environmental pollution. Thus, the aim of this research is to obtain silver nanoparticles through thyme, rosemary and daphne extracts and observe their antibacterial properties in order to produce a biodegradable antibacterial food package by using green polymers with the successful extracts. First, the herb extracts were obtained and used for obtaining silver nanoparticles. The antibacterial effects of the extract + silver nanoparticles were tested on S. aureus, E. coli, B. subtilis, V parahaemolyticus bacteria using the disk diffusion test. As a result, we observed that the inhibition effects of thyme and daphne extracts on bacteria are higher compared to other extracts. Therefore, we produced three different food packages using thyme extract and green polymers (starch, PVA and HEC). These packages were used on meat and cheese. As a result of the research conducted, we determined that the use of food packages containing PVA+HEC+Thyme is more beneficial than using packages that take too long to recycle and are unhealthy and harmful to nature.

Keywords: Recyclable, Biodegradable, PVA, HEC, Thyme

INTRODUCTION

In the food industry, materials that help to preserve food without spoilage, provide cheap and secure transportation, increase the durability of the product and protect the product inside, facilitate its marketing and ensure ease of use in terms of loading, unloading and stocking are called Package. (Üçüncü, 2007). One of the most commonly used materials in food packaging is plastics. Plastics are mostly produced from petroleum-derived materials, yet the recycling of these materials takes a long time and it high in cost. On a different note, plastic packaging is also one of the most important causes of environmental pollution and adversely affects human health (Davis and Song 2006). For example, dioxin is released during the production and recycling of PVC (Polyvinyl chloride), an artificial polymer in the structure of plastic packaging. In the case this substance enters the human body through the food chain (even in very small doses), it will cause problems in the immune and reproductive system (www.academia.edu). The use of renewable resources is increasing in the production of biodegradable food packaging materials due to the increase in environmental pollution, limited oil resources and adverse effects of these packaging on health (Namazi et. al., 2011). Among the renewable sources used are polymers such as cellulose, starch, protein (Çelebi and Dehmen 2013), as well as green polymers such as polycaprolactone (PCL), chitosan, polyvinyl alcohol (PVA), sodium alginate (Pereira et al., 2014; Rahman et al. 2017), with their usage and studies on them increased. These substances are natural polymers that are highly successful in terms of gel and film production and are not harmful to human health. Today, the total annual income

of edible polymers is reported to exceed $100 million. In terms of producing food packages, as well as their storage and easy transportation, it is also important to take into consideration the protection of the product against microorganisms. Generally, chemicals are used for this purpose. However, it is a well-known fact that chemical substances pose danger to human health and the environment. As a matter of fact, in terms of hygiene, in addition to the use of the actual material, the use of natural materials also comes into play. In this case, the first such materials that come to mind are medicinal plants. In recent years, due to the harmful side effects of substances of synthetic origin, especially the resistance of microorganisms to drugs used as antimicrobial and antibacterial, has boosted the importance of natural herbal resources and medicinal plants having antibacterial properties (Nakipoğlu and Otan 1992). Furthermore, one of the most important changes that occur during the preparation and consumption of foods is oxidation. Antioxidants help maintain product quality by preventing the oxidation of lipids, the basic ingredients of food. For this purpose, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylate hydroxytoluene (BHT), propyl gallate (PG), tertiary hydroxyquinone (TBHQ) and natural antioxidants such as α-tocopherol acetate, β-carotene and Vitamin C have been widely used in the food industry for many years. Informed about the possibility that synthetic antioxidants may have toxic effects on human health and, therefore, limiting or prohibiting their use. Health authorities' explanation of herbal antioxidants as reliable products and conscious consumers' preference for natural products has given rise to the use of herbal products. It has been demonstrated that flavonoids, phenolic compounds and their derivatives are effective in preventing autooxidation (Basmacıoğlu Malayoğlu, 2010). As a result of the academic research conducted, thyme was preferred as the first plant as it prevents the acting and spoilage of the antioxidant substances, which need to be stored for a certain period of time, by adding them into the foodstuffs or by putting them in the container they are packaged in (Azaz et. al., 2004). The recent years in particular have seen an increase in the number of studies on the use of medicinal and aromatic plants such as sage, thyme, rosemary and clove rich in phenolic compounds as a preservative in foods. Among these, rosemary has been studied intensively and is the only commercial product offered as an antioxidant in Europe and the USA. Hence, rosemary has been chosen as the second plant. The laurel (Schnaubelt 1999, Wyk and Wink 2004), which stimulates appetite, facilitates digestion, sheds intestinal worms, prevents tooth decay, is germ-killing, mucolytic, antiviral, antifungal, antibacterial, works as a muscle relaxant and is insecticidal, has been chosen as the third plant. When studies conducted using medicinal plants were examined, it was revealed that these plants were actively forming silver nanoparticles (Farghaly and Nafad, 2015; Gholami et. al., 2018; Heidari et. al., 2018). Nanoparticles is the general name given to particles ranging in size from 0.1 nm to 100 nm (Beykaya & Çağlar, 2016). The use of nanoscale materials is quite common. These are gold, silver, zinc, copper, nickel, palladium etc. and are widely used in the medical field, food industry, pharmacology, optics, electricity, textiles, bioremediation and agriculture (Board 2014; Fernandez et. al., 2016; Azarang et. al., 2014). The aim of this study is to achieve silver nanoparticles by obtaining the extracts of plants such as thyme, rosemary and laurel in order to investigate the antimicrobial properties of the extracts and nanoparticles obtained with the objective to produce biodegradable antibacterial food packaging using green polymers with successful extracts.

Method

2.1. Supply of Herbs

The herbs used in this study, such as Laurel (Laurus nobilis), rosemary (Rosmarinus officinalis) thyme (Thymus vulgaris), were obtained from the Medicinal Plants Garden. (Figure 1)

Figure 1: The plant samples used in the study.

2.2. Preparation of Plant Extractions

As the purpose of the study is the use of natural substances, the brewing method was preferred at this stage. Dried plants were washed with distilled water, cut into small pieces with a scalpel and scissors, and then thoroughly crushed with a blender. Thyme, rosemary and laurel samples were weighed to 5 grams and placed in glass beakers containing 50 ml of distilled water. The beakers were placed in a magnetic stirrer and stirred for 24 hours at an appropriate temperature (Figure 2). After the cooling process, the plant extract was filtered into plastic tubes using a Whatman filter paper. Plant extracts taken into tubes were centrifuged at 2500 rpm for 5 minutes to obtain the desired extract.

Figure 2: Process of obtaining plant extracts.

2.3. Obtaining Silver Nanoparticles

20 ml of the thyme, rosemary and laurel extracts were put in glass beakers and placed in a magnetic stirrer. The mixing process was continued by adding 10 ml of silver nitrate to each beaker; a sample was taken every 30 minutes (three times) and examined in the spectrophotometer (Figure 3). The change of color in the plant extract is a symptom of nanoparticle formation (Gupta, 2017).

Figure 3: Formation of silver nanoparticles using plant extracts.

The spectrophotometer was measured at 380-500 nm UV and the absorbance values of the changes were measured (Figure 4).

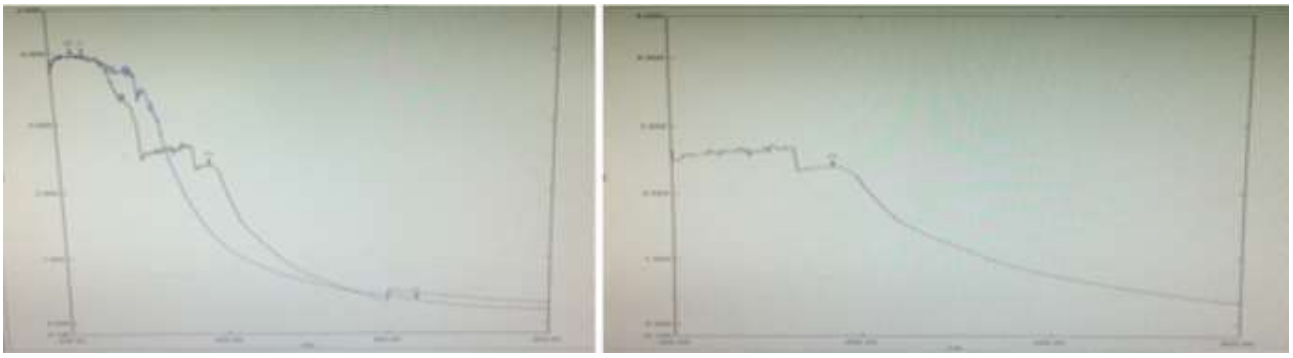

Figure 4: Spectrophotometer results

Once the plant extract and the silver nitrate mixture were centrifuged (2500 rpm, 2 min), the pellet was removed. Pellet distilled water was vortexed briefly, filtered and washed properly. After this process was repeated seven times, the silver nanoparticles were dried in the incubator (70 ° C, 48 hours).

2.4. Media Preparation:

The media to be used to determine the effects of the extracts on microorganisms were prepared as follows: Buffered Peptone Water (BPW) medium preparation: 2.295 grams of medium was used for 90 ml. As four cultures were enlivened in this medium, 9.18 grams of medium was used to create 360 ml of medium. The medium prepared was sterilized in an autoclave (121 ° C, 15 min). Plate Count Agar (PCA) preparation: 26.5 grams of medium was used for 1 liter. Each medium was poured into 12.5 ml petri dishes. The prepared medium was sterilized in an autoclave (121 ° C, 15 min). Preparation of TCBS agar medium: This medium is used vibrio only. A total of 88 grams medium is used for one liter. The medium, which does not need to be

autoclaved, is mixed after adding to the water and prepared when the medium dissolves and begins to boil.

2.5. Investigation of Antibacterial Effects of Silver Nanoparticles with Plant Extracts:

This study was conducted in the Microbiology laboratory. The preferred measure of bacteria to be used in the study was +/- 2 grams. While selecting bacteria, priority was given to those that cause adverse effects on foods with the excessive increase in the number of bacteria. These bacteria are Staphylococcus aureus (ATCC25923), Escherichia coli (ATCC25922), Bacillus subtilis (ATCC6633), Vibrio parahaemolyticus (ATCC17802). Buffered peptone water (BPW) was used to stimulate S.aureus cultures. After preparing the medium and autoclaving, colonies that we produced (revitalized) using the Plate Count Agar (LabM) spreading plate method were incubated. The disc diffusion method was applied to the samples and kept at 37° C for 24 hours and the results were observed. Bacteria were incubated after sterilization in the BPW (90ml) autoclave to stimulate E.coli cultures. Bacteria were incubated using the PCA smear plate method 0.1 ml; the disc diffusion method was applied and samples were kept for 24 hours at 37 ° C, and the results were evaluated. The same method was applied to revive B.subtilis cultures and to observe the results. 90 ml BPW was used to stimulate the V. parahaemolyticus culture, which was prepared differently. In this bacterial group, the same procedures were repeated and sowing was performed, and the disc diffusion method was applied (Figure 5).

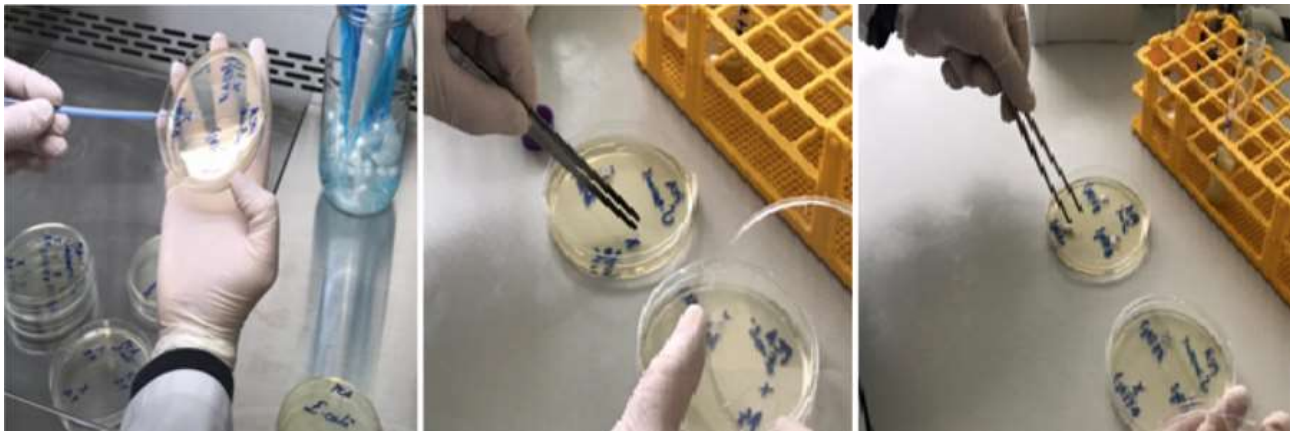

Figure 5: Bacterial cultivation and the disc diffusion method

In the disc diffusion method, the following steps were followed to test the extracts and the extract + silver nanoparticles. 1 ml of each sample to be tested was mixed with vortex and placed in petri dishes by absorbing the discs.

In the study, antibiotics were used as a positive control group, and the effect of silver nitrate alone was also investigated (Figure 6).

Figure 6: The disc diffusion method

2.6. Preparing Protective Film

This step was carried out after observing the positive effects of extracts on microorganisms. Three different films were produced to create a natural, biodegradable protective film. Natural polymers such as Starch, PVA and HEC were used in the preparation of these films. 5g PVA, 238ml distilled water, 10ml glycerol, 2ml thyme extract were used for the first film, while 3g PVA, 2g HEC, 238ml distilled water, 10ml glycerol, 2ml thyme extract, 3g PVA were used for the second film, and 2 g starch, 238 ml distilled water, 10 ml glycerol, 2 ml thyme extract were used for the third. These materials were placed in separate glass beakers and mixed in a magnetic stirrer at a suitable temperature for approximately 1-1.5 hours. They were then poured onto a flat tray when they reached the proper consistency (Figure 7). The trays were placed in a vacuum sterile cabinet to remove excess water.

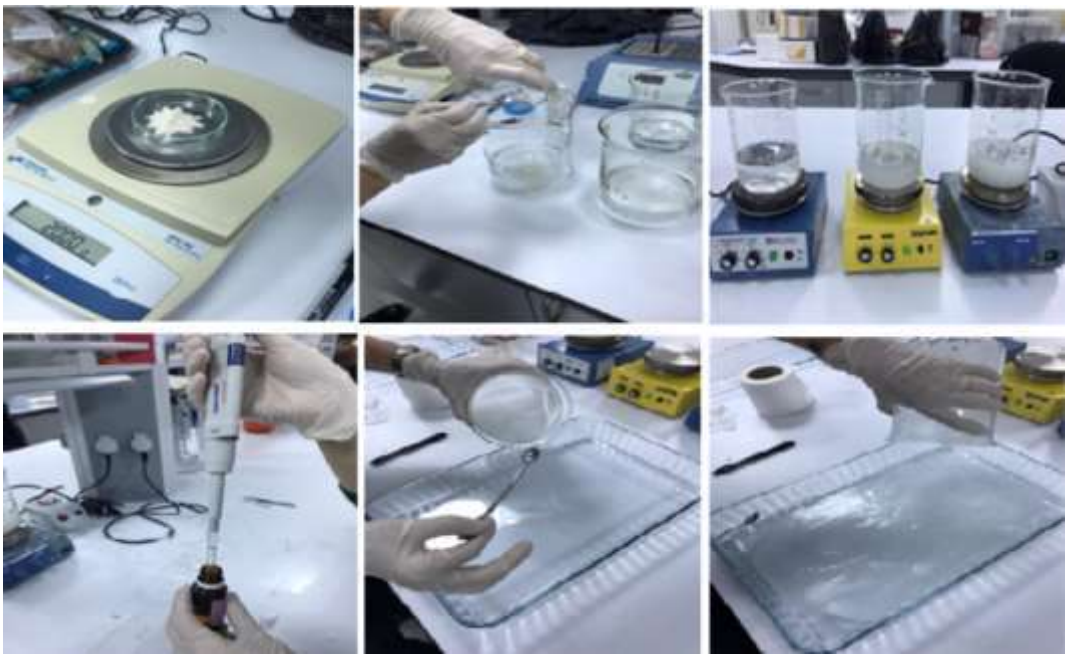

Figure 7: Preparation of protective film

2.7. Testing Protective Films on Food

It was observed that the films kept in the vacuum sterile cabin for 24 hours had the desired consistency. The films were tested using foods that deteriorate quickly, such as cheese and meat (Figures 8, 9, 0 and 1). Since films are considered as an alternative to stretch films, they were used in the stretch film study. Foods were packed with films and kept in the refrigerator for 2-3 hours at room temperature.

Figure 9: Film produced with PVA only

Figure 10: Film produced with PVA + starch

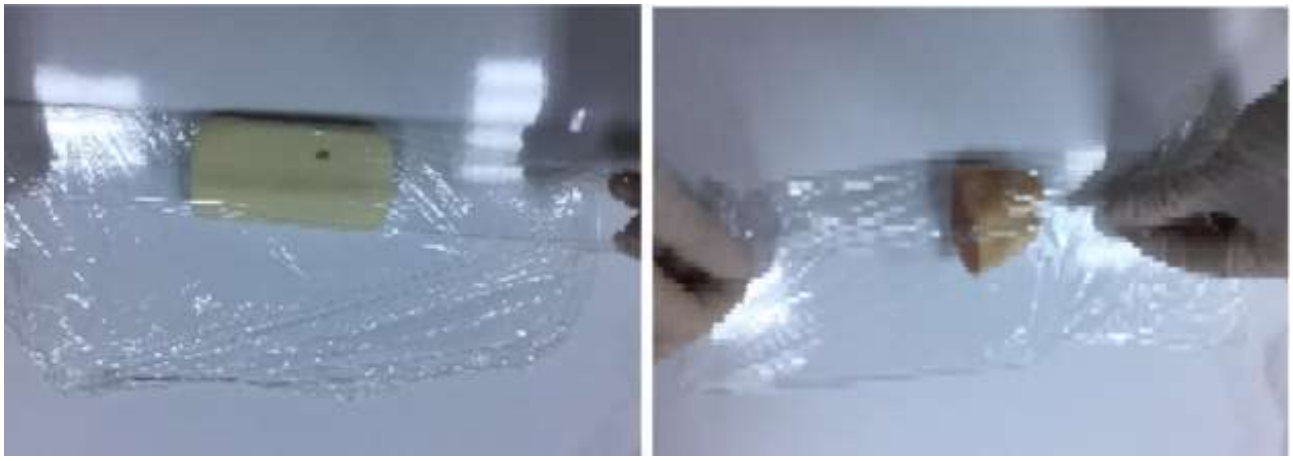

Figure 11: Film produced with PVA + HEC

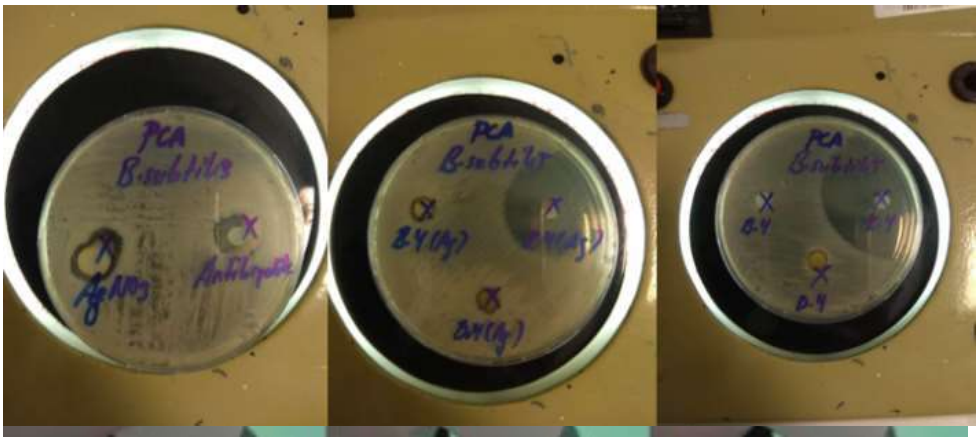

Figure 12: Stretch film

Findings

As a result of the microbiological study conducted with the prepared samples, the zone diameters formed in petri dishes were measured (Table 1, Figures 13, 14, 15 and 16).

Table 1: Inhibition zone diameters of the samples on four different bacteria

	B. subtilis	*Vibro p.*	*E. coli*	*S. aureus*
Daphne Extract	0 cm	0 cm	3 cm	3 cm
Thyme Extract	2.2 cm	3 cm	0 cm	0 cm
Rosemary Extract	0 cm	0 cm	0 cm	0 cm
Daphne + AgNO3	0.1 cm	0.7 cm	1 cm	1 cm
Thyme + AgNO3	1.2 cm	3.5 cm	0.1 cm	0 cm
Rosemary + AgN03	0.1 cm	0.9 cm	0.1 cm	0.3 cm
AgN03	0.5 cm	1 cm	0.4 cm	0.3 cm
Antibiotic	0.5 cm	1 cm	0.5 cm	0.5 cm

Figure 13: Inhibition effects of samples on Vibrio sp.

Figure 14: Inhibition effects of samples on E. coli

Figure 15: Inhibition effects of samples on S. aureus

Figure 16: Inhibition effects of samples on B. subtilis

While the prepared antibacterial films were removed from the trays, it was determined that only the film containing PVA is quite thin and looks like a stretch film; the PVA + starchy film is more flexible than others, and the film containing PVA + HEC is stronger than others. When foods packed with antibacterial, biodegradable films were observed, on the first day, it was determined that only the film produced using PVA melted on the meat. In the film produced with PVA + starch, melting was detected in a few spots. No melting or deterioration was detected in films produced with PVA + HEC (Figure 17).

Figure 17: Testing the films with food

4. Results and Discussion

Environmental pollution is one of the most serious problems we face today. In this sense, plastic bags are the most outstanding packaging that cause pollution. Unfortunately, they are not very easy to get rid of as the recycling of these substances takes a long time. Since these materials are mostly used for carrying and preserving food, the aim of this project has been to produce antibacterial biodegradable food packaging/film. In the studies conducted, medicinal plants such as thyme, rosemary, and laurel –which are easily found in nature and grow almost anywhere on earth– have been preferred. These extracts were also used to obtain silver nanoparticles and nanoparticles were obtained successfully. Samples containing the extract and extract + silver nanoparticles were tested on bacteria such as B. subtilis, Vibrio sp, E. coli and S. aureus, which had an adverse effect on food when their numbers increased. As a result of microbiological studies, it has been determined that thyme extract has high inhibition effects on B. subtilis and S. aureus, and daphne extract has high inhibition effects on Vibrio sp and E.

coli. The silver nanoparticles that we obtained using extracts were found to have no effect, leading to a 01-0.5 cm increase. Since thyme is easier to produce and find, sample films have been produced using thyme extract. PVA, Starch and HEC green polymers were used in film studies after determining which plant extract has positive effects on microorganisms. The purpose of trying these polymers is that they are biodegradable and have no negative effects on human health. PVC (polyvinyl chloride), which is generally used in packaging, is an artificial polymer and has a negative effect on human health due to the harmful chemical dioxin that occurs during its recycling process (www.academia.edu). The films were tested on cheese and meat. In the observations made, it was determined that the film containing PVA only started to melt on meat and cheese after a while (approximately 1 hour) due to PVA being soluble when contacted with water. It was observed that the film containing PVA + Starch is quite flexible during the packaging process. However, with the use of this film, deformation and contractions were observed, especially on certain parts of the meat. The film containing PVA + HEC resulted in no deformation or melting. Samples packed with the PVA + HEC film remained in the cabinet for 20 days with no deterioration observed. According to the results of the study, it has been concluded that the usage of biodegradable packages prepared with PVA + HEC instead of packaging prepared using PVC can contribute to the reduction of environmental pollution, while alternative products can be produced by using medicinal plants such as thyme, laurel and rosemary instead of different disinfectant chemicals.

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INVESTIGATION OF DANDELION (*TARAXACUM OFFICINALE*) PLANT AS AN ALTERNATIVE SOURCE FOR A NATURAL RUBBER PRODUCTION

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ABSTRACT

Rubber that is one of the most important polymers naturally produced by plants is a strategic raw material. Since they are used in many areas and natural rubber sources are highly destroyed, synthetic rubbers are produced especially using some petroleum-based polymers. In this study, it is intended to find a natural alternative to the rubber trees that are endangered and to the synthetic rubbers that are difficult to recycle and expensive. In this study, first, *T. officinale* plant was collected from natural areas through such a way that the roots of the plant were not damaged. After the well-washed plant roots were broken into small pieces, they were kept in different solvents for 24 hours. After they filtered and formic acid was added, the prepared samples were kept for 24 hours. The part sinking to the bottom was taken and dried in a fume hood. Since the obtained material was in a small amount, the test paste was prepared by using 100 g of the obtained rubber, 100 g natural rubber from rubber tree, 1 % sulfur and 2 % extender material. After the paste had reached the desired consistence, it was taken into hot press machine and the vulcanization process was completed (at 140 °C, for 7 minutes). Because too little substance in less variety was added into the molded material, vulcanization curve test was only performed by the rheometer machine. As a result of the study, it was determined that by adding some substances to the structure of the obtained rubber during the vulcanization according to the desired purpose, it can be used in various fields, and thus, *T. offcinale* can be an alternative natural rubber source.

Keywords: *Taraxacum officinale*, rubber, synthetic, recycling

1. Introduction

The rubber (cis-1, 4-polyisoprene), which is one of the most important polymers naturally produced by plants, is a strategic raw material. It is used as a raw material in about 40,000 products including more than 400 medicinal products (Mooibroek et al., 2000). The natural rubber, which is used in automobile parts, automobile tires and many tools and equipment in daily life, is an indispensable engineering material of the modern life (Vahapoğlu, 2007). Rubber was first used by Meso-Americans in the 1600s and was produced from Panama Rubber tree (*Castilla elastica* Sesse). The produced material was named as "cauchu" (weeping wood) by Meso-Americans (Hosler et al., 1999). Then, Aztecs, Mayans and Mexicans used rubber in the production of waterproof clothes, footwear and coating (Evans, 2008). While the rubber was used in ball production by the Spanish and in the production of waterproof tire fabric, hose, car bumper, tire, etc. by British, some problems (softening or hardening according to ambient temperature) were experienced. However, these problems were eliminated by the development of the vulcanization method found by Goodyear (Bell, 2013).

The source of the rubber, which is the only commercial natural resource, is *Hevea brasiliensis*, an endemic species (Brazilian rubber tree). The rubber obtained from this plant has

high performance in terms of flexibility, elasticity, wear resistance, heat distribution and impact resistance properties (Mooibroek et al., 2000). Due to these features and high usage areas, the demand for rubber increases day by day and millions of hectares of tropical forests having biological diversity are destroyed in order for meeting with these demands (Warren-Thomas et al. 2015). Also, it should not be forgotten that trusting in a single plant species has some disadvantages and potential dangers. The most of plants grown consist of cloned individuals. This situation affects the genetic diversity negatively and increases the susceptibility to pathogens (Bell, 2013). For example, cultivated trees in South America have been greatly damaged due to the leaf blight (Le Guen et al., 2004).

Synthetic rubbers are produced using various polymers (styrene-butadiene, etc.) in order to meet the need and prevent damage. Most of the rubber products used today are petroleum based. But, compared to the natural rubber, the produced synthetic rubber does not have high performance in terms of various properties (flexibility, impact resistance, etc.). Various chemicals are used to improve the performance and synthetic products are not recyclable. Performance is very important especially for medical materials and aircrafts (Bell, 2013). Therefore, natural rubber-producing sources need to be protected and developed or potential alternative rubber sources should be found (Iaffaldano et al., 2018). *Asclepias* spp. (buttercup, verbena, milkweed), *Castilla spp.* (rubber plant), *Euphorbia spp.* (spurge), *Ficus spp.* (rubber tree), *Landolphia ssp., Solidago ssp.* (goldenrod), *Taraxacum ssp.* (dandelion), *Parthenium ssp.* (guayule) etc. plants are proposed as natural sources for rubber production (Bell, 2013; Mooibroek et al., 2000). Among them, *Parthenium argentatum* (guayule) and *Taraxacum kok-saghyz* (Kazakh dandelion = Russian dandelion) come to the fore in terms of rubber yield (Iaffaldano et al., 2018).

T. kok-saghyz, a single-year plant, was defined as a latex producer in the 1930s (Ulmann, 1951). It produces latex in plant roots. Numerous studies related to this plant have been carried out and it has been determined that latex production of the plant is better than the other species but its competitiveness with weeds is weak. Therefore, studies such as gene transfer, cloning (Schmidt, 2009, 2010) and hybridization with *T. officinale* are going on (Iaffaldano et al., 2018). *Taraxacum kok-saghyz* is not a species that grows naturally in our country (Turkey); therefore, its culture must be done. On the other side, *Taraxacum officinale* is a common species that grows naturally in our country. *T. officinalis*, which is multi-year, is known for its pharmacological effect and the conducted studies are generally for this purpose. The plant has antioxidant, antifertility, hepatoprotective, anti-inflammatory, antitumor, choleretic, diuretic and antirheumatic effects (Baba et al., 1981; Jeon et al., 2008; Tahtamouni et al., 2011). It is used by the public for fever, stomachache and headache (You et al., 2010). Also, its racemes, leaves and roots are used as food, its roots are dried and consumed as coffee and used as a flavoring agent in various desserts, beverages and pastries. The studies done with this plant are quite limited in our country. Vahapoğlu (2007) gave place to this plant while giving information about rubber materials in his study. Yıldırım (2016) investigated the development of individuals under heat stress by adding plant extract to forage. Kaplan et al. (2018) used it in the production of tincture.

The Purpose of the Project

In this study, it is aimed to obtain rubber from *T. officinale* plant and to produce something from this rubber in order to contribute to the production of natural rubber, which has become a global problem and various alternatives are searched for it, and to produce healthy and natural substances instead of non-recycled synthetic products.

2. Method

2.1. Collecting of the Plant Material

In the study, *T. officinale* plant collected from various natural areas was used as material (Figure 1).

Figure 1: Collecting of material

2.2. Preparation of the Plant for Rubber Production

The roots of the collected plants were washed thoroughly with tap water and then the roots were cut into small pieces by means of knife and scalpel (Figure 2).

Figure 2: Washing and breaking up the roots

Because the root parts were hard, pestle was used to minimizing the parts better (to increase the surface). The crushed pieces were weighed and taken into 3 different glass flasks (500 g for each glass). Ethyl alcohol was added to the first glass flask, chloroform was added to the second glass flask, and acetone was added to the third glass flask (up to 1000 ml). The flasks were covered with aluminum foil and kept waiting at room temperature for 24 hours (Figure 3).

Figure 3: Crushing the roots and putting them in solvents

2.3. Obtaining of the Rubber

Samples were separately filtered fist through metal, then through a plastic strainer into glass flasks and formic acid (50 ml) was added on them. The glass flasks, whose mouths were closed, were waited for 12 hours in a fume hood. It was checked whether the rubber sank or not by looking at the bottom of the flask. The excess amount of the solvent and formic acid in the glass flasks was collected and the material accumulated at the bottom was taken into the petri dishes. In order to remove the liquid in the samples, the petri dishes were kept waiting in the fume hood (Figure 4).

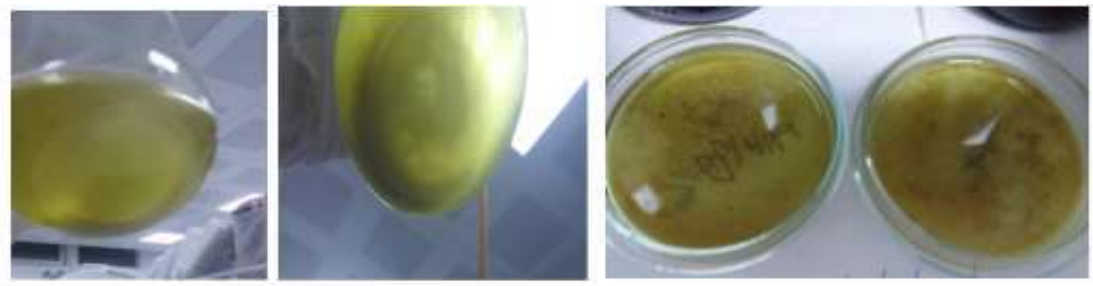

Figure 4: Obtaining of the rubber

2.4. Vulcanization

The purpose of this process: All rubber types, whether natural or artificial, are a part of the general polymer class named elastomer. The polymeric (macromolecules) materials, which can be extended to at least two times the original length at the room temperature and which are able to return to the original state when the force that provides this elongation is removed, are called as the elastomer. The elastomers have dual bonds that provide flexibility. Properties of the elastomers are not suitable for commercial use in the raw state, that is, before any chemical treatment or vulcanization. Therefore, elastomers are generally vulcanized by using various chemicals. Through this way, the undesirable properties of the elastomers are eliminated and they were turned into a very suitable material for commercial use. Vulcanization is chemically called as cross-linking (Evcin, 2017).

Under normal conditions (during production), 100 gram of extender material (X, Y, Z substances) and 100 gram sulfur are added for 100 kg paste. In the preparation of the test paste, the formula used in the production was not applied one-to-one. The reason of this is that the desired properties of the produced rubber are previously determined and which material will be added and in which amount is calculated according to these properties. The test paste in the study was prepared by using 100 g of the obtained rubber, 100 g natural rubber from rubber tree, 1% sulfur and 2% extender material. Since the available rubber amount is little, an equal amount of the natural rubber obtained from rubber tree is used. Firstly, natural rubber was placed in the machine and it was ensured to become flat shape by heating via friction. In order for a good mixture in the paste, the produced rubber was added to the natural rubber step by step and the process at the machine was continued until the paste reached the desired consistency (Figure 5).

Figure 5: Obtaining of Rubber Paste

After the paste reached the desired consistence, it was taken into hot press machine and vulcanization was performed in the press die (140 °C, 7 minutes). The produced rubber was molded; various materials can be added, and the desired shapes can be given according to using area (Figure 6).

Figure 6: Vulcanization and molding of rubber paste

2.5. Analysis

Vulcanization values, wearing, tensile-rupture strength, ash determination, aging determination etc. tests are applied to the rubbers produced for specific purposes. Information about these tests was obtained from the R & D Laboratory of a factory and the test procedures were carried out there. In the study, only the vulcanization values test was performed since much additional material (enhancing strength-flexibility, more extender material etc.) were not added. At this stage, the material was placed in the rheometer machine and the vulcanization curve was followed on the screen. If the curve is increasing, it means that it is vulcanized. When the curve starts to decrease, it means that it is deteriorated.

3. Results

- In the first step of the study, natural areas in our environment were visited and the areas where *T. offcinale* is common were determined. Plenty of samples were collected from the Namık Kemal university campus. It was seen that the latex was flowing immediately if the root parts were damaged when collecting these samples. Therefore, they were collected carefully. Compared to the results of three different solvents used in the study; it was determined that in the samples obtained in acetone from the same amount of material, there was no precipitation when formic acid was added and there was more precipitation (rubber) when ethyl alcohol was used than chloroform usage.
- Plants were collected several times during the study. The samples we collected one day after raining were observed to be more developed, soft and latex rich.

- It has been understood that natural rubber which is obtained as a result of the application of ethyl alcohol and formic acid cannot be used directly in anywhere and vulcanization process had to be applied; then, operations were applied about this issue. In vulcanization, the baking operation with sulfur is carried out and during this process, various substances are added to provide it more durable, more flexible, etc. properties. In this study, only one locomotive extender material was added to the paste, the other materials were not added. The produced natural rubber was first pulped in the machines used for this operation and the baking process (vulcanization) was applied by using hot press. After this stage, the rubber became ready for use in certain areas. It can be easily moved to any place where it will be used in mold form.
- As a result of the vulcanization curve test, it has been seen that obtained mold rubber started to deteriorate in a short time (Figure 7).

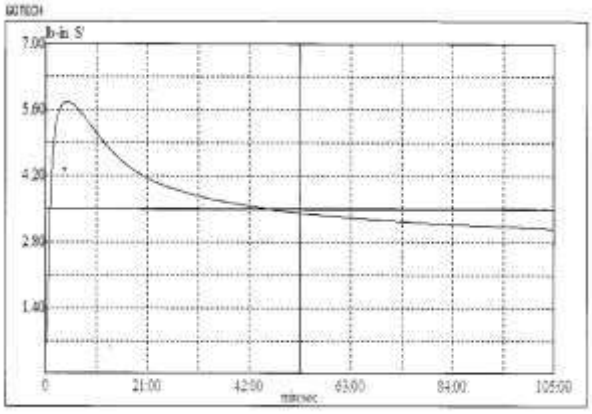

Figure 7. The results of the vulcanization curve test The results of original vulcanization curve test

4. Conclusions and Discussions

Rubber is an important green polymer (biopolymer). Polymers are materials that have many application areas and have great importance in human life. Today, since natural polymers cannot meet the requirements, synthetic (industrial) polymers are produced. In contrast to their high potential and characteristics, industrial polymers creates contamination due to the fact that they cannot be eliminated by a natural process and it can only be removed by a result of costly fragmentation process. Moreover, the fact that they are obtained from exhaustible resources, such as petroleum resources, has led to new searches in the polymer sector in terms of continuity and environmental compatibility (Hazar Yoruç & Uğraşkan, 2017). Damaging of the rubber trees due to the destruction of nature has also triggered the search (Warren-Thomas et al., 2015). *Parthenium argentatum* (guayule) and *Taraxacum kok-saghyz* (Kazakh dandelion = Russian dandelion) plants have come into prominence in this search (Mooibroek et al., 2000; Bell, 2013) and the yield of guayule was increased by plant development studies (Durmuş et al., 2005). The same processes are also carried out for *T. kok-saghyz*. Based on these studies, it was decided to study with *T. officinale* since it grows in large areas naturally in our country (Turkey).

In the study, it was observed that when the plants used as material were collected after rain, the roots were easily broken into pieces and more rubber was obtained. It was been seen that use of ethyl alcohol or chloroform as the solvent were useful. Here, another important point is the collection time of the plant. The plants were collected at the end of the summer, beginning autumn. If the collection process is carried out in the spring, the amount of obtained rubber, that is amount of the product, can be increased. The reason of this is that the rubber is a secondary metabolite for the plant. The amount of secondary metabolite, the included substances, and the plant parts can vary depending on many factors such as the harvesting season, used methods and techniques, plant development stage and geographical area (Burts, 2004).

Natural rubber obtained from *T. officinale* was not used directly on a material like other rubbers and it was subjected to vulcanization process. Today, modern vulcanization processes are carried out at temperatures of 140-180 °C. During these processes, various additives are added to improve the physical and chemical properties of the rubber. 3 of these additives are very important. These are the additives that shorten the vulcanization time [inorganic accelerators (lead oxide, magnesium, calcium), organic accelerators, ultra-accelerators], antioxidants which increase the strength and carbon black which increases the tearing-friction resistance (Vahapoğlu, 2007). The amount of sulfur (0.5-50 %) added during the vulcanization affects the properties of the produced rubber; the rubber hardened when the amount of sulfur increases. For example, rigid rubber is obtained by performing vulcanization with 30-50% sulfur (Hazar Yoruc & Ugaşkan, 2017). In this study, only 1% sulfur and 2% extender material were added during vulcanization. Therefore, as a result of the vulcanization curve test, it has been observed that the obtained rubber began to deteriorate in a short time. This result was also evaluated by the authorities in the R & D department. It was determined that the rubber obtained by increasing the amount of extender material and sulfur with adding other materials can be used in various areas.

On the other hand, the obtained rubber is not a pure rubber. Other herbal substances in the structure may also have affected the vulcanization curve test. Various methods have been applied in order to obtain pure rubber in the literature (Bushman et al., 2006; Bell, 2018). However, a wide variety of chemicals and laboratory environments are needed to apply these methods.

The rubber, which has a very wide usage area and market, is today generally produced synthetically from petroleum-based products. However, these products do not have the characteristics of natural rubber and a lot of time and money are spent for their recycling. In addition, some of them are non-recyclable. Natural rubber sources have lost their previous yield due to destruction. Therefore, this study is very important. As a result of the study, it was determined that obtained rubber can be used in various areas by adding some substances to its structure during vulcanization according to the desired purpose, so that *T. offcinale* may be an alternative source of natural rubber. It is thought that the obtained data will be evaluated by experts in the field and the study will be improved.

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KAIZEN APPLICATIONS AS LEAN MANAGEMENT IN INDUSTRY – CASE STUDY

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ABSTRACT

This study focused on developing production stages as much as required in processes those were performed for an exclusive company which works in İstanbul/TURKEY. So as to accomplish this, some problems of production were inspected via Lean Management methods and new ways for improvement of products were done and applied. Necessary studies were performed via lean system which aims for developing efficiency in recent industry world such as KAIZEN. In order to apply Kaizen effectively, Why-why analyse and PDCA (Plan-Do-Check-Act) methods were used. The kaizen's goal was to reduce rate of problems occurred during production and gain more successful products. One of the applied KAIZEN methods was explained with details in this study.

Keywords: *Lean Philosophy, Kaizen, Problem, Product.*

INTRODUCTION

The package, which was originally used to preserve and transport the product within its sole function, has now become a visual tool in addition to keeping the product clean. It is also effective in terms of maintaining the product in healthy conditions, following the barcodes on it and including the necessary information and brand details. In summary, it is not only a carrier for the enclosed product but also a part of the product that is suitable for that product. Food packaging is especially important for keeping the product fresh and healthy and providing information about the product. Because these features play a very important role for consumer to choose the product. In current years, competition in the packaging industry all around the world is very demanding. In case of inspecting industry reports it has been shown that the sector is still responsive. In 2017, Veres & Marian examined the effects of Japanese management model implementation in their work. Japanese model management systems have recently been adopted (Veres, C. & Marian, L., & Moica, S., 2017). After the Second World War, these management models have a huge impact on the development of the Japanese economy in the form of miracles. Kaizen, which means continuous development, has emerged as one of these management systems. Japanese words Kai (change) and Zen (Better) combine to form Kaizen (Continuous Improvement). The main purpose of the Kaizen method is to improve the process rather than the results. In short periods, results are improved. In long periods, improvement is achieved. Via this study, Kaizen is applied for solving problems which occur during production in a company in Istanbul/Turkey. Firstly, problems were defined as three parts. Then, in comply with lean philosopy, applied Kaizen was determined. Studies were recorded with Kaizen forms as well.

MATERIAL AND METHODS

History of Lean Management

In the world, production systems that demanding intensive labor were used until 1920. In the following years, expanding market volume and mass production forced producers to produce more products with less work. In this process, the mass production companies were the leaders in the markets and surpassed all their competitors. After World War II, Japan was in a very bad financial situation. In addition to this, production costs were as high as possible. In order to compete with Western and American producers who were working successfully in the mass production system, a new production system was needed to reduce costs. In this context, Toyota officials began to look for a way to reduce costs in order to cope with the market (Liker, 2014). With the studies, it was determined that stocks and costs increased by mass production systems and product types became monotonized. In this period, it was seen that increasing variety of products would bring advantage in the market by reducing stocks and lowering costs. The customer's view of quality changed over time and gained a new sight. They requested the products to be cheaper and specific for them. At Toyota company, Eichi Toyoda and Taichi Ohno began establishing a new production philosophy in the 1950s. In 1978, it was published as Toyota Production System ,which was new system, by Taichi Ohno. These advanced techniques in the book were so called "Lean Manufacturing" for the first time by John Krafcick, a researcher at MIT. The use of the definition of "Lean in this developed system is due to the fact that it is a simple system that produces more value with more limited resources than mass production. Book, 'The Machine That Changed The World', was published in 1990, making lean system known worldwide (Womack, J. P., Jones, D. T., & Roos, D., 2007).

Kaizen

One of the lean management methods is Kaizen. With the combination of Japanese words Kai (change) and Zen (Better), Kaizen (Continuous Improvement), the primary objective of the method is to improve production processes with small but effective and efficient changes (Sonobe, T., 2014). Kaizen is a method that gives priority to the process in production because it has aim for the improvement of processes.

To summarize Kaizen:

- Continuous improvement activities involving senior management, executives and all employees.

- Existing situations are not enough and it teaches to identify problems.

- Continuous change and improvement is always expected.

- Instead of big and uncertain steps, small and solid steps lead to the target.

Although they are usually confused with each other, Innovation and Kaizen are complementary but very different concepts. When innovation is mentioned, radical changes should come to mind. That is, a complete renovation of an existing system. Kaizen, on the other hand, is a small but effective change to make the existing system work better. Significant investments and the latest technologies are used in a short time for innovation. In contrast to innovation, small investments are made with reasonable investments to ensure continuous development for kaizen.

As a brief, they are defined as such:

- Innovation is a method that requires high investments in a short period of time, at the senior management level and uses new technologies.

- Kaizen is a method that does not require the participation of all teams and new technologies in a long process, it is done with small investments.

Henry Ford is the key character of innovation. As for kaizen, Taichi Ohno is the key character (Košturiak J, Boledovič Ľ, Kriťak J, Marek M., 2010).

There three types of Kaizen as mentioned:

1. Before / After Kaizen (Point Kaizen)
2. Kobetsu Kaizen
3. Kaikaku Kaizen (System Kaizen)

When there is an application that starts for Kaizen, there are some methods for efficient application.

PDCA Cycle

PDCA (Plan, do, check, act) is used in Kaizen processes. It was a cycle that first appeared in 1939 by W.A.Shewhart as "Define - Produce - Control". It was developed by W.E.Deming and became PDCA, which is defined as one of the quality activities (Santos, H., 2018). Via this method, requested improvement activities are carried out, control of the obtained results and all activities that make improvements become permanent. The PDCA cycle implements solution-oriented tools such as Pareto analysis, why-why analysis, and Ishikawa diagram (Brunet, A.P., New, S., 2003).

Figure 1: PDCA Cycle

PDCA cycle (shown as figure 1) with basic definitions:

- Plan: The problem is identified. The current situation is determined by analysis. The data required for the improvement are collected.

- Do: After planning, the application is started for improvements.

- Check: It is determined whether the planned improvement has been carried out successfully or not.

- Act: Necessary measures are taken to maintain the improvement (Higuchi, Y., & Nam, H.V., & Sonobe, T., 2015).

Ishikawa Diagram

This diagram found by Kaoru Ishikawa is known as fishbone because of its shape. It is Also known as cause-effect diagram. It was revealed in 1943. It is a diagram used to identify, classify,

and find the connection between the causes of the problem when faced with a problem. With the Ishikawa diagram, the whole team focuses on the problems and their knowledge and experience are utilized. It improves communication between team and employees. In the Ishikawa diagram, 6M is used when creating factors. 6M: Man, Machine, Measurement, Material, Method, Mother nature.

Ishikawa diagram steps:

- At first, the problem is identified and it is written on the fish's head tab on diagram.

- Then, factors affecting the problem are identified and written under the relevant sections.

- Different factors can be linked together by writing the same reasons. For example; The cause of a problem can be caused by both human and machine.

- Finally, the problems begin to be solved by voting of the team to determine the most important root causes of them.

Why-why analysis

It is also known as 5 why analysis or why-why analysis. It is a technique to ask until you find the root cause of a problem encountered. In order to get answers that start with 'because' about a problem, we need to ask "why ". It is found the real root cause of the problem with this technique and at the same time discover the connections between different causes.

Pareto analysis

Pareto analysis is a technique invented by the Italian economist and mathematician Vilfredo Pareto. Also it is known as the 80/20 technique. Examining wealth and income distribution of 19th century England, Pareto found that 80% of the country's wealth belongs to 20% of the population. He then studied research in other countries, including his own country, Italy, and he was surprised to see a very similar rate. According to this analysis, 80% of the results exist with the effect of only 20% of the inputs, and if the most important 20% of a job is done very well, 80% of the work will be done too.

Pareto analysis steps:

- Problems are identified and listed in categories.

- The percentages of the categories are determined and sorted from large to small.

- Cumulative distributions are calculated by saving the percentages in the graph. The lorenz curve is drawn as a line showing percentages.

CASE STUDY

The Kaizen study performed for this packaging company is called as " Kaizen for joint sensor". The packaging products manufactured in the company are wrapped around rolls. There are various defects in the packages on these rolls which reach to customers. These defects are such as black dots on package, printing defects, blade marks, lamination and non-adhesive areas (Shown as figure 2). For this reason, complaints are received from the customers every year and Kaizen study was carried out to reduce them. In Kaizen study, "Joint control" was applied to prevent these faulty products to reach the customers. Production in the packaging company starts primarily in extrusion process (Shown as figure 3). The products from the extrusion process are sent to printing process as next process (if there is no printing process, it is directly passed to the lamination process). In the printing process, when the related design is printed on the products, they are sent to the lamination process (Shown as figure 4). Here, the required

layers are laminated according to the structure of the material. For example: Polyethylene-Metalized Pet-Polyethylene structure has 3 layers and lamination process is performed by adding glue-catalyst between the layers. After the lamination process, the final process is slitting process, where the products are slitted as requested amount.

Figure 2: Faulty product

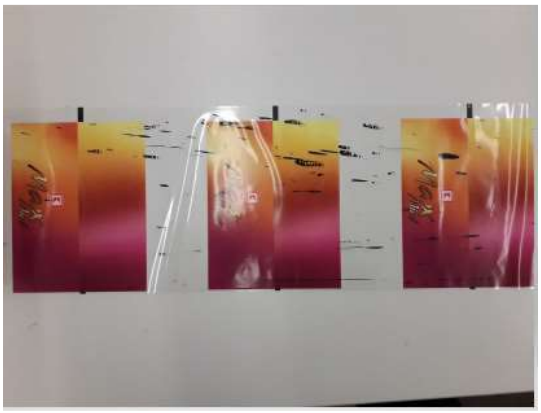


Figure 3: Extrusion and printing processes

Figure 4. Lamination and slitting processes

What is Joint?

So as to show detected problems of all processes when they are at next process, it is made coloured joint/flag (Shown as figure 5). The joint is made in order to determine problems correctly and avoid sending problematic parts to customer via cleaning them. Operators make the joint in accordance with procedure arranged for each process (It is indicated for each process). The joint must easily be seen at the edge of reel and half of it must be out of reel. It must not fall down from reel. As soon as production operators detect the defect, they make the first mark with the appropriate additional joint (non-adhesive area, printing defect, blade mark, etc.). Then these problematic parts are cleaned at slitting process.

Figure 5. Joints used inside of reel

2 types of joints are used:

- Aluminum joint (used by printing and extruder department),

- Metallized joint (used by lamination unit).

These joints must be detected by the ‘metal sensors’ shown in the Figure 6. The sensors are on the slitting machine. As soon as the machine starts, the sensor automatically approaches the packaging film and the metallized joint is automatically captured by the machine, alarm sounds on and “Automatic stop ”as shown in the figure 7 stops the operation of the machine. Operators then clean relevant parts with the joint and ensures that clean products are delivered to the customer.

Figure 6. Metal sensor

Figure 7. Automatic stop moment of machine

When the reel is inserted, the metal sensors are placed by the operator where the joints will pass so that they can detect the joints. If the sensors are not placed in the right place, they cannot be monitored visually by the operator because the reels rotate too fast and therefore the problematic parts that must be cleaned are sent to the customers. To prevent this, the metal sensor must be placed in the appropriate place.

Joint Sensor Solution

In order to solve the problem here, the fork sensor shown in Figure 8 is added to detect the film so that the metal sensor can approach and stop at the right place as soon as the machine starts. This will ensure that the metal sensor is exactly where it should be, and so the problematic parts will not go to the customer. In order to identify the problem and solution, the why-why analysis and PDCA method were used.

Figure 8. Fork sensor

In order to check whether the fork sensors were operating effectively at high speeds while the machine was running, tests were carried out at 500 m / min on average for 2 days. In all tests shown as table 1, the machine received a signal and the result was positive.

Table 1.

Date	Machine	Speed	Joint	Result
1.Day	8	450	A type	Positive
1.Day	8	300	A type	Positive
1.Day	8	290	A type	Positive
1.Day	8	500	B type	Positive
1.Day	8	500	B type	Positive
1.Day	8	500	B type	Positive
1.Day	8	500	B type	Positive
1.Day	8	445	B type	Positive
1.Day	8	420	B type	Positive
2.Day	8	300	B type	Positive
2.Day	8	500	B type	Positive
2.Day	8	460	B type	Positive
2.Day	8	580	C type	Positive
2.Day	8	500	C type	Positive
2.Day	8	500	C type	Positive

• ***Before the Kaizen Work***

Because of the metal sensors could not be placed in the right place, the problematic parts were sent to the customers before they could be cleaned. As a result of this, complaints were received from the customers.Credit notes were made to the customers due to the complaints received and at the same time these complaints caused bad reputation for the company.

• ***After the Kaizen Work:***

Fork sensors were installed onto the machine and the problematic areas, on which joints are made, were caught and they were cleaned. Clear products without any kind of defect have been sent to the customers. Credit notes have been reduced and customer satisfaction has been gained.

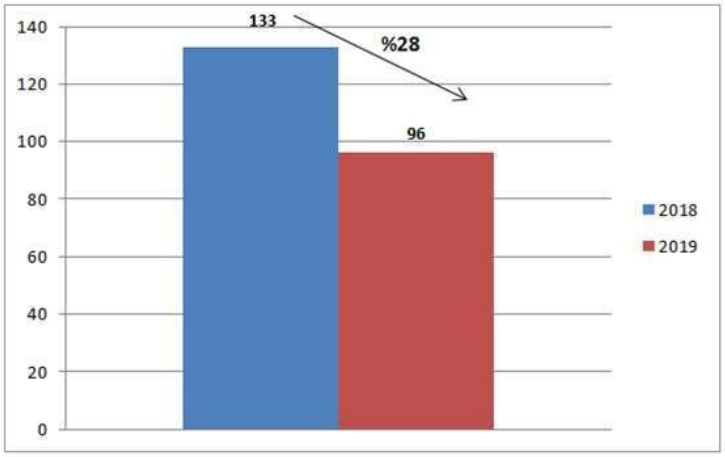
The study was recorded as before-after kaizen form as shown in Figure 9.

Figure 9. Before-After Kaizen Form for Joint Sensor

CONCLUSIONS

Although there is no need for statistical data in before and after kaizen, the related study, led to improvements in the last process as slitting proces and 28% reduction in customer complaints from this section was achieved compared to 2018 as shown in the figure 10.

Figure 10. Comparation of customer complaints



Each employee in the lean production team is responsible for recognizing defective products during production, ensuring production in targeted periods, ensuring safe and proper use of the machines and inventories, and recognizing abnormalities occurred during production. Definition of these responsibilities can be achieved by standardizing the work performed and identifying who are the responsibles in each department. The standards are determined directly with the people who perform the work. Since all employees in the departments are given opportunity to develop themselves and they are esteemed with the opportunity to evaluate these ideas, the employees do not refrain from taking responsibility and development. This provides an active and live suggestion system at all times. Through improvements and training, workers become more skillful and get more effective thinking. One of the most solid subject of lean philosophy is the relationship of trust. Establishing trust between employees and management,

the system provides the most suitable ground for improvements. The most important indicator of the value given to employees is trust (Altınbalık,T,&Karakaya,Ş., 2019).

Lean production cannot be accomplished only by using certain techniques in the field of production. All units of the enterprise, namely R & D, Product, Quality, Purchasing, Sales and Marketing, Human Resources, Inventory Control and Shipment carry out together. Wastes should be revealed by joint studies and the capacity consumed should be determined. To keep this capacity at minimum, a common business development plan should be developed. In some cases, it may be necessary to reorganize on the basis of a value stream. Although it is very important to get the top management to lead and provide motivation in the processes, lean production can only be learned and improved through applications only. Therefore, as soon as the first momentum is gained, applications should be started as soon as possible and motivation should be created by giving priority to the successful applications (Hambach, J.,2017).

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FIGURES

Figure 2

https://images.app.goo.gl/WQHGpP1CiaX7BoXw6, 2019
https://images.app.goo.gl/ZpEQ2WEuRmszzfE49, 2019

Figure 3

https://images.app.goo.gl/qwjNkhky5SymBuZr7, 2019
https://images.app.goo.gl/4bBHQECR6R8PKzin8, 2019

EFFECTS OF RUMEN PROTECTED CHOLINE SUPPLEMENTATION ON DAIRY CATTLE

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ABSTRACT

Trimethyl-β-hydroxyethylammonium, a vitamin like ingredient also known as choline is a vital nutrient for health, reproduction as well as production performance for animals. During milk production it has been prescribed as limiting nutrient in dairy cow's ration. As such supplementation of choline in ruminants ration leads to ruminal degradation; that's why it is recommended to supplement choline in rumen protected form. Phosphatidylcholine is made-up of choline which helps in formation of lipoprotein of very low density. Fatty liver can be caused, due to deficiency of choline due to export of triglycerides from liver. It is supplemented in dairy cows' diet 3 weeks before and 4 weeks after parturition to subside over energy requirement during this critical transition phase. It helps to overcome negative energy and hormonal balance in transitional phase of dairy cattle. It helps to improved fat, energy corrected milk, proteins, to overcome risks of mastitis and retained placenta, improves milk production along with composition and reproduction performance. Rumen protected choline (RPC) supplementation during transition period reduces inflammation indicator leading to decreased rectal temperature as well as reduced fibrinogen and haptoglobin concentration in plasma. Combination of RPC along with L-carnitine resulted in higher liver health index as well as reproduction index. Blend of RPC and long chain fatty acid calcium salts increases concentration of total monounsaturated fatty acid and total *cis* poly unsaturated fatty acid as well as omega-3 fatty acid. The optimum level of RPC supplementation in most trials is 12.9 g/d to obtain maximum health, production as well as reproduction potential. This review describes the influence of RPC on animal production, reproduction and biological health parameters of dairy cattle.

Keywords: Rumen protected choline, transition phase, dairy cattle, postpartum complications

Effect on production performance:

Quality as well as quantity of milk produced in animal production especially dairy sector are considered as back bone traits. Increase in milk production by supplementing RPC can be attributed to reduced chances of fatty liver (Cooke et al., 2007; Zenobi et al., 2018), retained placenta (Lima et al., 2012), mastitis (Arshad et al., 2020), inflammation (Sun et al., 2016; Vailati-Riboni et al., 2017) and increased digestibility along with volatile fatty acids (Mohsen et al., 2011). An increase of 0.20 % in milk fat content, 0.16 kg/d milk fat yield and 3.1 kg/d energy corrected milk was observed by Bollatti et al. (2020) as result of 12.9 g/d supplementation of choline. Supplementation of choline in rumen protected form resulted in

7% increased production of milk compared with control diets (Baldi et al., 2006). Similarly, other studies (Mohsen et al., 2011; Scheer et al., 2002) concluded enhanced milk yield and fat corrected milk (Soltan et al., 2012; Lima et al., 2007). Contrarily, some studies (Nardi et al., 2012; Leiva et al., 2015; Pineda and Cardosa, 2015) reported that supplementation of RPC did not enhanced fat corrected milk and overall milk production. These variation in results can be accredited to dissimilarity in parity (Davidson et al., 2008), breed (Guretzky et al., 2006), body condition score (Zahra et al., 2006), supplementation (Ardalan et al., 2010), method (Sales et al., 2010), stage of lactation. Increase in milk protein, lactose, fat, total solids and solid not fat was observed with supplementation of RPC (Mohsen et al., 2011; Nardi et al., 2012; Leiva et al., 2015). It can be imputed to role of choline in phospholipid formation which helps in absorption of lipid and translocation towards mammary gland.

Effect on reproduction performance:

Arshad et al. (2020) stated that supplementation of RPC during transition period of dairy cows resulted in reduced chances of retained placenta and mastitis. There were reduced inflammatory indicators and rectal temperature observation with addition of RPC in dairy cow's ration (Zenobi at al., 2020). Supplementation of RPC and rumen protected methionine may be advantageous for long term health of reproduction and immune response of uterus (Skenandore et al., 2017). Holstein dairy cow management is mainly dependent on reproduction indices, incorporation of RPC and L-carnitine could potentially enhance reproduction indices (Pirestani et al., 2018). Zenobi at al. (2018) reported that inclusion of RPC, 3 weeks before and after pregnancy increases chances of conception rate at first artificial insemination. Incorporation of RPC in dairy cows leads to more chances of twining at birth as compared to those cows fed control diet (Guretzky et al., 2006).

Effect on health status:

Supplementation of RPC in transition phase resulted in reduced inflammatory indicators (lower rectal temperature), postpartum fibrinogen, prepartum haptoglobin and less production of tissue necrosis factor (Zenobi et al., 2020). Whereas oxidative burst and blood neutrophil proportion enduring phagocytosis increases, indicating enhanced immune function (innate cellular). Improved innate cellular immunity is indicator of better health status. Inclusion of RPC in dairy cattle ration reduces chances of hypocalcemia (Bollatti et al., 2020) and reduced aspartate aminotransferase indicating better liver health (Pirestani et al., 2018) results in better health status.

Reduced morbidity, mastitis and retained placenta were observed in cattle's fed RPC (Suksombat et al., 2012). Heifers produced from cattle fed RPC shows better growth performance especially daily gain (Zenobi et al., 2018). Addition of RPC in cattle feed resulted in increased infiltration of hepatic fatty acids and enhanced transportation of very low-density lipopolysaccharides leading to less accumulation of fatty acids in liver with reduced fatty liver syndrome. Reduced incidences of fatty liver syndrome are an indicator of better animal health index with optimal production performance (Zenobi et al., 2018).

CONCLUSION

Inclusion of choline in the form of RPC in dairy cattle feed increases production performance (milk production, fat, energy corrected milk), improves reproduction performance (reduced retained placenta cases, better result of conception through artificial insemination, less inflammatory indicators) and better health index (less chances of mastitis, hypocalcemia, fatty liver, overall reduced metabolic disorders, increased growth performance of heifers produced from RPC supplemented cattle's, increased innate immunity). It is recommended to supplement RPC in dairy cattle feed 3 weeks before and after parturition for optimum production, reproduction and health performance.

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ASSESSMENT OF CONTAMINATION BY METALLIC TRACE ELEMENTS IN SOLE *SOLEA SOLEA* (LINNAEUS, 1758) FISHED IN THE BAY OF GHAZAOUET (NW ALGERIA)

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ABSTRACT

The present work consists in evaluating the quality of the West Algerian coast using a biological approach based on the estimation of the contents and the bioavailability of two metallic trace elements copper and lead in a teleostean fish: *Solea solea* (Linnaeus, 1758), fished in the bay of Ghazaouet. Sampling was spread over a period of six months on 100 individuals, including 45 females and 55 males. The metal analysis was carried out on the whole population, two organs were removed. The determination of metals was carried out by flame atomic absorption spectrophotometry (S.A.A). The comparative analysis of the mean lead and copper concentrations in the branchial and muscular tissues show that the importance of the metallic accumulation is in the following decreasing order: gills > muscle. The results that we have recorded indicate that there is a downward trend in the average levels of the two pollutants during the winter period compared to the fall period. The seasonal factor is therefore important and numerous studies have shown that the metal concentrations measured in marine species vary seasonally. We conclude that the sole coming from Ghazaouet bay does not seem to present a real danger for the consumer compared to the Maximum Acceptable Dose (D.M.A), it should be remembered that these micropollutants have a cumulative effect through the trophic chain, and that they have a long-term harmful effect on public health.

Key words : *Solea solea*; Metallic pollution; S.A.A; D.M.A; Bay of Ghazaouet.

INTRODUCTION

The aquatic ecosystem is increasingly threatened by various sources of pollution which risk reducing its economic potential and having negative repercussions on human health. More than ever, at the end of the millennium, the fight against water pollution is at the center of discussions and debates on a global scale. Hundreds of titles devoted to this subject have been found in the press, hundreds of seminars, meetings, congresses have taken place to discuss it, not to mention awareness campaigns on the serious pollution problems suffered by the various ecosystems (El Morhit et al., 2009). Among the chemical substances likely to constitute a danger to aquatic life in general, we point out heavy metals, some of which, such as lead, cadmium. These elements are very toxic and increasingly used in industrial sectors. Certain non-toxic metals such as zinc and copper become toxic due to their bioaccumulative power and their persistence in the aquatic environment and in particular in the sediments given their non-biological degradation

(Bouhadiba., 2018). The Ghazaouet region has a more or less diversified industrial fabric, represented mainly by the ALZINC unit (Algerian Zinc Company) which is a subsidiary of the METANOF company. The purpose of this unit is the production and marketing of zinc and its alloys, sulfuric acid, cathodic copper (Benguedda., 2012). The marine environment of Ghazaouet receives industrial wastewater recycled from this zinc electrolysis unit, with discharges loaded with heavy metals (D.P.R.H.T., 2004). The present work consists in evaluating the state of the water quality of the Algerian west coast based on the chemical contaminants (Cu and Pb) present in the muscle and the gills of Sole *Solea solea* fished in the bay of Ghazaouet.

Material and methods

1- Study site

Algeria has a coastline of around 1,622 km, from the Algerian-Moroccan border in the west to the Algerian-Tunisian border in the east. This coastline is characterized by a reduced continental shelf with the exception of the extreme west region (Ghazaouet, Beni saf and Bouzedjar) and the region of El Kala in the extreme east (Bekada., 2015).

The bay of Ghazaouet (figure 01) is located at the western end of Algeria. It is 80 km north of the wilaya of Tlemcen and 50 km from the Moroccan border. Apart from the site sheltering the port and the old urban center, the entire coast consists of very steep cliffs. Geologically, the Traras massif is the main structural unit of the coastal zone (Benguedda et *al*., 2011).

Figure 01 : The study area: Ghazaouet bay (Google map., 2019).

2- Biological Model

The common sole (*Solea solea*, Linnaeus, 1758) (Figure 02) is a teleost belonging to the order Pleuronectiformes and to the family Soléidae. It is present mainly on the Atlantic coasts of Europe and North Africa as well as in the Mediterranean (Quéro et *al*., 1997). Like most flatfish, it is a benthic species that prefers estuarine coastal areas with sandy or muddy bottoms (Le Pape et *al*., 2003)

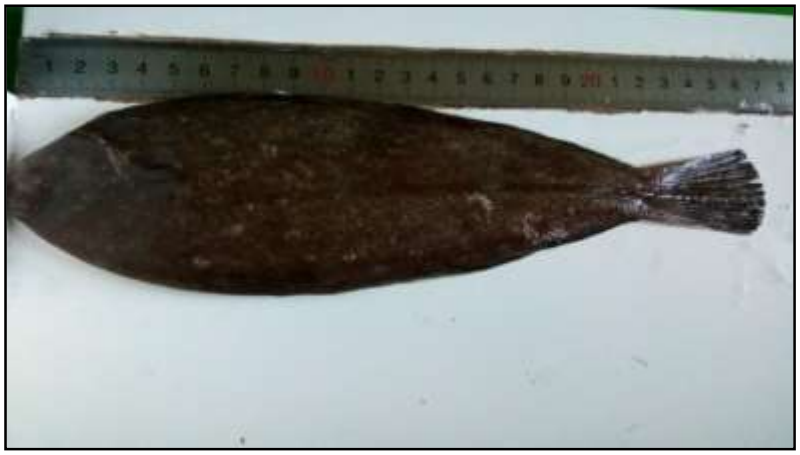

Figure 02 : Photo of the Common Sole *Solea solea* (Linnaeus, 1758).

Sampling was carried out monthly from October 2018 until March 2019, 100 individuals (55 males and 45 females), were treated on the same day. The two organs were removed: muscle and gills.

3- Analysis of heavy metals (Pb and Cu)

Before performing the metal analysis in the various samples, they must first be subjected to mineralization, that is to say destruction of the organic matter by acid attack (pure nitric acid HNO3). The flesh of the individuals is put in an oven at 60° C until a constant weight is obtained (from 24 to 72 h), then the biological samples are mechanically crushed, the latter are weighed and placed in small teflon tubes. 0,2 g of sample is taken for each replica, 4 ml of pure HNO3 are added to them. The mixture is left at room temperature overnight, afterwards they are placed in an oven at 90° C. for 3 h. The minerals are then filtered with wattman filter paper (Chiffoleau et *al*., 2001).The metals are determined by an atomic absorbance spectrophotometer of the Perkin Elmer precisely AAnalyst 400 atomic absorption spectrometer type.

Results

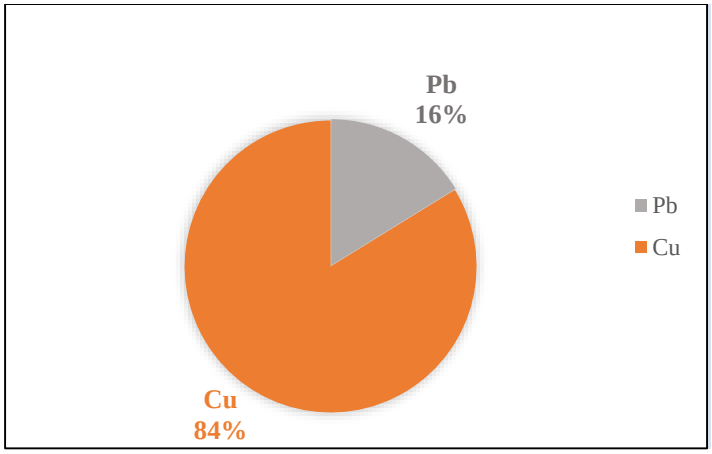

Figure 03: The percentage of heavy metals in the bay of Ghazaouet.

Figure 03 represents the percentage of the average concentrations of heavy metals, lead and copper in sole *Solea solea* fished in the bay of Ghazaouet.

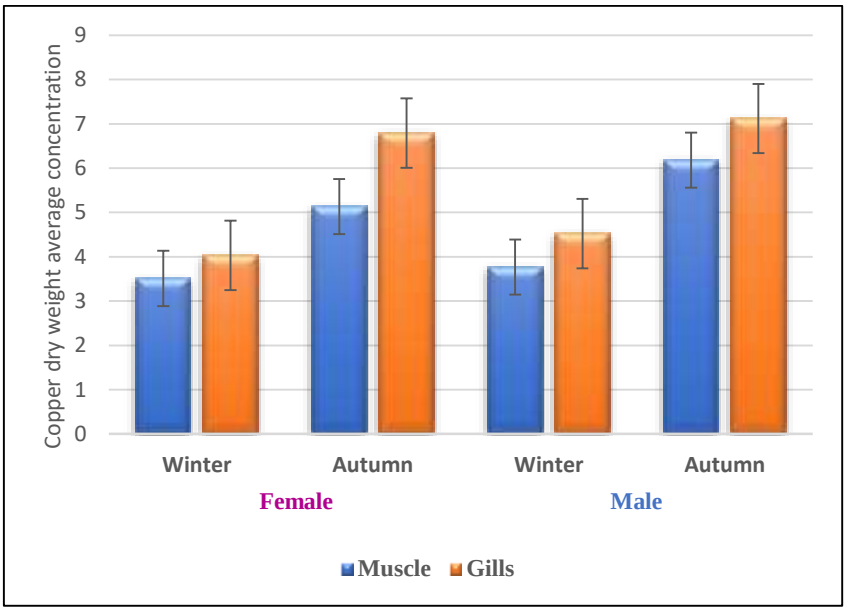

Figure 04: Average seasonal evaluation of copper contents as a function of organs in sole fished in the Bay of Ghazaouet.

The graphs in figure 04 represent the average copper concentrations as a function of the seasons in males and females of the sole *Solea solea* fished in the bay of Ghazaouet

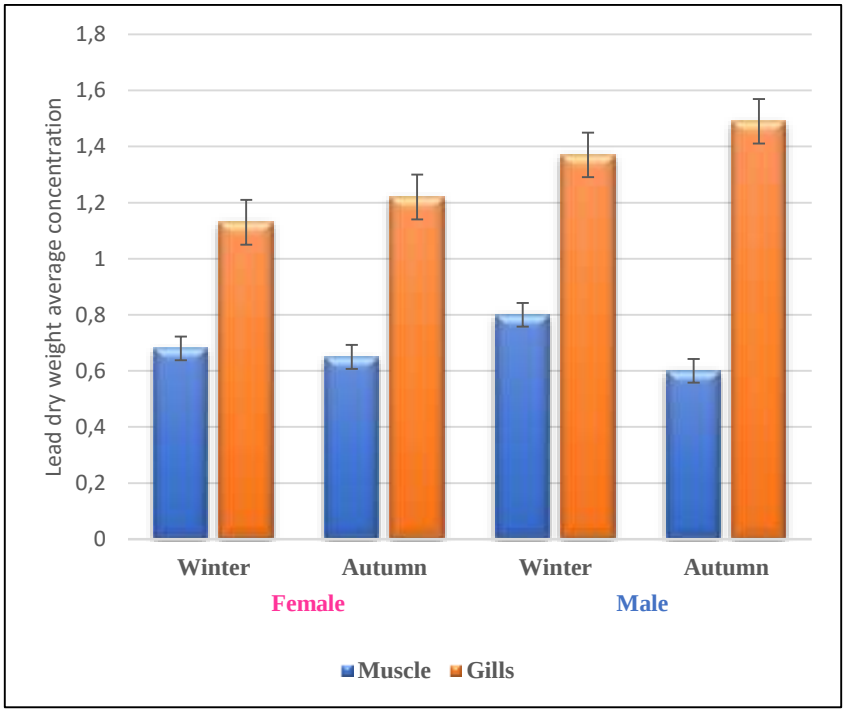

Figure 05: Average seasonal evaluation of lead contents according to the organs of the sole fished in the bay of Ghazaouet.

The graphs in figure 05 represent the average lead concentrations as a function of the seasons in males and females of the sole *Solea solea* caught in the bay of Ghazaouet

Table 01 : Comparison of the average heavy metal content in sole *Solea solea* muscle and the maximum authorized doses (D.M.A)

(a) G.I.P.P.M (1973) [Groupe Interministériel des Problèmes de Pollution de la Mer]
(b) CSHPF (1990) [Conseil Supérieur d'Hygiène Publique de France]
(c) CNRMS d'Australie (1992) [Conseil National pour la Recherche Médicale et de la Santé]

		Pb	Cu
Present study	Sole *Solea solea*	0,68 µg/g	4,65 µg/g
Fish (D.M.A)		0,3 à 6 mg/kg (a) (b)	5 mg/g P.S (c)

Table 01 shows the comparison of the average concentrations obtained of the four heavy metals (Pb and Cu) in the muscle of the sole *Solea solea* in the bay of Ghazaouet compared to the average admissible doses (D.M.A).

Discussion

The choice fell on these two organs because the gills are external organs in direct contact with water and play a key role in respiratory phenomena in fish. In the event of pollution, the respiratory processes can be disturbed (Fekhaoui., 1983). The muscle (the flesh) being the most edible part of the fish.

The results obtained show the presence of heterogeneous values of metallic traces. The most important contamination concerns copper, which represents 84%, followed by 16% lead (figure 03). The results obtained by (Benguedda et *al.*, 2011; Borsali et *al.*, 2014; Bouhadiba et *al.*, 2017) go in the same direction as ours.

The comparative analysis of the average concentrations of lead and copper in muscle and gill tissue shows that the importance of metallic accumulation is in the following descending order: gills > muscle (Figures 04 and 05). These results agree perfectly with other studies (El Morhit et *al.*, 2009; Belhoucine et *al.*, 2014; Borsali et *al.*, 2014).

he results we have recorded indicate that there is an increasing trend in the average levels of the two pollutants during the autumn period compared to the winter period (Figure 04 and 05). The seasonal factor is therefore important and many studies have shown that the concentrations of metals measured in marine species vary according to the seasons (Borsali., 2015)

The results reported in this work show a difference between the average accumulation of metals in male tissues compared to female tissues (Figure 04 and 05). Gender is one of the predominant biological factors, partly because of the difference in growth between males and females (Belhoucine et *al.*, 2014).

It should be noted that the average doses of heavy metals found in the fillet of the sample studied (sole) compared with the tolerated thresholds provided by the literature on D.M.A (Table 01) are not immediately worrying.

Conclusion

The results obtained during our study reflect the fluctuations in the contamination of the coast of Ghazaouet by heavy metals, and although a diagnosis is difficult to establish within a period of six months, our data perfectly illustrate the degradation of this environment. sailor and the danger he represents. metal pollution matter. We conclude that the sole from the bay of Ghzaouet does not seem to present a real danger for the consumer, it should be remembered that these micropollutants have a cumulative effect through the trophic chain, and that they have a detrimental effect in the long term on public health.

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EFFECTS OF QUINCE ROOTSTOCKS ON PHENOLOGICAL PROPERTIES AND FRUIT SET RATES IN HAFIF CUKURGÖBEK LOQUAT CULTIVAR

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Abstract

In the research, it was aimed to determine the effects of different quince rootstocks on phenological stages and inflorescence properties besides to the success of obtaining dwarf loquat nursery, which was a requirement of intensive loquat cultivation. For this purpose, two years old tree of Hafif Çukurgöbek (HÇG) loquat cultivar budded on BA-29, Quince-A and Quince-C quince rootstocks was used. The experimental plants were planted at high density with planting spaces of 1.0 m x 0.5 m in January 2017. The plants were irrigated by drip irrigation since their transplantation. The experiment was arranged according to a completely randomized designed with 5 replications and 6 plants were used in each replicate. In the study, effects of the rootstocks on the flowering periods, the inflorescence characteristics, flowering and fruit set rates of the cultivar were evaluated. Flowering period of the Hafif Çukurgöbek/Quince rootstock combination were completed between 12 Nov. and 26 Jan. The first flowering was observed on the plants with Quince-C rootstock and Quince-A, plants with BA-29 rootstock began flowering later. Plants with Quince-C rootstocks reached full bloom seven days before the other rootstocks. In terms of the fruit set, BA-29 and Quince-C rootstocks were found earlier than the Quince-A rootstocks. The earliest fruit ripening was observed on plants with BA-29 rootstock (18 May). Blossoming percentages were significantly affected by rootstocks. The highest blossoming rate was observed on Quince-C (97.77%), followed by BA-29 (88.90%). Effect of the rootstocks on initial fruit set, fruit set and final fruit set rates were found to be statistically significant at $P < 0.01$ level. The highest rates were taken in plants with BA-29 rootstock and this was followed by Quince-C. As a result, in terms of the several parameters, Quince-C and BA-29 rootstocks were found better than the Quince-A.

Key words: Loquat, dwarf rootstock, flowering, fruit set

INTRODUCTION

Loquat seedlings, quince, and hawthorn can be used as rootstocks for loquat (Polat, 1995). Loquat seedlings are preferred over quince or pyracantha rootstocks under most conditions. Quince and pyracantha may cause extreme dwarfing–to less than 8 ft (2.5 in) (Crane and Caldeira 2006). Loquat is a large tree which restricts the number of trees that can be planted per unit area and makes it necessary to harvest fruit with ladders. The use of dwarfing rootstocks using quince (*Cydonia oblonga*) is one method to reduce tree size, facilitate harvest, and increase early yield, but compatibility of loquat and quince is low (Janick, 2011).

The use of dwarfing rootstocks in loquat production reduces tree size making it possible to plant more trees per unit area and thereby increase early yield (Polat et al., 2004), facilitate harvest, and reduce costs (Polat et al., 2003). Quince rootstocks slow scion growth reducing tree size by 20 to 25% as compared to loquat seedlings, increase earliness, and increase fruit quality and size (Demir, 1987; Polat and Kaşka, 1992a, b; Polat, 1995). We suggest dwarfing, clonal rootstocks such as Quince-A and BA-29 for loquat production. This species allows growers to obtain smaller and more compact trees.

In the research, it was aimed to determine the effects of different quince rootstocks on phenological stages, and inflorescence properties besides to the success of obtaining dwarf loquat nursery, which is a requirement of intensive loquat cultivation.

MATERIAL AND METHOD

This research was carried out in 2019 at experimental plot of Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay, Turkey. In the study, two-year-old plants of Hafif Çukurgöbek loquat cultivar budded on BA-29, Quince-A and Quince-C quince rootstocks was used. The experimental plants were planted at high density with planting spaces of 1.0 m x 0.5 m in January 2017. The experiment was arranged according to a completely randomized designed with 5 replications and 6 plants were used in each replicate. In the study, effects of the rootstocks on the flowering periods, the inflorescence characteristics, flowering and fruit set rates of the cultivar were evaluated. Data were taken on date of first blossoming, full bloom, end of blossoming and harvest time. We considered the beginning of flowering as the date when 5% of the flowers were open; 70% as full bloom and 90% petal drop as the end of blossoming. To determine the flowering periods and the fruit set rate of the cultivar according to rootstocks, a branch was selected randomly from the four sides of the canopy and they were tagged accordingly. All flower buds were counted at the pre-blossom phase at each of these selected branches to carry out phenological observations. The flowers of each tagged branch were counted and % blossom rate was calculated by taking the proportion of the flower amount to bud amount during the observations made between the phase when blossoming starts and ends. 10 days after the end of blossoming, the number of initial fruits was found and by dividing this number by flower amount, % initial fruit set was determined. Percentage final fruit set was determined by taking the proportion of the amount of fruit during the maturing period to the amount of flowers. In addition, the productivity of these cultivars was calculated by taking the proportion of the amount of harvested fruit to the total amount of flowers. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The means were separated by Tukey's Honestly Significant Difference (HSD) method at $p = 0.01$.

RESULT AND DISCUSSION

Inflorescence Characteristics and Fruit Set

The highest values in terms of the average lenght of terminal panicle, the average number of secondary panicle on the terminal panicle, and the number of flower buds in the inflorescence (bud/per cluster) were obtained from the plants raised on Quince-C rootstock (Table 1). In terms

of the tree parameters, these differences between rootstocks were found to be statistically significant at P < 0.01 level.

Tablo 1**.** The effects of some Quince rootstocks on inflorescence characteristics of the Hafif Çukurgöbek cultivar.

Rootstocks	The average lenght of terminal panicle	No. of secondary axis per main panicle axis	No.of flower bud per cluster	No.of opened flower per cluster
Quince-A	15.64 b [(x)]	22.61 b	141.14 c	106.46 c
Ouince-C	17.89 a	26.06 a	206.98 a	196.16 a
BA-29	16.38 ab	20.94 b	182.77 b	162.49 b
HSD%1	1.71	1.93	3.26	2.51

[(x)]Means within a column followed by different letter are significantly at the 1% by Tukey test.

Blossoming percentages were significantly affected by rootstocks. The highest blossoming rate was observed on Quince-C (97.77%), followed by BA-29 (88.90%). The lowest blossoming rate was measured in plants with Quince-A rootstock (75.43%). Effect of the rootstocks on initial fruit set, fruit set and final fruit set rates were found to be statistically significant at P < 0.01 level. The highest rates were taken in plants with BA-29 rootstock and this was followed by Quince-C. The lowest values were measured in plants with Quince-A rootstock. The differences between the rootstocks for all these parameters were statistically significant at p<0.01 (Table 2).

Tablo 2**.** The effects of some Quince rootstocks on Blossoming Rate and fruit set rates of the Hafif Çukurgöbek cultivar.

Rootstocks	Blossoming Rate (%)	Inital fruit set (%)	Fruit set (%)	Final fruit set (%)
Quince-A	75.43 b[(x)]	8.05 c	3.80 b	2.64 b
Ouince-C	94.77 a	9.63 b	4.93 ab	4.23 a
BA-29	88.90 a	11.98 a	6.08 a	4.50 a
HSD%1	7.69	1.62	1.48	1.28

[(x)]Means within a column followed by different letter are significantly at the 1% by Tukey test.

Phenological Data

The results related to the effects of the Quince rootstocks used in the experiment on the phenological stage of the Hafif Çukurgöbek cultivar are given in Table 3.

Tablo 3**.** The effects of the Quince rootstocks on the phenological stage of the Hafif Çukurgöbek cultivar

Rootstocks	The phenological stages					
	Bud swelling	Beginning of blossoming	Full bloom	End of blossoming	Fruit set	Maturity period
Quince-A	15 November	1 December	7 January	26 January	18 February	21 May
Quince C	12 November	1 December	28 December	7 January	11 February	21 May
BA-29	15 November	8 December	7 January	17 January	11 February	18 May

Flowering period of the Hafif Çukurgöbek/Quince rootstock combination were completed between 12 Nov. and 26 Jan. (Table 3). The first flowering was observed on the plants with Quince-C rootstock and Quince-A, plants with BA-29 rootstock began flowering later. Plants with Quince-C rootstocks reached full bloom 7-8 days before the other rootstocks. In terms of the fruit set, BA-29 and Quince-C rootstocks were found earlier than the Quince-A rootstocks. The earliest fruit ripening was observed on plants with BA-29 rootstock (18 May) (Table 3).

CONCLUSIONS

As result, preliminary data obtained from this research show those quince rootstocks as dwarf rootstock in intensive loquat cultivation can be used. In terms of the several parameters, Quince-C and BA-29 rootstocks were found better than the Quince-A. The earliest fruit ripening was observed on plants with BA-29 rootstock. However, it is of great benefit to continue the further work to make a definitive judgment about the possibilities of using BA-29, Quince-A and Quince-C rootstocks as new dwarfing rootstocks.

Acknowledgements

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DETERMINATION OF BUDDING SUCCESS IN LOQUAT BUDDED ON HAWTHORN ROOTSTOCK

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Abstract

The purpose of the work was to find out the success of budding of loquat *(Eriobotrya japonica Lindl*.) on hawthorn (*Crataegus* spp) rootstock and the effect of this rootstock on the growth of the nursery plants. For this purpose, Hafif Çukurgöbek loquat cultivar was budded on the hawthorn rootstocks with the chip budding method on 6th May and 24th June, 2020. Loquat seedling was used as the control rootstock in the study. The experiment was laid out in a split-plot experimental design with nine replications and ten plants (five plant in June) per replication. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The means were separated by Tukey's Honestly Significant Difference (HSD) method at p=0.01. The average success rate of budding was better in June (78.39%) than in May (28.43%). In the both budding periods, it was found that buddings on loquat seedling (respectively, 50.0% and 88.6%) were more successful than the ones on hawthorn (respectively, 6.86 % and 68.18 %). However, the highest bud sprout rate (100 %) was taken from the buddings which were done on hawthorn rootstock in June. Budding performed in June gave the higher values (86.53%) of bud sprout rate than the budding done in May (35.87 %). As result, preliminary data obtained from this research show hawthorn rootstock as dwarf rootstock in intensive loquat cultivation can be used. However, researches need to be continued, especially to determine all the characteristics regarding yield and fruit quality.

Keywords: *Loquat, hawthorn, budding success, high density.*

INTRODUCTION

Loquat is a large tree which restricts the number of trees that can be planted per unit area and makes it necessary to harvest fruit with ladders. Most loquats are grown on loquat seedlings and the genetic variability of rootstocks probably contributes to variability of performance in grafted trees (Janick, 2011). The use of dwarfing rootstocks using quince (*Cydonia oblonga*) and hawthorn (*Crataegus spp.* L.) are one method to reduce tree size, facilitate harvest, and increase early yield.

There is some information in the literature that hawthorn can be used as rootstocks for loquat (Polat and Kaşka, 1992; Polat, 1995; Polat, 2007; Polat, 2018), however only two studies (Jamil et al., 2012; Polat, 2020) on the use of hawthorn rootstocks in loquat cultivation has been found. A study by Jamil et al., (2012) was conducted during two successive growing seasons 2009 and 2010, in Iraq. Hawthorn trees of 25-30 years old, grown naturally in the region were used as rootstock. Loquat cultivar was budded on the wild hawthorn trees in mid-May, early June, and

mid-June during two successive seasons. The maximum value of percent budding success (80.00%) was recorded when budding was done in mid-May 2010, followed by budding in mid-May 2009 (79.30%). In another study conducted by Polat (2020), in first year, Sayda loquat cultivar was budded on hawthorn rootstocks (2-3 years old) with chip budding method on 17 March, and 2 June, 2017, however, budding success was not achieved. In second year, Hafif Çukurgöbek loquat cultivar was budded on hawthorn rootstocks (1-2 years old) with chip budding method on February 8, 2018. Budding success was very low (10.58 %). Thus, they were repeated with the same scion type by using the "T" budding method in May 10, 2018. Budding success was not achieved in this period.

The purpose of the work was to find out the success of budding of loquat on hawthorn rootstock and the effect of this rootstock on the growth of the nursery plants.

MATERIAL AND METHOD

This research was carried out in 2020 at experimental plot of Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Antakya, Hatay, Turkey. Hafif Çukurgöbek loquat cultivar was budded on the hawthorn rootstocks with the chip budding method on 6th May and 24th June, 2020. Loquat seedling was used as the control rootstock in the study. The experiment was laid out in a split-plot experimental design with nine replications and ten plants (five plant in June) per replication. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The means were separated by Tukey's Honestly Significant Difference (HSD) method at p=0.01.

RESULT AND DISCUSSION

The success rates of budding made on 6 May and 24 June 2020 are presented in Table 1. In the both rootstocks, the budding success rates were found higher in June buddings than in May ones. The average budding success rate was higher in June (78.39%) than in May (28.43%). The differences between the budding periods were statistically significant at p<0.01. In the both budding periods, it was found that buddings on loquat seedling (respectively, 50.00% and 88.60%) were more successful than the ones on hawthorn (respectively, 6.86% and 68.18%). The differences between the rootstocks were statistically significant at p<0.01.

Table 1. The bud take rates of budding made in different periods

Rootstocks	Budding period		Mean(%) (Rootstock)
	06 May, 2020	24 June, 2020	
Hawthorn	6.86 d$^{(y)}$	68.18 b	37.52 B$^{(x)}$
Loquat seedling	50.00 c	88.60 a	69.30 A
Mean(%) (Budding period)	28.43 B$^{(x)}$	78.39 A	

$^{(x)}$Means followed by different capital letters are for rootstocks and budding dates and indicate significant difference by Tukey's test at 0.01.

$^{(y)}$Means followed by different lowercase letters are for rootstock x budding dates interaction and indicate significant difference by Tukey's test at 0.01.

However, the highest bud sprout rate (100.0 %) was taken from the buddings which were done on hawthorn rootstock on 24 June, 2020. Budding performed in June gave the higher values (78.39%) of bud sprout rate than the budding was done 6 May (28.43 %)(Table 2).

Table 2. The bud sprout rates of budding made in different periods

Rootstocks	Budding period		Mean(%) (rootstock)
	06 May, 2020	24 June, 2020	
Hawthorn	42.85 c[(y)]	100.00 a	71.42 A[(x)]
Loquat seedling	28.90 d	73.07 b	50.98 B
Mean(%) (Budding period)	35.87 B	86.53 A	

[(x)]Means followed by different capital letters are for rootstocks and budding dates and indicate significant difference by Tukey's test at 0.01.

[(y)]Means followed by different lowercase letters are for rootstock x budding dates interaction and indicate significant difference by Tukey's test at 0.01.

Rootstock × budding period interaction was statistically significant at p=0.01 indicating that rootstocks are affected differently with budding period. In two budding periods, loquat seedling had the highest bud-take rates while the highest bud-sprout rates were observed in hawthorn. The budding success rates obtained in our study were lower than values of Jamil et al. (2012), but higher than that of Polat (2020).

CONCLUSION

Reducing of vegetative growth is very important in loquat cultivation because of its very high tree size. The most effective method for this is the use of dwarfing rootstocks such as quince (*Cydonia oblonga*) and hawthorn (*Crataegus* spp). The use of dwarfing rootstocks in loquat production reduces tree size making it possible to plant more trees per unit area and thereby increase early yield facilitate harvest, and reduce costs. However, there are not enough studies on using hawthorn as rootstock for loquats. The present investigation was taken up to study, the determined of the success of budding of loquat on hawthorn rootstock and it is one of the first studies in our country. As result, preliminary data obtained from this research have shown that can use of hawthorn as rootstock in loquat cultivation. However, researches need to be continued, especially to increase budding success rates on hawthorn rootstocks.

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FIRE EFFECTS ON CORK OAK WOODS STRUCTURE AND DIVERSITY IN ALGERIA

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Abstract

Little is known about the effects of fire on vegetation dynamic or about fire-plant trait interactions in the forests of Algeria, where disturbance by Wildfires is generally frequent and intense. We focused on Chrea National Park and choose cork oak (*Quercus suber* L.) ecosystem because of its high economic and ecological importance. Sixty-one plots were established taking into account the height and density of the woody species. For each plot, floristic composition and environmental variables were evaluated. Furthermore, easily discernible traits of species (life forms, dispersal mode, Grime's strategies and regenerative traits) were used to analyse fire effects on functional composition. TWINSPAN (Two-Way Indicator Species Analysis) results revealed that the current landscape is a mosaic of five vegetation types, with two types of woodlands and three types of shrublands. According to the CCA (Canonical Correspondence Analysis) results, these vegetation types were significantly correlated with a dynamic gradient caused by fire disturbance, which masks the effects of the dominant mesological factors (altitude and exposure). Moreover, fire effects are strongly dependant on species' biological and life history traits. In fact, PCA (Principal Component Analysis) indicated that the abundance of seeders, ruderal stress-toleros species, therophytes, and Anemochorous species was associated with recent and/or recurrent burned shrublands. Conversely, competitive stress-tolerant species, phanerophytes, zoochorous species and resprouters decreased with increases in fire recurrence and reductions in time since the last fire. Further measures for the conservation of forest ecosystems against wildfires, in the protected areas of Algeria, must be undertaken.

Keywords: summer wildfires, cork oak woodlands, regressive dynamic, plant traits, conservation

INTRODUCTION

Cork oak (*Quercus suber* L., Fagaceae) is an endemic species of the western Mediterranean Basin where an oceanic Mediterranean climate rules (Quézel and Médail, 2003). In Algeria, this species covers an area of 410 000 ha, or 18.1% of its world area (Silva and Catry, 2006). The largest cork oak forests are located in the north-eastern Algiers, with other smaller forested regions in the centre and the north-western (Zéraia, 1982). This sclerophyllous tree favours acidic soils (Seigue, 1985) and grows from sea level up to 1500 m (Khelifi, 1987).

However, in recent decades, wildfires are causing a serious cork oak forest area regression in this country with biodiversity loss and alteration of ecosystem services. Wildfires are one of the main disturbances shaping the current Mediterranean forest landscapes (Pausas et al., 2008). In this region, the traits of the species are essential for understanding plant evolution, vegetation dynamics, and vegetation responses to disturbances such as fire (Paula et al., 2009).
Most of the studies on the *Quecus suber* L. woodlands have been conducted in the north-eastern part of Algeria, including studies related to the relationship between floristic composition and fire regime at lower elevations (Ouelmouhoub and Benhouhou, 2007; Bekdouche, 2010), and the impact of anthropogenic disturbances on the vegetation of a coastal cork oak forest (Zeddam et al., 2007). However, no studies have examined the influence of fire on the vegetation dynamic and functional diversity in the *Quecus suber* L. woodlands of the Chréa Mountain National Forest Park, where topography-derived variables such as altitude, aspect, and slope, and recurrent fires are the main factors shaping plant communities and landscape.
Thus, our objectives were to identify the current dynamic stages of cork oak forest and analyzing their relationships with environmental variables; to determine the main functional groups pertaining to these plant communities; and to test the hypothesis that the traits of species determine their responses to environmental factors or disturbances.

Material and Methods

Study area

The study area is part of the Chrea massif National Park, it is situated about 42 Km Southwest Algiers (2°46'25''- 2°53'9''E, 36°25'22''- 36°28'28''N) (Fig.1), and located at the Central Tell Atlas, also known under the name Blidean Atlas.
The climate there is Mediterranean with wet temperate winters and dry hot summers. Mean annual precipitation ranges from 746 to 1065 mm per year, and mean annual temperature ranges from 14,4 to 18,3°C.
The altitude ranges between 500 and 1000 m. In this area, acidic soils promoted the development of a specific vegetation types dominated by cork oak.

Figure 1. Geographic situation of the study area

Sampling strategy

In order to select plots with the dominant physiognomic vegetation types we studied the structure and composition of vegetation in 61 plots. Each plot covered 100 m^2, this area most commonly used to sample the cork oak formations of Algeria (Zéraia, 1982; Ouelmouhoub and Benhouhou, 2007; Zidam et al., 2007).

Plants were surveyed in spring, when the maximum number of species can be identified, thus, a total of 173 plant species were recorded in 61 plots. The abundance (percent cover) of all these species was evaluated using a phytosociological scale based on Braun-Blanquet (1952) method.
Eight environmental variables were measured and recorded for each plot: altitude, slope and exposure, cover of bare soil, litter (loose, dense or woody), ground layer (herbaceous species), understory layer (shrubs and small trees) and the overstory layer (trees).

Collection of plant attribute
According to Schaffauser et al. (2011), the main plant attributes that might be affected by fire are : Raunkiaer's life forms (Raunkiaer, 1934), Grime's strategies or vital types (Grime, 1977), mode of dispersal (Van der Pijl, 1982), and post-fire regenerative types (Paula et al., 2009).

Data analysis
The TWINSPAN (Two-Way Indicator Species Analysis) classification and CCA (Canonical Correspondence Analysis) ordination method (Ter-Braak, 1986) were used to identify plant community types and to determine their relationship to environmental variables. In order to facilitate the reading of numerical analyzes, species were coded (the first three letters of the genus and the first three letters of the species). In addition, a PCA (Principal Component Analysis) was calculated on the "plant attribute types (presence/absence) x plots" data matrix.

Results

Plant community diversity
TWINSPAN classified the 61 plots into five clusters, corresponding to five vegetation types (Fig. 2). These types represent the main dynamic stages of the cork oak vegetation, in the Chréa massif, under different fire regimes and fire severity.

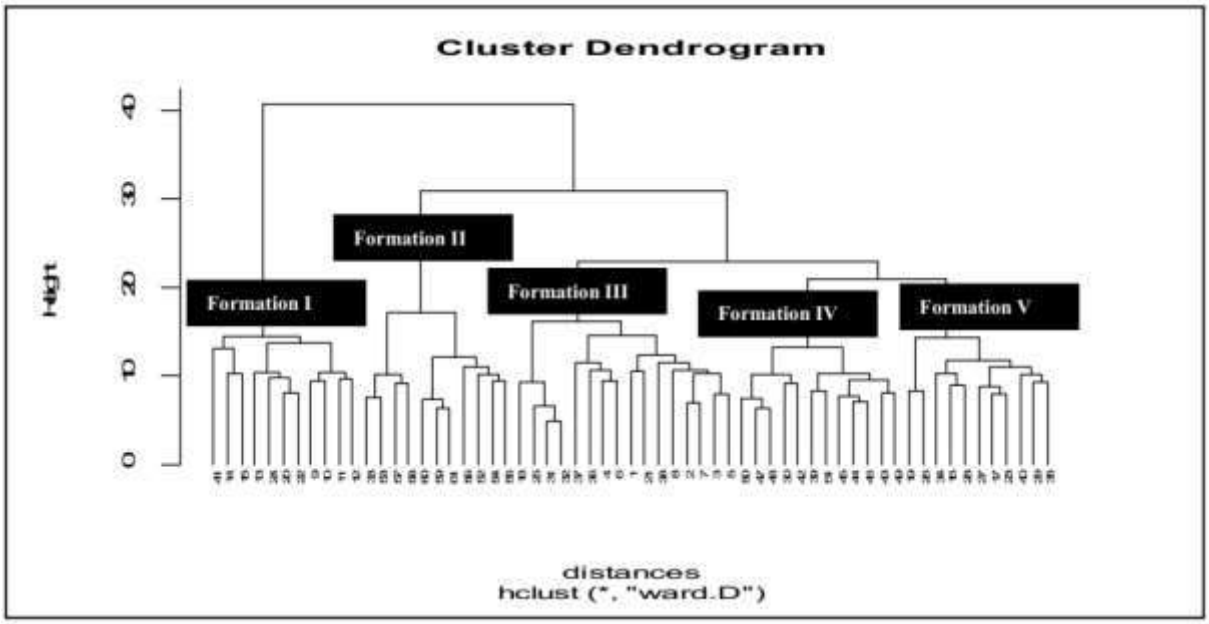

Figure 2. The dendrogram of TWINSPAN results for the 61 vegetation plots in the study area. Roman numerals I – V refer to the five plant formations

-Low and spars shrublands (mean height 0,5m) dominated by *Genista tricuspidata* Desf. and *Cistus salviifolius* L. (Formation I**),** with rare scattered *Quercus suber* L. individuals and patches of bare soil and herbaceous species. This post-fire natural plant formation, subject to

recent and recurrent burning, is disturbed from 750 to 900 m, on southern and western slopes of 5 - 25°.

-Medium shrublands (mean height 2 m) dominated by the shrubs *Calicotome spinosa* (L.) Link., *Cistus salviifolius* L., and *Erica arborea* L. (Formation II**)** which constituted a dense and continuous layer, with a low density of cork oak trees**.** This is a post-forest natural formation, subject to recurrent burning but generally before 15 years (i.e. intermediate fire recurrence), and disturbed from 500 to 750 m, on northern and western slopes of 5 - 15°.

-High shrublands (mean height 2-4 m) dominated by *Calicotome spinosa* (L.) Link., and *Erica arborea* L. (Formation III), with some *Quercus suber* L. trees, unburned since 20 years (i.e. intermediate fire recurrence). This is a post-forest natural plant formation, disturbed from 600 to 900 m, on southern and western slopes of 15 - 35°.

-Pure woodlands dominated by *Quercus suber* L. (Formation IV), unburned since at least 50 years (i.e. low fire recurrence). This is a mature natural pure forest formation, disturbed from 650 to 800 m, on northern and eastern slopes of 15 – 45°, and deep forest soil.

-Mixed oak woodlands dominated by *Quercus suber* L. with Quercus *ilex* subsp. ballota (Desf.) Samp. (Formation V), unburned since at least 50 years (i.e. low fire recurrence). This is a natural mixed forest formation, disturbed from 800 to 1000 m, on northern slopes of 15 – 35°, and deep forest soil.

Plant communities and related environment

The canonical correspondence analysis (CCA) was performed on the floristic data matrix (173 species × 61 plots) and the environmental data matrix (8variables × 61 plots).

Ordination results showed that the first CCA axis was significantly correlated with a dynamic gradient; tree cover and litter cover had a positive correlation and herb cover had a negative correlation (table 1, Fig. 3). The first CCA axis was also negatively correlated with exposure, which increases from the northern slopes (positive part) to the southern slopes (negative part).

The second CCA axis was significantly correlated with topography derived variables. The altitude and slope had a positive correlation and increase gradually along this axis from the negative part to the positive part. Moreover, shrub cover had a negative correlation with the second CCA axis (table 1, Fig. 3).

Table 1. Inter-set correlation coefficients of environmental variables with CCA axes in the study area

Environmental variables	**CCA1**	**CCA2**	**CCA3**
Altitude (Alt)	-0.394	0.638***	0.339
Exposure (Exp)	-0.515**	-0.097	-0.733***
Slope (Slo)	0.240	0.417*	0.135
Tree cover (Tre)	0.711***	0.590***	-0.056
Shrub cover (Shr)	0.120	-0.639***	0.197
Herb cover (Her)	-0.890***	0.028	0.159
Litter cover (Lit)	0.733***	0.023	-0.091
Bare soil cover (Soi)	-0.094	0.013	-0.079

* P<0.05, ** P<0.01, *** P<0.001

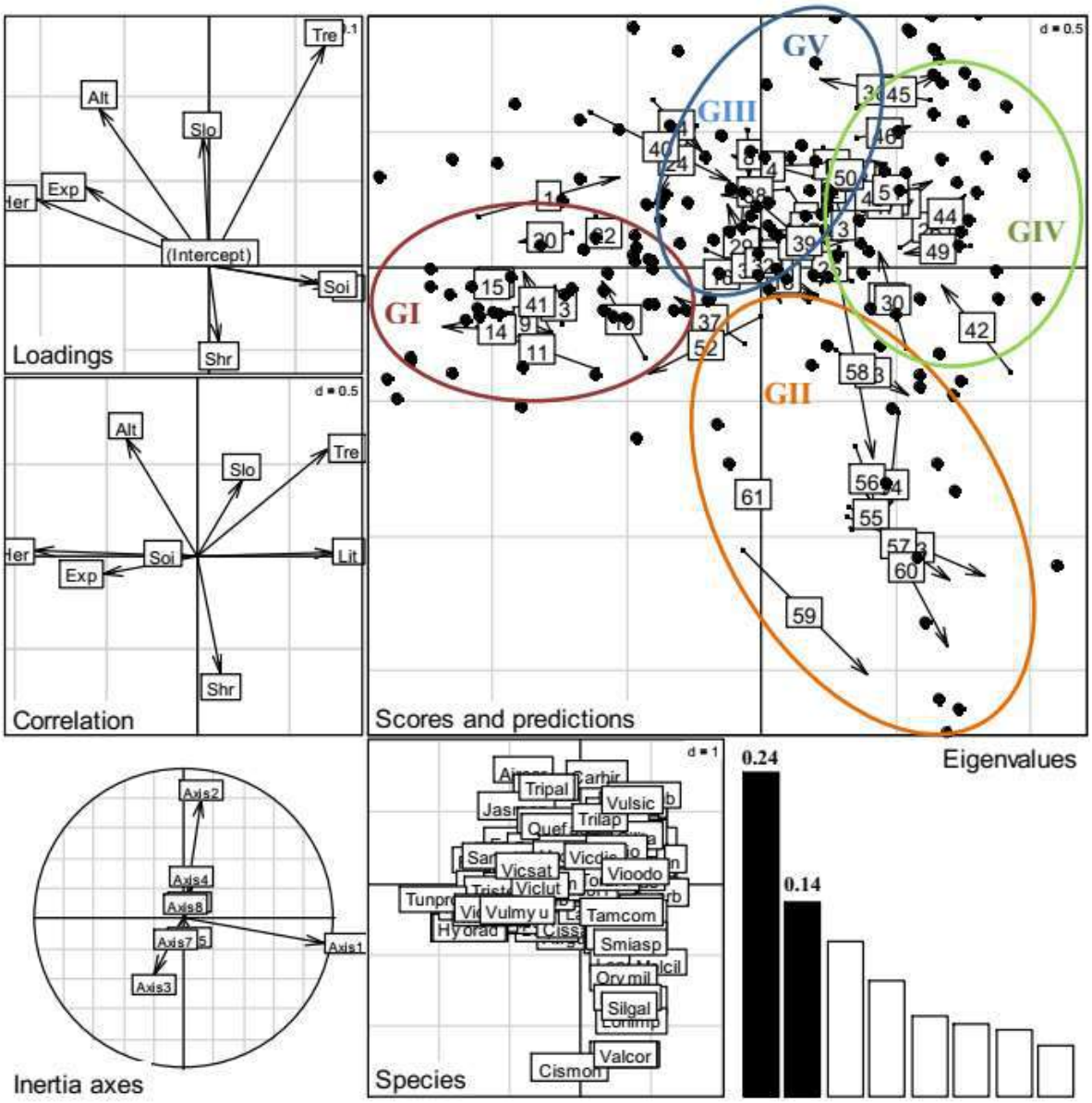

Figure 3. Plane1 / 2 of the CCA conducted on the vegetation and environmental variables. See text for the abbreviations of variables and species name. Roman numerals I – V refer to the five plant formations

The distribution of vegetation types on the CCA ordination map was most related to recent fire history, but also to topography derived variables. On the positive part of the first axis, plots made in the pure cork oak woodlands (formation IV), that had not burned since at least 50 years, were grouped around tree cover and litter cover (Fig. 3). This mature plant community is characterized by the presence of woody species revealing a forest environment such as *Rhamnus alaternus* L., *Laurus nobilis* L., and *Prunus avium* (L.) L. In contrast, on the negative part of the first axis the plots found around herb cover and exposure represented the low spars shrublands of *Genista tricuspidata* Desf. and *Cistus salviifolius* L., that had recently and recurrently burned (Formation I). This post-fire plant formation dominated on southern and western slopes, and characterized by the dominance of thermophilous herbaceous species such as: *Gastridium ventricosum* (Gouan) Schinz & Thell., *Lagurus ovatus* L., *Echinops spinosus* L., *Pallenis spinosa* (L.) Cass., *Vicia onobrychioides* L., and *Trifolium arvense* L.

Among the second axis the altitude and slope decreased gradually from the positive part to the negative part. The plots found around these two variables corresponded to the high shrublands dominated by *Calicotome spinosa* (L.) Link., and *Erica arborea* L. (Formation III) and the mixed oak woodlands dominated by *Quercus suber* L. with Quercus *ilex* subsp. Ballota (Desf.) Samp. (Formation V), which can develop until 900 m and 1000 m respectively. The altitudinal character of these two vegetation types was indicated by the presence of the phanerophytes *Quercus coccifera* L. and *Quercus canariensis* Willd. These plots opposed to those conducted on the medium shrublands of *Calicotome spinosa* (L.) Link., *Cistus salviifolius* L., and *Erica arborea* L. (Formation II**)** characterised by a dense and continuous shrub layer**,** and which developed at less than 750 m of altitude. These thermophylous conditions were indicated by the abundance of *Phillyrea angustifolia* L., and *Cistus monspeliensis* L.

Functional diversity

The Principal Component Analysis (PCA) was calculated on the species traits data matrix (18 plant attribute types (presence/absence) x 61 plots). The most contributor variables for the positive and negative poles of the first three axes are indicated in the table 2. The figure 4 shows the level of correlation of the variables.

Table 2. Inter-set correlation coefficients of types of plant attributes with PCA axes in the study area

Modalities of plant attribute	**PCA1**	**PCA2**	**PCA3**
Therophytes (Th)	-0.895***	0.293	-0.106
Geophytes (Ge)	-0.108	-0.707***	0.218
Hemicryptophytes (He)	-0.852***	-0.079	0.157
Chamaephytes (Ch)	-0.197	0.223	0.708***
Phanerophytes (Ph)	0.169	-0.903***	-0.121
Competitives (C)	-0.046	-0.628***	0.200
Stress-tolerants (S)	-0.607***	-0.362	0.382
Ruderals (R)	-0.502**	-0.065	-0.360
Competitive stress-tolerants (CS)	-0.207	-0.811***	0.036
Competitive-ruderals (CR)	-0.250	-0.047	-0.604***
Ruderal stress-tolerants (SR)	-0.873***	0.302	-0.111
Triple strategie (CSR)	0.045	0.380	-0.205
Anemochorous (Ane)	-0.891***	0.312	0.214
Autochorous (Aut)	-0.299	-0.038	-0.519***
Barochorous (Bar)	-0.449**	-0.505***	-0.160
Zoochorous (Zoo)	-0.258	-0.836***	-0.148
Resprouters (Res)	-0.200	-0.827***	0.018
Seeders (See)	-0.889***	0.271	0.022

* P<0.05, ** P<0.01, *** P<0.001

According to the PCA results (figure 4), two main functional groups can be identified. The first one with the traits: seeders (See), ruderal stress-toleros (SR), therophytes (Th), and wind dispersed species (Ane) was associated with post-fire plant communities of spars and low shrublands recently and/or recurrently burned.

The second functional group corresponded to unburned plots (i.e. mature cork oak woodlands and mixed oak woodlands) with as plant traits: competitive stress-tolerant species (CS), zoochorous species (Zoo), phanerophytes (Ph), and resprouters species (Res).

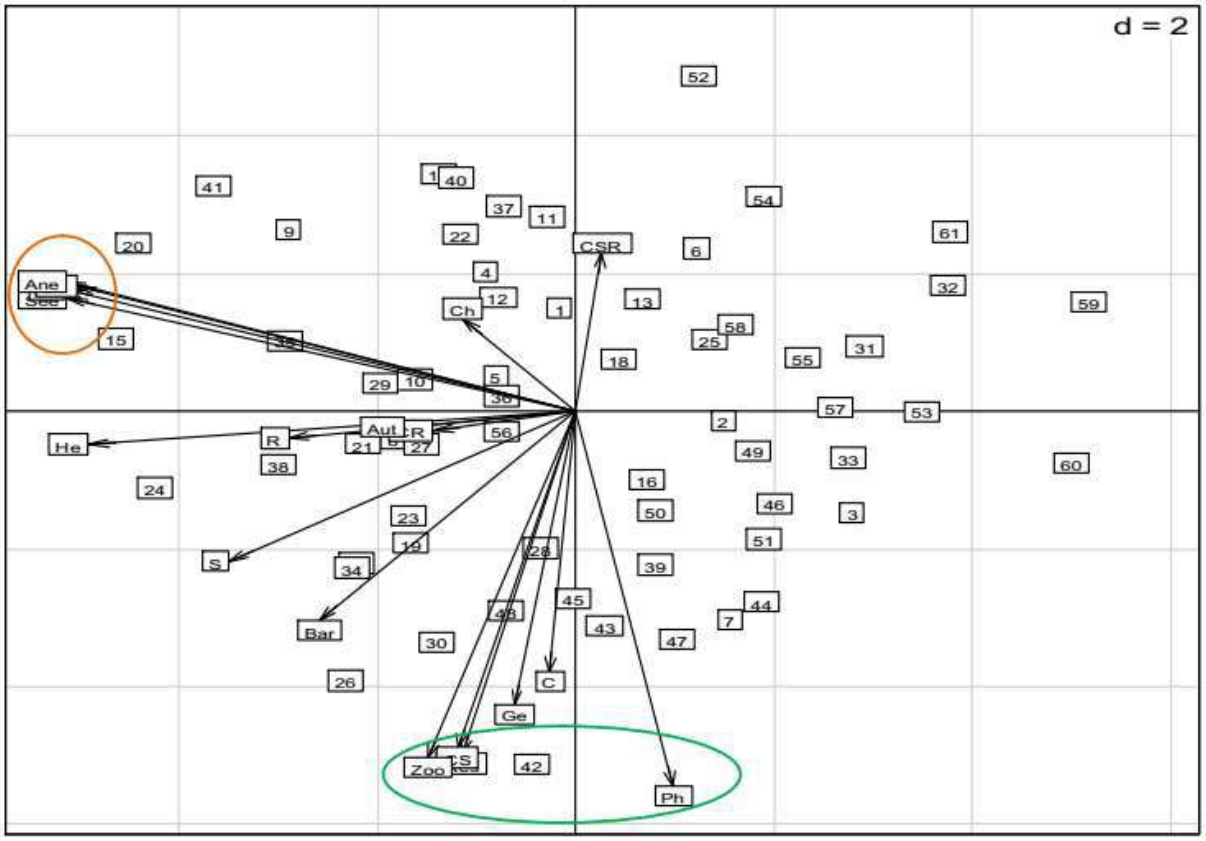

Figure 4. Representation of the 2 first axes of the Principal Component Analysis (PCA)

Discussion

Fire-Vegetation dynamic

In the Chréa National Park, fire involves a structural simplification of the vegetation, due to the decrease in the cover of tree layer. Hence, the reduce in competition intensity after this disturbance favour herbaceous species, potentially inducing a positive herb–fire feedback (Delitti et al., 2005; Eugenio and Lloret, 2004). Moreover, the expansion of low shrubs *Cistus salviifolius* L. and *Genista tricuspidata* Desf. into the post-fire plant communities (formation I) can be explained by the recurrent fires at short intervals (Schaffausser et al., 2011), Cistus salvifolius L. occupies areas where fire severity was medium or high (Francos et al., 2016).

Thus, after fire disturbance vegetation uses different strategies to resist fire and that it is able to re-establish itself quickly, especially from burned seed source. This resilience is critical to protect the soil from water and wind erosion in the shirt-term (Francos et al., 2016). In the post-forest plant communities (formation II and formation III), higher temperatures due to the canopy opening allowing high shrubs *Calicotome spinosa* (L.) Link., and *Erica arborea* L. to establish and gain dominance. Furthermore, intermediate fire recurrence leads to multi-layered fuels with trees and shrubs interconnected.

In cork oak woodlands and Mediterranean shrublands, differences in vegetation structure induce changes in fire risk. In fact, fire behaviour and fire risk depend on the stage of development of the vegetation (Baeza et al., 2006 ; Schaffhauser et al., 2015), and the local topographic and meteorological conditions (Riccardi et al., 2007). According to the present study, the risk of fire is high in the three shrubland formations. This risk is in relationship with high fuel accumulation (dense and continuous shrub layer) and flammability in these plant communities, and the local topographic derived variables; especially hot aspect and low elevations. In the Chréa massif, it is well known that southern and western exposures are more

exposed to wildfires than fresh exposures, north and east (Halimi, 1980). Furthermore, at low elevations, summer wildfires in the shrublands have very high intensity due to harsh meteorological conditions.
In contrast, this risk is low in mature cork woodlands because of the high horizontal connection between tree canopies and low vertical connectivity between understory shrubs and the tree overstory (Schaffausser et al., 2011). Moreover, fresh exposures limit fire spreading and fire severity on the massif.

Fire-functional diversity

In the *Quercus suber* L. woods of the Chréa National Park, the responses of the plant species to the fire disturbance are strongly correlated with their life traits. The PCA results are consistent with those found by Schaffausser et al. (2012) in *Quercus suber* woodlands and maquis of Southern France. In fact, the importance of competitive stress-tolerant species, zoochorous species, phanerophytes, and resprouters species decreases with increases in fire regime and reduction in time since the last fire. Nevertheless, increasing in fire recurrence was accompanied by the abundance of seeders, ruderal stress-tolerant species, therophytes, and wind dispersed species.
The abundance of seeders species in recent burned areas is due to the fact that this disturbance stimulates the emergence of pyrophyte seeders (Kunholtz-Lordat, 1938; Schaffausser et al., 2012), especially from burned seed source, whereas the ability of resprouters to recover areal parts declines because of the depletion of storage organs or meristems by successive fires (Vilà and Terradas, 1995; Iwasa and Kubo, 1997). Furthermore, the temporary increase in light and nutriment availability in the upper soil layers after fire, favours ruderal species (Ferran et al., 2005). This opening vegetation also constitutes a favour environment for wind-dispersed species and disadvantages zoochorous species (Schaffausser et al., 2012).

Conclusion

In the Chréa National Park, strong interactions exist between the vegetation (structure, composition), microsite characteristics and recent fire history. The mosaic of vegetation studied here included five vegetation types, with three types of shrublands and two types of woodlands, each corresponding to a well-defined dynamic stage under fire regime.
According to the present study, high fire risk was observed in the post-fire and post-forest plant communities; these vegetation types are very dominant in the studied area, making fire the main disturbance of cork oak forests. Reducing fire risk and fire damage is thus primordial for maintaining *Quercus suber* L. stands of the Chréa National Park. Shrub-clearing by prescribed fires is an efficient measure to fight intense summer wildfires; this technique has no negative effects on soil or forest biodiversity (Pereira, 2011). The conservation of mature woodlands would also help as they are seed sources for regeneration of shrublands from seeds.

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ANTIOXIDANT AND ANTICHOLINESTERASE EFFECTS OF *PHAEOLUS SCHWEINITZII* AND *PHELLINUS IGNIARIUS MUSHROOMS*

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ABSTRACT

Free radicals are produced from oxygen during aerobic respiration and excessive formation and accumulation cause oxidative stress. It has been reported in previous studies that oxidative stress causes various diseases and antioxidants help to prevent or reduce the damages caused by oxidative stress. AChE and BChE inhibition theory is very popular among the other strategies in the treatment for Alzheimer's disease (AD) and also it is well known that antioxidants have an important role in the treatment of AD. Antioxidant and anticholinesterase effects of the hexane and methanol extracts of *P. schweinitzii* and *P. igniarius* mushrooms were examined in this study. β-carotene-linoleic acid, $DPPH^{\bullet}$ scavenging, $ABTS^{\bullet+}$ scavenging, CUPRAC and metal chelating assays were used for testing of antioxidant effect. Also, Ellman method was used for anticholinesterase effect. The best antioxidant effect was recorded in *P. schweinitzii* methanol extract in β-carotene-linoleic acid (IC_{50}: 1.16±0.14 µg/mL), $DPPH^{\bullet}$ scavenging (80.56±0.53 %), $ABTS^{\bullet+}$ scavenging (IC_{50}: 2.96±0.15 µg/mL) and CUPRAC ($A_{0.50}$: 5.26±0.19 µg/mL) assays except metal chelating assay. At 200 µg/mL concentration, *P. schweinitzii* methanol extract was found as the best inhibitor against AChE (56.38±0.09 %) and BChE (85.64±0.16 %).

Keywords: *Phaeolus schweinitzii, Phellinus igniarius, Antioxidant, Anticholinesterase activity*

INTRODUCTION

Oxidation reactions are of great importance for vital activities, but can lead to damaging consequences. Low antioxidant level, inhibition of antioxidant enzymes and excessive free radical production cause oxidative stress and these stress conditions may be mild or cause serious disorders (Babior and Woodman, 1990). Many studies have been conducted in recent years to prevent uncontrollable oxidative stress and it has been demonstrated that oxidative stress has an important role in the onset and development stages of atherosclerosis, cancer, chronic inflammation, diabetes, aging and other pathological diseases (Beckand and Levander, 1998; Frei, 1994). In addition, the weakening of the antioxidant defence system due to oxidative stress is thought to be effective in the formation of neurodegenerative diseases such as Alzheimer's, Parkinson's, brain tumor (Good et al., 1994; Rao et al., 2000).

Enzyme inhibition theory is one of the most accepted strategies in the treatment of many health problems today (Amtul et al., 2002). Since this theory is based on inhibiting key enzymes to alleviate disease symptoms, a large number of synthetic enzyme inhibitor drugs have been

produced. However, the negative aspects of these drugs such as limited effects and side effects increase the importance of natural enzyme inhibitors (Alkan et al., 2017). Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are enzymes linked with Alzheimer's disease (AD), and synthetic inhibitors are used in the treatment of this diseases (Feng and Wang, 2012). However, studies to detect natural inhibitors instead of synthetic ones are becoming more and more popular.

Although mushrooms have been used by humans for food and medicinal purposes for many years, scientific interest in studying their bioactive properties has increased in recent years. The aim of this is to determine antioxidant and anticholinesterase activities of the hexane and methanol extracts of *Phaeolus schweinitzii* and *Phellinus* mushroom species.

Material and Methods

Mushroom materials

Phaeolus schweinitzii (Fr.) Pat and *Phellinus igniarius* (L.) Quél mushroom species were harvested from Fethiye, Muğla, Turkey in 2016. The specimens with voucher numbers have been deposited at Natural Products Laboratory of Muğla Sıtkı Koçman University Fungarium.

Extraction

The dried mushroom samples were macerated with *n*-hexane at room temperature. After filtration, the solvent was evaporated under vacuum by an evaporator to obtain hexane extracts. The mushroom residue dried and then was macerated with methanol at room temperature. After filtration, the solvent was evaporated under vacuum by an evaporator to obtain methanol extracts. All extracts were stored at +4°C for further tests.

Determination of antioxidant activity

β-carotene-linoleic acid, $DPPH^{\bullet}$ scavenging, $ABTS^{\bullet+}$ scavenging, CUPRAC and metal chelating were performed for measurement of antioxidant activities of the extracts (Çayan et al., 2019). The graph of the inhibition percentage (%) versus the concentration (µg/mL) was used to calculate the IC_{50} values of the extracts. The sample concentration showing 0.50 absorbance ($A_{0.5}$) was calculated from the CUPRAC absorbance against the sample concentration. The antioxidant activity results were stated as 50 % inhibition concentration (IC_{50}) for β-carotene-linoleic acid and ABTS scavenging assays; inhibition percentage (%) at 400 µg/mL concentration for metal chelating and DPPH scavenging assays; $A_{0.50}$ which corresponds to the concentration producing 0.500 absorbance for CUPRAC assay.

Determination of anticholinesterase activity

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of the extracts were carried out as reported in our previous study (Deveci et al., 2019). Galantamine was used as standards. The enzyme inhibitory activity results were stated as inhibition percentage (%) at 200 µg/mL concentration.

Statistical analysis

Antioxidant and anticholinesterase activities results were the average of three parallel sample measurements. The data were registered as the mean ± S.E.M. Student's t test was used to determine significant differences between the means and p values <0.05 were accepted as significant.

Results and Discussion

Since the antioxidant action mechanisms differ, single method cannot clearly define the antioxidant activity of the samples, so it is necessary to use more than one method for all the antioxidant activities of the extracts (Lü et al., 2010). For this reason, antioxidant activities of the mushroom extracts were tested by using five different spectrophotometric assays. β-carotene-linoleic acid, CUPRAC and metal chelating activity results are summarized in Table 1 and $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activity results are given in Table 2. According to obtained results the methanol extracts showed higher antioxidant activity than the hexane extracts. The highest antioxidant activity was found in *P. schweinitzii* methanol extract in β-carotene-linoleic acid (IC_{50}: 1.16±0.14 µg/mL), $DPPH^{\bullet}$ scavenging (80.56±0.53 % at 400 µg/mL concentration), $ABTS^{\bullet+}$ scavenging (IC_{50}: 2.96±0.15 µg/mL) and CUPRAC ($A_{0.50}$: 5.26±0.19 µg/mL) assays except metal chelating assay.

Table 1. Antioxidant activities of the extracts of the mushroom species by β-carotene-linoleic acid, CUPRAC and metal chelating assays

		Antioxidant Activity		
		β-Carotene-linoleic acid	**CUPRAC**	**Metal chelating**
Mushrooms	**Extracts**	**IC_{50} (µg /mL)[a]**	**$A_{0.50}$ (µg/mL)[b]**	**Inhibition (%)[c]**
P. schweinitzii	Hexane	243.63±0.49	193.75±0.50	NA[d]
	Methanol	1.16±0.14	5.26±0.19	NA[d]
P. igniarius	Hexane	47.21±0.67[b]	263.23±0.18	31.63±0.76
	Methanol	7.05±0.40	142.37±0.09	52.09±0.09
Standards	α-Tocopherol	2.10±0.08	66.72±0.81	NT[e]
	BHA	1.34±0.04	24.40±0.69	NT[e]
	EDTA	NT[e]	NT[e]	95.20±0.13

[a]: IC_{50} values represent the means ± SEM of three parallel measurements *($p < 0.05$).*
[b]: $A_{0.50}$ values represent the means ± SEM of three parallel measurements *($p < 0.05$).*
[c]: Inhibition % of 400 µg/mL concentration of the mushroom extracts *($p < 0.05$).*
[d]: NA: not active.
[e]: NT: not tested.

When *P. schweinitzii* mushroom showed no metal chelating activity, *P. igniarius* methanol extract showed moderate metal chelating activity with inhibition value of 52.09±0.09 % at 400 µg/mL concentration. Moreover, *P. schweinitzii* methanol extract indicated higher antioxidant activity than α-tocopherol and BHA in β-carotene-linoleic acid, CUPRAC and $ABTS^{\bullet+}$ scavenging assays.

Table 2. Antioxidant activities of the extracts of the mushroom species by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging assays

		Antioxidant Activity	
		$DPPH^{\bullet}$	**$ABTS^{\bullet+}$**
Mushrooms	**Extracts**	**Inhibition (%)[a]**	**IC_{50} (µg/mL)[b]**
P. schweinitzii	Hexane	11.28±0.95	20.56±0.76[a]
	Methanol	80.56±0.53	2.96±0.15
P. igniarius	Hexane	5.39±0.78	18.86±0.08[a]
	Methanol	76.34±0.09	82.65±0.59
Standards	α-Tocopherol	97.79±0.15	38.51±0.54
	BHA	94.74±0.15	11.82±0.09

[a]: Inhibition % of 400 µg/mL concentration of the mushroom extracts ($p < 0.05$).
[b]: IC_{50} values represent the means ± SEM of three parallel measurements ($p < 0.05$).

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders that frequently cause dementia and affect the middle- to old-aged individuals. This disease is

characterized by a progressive cognitive decline leading to social or occupational disability. One of the most accepted treatment strategies in treating of AD is the inhibition of the AChE and BChE (Castellani et al., 2010). Anticholinesterase activities of the mushroom extracts were tested by using Ellman method and the results are presented in Table 3. The highest inhibitory activity against AChE was found in *P. schweinitzii* methanol extract (56.38±0.09 %) and followed by *P. igniarius* hexane extract (56.32±0.61 %) at 200 µg/mL concentration Against BChE, *P. schweinitzii* hexane and methanol extracts showed superior inhibitory activity with inhibition values of 75.68±0.23 % and 85.64±0.16 % at 200 µg/mL concentration, respectively.

Table 3. Anticholinesterase activities of the extracts of mushroom species[a]

		Anticholinesterase activity	
Mushrooms	**Extracts**	**AChE**	**BChE**
P. schweinitzii	Hexane	44.09±0.40	75.68±0.23
	Methanol	56.38±0.09	85.64±0.16
P. igniarius	Hexane	56.32±0.61	29.07±0.83
	Methanol	8.40±0.15	54.27±0.63
Standard	Galantamine	80.41±0.98	82.23±2.67

[a]: Inhibition % of 200 µg/mL concentration of mushroom extracts ($p < 0.05$).

Previously, antioxidant activities of the methanol and water extracts of *P. igniarius* were reported by using reducing power, metal chelating, DPPH scavenging and superoxide anion scavenging assays (Lung et al., 2010). In the study of Seephonkai et al. (2011), water, 50 % ethanol, 80 % ethanol, ethanol and ethyl acetate extracts of *P. igniarius* were tested for DPPH scavenging activity. DPPH and ABTS scavenging activities, ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC) of the various extracts of *P. schweinitzii* were investigated by Smolskaite et al. (2015). Our results are consistent with previous studies. Also, this is the first study about anticholinesterase activities of the studied mushroom species.

Conclusions

In this study, antioxidant and anticholinesterase activities of the hexane and methanol extracts of *P. schweinitzii* and *P. igniarius*, considered as medicinally important mushrooms, were investigated. *P. schweinitzii* methanol extract indicated higher antioxidant activity than standards in β-carotene-linoleic acid, CUPRAC and $ABTS^{\bullet+}$ scavenging assays. Furthermore, *P. schweinitzii* methanol exhibited stronger BChE inhibitory than galantamine used as standard. This study suggests that studied mushroom extracts could be used as promising antioxidant and anticholinesterase agents with the highest bioactive properties.

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EVALUATION OF FORAGE PEA LINES IN REGARD TO EARLINESS AND GRAIN YIELD

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ABSTRACT

Five hybrid lines of forage peas (No. 14, No. 6, No. 12A, No. 13 and No. 9) and the Mir variety (standard) were studied in terms of ripeness and yield of grain and green mass. The study was performed in the experimental field of Institute of Forage Crops - Pleven, Bulgaria (2016-2018). The occurrence of the phenological stages beginning of flowering, full flowering, and technical maturity (days) was monitored. At the full bottom pods stage the green mass yield (kg/da), and at the technical maturity stage the grain yield (kg/da) were recorded. The line No. 14 (vegetation period 218 days) was found to develop faster than other lines. Plants from this line enter the flowering stage much earlier than the Mir variety and reach technological maturity at the earliest. At ripeness, the lines are arranged: line No. 14 (218 days), line No. 12A (222 days), Mir variety (224 days), line No. 6 (225 days) and line No. 13 (233 days). Lines No. 13 and PL managed in the most unfavorable environment to fully manifest their biological potential and to accumulate more green mass (2007 and 2059 kg/da, respectively). In terms of grain yield, line No. 14 exceeds the standard by 35.42% on average over the period. Grain yield after it, lines No. 6 and 12A are ranked, with an excess of 21.53% and 20.83% respectively. For breeding purposes line No. 14 may be used in terms of ripeness, lines No. 14, 2A and 6 in terms of grain yield. The study is a contribution to the selection for the creation of new genotypes of forage peas with pronounced ripeness, increased ecological stability of both, green mass and grains.

Keywords: Plasticity, Productivity, Ripeness, Stability, Variety

INTRODUCTION

Pea is one of the most common annual legumes and is characterized by a wide variety of forms and uses. This crop is cultivated in virtually every agricultural region in the world (Gordeev, 2008; Kadermas, 2014). The variety has always played an important role in increasing the yield of agricultural crops. Modern agricultural production has high requirements for the variety, the main ones being high and stable for years of production. Therefore, productivity is the main criterion for the effectiveness of the varieties and lines studied. Variety characteristics are of great importance for improving the quality of the grain also (Romanenko, 2005; Markin and Yakovleva, 2009). Sown areas of peas have increased in recent years, which is associated with an increase in its demand worldwide (Zavalin and Bezgodova, 2007; Golopyatov et al., 2011; Golopyatov, 2017). The aim of the study was to analyze lines of forage

pea to create new genotypes with increased ecological stability of grain yield and green mass yield and ripeness.

Material and Methods

A three-year competitive variety experiment was conducted on the experimental field of the Institute of Forage Crops - Pleven, Bulgaria with five hybrid lines of forage peas (No. 14, No.6, No. 12A, No. 13 and No. 9) and Mir variety as a standard. The seeds were sown in plots in six repetitions with a plot size of 10 m2. The phenological stages of the varieties and lines were recorded – beginning of flowering, full flowering and technical maturity (days). At the full bottom pods stage the green mass yield (kg/da), and at the technical maturity stage the grain yield (kg/da) were recorded.

Experimental data are presented by year and average over the study period. They were statistically processed using the GENES 2009.7.0 computer software for Windows XP (Cruz, 2009) and STATGRAPHICS Plus for Windows Version 2.1.

Results

It is common knowledge that the level of plant productivity depends on the interaction of the genotype with environmental factors. Essential for the formation of productivity in legumes is sufficient moisture during the growing season and the required amount of active temperature.

During the study period, favorable conditions for pea development were 2017 and 2018, when the varieties and lines tested were able to reach their biological potential to a great extent (Table 1). At the same time, it should be noted that different genotypes respond differently to stressful situations.

Table 1. Meteorological conditions during the vegetation of pea (2016-2018)

Year	Daily average temperature, °C						Rainfall, mm					
	III	IV	V	VI	VII	av.	III	IV	V	VI	VII	av.
2016	8.5	15.3	16.4	23.0	24.6	17.6	76.6	73.1	76.5	45.8	7.8	279.8
2017	10.3	12.2	17.0	23.0	24.0	17.3	46.1	37.5	155.0	44.8	155.9	439.3
2018	5.3	16.9	19.6	21.8	23.0	17.3	98.1	19.6	47.7	155.2	119.0	439.6

Phenological observations

The observed phenological differences between the lines, although genetically determined, are due primarily to changes in climatic conditions during the year. The differences in the occurrence and duration of the individual phenological stages, especially the later ones, are maintained until the end of the growing season, following the individual biological characteristics of the plants. Line No. 14 is developing faster than other lines. Plants from this line have entered the beginning of flowering stage between 06.04.2017 and 18.04.2018, much earlier than the Mir variety (between 12.04.2016 and 27.04.2018). The line No. 13 can be identified as the latest flowering line, which in 2018 has flowered 16 days later than line No. 14 (04.05.2018).

It was found that lines No. 6 and 12A did not differ in the rate of development with respect to the Mir standard (225 days) (Table 2). They complete their vegetation for 226 and 224 days, respectively, and reach the earliest maturity, after line No. 14 (218 days). The vegetation period for lines No. 9 and No. 13 is longer (228-232 days) than Mir variety. Minor differences in the occurrence of the individual phenological stages, in the duration of flowering and ripening, as well as in the duration of the growing season, were found between lines 12A and Mir variety.

Table 2. Phenological development of lines and varieties of winter forage peas

Phenological stages/ Variety (line)	Data of sowing	Beginning of flowering	Full flowering	Technical maturity	Vegetation period (days)
Mir	04.11.2015	12.04.2016	20.04.2016	13.06.2016	221
	02.11.2016	20.04.2017	28.04.2017	19.06.2017	225
	02.11.2017	27.04.2018	02.05.2018	15.06.2018	225
№9	04.11.2015	18.04.2016	09.05.2016	20.06.2016	228
	02.11.2016	25.04.2017	08.05.2017	23.06.2017	229
	02.11.2017	27.04.2018	02.05.2018	18.06.2018	228
№6	04.11.2015	15.04.2016	25.04.2016	15.06.2016	223
	02.11.2016	20.04.2017	26.04.2017	19.06.2017	225
	02.11.2017	30.04.2018	08.05.2018	16.06.2018	226
№12A	04.11.2015	12.04.2016	20.04.2016	13.06.2016	221
	02.11.2016	18.04.2017	21.04.2017	15.06.2017	221
	02.11.2017	27.04.2018	02.05.2018	14.06.2018	224
№14	04.11.2015	07.04.2016	15.04.2016	08.6.2016	218
	02.11.2016	06.04.2017	14.04.2017	12.06.2017	218
	02.11.2017	18.04.2018	30.04.2018	08.06.2018	218
№13	04.11.2015	25.04.2016	09.05.2016	24.06.2016	234
	02.11.2016	27.04.2017	12.05.2017	26.06.2017	232
	02.11.2017	04.05.2018	14.05.2018	22.06.2018	232

Analysis of variance

The results of the two-factor analysis of variance show that the conditions of the year (B) - 86.8% have the greatest influence on the yield. The share of influence of variety (A) on yield formation was found 9.8% (Table 3). According to the traits studied, there is a reliable

interaction between the genotype and the environment, which is a prerequisite for a more detailed analysis of the nature of the observed interaction.

Table 3. Analysis of variance for green mass yield and grain yield

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
green mass yield					
Variety/lines (A)	549175	4	137294	6.97	0.0102
Years (B)	1.25092E7	2	6.2546E6	317.45	0.0000
RESIDUAL	157621	8	19702.6		
TOTAL(CORRECTED)		14			
grain yield					
Variety/lines (A)	10057.60	5	2011.52	4.78	0.0172
Years (B)	12318.10	2	6159.06	14.63	0.0011
RESIDUAL	4208.56	10	420.85		
TOTAL(CORRECTED)	26584.30	17			

Dry conditions in the growing area often coincide with the beginning of the critical stage of legume organogenesis - the formation of generative organs. The lack of moisture during this period, in combination with very high temperatures, leads to a decrease in the values of the productivity elements, such as the number of pods and seeds per plant, the number of seeds per pod and the mass per 1000 seeds, and hence the yield, respectively

As a complex quantitative trait, the formation of fresh biomass is directly dependent on changing climatic conditions. Lines No. 13 and PL managed in the unfavorable environment to fully exert their biological potential and to accumulate more green mass (2007 and 2059 kg/da) (Table 4). In this respect the PL line is different from the other lines, it also exceeds the Mir variety (1916 kg/da), although it is not significantly different from it. The last position of line No. 14 (1514 kg/da) is due not to the fact that it is less leafy, but to the shorter growing season, during which it fails to produce significant green biomass, and the low plants.

The PL line exhibits some fluctuation in grain yield stability. The numerical expressions on the PL line give reason to believe that it is significantly affected by changes in climatic conditions. Line No. 14, line No. 6 and line No. 12A show grain yield stability over the years. Grain yield averaged over the study period from these lines was 195 kg/da (line No. 14), 175 kg/da (line No. 6), 174 kg/da (line No. 12A). Line No. 13 has very good yields on green mass, but shows low environmental stability in grain yield.

The results obtained prove the close relationship between grain yield and rainfall during the growing season, where the correlation coefficient is quite high (r = 0.75-0.89). The results of the mathematical analysis reveal the existence of a close relationship between the amount of rainfall during flowering and grain production (r = 0.73-0.85). Pea productivity depends more on the amount of rainfall that falls during flowering and seed filling, in which case the

correlation coefficients are r = 0.76-0.84. A similar relationship is observed between the yield of the varieties tested and the temperature values. The seed productivity of a genotype consists of a harmonious combination of all the elements of its components. Analyzing the morphological productive elements and the impact of climatic factors Vozian et al. (2007) found a slight dependence of the number of pods per plant with temperature values (r = 0.01-0.24).

Table 4. Green mass yield (kg/da) and grain yield (kg/da)

Variety/ lines	2016	%	2017	%	2018	%	Average for the period	%
	green mass yield							
Mir	2680b	100	2439c	100	629bc	100	1916b	100
№PL	2867d	107	2705e	111	605bc	96	2059b	107
№12A	2737c	102	2254b	92	596b	95	1862b	97
№14	2143a	80	1951a	80	450a	72	1514a	79
№13	2873d	107	2468d	101	679c	108	2007b	105
	grain yield							
Mir	169ab	100	155ab	100	107b	100	144ab	100
№PL	151ab	89	139ab	90	150f	140	147ab	102
№12A	209ab	124	192ab	124	121d	113	174bc	121
№14	231b	137	212b	137	141e	132	195c	135
№13	140a	83	128a	83	104a	97	124a	86
№6	211ab	125	194ab	125	119c	111	175bc	122

Line No. 14 has a vegetation period of 218 days, with the highest grain yield (195 kg/da) and ripeness. Plants reach technological maturity early. Grain yield exceeds that of the control by over 30% during the three years of the study, incl. in the most unfavorable weather conditions. The average for the period the exceeding was 35.42%. After it, at grain yield are line No. 6 and line No. 12A, with the excess over the control being 21.53% and 20.83%, respectively.

According to Vozian et al. (2017) drought is one of the most dangerous natural phenomena in the climate due to the uneven distribution of atmospheric precipitation against the background of high air temperatures. The author finds that the likelihood of severe droughts during the growing season is 11-41%.

Conclusions

When evaluating winter forage pea lines with respect to the duration of the growing season and the yield of green mass and grain, it was found that line No. 14 (vegetation period 218 days) develop faster than other lines. Plants from this line enter the flowering stage much earlier than the Mir variety and reach technological maturity at the earliest. At ripeness, the lines are arranged: line No. 14 (218 days), line No. 12A (222 days), Mir variety Мир (224 days), line No. 6 (225 days) and line No. 13 (233 days). Lines No. 13 and PL managed in the most unfavorable environment to fully manifest their biological potential and to accumulate more green mass (2007 and 2059 kg/da, respectively). In terms of grain yield, line No. 14 exceeds the standard by 35.42% on average over the period. Grain yield, after it lines No. 6 and 12A are ranked, with an excess of 21.53% and 20.83%, respectively. For breeding purposes line No. 14 may be used in terms of ripeness, lines No. 14, 2A and 6 in terms of grain yield. The study is a contribution to the selection for the creation of new genotypes of forage peas with pronounced ripeness, increased ecological stability of both, grain yield and green mass.

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IN VITRO SCREENING OF COMMON BREAD WHEAT (*Triticum aestivum* L.) CULTIVARS IN TURKEY FOR HIGH REGENERATION

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ABSTRACT

In this research, 19 winter wheat cultivars were used. These genotypes were used in our experiment since they have high adaptation ability to the biotic and abiotic stress factors and used as parents for wheat breeding, were investigated in terms of tissue culture parameters using mature embryos. Mature embryos were aseptically removed from soaked seeds and placed scutellum up in petri dishes contains 2 mg l-1 2,4-D as callus induction media, and then they were left at dark. After 14 days, developed calli were transferred in the dishes to MS-0 mediums for the regeneration. When mature embryos of 19 winter wheat registered cultivars were checked, significant differences were observed in "Callus Induction", "Callus Weight", "Regeneration Capacity" and "Culture Efficiency" parameters. The aim of using these parameters is to determine the susceptibility of genotypes to tissue culture. So, in terms of these parameters have the highest varieties value (96.7%, 0.76 g, 100.0% and 96.7%), for Bayraktar-2000 and (100.0%, 1.01 g, 100.0% and 100.0%) for Kate A-1 and a remarkable genotypic effect was detected on the tissue culture parameters responses among the used genotypes in this experiment. On the other hand, it has been seen that there is remarkable germplasm transferring ability among them, and this/these potential(s) allow a possible direct gene transfer possibility(ies) for desired trait(s) using with advanced molecular and biotechnological methods such as biolistic, etc. in the future.

Key words: winter wheat, *Triticum aestivum* L., embryo culture, regeneration

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is a staple plant for the human nutrition due to its adaptation ability to grow under different climatic and soil conditions, high nutritional content, its utilization as a raw material in many fields of industry in addition to its nutritional value for humans and animal, and its convenient cultivation, transportation, and storage. In addition to being the most cultivated crop in Turkey and the world, it takes up about 39% of the cultivated agricultural areas every year (Anonymous, 2019).

In addition to reaching their natural borders both in the world and in our country, agricultural areas are shrinking rapidly for reasons such as erroneous agricultural activities, reduced productivity due to erosion, and misuse of agricultural areas. In order to meet the nutritional needs of the growing population, it is inevitable to increase the crop yield per unit area. In recent years, plant breeding efforts toward developing high quality, high yield, biotic and abiotic stress tolerant varieties have been redoubled.

Today, plant breeding programs are designed as traditional/classical, and second biotechnological. The first one has more time consumer and labor-intensive activities than the other. Joint efforts and the use of biotechnological techniques in overcoming the problems

arising in traditional breeding works will significantly contribute to the development of new varieties (Ozgen et al., 2005).

As known, tissue culture is the first necessary step for the successful application of biotechnological techniques and the main goal of its to ensure healthy and high-level plant regeneration and rapid multiplication ability. Achieving success in callus induction and plant regeneration, an important step in gene transfer through. Biotechnological techniques depend on many factors such as the content of the culture medium, genotype, explant source and age, growth regulators and used methods, relevant abiotic and biotic stress factors such as temperature, pH, light density, etc. (Tuberosa et al., 1988; Ahmet and Adak 2007; Farshadfar et al., 2012a; Ozgen et al., 2017).

Embryo culture is the most commonly used plant tissue culture method in cereals and it is defined as the isolation of embryos from seeds and ovules from the plants (mostly high ones) followed the culture in certain mediums (Hakam et al., 2016). Embryo culture has two applications; embryo culture of mature and immature seeds. The limitations in obtaining immature embryos make the use of this explant difficult. Since mature embryos are easy to obtain everytime, they find extensive use in tissue culture studies (Chen et al., 2006).

Another important factor for winter wheat ability to produce callus and regenerate in tissue culture conditions is genotype (Przetakiewicz et al., 2003; Zale et al., 2003; Pellegrineschi et al., 2004; Şehirali and Ozgen, 2015). In our experiment, it was concluded that a strong genotypic influence has been observed. This effect can be explained as nuclear or cytoplasmic effects', and one or several chromosomes are responsible for this as mentioned by Ozgen et al. (2001). The main aim of this study is to determine the ability of the 19 winter wheat (*Triticum aestivum* L.) cultivars which are widely grown in Turkey and used as parents for wheat breeding to be able to find out their callus induction and plant regeneration capacity or potential from the mature embryos with the tissue culture experiment under the, and to identify their potential for future and possible gene transfer or genetic studies.

MATERIAL AND METHOD

This research was carried out at the Ankara University, Biotechnology Laboratory, 2018 between 2019 years and the mature embryos of 19 winter wheat genotypes (Bayraktar-2000 Bezostaja 1, Bolal-2973, Demir 2000, Doğu 88, Gerek 79, Göksu 99, Gün 91, İkizce-96, Kate A-1, Kenanbey, Kınacı-97, Kıraç 66, Lancer, Lütfibey, Pehlivan, Sönmez 2001, Tosunbey and Zencirci-2002) were used.

Winter wheat cultivars' seeds were sterilized with 70% (v/v) ethanol for 5 min, washed 2-3 times with sterile water, shaken for 25 min with Sodium Hypochlorite (NaClO), and washed several times with sterile water. The seeds were then soaked in sterile water for 2 h at 33°C. Afterward, mature embryos were aseptically seperated from the endosperm of soaked seeds for callus initiation. Obtained embryos were placed, with the scutellum up, in petri dishes containing 4,43 g l^{-1} MS (Murashige and Skoog 1962), 20 g l^{-1} sucrose, 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 7 g l^{-1} agar. The petri dishes were held at 25 ±1°C in darkness for 2 weeks (Ozgen et al., 2017).

Embryogenic calli was transferred to the same hormone-free medium for root and shoot growth. Callus weight parameter were gauged before calli were transferred to regeneration medium. The transferred calli were grown under 16 h/8 h (light/dark) photoperiod at 25±1 °C. Regeneration rates were determined by counting green dotted calli after 4 weeks. Culture efficiency is determined based on the proportion of regenerated callus obtained from cultured

mature embryos. Regenerative shoots were transferred to jars with the same medium when they grow 10-40 mm. Regenerants for rooting were exposed to 50 W cool White fluorescent light (50 µmo m^2 s^{-1}) at 25±1 °C under 16 h/8 h (light/dark) photoperiod for 4 weeks. All media were adjusted to pH 5.8 and autoclaved for 30 min at 121°C and 1.1 kg cm^{-2} pressure (Ozgen et al., 1998).

Analyzed tissue culture parameters respectively "Callus Induction" (%), "Callus Weight" (g), Regeneration Capacity" (%) [(number of nodular calli with green spots/ number of calli induced x 100)], and "Culture Efficiency" (%) [(number of nodular calli with green spots/number of embryos cultured x 100)] (Ozgen et al., 2017).

Statistical Analysis

The present experiment was adjusted as a completely randomized design for each genotype with three replicates. Each petri dishes containing 10 embryos were considered the units of replication, and the effects of genotypes on culture responses were determined by analysis of variance (One-tailed of ANOVA) and mean values comparison(s) (Steele 1980), and analysis of the Duncan test was carried out with MSTAT-C. Similarly, correlation coefficients were calculated with this software among the investigated traits.

RESULTS

The results of the variance analysis carried out via the data obtained from the parameters examined in the tissue culture conditions from the wheat genotypes used in the experiments are shown in Table 1. When Table 1 is examined, it is seen that the difference between the varieties in terms of "callus induction", "callus weight", "regeneration capacity" and "culture efficiency" is statistically significant at a level of P<0.01. Results of the Duncan test that was conducted to determine the differences among the cultivars are also given in Table 2.

Table 1. ANOVA results for the response to tissue culture parameters of 19 bread wheat cultivars

Variation Resources	**df**	**F values**			
		Callus Induction	**Callus Weight**	**Regeneration Capacity**	**Culture Efficiency**
Genotype	18	17.619**	19.406**	8.569**	15.328**
Error	38	-	-	-	
Total	56	-	-	-	

**: Significantly different from zero at 0.01

The callus induction from mature embryos was observed on the third day of cultivation, and this duration varied depending on the genotype. Calli obtained from genotypes formed two types of forms: white colored, loose - watery and dark cream colored, compact - solid form. The callus induction rate and callus weights of the varieties were calculated based on the the data obtained on the 14th day of experiment and mean diameter of callus was measured as 4-5 mm. It has been determined that the callus induction varies depending on the genotype.

As shown in Table 2, where the averages of the varieties are compared, calli induction rates of genotypes ranged from 50% to 100%. The highest callus rate (100%) was maintained by Gerek-79, Kate-1, Lancer, Sönmez-2001 and Zencirci-2002 cultivars while the lowest callus rate

(50%) occurred in the Pehlivan. Using Duncan test, the genotypes were classified under 4 different classes in terms of callus induction. The callus means weights of the genotypes were measured as 0.85 g Lütfibey had the highest callus weight with 1.20 g, while the Pehlivan showed the lowest weight of callus with 0.20 g. Cultivars were categorized into 10 different groups according to their callus weights. With the data obtained from the callus weight parameter, it has been determined that this parameter varies significantly between genotypes.

Table 2. The mean values of bread wheat cultivars in tissue culture parameters

Cultivars	Callus Induction (%)	Callus Weight (g)	Regeneration Capacity (%)	Culture Efficiency (%)
Bayraktar-2000	96.7 a[1]	0.76 fg	100.0 a	96.7 ab
Bezostaja-1	96.7 a	1.14 ab	96.7 ab	93.3 a-c
Bolal-2973	53.3 d	0.56 hi	33.3 f	16.7 f
Demir-2000	93.3 ab	0.97 b-d	89.6 a-d	83.3 a-d
Doğu-88	93.3 ab	0.44 i	57.4 e	53.3 e
Gerek-79	100.0 a	0.91 c-f	93.3 a-c	93.3 a-c
Göksu-99	80.0 c	0.68 gh	86.8 a-d	76.7 cd
Gün-91	83.3 bc	0.99 b-d	96.3 ab	80.0 b-d
İkizce-96	96.7 a	0.78 e-g	72.6 de	70.0 e
Kate A-1	100.0 a	1.01 bc	100.0 a	100.0 a
Kenanbey	93.3 ab	1.02 bc	96.7 ab	90.0 a-c
Kınacı-97	96.7 a	0.98 b-d	89.3 a-d	86.7 a-d
Kıraç-66	93.3 ab	0.82 d-g	82.6 a-d	76.7 cd
Lancer	100.0 a	0.74 fg	76.7 cd	76.7 cd
Lütfibey	96.7 a	1.20 a	86.7 a-d	89.3a-c
Pehlivan	50.0 d	0.20 j	87.8 a-d	43.3 e
Sönmez-2001	100.0 a	0.98 b-d	96.7 ab	96.7 ab
Tosunbey	96.7 a	0.95 c-e	96.3 ab	96.7 ab
Zencirci-2002	100.0 a	0.95 c-e	80.0 b-d	80.0 b-d
Total	90.5±2.0	0.85±0.03	85.2±2.4	78.9±5.8

[1]: means followed by the different letters are significantly different at the 0.05 probability level

The calli that had been transferred to regeneration medium started to form green spot rapidly on them under 16 h/8 h (light/dark) photoperiod conditions. In this hormone-free regeneration medium, a large number of shoots and roots were obtained from the calli, varying according to the genotypes (Figure 1). The regeneration capacities of the varieties ranged from 33% to 100%. Regeneration capacity was the highest (100%) in the Bayraktar-2000 and Kate A-1 (Figure 2) and the lowest (33.3%) in the Bolal-2973. In Duncan test, varieties were categorized into 6 different groups according to their regeneration capacities.

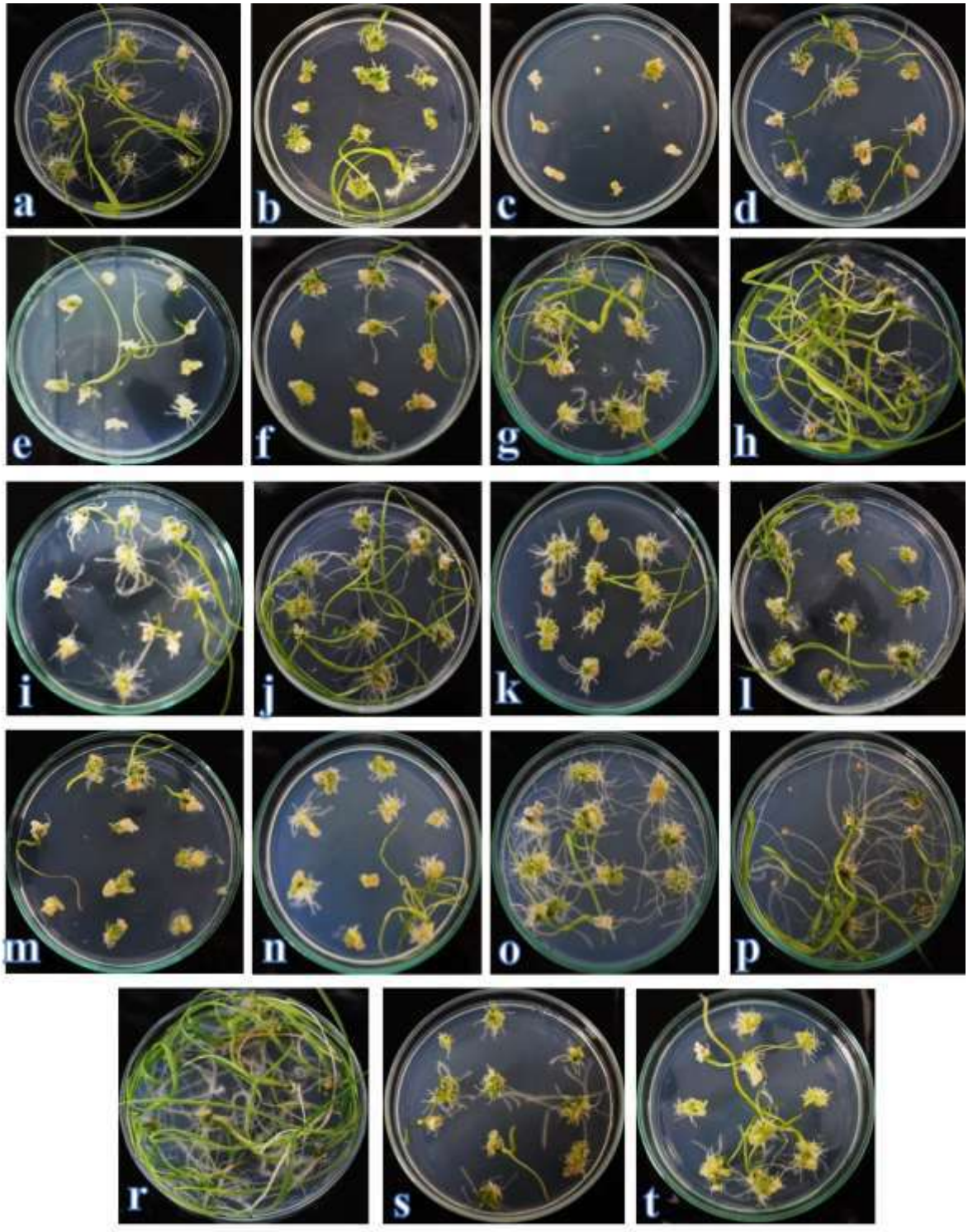

Figure 1. 4 week regenerants of bread wheat genotypes (a: Bayraktar-2000, b: Bezostaja-1, c: Bolal-2973, d: Demir-2000, e: Doğu-88, f: Gerek-79, g: Göksu-99, h: Gün-91, i: İkizce-96, j: Kate A-1, k: Kenanbey, l: Kınacı-97, m: Kıraç-66, n: Lancer, o: Lütfibey, p: Pehlivan, r: Sönmez-2001, s: Tosunbey, t: Zencirci-2002).

Figure 2. 8 weeks regenerants of bread wheat cultivars with higher tissue culture response (a: Bayraktar-2000, b: Bezostaja-1, c: Gerek-79, d: Kate A-1, e: Sönmez 2001, f: Tosunbey).

In terms of culture effect values; Bolal-2973 showed the lowest culture effect value (16.7%) while Kate A-1 showed the highest (100%). The average culture effect of wheat cultivars was calculated as 78.9% and categorized under 6 different groups using Duncan test.

The dendogram showing the proximity-distance degrees of bread wheat varieties in terms of the investigated properties is given in Figure 3. According to the results, genotypes were divided into 4 main clusters. Sönmez-2001 and Tosunbey varieties were the genotypes closest to each other in terms of the characteristics studied. Bayraktar-2000 and Bolal-2973 were the most different genotypes in terms of these characteristics.

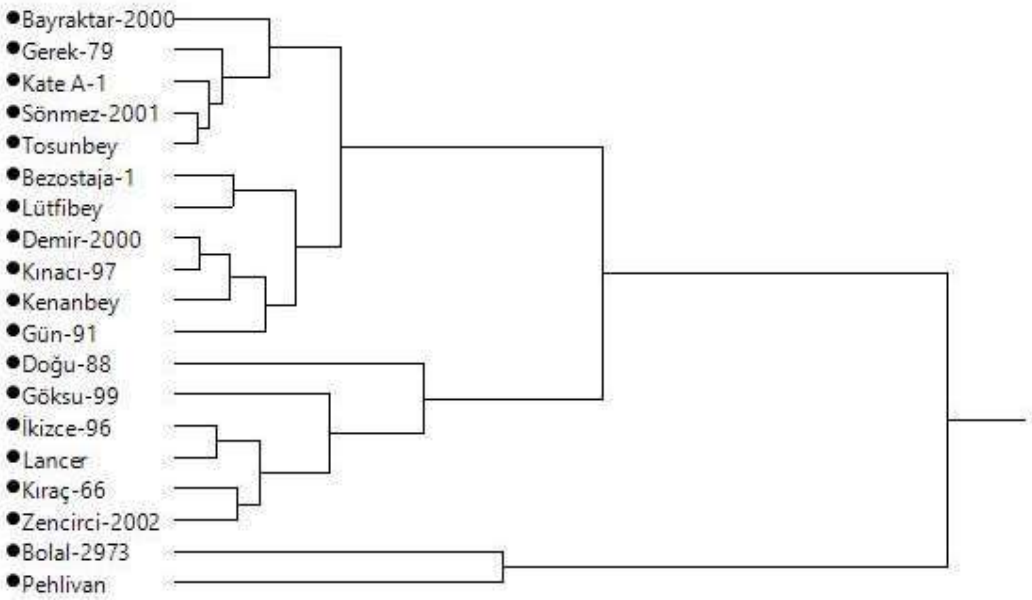

Figure 3. Dendrogram based on tissue culture parameters of 19 bread wheat cultivars

The results of the correlation analysis to determine the relationship between tissue culture parameters obtained from wheat varieties are given in Table 3. When Table 3 is examined, it is seen that there are positive and statistically meaningful correlations among all the characters. Particularly the correlation between regeneration capacity and culture efficiency (r= 0.835**) and callus induction and culture efficiency (r= 0.744**) were found to be the highest (Table 3).

Table 3. Correlations between different characters in callus cultures from mature embryos of bread wheat genotypes

Characters	**Correlation coefficients between characters**			
	1	2	3	4
(1) Callus Induction (%)	-	0.679**	0.381*	0.744**
(2) Callus Weight (g)	-	-	0.428*	0.706**
(3) Regeneration Capacity (%)	-	-	-	0.835**
(4) Culture Efficiency (%)	-	-	-	-

*,** : Significantly different from zero at 0.05 and 0.01 probability, respectively.

DISCUSSION

The obtained results of our study, which have revealed the fact that callus induction varies considerably even among the genotypes of the same species, indicate that genotype has a significant impact on callus induction. As a matter of fact, previous studies on wheat also affirmed that callus induction is related to the genotype (Ozgen et al., 1996; Zale et al., 2003;

Grigoryeva and Shletser, 2006; Ahmet and Adak, 2007; Farshadfar et al., 2012a; Aadel et al., 2016; Ozgen et al., 2017; Malik et al., 2017).

Our findings indicate that the regeneration capacity varies depending on the genotype. Our results are consistent with other findings (Ozgen et al., 1996; Ozgen et al., 2001; Bajji et al., 2000; Gonzales et al., 2001; Przetakiewicz et al., 2003; Pellegrineschi et al., 2004; Zale et al., 2004; Grigoryeva and Shletser 2006; Ahmet and Adak 2007; Farshadfar et al., 2012a; Farshadfar et al., 2012b; İbrahim 2012; Delporte et al., 2014; Aadel et al., 2016; Ozgen et al., 2017; Malik et al., 2017).

Our results indicating that the culture effect varies according to the genotype are in parallel with other similar studies (Ozgen et al., 2001; Birsin et al., 2001; Haliloglu 2002; Birsin and Ozgen, 2004; Ahmet and Adak, 2007; Ozgen et al., 2017). With the relationship we determined between the tissue culture parameters examined, our results are compatible with previous studies on wheat (*Triticum aestivum*) (Ozgen et al., 1998; Sayar et al., 1999; Ahmet and Adak 2007), oats (*Avena sativa*) (Birsin et al., 2001), triticale (Birsin and Ozgen, 2004) and maize *(Zea mays*) (Akınyosoye et al., 2014).

CONCLUSION

In conclusion; what is sought in plant tissue cultures is for a genotype to have a high callus induction and plant regeneration capacity. In the study, the tissue culture responses of 19 cultivars, which are vital for common wheat agriculture in Turkey, were measured using mature embryos. Based on the observations and the data obtained from the experiments, the varieties with the highest regeneration ability in tissue culture conditions were determined to be Bayraktar-2000 (96.7%, 0.76 g, 100.0% and 96.7%) and Kate A-1 (100.0%, 1.01 g, 100.0% and 100.0%). It can be interpreted that the direct gene transfer potentials of these varieties are higher than other common wheat varieties and that these varieties can form a basis for the development of resistant varieties using biotechnological methods in the forthcoming periods.

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INCREASED INSECTICIDE RESISTANCE LEVELS REVEAL HIGH INSECTICIDE PRESSURE ON *AN. CLAVIGER* POPULATIONS COLLECTED FROM THE AEGEAN, MEDITERRANEAN AND SOUTH EAST REGIONS OF TURKEY

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ABSTRACT

Anopheles claviger, a malaria vector species of Turkey, have a significant public health importance. It is aimed to determine the insecticide resistance status in *Anopheles claviger* populations in the Aegean, Mediterranean and Southeastern Anatolia regions of Turkey. A total of 9 populations were analysed from 3 different geographical regions. Bioassay results showed that all *An. claviger* populations were resistant to DDT. Most of the populations were resistant or at least possible resistant to malathion and propoxur. Some of the populations begun to gain resistance against permethrin. Biochemical analyses results indicated that glutathione-S-transferases and mixed function oxidases might be responsible from the mechanisms of DDT resistance; esterases and acetylcholinesterase might be responsible for malathion and propoxur resistance; mixed function oxidases and esterases might be responsible for permethrin resistance in populations sampled from the study area. Additionally, increased acetylcholinesterase insensitivity was detected in most of the *An. claviger* populations. Overall results indicated the presence of multiple resistance mechanisms in Turkish *An. claviger* field populations suggesting that populations might gain resistance against all possible insecticide in the future. Therefore, insecticide resistance management strategies are urgently needed for effective vector control efforts.

Keywords: *An. claviger, insecticide resistance, GST, Acetylcholinesterase, MFO, esterase*

INTRODUCTION

Although effective control efforts have reduced malaria mortality in many parts of the world, malaria still remains a serious threat to people (Lu et al., 2014). It was responsible for 219 million new cases with 435.000 deaths worldwide, of which 92% of Africa, 7% of Southeast Asia, 2% in the Eastern Mediterranean Region (WHO, 2018). As well as other parts of the world, Turkey has suffered from malaria for long years (Piyal et al., 2013). However, effective control efforts have reduced the malaria cases after the Turkish Ministry of Health declared the Tashkent declarations in 2005. There have been no reported indigenous cases in Turkey since 2010 except for cases derived from foreign cases that reported in 2012 and 2014 (WHO, 2019).

There are four major classes of insecticides used intensively in malaria control: pyrethroids (PY), organochlorines (OC), carbamates (CB) and organophosphates (OP). Extensive use of chemical insecticides either in the past and or currently has resulted in insecticide resistance problems of different mosquito populations. Numerous researchers have reported the occurrence of insecticide resistance in mosquito populations in Turkey (Akıner et

al., 2013; Taşkın et al., 2015; Yavaşoğlu et al., 2019). These studies have shown different resistance levels in different mosquito species and populations in different regions of Turkey. Regular monitoring and surveillance for insecticide resistance provide valuable information for effective resistant management. Insecticide resistance mechanisms are divided in to four groups: 1. physiological behaviour 2. behavioural resistance 3. metabolic resistance 4. target site mutations. Last two groups are the most important resistant types seen in mosquito populations. Metabolic resistance mechanisms consisted of increased detoxification enzyme levels and conferred by mixed function oxidases (MFOs), glutathione *S*-transferases (GSTs), and non-specific esterases (NSEs) (Zhong et al. 2013; Safi et al. 2017) while target site mutations include mutations in voltage-gated channel, acetylcholinesterase (AChE) and γ-aminobutyric acid (GABA) receptor genes (Hemingway et al., 2004). Esterases are known to take a role in OP, CB and PY resistance (Hemingway and Ranson, 2000) while MFOs are responsible of PY resistance (Yahuedo et al., 2016). The Association between GST acitivity and DDT resistance has also shown by several researchers (Polson et al., 2011; Cisse et al., 2015).

Anopheles claviger is widely distributed in the Palearctic region (Becker et al., 2003). It has been found up to 2300 meters in all geographical regions of Turkey. *An. claviger* bite mostly large mammals however it also feeds on human in the absence of animal resources. Thus, their importance for malaria vectors varies between distribution area. Although epidemiological importance of *An. claviger* is not large due to small populations, it is a well-known principal malaria vector in the eastern Mediterranean region (Şimşek, 2006). Besides being malaria vector, *An. claviger* has medical and veterinary importance since it can be vector of Tularemia and Tahyna virus.

In Turkey, there are some reports regarding the insecticide resistance of different mosquito vector species (Akıner et al., 2013; Akıner, 2014; Taşkın et al., 2015; Yavaşoğlu et al., 2019; Guz et al., 2020). However, there have been nor reported data set of insecticide resistance in *An. claviger* populations in any geographical regions of Turkey. In this study, we aimed to examine the current status of insecticide resistance and mechanisms in field-collected adult *An. claviger* mosquitoes from the Mediterranean, Aegean and Southeastern Anatolia regions of Turkey. Insecticide susceptibility tests were performed on adult female mosquitoes to determine resistance status. Metabolic detoxification enzyme (MFO, GST, and NSE) activities were examined to determine resistance mechanisms.

Material and Methods

Sampling and laboratory colonisation

Larvae and adult samples of *An. claviger* were collected from 9 locations of the Mediterranean (Adana-Tufanbeyli, Antalya-Alanya and Kahramanmaraş-Türkoğlu) Aegean (Aydın-Karacasu, Muğla-Dalaman, İzmir-Menemen) and Southeast Anatolia (Şanlıurfa-Siverek, Mardin-Savur, Adıyaman-Kahta) regions of Turkey between April and September 2015. Larval sampling was performed by using larval dippers while adults were caught by mouth aspirators from resting sites of *An. claviger*. Morphological identifications have been performed by using an identification key suggested by Becker et al. (2003). Larval samples were reared to adults under the standard insectarium conditions at 26-28^{o}C, 12:12 h photoperiod and 70-80% relative humidity. An unfed 3-5 day old F_1 generation females were used for all bioassays and biochemical assays. Samples were kept in a -80^{o}C freezer until biochemical analyses have been carried out. Susceptible *An. sacharovi* individuals which had not been exposed to insecticides for approximately 20 years were obtained from Aydın Adnan Menderes University used as a reference strain in biochemical analyses.

WHO's susceptibility bioassay

Insecticide resistance levels was determined against DDT (4%), malathion (5%), propoxur (0.1%) and permethrin (0.75%) using WHO diagnostic bioassay tube test by following WHO (2016). Each assay tube included 20 adult mosquitoes in triplicates. Mosquitoes were exposure to each insecticide-impregnated papers for one hour and then transferred into the holding tubes without any exposure to insecticides and fed on 10% sugar solution for 24 h. A susceptible reference strain was exposed to insecticide free papers and after 24 h, mortality rates were calculated based on the mean values of three replicates. Populations were considered as susceptible if the mortality rates were ≥ 98%, possible resistance if mortality rates were 90–97% and confirmed resistance if mortality rates were lower than 90% as suggested by WHO (2016).

Biochemical analysis

A total of 270 *An. claviger* females were included in biochemical analysis. First of all, mosquito homogenates were created individually in 250 µL 50 mM sodium phosphate buffer by using homogenizer onto the ice to avoid from heat denaturation. All measurements were carried out by spectrophotometric analysis in 96 well microtiter plates through microplate reader. Bradford assay were followed for assessment of protein content (Bradford, 1976). The protein absorbance was read at 595 nm. Standard curve bovine serum albumin with different concentrations were prepared for the calculation of total protein content. All biochemical analyses including non-specific esterase (NSE), mixed function oxidase (MFO), glutathione S-transferase (GST) and Acetylcholinesterase (AChE) assay were carried out by following the test procedure provided by WHO 1998. α-naphthyl acetate, β-naphthyl acetate and p-nitrophenyl acetate were used as substrates of esterase enzyme for the calculation of non-specific esterase activity. Specific enzyme activities were stated as *n* moles α-napthol/min/mg protein, *β-napthol/min/mg protein* and p-napthol/min/mg were calculated against standard curves of *α* and *β*napthol acetate and 4-nitrophenol acetate (WHO, 1998). MFO level was calculated using heme-peroxidase assay based on heme-protein amount (Brogdon et al., 1997; WHO, 1998). A standard curve of cytochrome-c protein was created to calculate heme protein content which is related with MFO levels. Glutathione-S-transferase assay were performed by following the instructions of WHO, 1998 and the extinction coefficient (ε): 4.39 mM^{-1} was used to calculate specific GST enzyme activities. Similarly, WHO (1998) were followed for the detection of sensitive and insensitive Acetylcholinesterase (AchE) levels again. AchE inhibition rate was calculated based on well ODs. Populations with a % value greater than 30% were stated as sensitive to insecticides in terms of AchE levels.

Calculations and statistical evaluation

Mean Mortality rates and ANOVA analysis followed by Tukey's HSD test were performed through Statistica version 12.0. Median enzymatic activities were calculated through Kruskal-Wallis non-parametric test using Statistica version 12.0.

Results

Bioassay results

Bioassay results indicated that mortality rates varied between 68.3% and 88.3% for DDT and all *An. claviger* populations were resistant to DDT. Mortality rates changed between 71.6% and 91.6 for malathion; 76.6% and 95% for propoxur. Most of the populations were resistant to malathion and propoxur while some of them were possible resistant to those insecticides. All of the Mediterranean population were still susceptible to permethrin while

some populations have begun to have resistance against permethrin in the Aegean and Southeastern regions (Table 1). ANOVA results showed statistically difference between the Mediterranean and Southeastern populations in DDT, malathion and propoxur mortality rates (p<0.05). Mortality rates for propoxur were also statistically different between the Aegean and Southeastern populations (p<0.05).

Table 1. WHO insecticide susceptibility test results of *An. claviger* populations of the Mediterranean, Aegean and Southeastern Anatolia regions. Calculations are based on the mean results of three replicates.

Geographical region	Sampling localities		DDT (%4)	Malathion (%5)	Propoxur (0.1)	Permethrin (%0.75)	Control
			Percentage mortality (%)				
Southeast region	Adıyaman	Kahta	70*	75*	86*	98***	1
	Mardin	Savur	71*	75*	78*	96**	1
	Şanlıurfa	Siverek	70*	71*	76**	96**	1
Mediterranean region	Adana	Tufanbeyli	68*	75*	83*	98***	0
	Antalya	Alanya	86*	88*	95**	100***	0
	Kahramanmaraş	Türkoğlu	88*	91**	93**	100***	0
Aegean region	Aydın	Karacasu	73*	78*	86*	95**	1
	İzmir	Menemen	81*	88*	91**	100***	0
	Muğla	Dalaman	73*	76*	85*	98***	0

C: Control; * *P* Resistant; ** Possible resistant; *** Susceptible

Biochemical analysis results

Biochemical analyses results revealed that all of the populations had higher median alpha and beta esterase activity in relation to the reference strain except the Kahta population (p< 0.05). Similarly, all of the populations had increased median PNPA and MFO rates in relation to the reference strain (p<0.05). The only populations in which the median GST activity was not significantly higher than reference strain was the Tufanbeyli population. All of the populations had higher AChE inhibition rates comparing to the reference strain and all of the populations had insensitive AChE rates expressed higher than 30% critical level except the Karacasu population (p< 0.05) (Table 2 and 3).

Table 2. Median esterase activities of *An. claviger* populations from the Mediterranean, Southeastern Anatolia and Aegean region.

Region	City	Locality	Median levels of enzyme activities					
			α-Esterase (α napthol/min/mg protein)		β -Esterase (β napthol/min/mg protein)		pNPA (p napthol/min/mg protein)	
			Median	SD[1]	Median	SD	Median	SD
Southeast region	Adıyaman	Kahta	0.10	0.04	0.10	0.04	0.07*	0.05
	Mardin	Savur	0.14*	0.03	0.13*	0.03	0.11*	0.33
	Şanlıurfa	Siverek	0.14*	0.04	0.13*	0.04	0.07*	0.06
Mediterranean region	Adana	Tufanbeyli	0.99*	0.48	0.89*	0.36	0.11*	0.08
	Antalya	Alanya	0.72*	1.03	0.96*	0.53	0.10*	0.15
	Kahramanmaraş	Türkoğlu	0.14*	0.04	0.13*	0.04	0.07*	0.06
Aegean region	Aydın	Karacasu	0.15*	0.04	0.14*	0.04	0.11*	0.19
	İzmir	Menemen	0.33*	1.06	0.89*	0.39	0.11*	0.05
	Muğla	Dalaman	0.13*	0.28	0.12*	0.05	0.10*	0.04
Control			0.02	0.006	0.02	0.003	0.01	0.005

1 S.D. standard deviation; * P<0.05

Table 3. Median oxidase, GST and acetylcholinesterase (%) levels of *An. claviger* populations from the Mediterranean, Southeastern Anatolia and Aegean region.

Region	City	Locality	Median levels of enzyme activities					
			Oxidase (n mole cytochrome-c/mg protein)		GST (nmole GSH/min/mg protein)		ASCHI (% inhibition)	
			Median	SD[1]	Median	SD	Median	SD
Southeast region	Adıyaman	Kahta	0.27*	0.15	0.23*	0.05	39.8*	7.10
	Mardin	Savur	0.27*	0.18	0.29*	0.08	30.9*	9.25
	Şanlıurfa	Siverek	0.33*	0.16	0.27*	0.04	36.1*	10.0
Mediterranean region	Adana	Tufanbeyli	0.19*	0.12	0.04	0.01	34.4*	4.40
	Antalya	Alanya	0.25*	0.17	0.60*	0.60	31.6*	14.0
	Kahramanmaraş	Türkoğlu	0.29*	0.25	0.16*	0.10	31.4*	5.98
Aegean region	Aydın	Karacasu	0.61*	0.22	0.27*	0.06	27.6	9.54
	İzmir	Menemen	0.20*	0.09	0.49*	0.59	33.3*	4.61
	Muğla	Dalaman	0.44*	0.23	0.33*	0.09	60.06*	3.54
Control			0.006	0.001	0.008	0.003	7.08	2.36

1 S.D. standard deviation; * $P<0.05$

Discussion

Anopheles claviger (Diptera: Culicidae) is widely distributed throughout Anatolian plateu and well-known principal malaria vector in the eastern Mediterranean region. It is also an important vector of Tularemia and Tahyna virus, as well as malaria (Şimşek, 2006). A significant malaria case reduction wast achieved by the Turkish Ministry of Health after Tashkent declarations in 2005. However, 218 malaria cases were reported in 2012 in Mardin and although there have been no reported indigenous cases since 2016, Turkey has not certified as malaria-free country yet (WHO, 2019). Synthetic insecticide based vector-control is still the main prevention and elimination method of vector control diseases in Turkey. Thus, understanding the current status of insecticide resistance is crucial for the deployment of effective insecticide types. Even though several researchers revealed the insecticide resistance status to various insecticides, there have been no reported data set regarding the insecticide resistance status in *An. claviger* populations in Turkey, to date. In this study, the resistance to multiple insecticides was investigated in *An. claviger* populations from the Mediterranean, Aegean and Southeast Anatolia regions of Turkey. The results indicated that *An. claviger* populations had developed resistance to three classes of insecticides: organochlorines (DDT), carbamates (propoxur), organophosphates (malathion) and pyrethroids (permethrin). An increased DDT, propoxur, bendiocarb, malathion, propoxur, permethrin and deltamethrin resistance in other malaria vectors *An. sacharovi* and *An. superpictus* was also evidenced in the study reported by Yavaşoğlu et al. (2019). Multiple insecticide resistance in different *An. claviger populations* suggests that the insecticides currently used for vector control may only be partially effective or even ineffective. This result reveals the need for urgent implementation of insecticide resistance management strategies. Multiple insecticide resistance in another malaria vector, *An. maculipennis* populations has been reported in Birecik, Beysehir, Cankiri, Avariz, Tatarkoy and Seremkoy in 2007 and 2008 (Akıner et al., 2013). That study fills the gap in *An. claviger* resistance data in three geographical regions of Turkey. The data set collected will also be valuable in monitoring the insecticide resistance in different *An. claviger* populations. Understanding resistance mechanisms and monitoring resistance patterns are valuable to manage insecticide resistance in field populations of vector species.

This study has indicated the involvement of metabolic detoxification in resistance of *An. claviger* populations. All of the populations exhibited significantly higher GST enzyme

activity compared with the susceptible laboratory strain except the Adana-Tufanbeyli population. Previous studies showed the relationship between DDT resistance and alterations in GST enzyme levels in different *Anopheles* mosquito populations (Yavaşoğlu et al., 2019; Zhong et al., 2013). The *kdr* mutation frequencies might be responsible of DDT resistance in Adana-Tufanbeyli populations. However, the main limitation is that study is the lackness of molecular data set. Underlying DDT resistance mechanisms of Tufanbeyli populations would be highlighted by *kdr* mutation frequencies. Thus, several researchers found different kinds of *kdr* mutant alleles in different *Anopheles* mosquito populations along the different regions of the world (Tan et al., 2012; Vergaeghen et al., 2010). Results showed all of the populations were resistant or at least possible resistant to malathion and propoxur. Several researchers have reported that OP and CB resistance is maintained by altered esterase and acetylcholinesterase levels (Peiris and Hemingway, 1993). In parallel to that, all of the populations had significantly higher non-specific esterase and acetylcholinesterase levels except the Kahta populations in which α-esterase and β-esterase and Karacasu population in which acetylcholinesterase levels were similar to control strain. Significantly higher esterase and acetylcholinesterase levels were associated by OP and CB resistance in other *Anopheles* mosquito populations by several researchers, similarly (Akıner et al. 2014; Qin et al., 2014). However, in Kahta and Karacasu populations might be explained by increased MFO levels as well as *Ace-1* mutations which is not covered and one of the main restriction of this study. The role of Ace-1 mutation has been shown by several researchers in different mosquito populations (Tmimi et al., 2018; Soltani et al., 2015). The Savur, Siverek and Karacasu populations have begun to gain permethrin according to the bioassay results. To date, many researchers suggested that increased MFO and NSE activity might be responsible for PY resistance in these populations in addition to the *kdr* mutation (Safi et al. 2017). In this study, both increased MFO and NSE levels underlie the permethrin resistance in *An. claviger* populations in study area. Synergists assays are effective tools to demonstrate the efficacy of any enzyme in resistance of different insecticides. Djouka et al. (2016) briefly reported the role of increased oxidase levels in PY resistance in *An. funestus* populations. The lackness of the synergistic assay data set is another restriction of that study to explain roles of enzymes in different insecticide resistance more reliably.

Overall results have indicated the field collected OC, OP, CB and PY resistant An. claviger populations exhibited higher MFO, GST and NSE enzyme activity compared with the susceptible laboratory strain. These results suggest that multiple mechanisms are involved in insecticide resistance in *An. claviger* populations collected from the study area. This is further evidence that massive use of insecticides for vector control and agricultural purposes has resulted in multiple insecticide resistance in *An. claviger* populations in different regions of the Turkey. Regular monitoring of resistance status is needed to confirm the underlying mechanisms of resistance and implementation of the effective insecticide types before these resistant individuals become dominant within the populations. The current data set will be valuable for monitoring of insecticide resistance spread and effective vector control efforts.

Conclusions

Bioassay results show high insecticide resistance in *An. claviger* populations demonstrating high insecticide pressure within these populations. Overall results indicate the presence of multiple resistance mechanisms in these populations. Therefore, insecticide resistance management strategies are urgently needed for effective vector control implementation otherwise populations might gain resistance against all possible insecticides in the future.

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OPTIMIZATION OF PROCESS PARAMETERS FOR ENZYME ASSISTED ULTRASONICATION EXTRACTION OF ZEAXANTHIN FROM *TRICHODESMIUM THIEBAUTII* (NIOT 152)

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ABSTRACT

The xanthophyll carotenoid pigment zeaxanthin (3, 3′dihydroxy β carotene) is a biomolecule of paramount importance in pharmaceutical, food and feed industry globally. The yellow pigment zeaxanthin is synthesized de nova by most of the bacteria, fungi and microalgae. The marine Cyanophycean microalgae *Trichodesmium thiebautii*, is capable of accumulating xanthophyll carotenoid zeaxanthin. The present study successfully demonstrated statistical optimization of novel enzyme assisted ultrasonic extraction (EAUE) of zeaxanthin from *T. thiebautii* (NIOT 152). Zeaxanthin extraction conditions were optimized using a two-step sequential statistical optimization involving Plackett Burman (PB) method for initial screening of crucial parameters followed by response surface methodology (RSM) for optimization of identified vital process parameters. PB experiments revealed three independent variables as important factors affecting zeaxanthin extraction, namely, enzyme concentration, sonication time and solid-liquid ratio, which were further optimized using RSM. The obtained data were subjected to two way ANOVA, multiple regression analysis and Fischer's F test and were fitted into a quadratic model and the significance was set at 5% level. The optimized parameters for EAUE of zeaxanthin extraction from *Trichodesmium thiebautii* were solid-liquid ratio (g/ml) of 1:63, ultrasonication time of 44.57 min and enzyme (hemicellulase) concentration of 32.66%. The results were validated using confirmatory experiments in large scale and a zeaxanthin content of 5.16 ± 0.34 mg g^{-1} was obtained, which was 2.8 fold higher than the conventional extraction (1.84 ± 0.12 mg g^{-1}). The model for maximizing the zeaxanthin extraction yield from *T. thiebautii* had a high coefficient of determination (R^2) of 0.9850, which further confirmed the reliability and precision of optimization experiments. The results showed that *T. thiebautii* (NIOT 152) could be a potential candidate for commercial exploitation of high value zeaxanthin pigment.

Keywords: *Trichodesmium thiebautii*, zeaxanthin, Plackett Burman, response surface methodology, ultrasonication.

INTRODUCTION

Carotenoids are naturally occurring pigments in the photosynthetic apparatus of higher plants and a wide variety of microbes inclusive of bacteria and microalgae (Kyriakopoulou et al., 2015). Zeaxanthin, β-carotene, lycopene, lutein are some of the carotenoids which enjoy huge demand in the global market due to various applications in commercial sector like food, feed and pharmaceuticals (Prommuak et al., 2013; Moeller et al., 2006). The natural pigment zeaxanthin is a highly valuable nutraceutical, because it acts as an integral component of eye and has a very crucial role in eye health (Murray et al., 2013). Several research studies have

demonstrated that zeaxanthin supplements could reverse age related macular degeneration (AMD), cataract, glare response and night blindness (Nishino et al., 2002; Bernstein et al., 2016). Furthermore, zeaxanthin along with lutein is administered in the feed of ornamental fishes to improve the color and texture of skin and gills (Sajilata et al., 2008). It is also used as supplementary feed in piggery and poultry to enhance the nutritional value and to impart characteristic yellow color to egg yolk (Sarnaik and Reena, 2018). Zeaxanthin is very well known for its antioxidant activity and delays ageing, therefore, used in cosmetics (Christen et al., 2008) as well. Recent research suggests zeaxanthin possess anti-cancer activity and also used to treat some auto-immune diseases (Fiedor and Burda, 2014).

Several microalgae synthesize zeaxanthin as a major photosynthetic pigment and one among them is *Trichodesmium* sp, which often forms blooms in Sea and appear as saw dust (Bergman et al., 2013). *Trichodesmium* occupies an important ecological niche in tropical and sub-tropical oceans and acts as a nitrogen harvesting apparatus for primary producers (Capone, 2001; Westberry and Siegel, 2006). Nitrogen fixation in *Trichodesmium* is a unique ability, as they fix atmospheric nitrogen without the use of heterocyst (Bertin et al., 2017).

Conventionally, xanthophylls carotenoids inclusive of zeaxanthin are extracted from several microalgae by maceration, sonication and Soxhlet methods (Wang and Weller, 2006). Traditional techniques suffer from the setback of high energy consumption, use of large volumes of toxic organic solvents; extended extraction time; low selectivity or low extraction yields and exposure of the extracts to excessive heat, light and oxygen. The superiority of different carotenoid extraction methods depends on their characteristics, including the complexity of materials, production cost, environmental effects and safety. Additionally, choosing a suitable extraction technique for extracting carotenoids is pivotal in determining the product quality and quantity that could meet the growing demands of nutraceutical industry (Zhang and Liu, 2008). Recently, environmental friendly techniques like ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE) and ulrasonication assisted enzymatic extraction (UAEE) have emerged as viable alternatives to traditional techniques in the food industry. UAE generates sufficient energy to give rise to collisions between suspended plant particles as in the case of classical extraction, by stirring due to cavitation (Chemat et al., 2011). Research evidences have also confirmed the positive aspects of low frequency ultrasonication on the activity of cellulase enzyme (De Carvalho et al., 2020). Hence the present study aimed at ultrasound-assisted enzymatic extraction (UAEE), which uses the synergistic interaction of enzymatic hydrolysis and ultra-sound, to meet the needs of industry. The major impact of UAEE is endorsed with acoustic cavitation, due to which the cell wall ruptures to aid in the diffusion of intracellular metabolites (Ofori-Boateng and Lee, 2013). Nevertheless, higher ultrasound intensity and prolonged sonication might lead to formation and accumulation of hydroxyl radicals during cavitation and streaming process and consequent reduction in carotenoid extraction (Araujo et al., 2013). Hence, ultrasonic temperature, time interval and density (solid to liquid ratio) are some of the important parameters that had to be optimized for efficient extraction of metabolites (Wilkhu et al., 2008).

Therefore the present study focused on the statistical optimization of extraction conditions of zeaxanthin from marine microalgae *Trichodesmium thiebautii* (NIOT 152). Initially one-variable–at-a-time (OVAT) experiments were performed for cell disruption process, followed by a two-step sequential statistical optimization procedure including Plackett-Burman method combined with response surface methodology to enhance the extraction efficiency of zeaxanthin. Eventhough, a few literatures are available regarding the production potential of zeaxanthin from *Trichodesmium* sp, none of them focused on the major objective of statistical optimization of process parameters to improve the rate of extraction efficiency of

zeaxanthin from *T. thiebautii*. Hence this investigation will be of greater significance for large scale exploitation of zeaxanthin from *Trichodesmium thiebautii*.

Materials and Methods

Algal dry biomass for zeaxanthin extraction

The Cyanophycean microalgae, *Trichodesmium thiebautii* (NIOT 152), used in this study, was received from microalgal culture collection of National Institute of Ocean Technology (NIOT), Chennai. The pure culture of blue green microalga, *Trichodesmium thiebautii* (NIOT-152) was cultured in large scale using 5 numbers of 20 L Nalgene carbouys with working volume of 15 L in ASN III growth medium (Rippka et al., 1979) prepared using filtered (0.22 µM Whatman No.1 filter paper) Sea water with 35% salinity (pH 8.0 ± 0.02). *Trichodesmium* cultures were grown under standard abiotic conditions like 140 µmol photon m^2 s^{-1} light intensity with alternate photoperiod of 14:10 light/dark regimes at 25 ± 1ºC with aeration and proper stirring to prevent cell aggregates (Bell et al., 2005). The stationary phase cultures were harvested using cooling centrifuge (Sigma 3K 30) at 6500 rpm for 15 min at 4°C and the wet biomass were freeze dried using lyophilizer (VirTis®) at -52º C. The lyophilized biomass were finely pulverized into a homogenized fine powder and then stored in cryovial tubes in deep freezer (Sanyo, Japan) at -40º C for further optimization studies for zeaxanthin extraction.

Reference extraction process of zeaxanthin using alkali digestion

A reference zeaxanthin extraction process without the use of enzyme and ultrasonication was used as control to compare and evaluate the significance of optimization. The alkali digestion method extracts almost 100% of zeaxanthin from microalgae (Chen et al., 2012). Zeaxanthin extraction from dried biomass of *T. thiebautii* (NIOT 152) was carried out as per the method adopted by Priyanka et al. (2019). A known volume (10 mg) of lyophilized algal biomass was subjected to alkali treatment using 10 M aqueous KOH (1 ml) amended with antioxidant (2.5% ascorbic acid). The algal extract was given heat treatment (60ºC) in a water bath until the pellet becomes colorless and the mixture was cooled in ice bath. The carotenoid pigment was extracted using 3:1 ratio of methanol : dichloromethane (9 ml) solvent mixture. The extract was centrifuged at 5000 x g for 15 min (Sigma). The experiments were conducted in triplicates without exposure to light, as zeaxanthin being photo-oxidative pigment. However, antioxidant (ascorbic acid) was added to improve the stability of zeaxanthin and to prevent degradation upon storage. The crude solvent extract containing zeaxanthin was concentrated in rotary evaporator (Thermo Fisher Scientific, BUCHI Rotavapor™, R-100) equipped with vacuum concentrator for removal of solvent and other debris, if any, and subjected to HPLC quantification.

Quantification of extracted zeaxanthin using HPLC

Zeaxanthin was quantified using reverse phase HPLC (Shimadzu, Japan) equipped with an auto sampler (LC 2010 CHT) and quaternary pump (LC 2010) along with programmable UV-Vis detector. Column used was phenomenex Luna C-18 column with a dimension of 4.6 mm x 250 mm and a particle size of 5 µm (Li et al., 2006). LC solutions software was used to retrieve experimental data three dimensionally, i.e., absorbance-time-wavelength. Mobile phase constituted was methanol / dichloromethane / acetonitrile / de-ionised water in the ratio 67.5 : 22.5 : 9.5 : 0.5 (v/v) and the flow rate was maintained at 1 mL per min. All solvents were of HPLC grade (Merck, Germany), filtered (0.22 µM) and degassed prior to use. Zeaxanthin standard and samples were filtered through a 0.22 µM PTFE syringe filter (acrodisc, Pal Gelman, Germany) prior to injection. The injection volume was 20 µl and zeaxanthin was

observed at a wavelength of 453 nm (Priyanka et al., 2019). Zeaxanthin present in the algal extract was quantified by comparing the peak area and correlation of retention times with the authentic zeaxanthin standard (Sigma Chemical Co., St. Louis, MO, USA) using standard calibration curves. The experimental data were appraised based on the scrutiny of observations in triplicates and expressed as mean value.

Optimization using One-variable-at-a time (OVAT) experiments

Single factor experiments were performed for cell disruption methods in order to screen the most suitable technique which favors efficient extraction of zeaxanthin (Alhattab et al., 2018). For the one–factor at a time experiments (OFAT) to optimize cell disruption method for improving zeaxanthin extraction, 10 mg of freeze-dried algal biomass of *T. thiebautii*, was blended with 10 ml of aqueous alkali (1M potassium hydroxide) and anti-oxidant (2.5% ascorbic acid). The alkali mixture was disrupted using various mechanical, physical and biological methods involving enzymes as detailed below. After each treatment, zeaxanthin extraction was performed using solvent extraction (90 ml of 3:1 ratio of methanol and dichloromethane) according to Li et al. (2006), with all other extraction parameters being uniform throughout the experiment. Subsequently the mixture was centrifuged (5000 rpm for 15 min) and quantified using HPLC. For all single factor experiments, 10 mg of freeze dried algal biomass was used. All the optimization experiments were carried out in darkness and in triplicates. The following cell disruption methods were tested:

Mechanical disruption: For mechanical method of cell disruption process, the algal biomass was finely macerated using a ceramic mortar and pestle and homogenized with aqueous alkali prior to solvent extraction.

Liquid nitrogen grinding: A pre-weighed freeze dried biomass was frozen with 10–15 ml of liquid nitrogen and subsequently homogenized with pestle for 2 min.

Bead beating: To the pre-weighed algal biomass, 1 mg of glass beads of 0.5 mm size (zirconia beads, Klausen Pty Ltd., Blaxland, NSW, Australia) was added in a glass centrifuge tube (15 ml) along with 1 ml aqueous KOH solution. The mixture was vortexed for 20 min, using a vortex (Remi) in 30-second bursts for 3 cycles to enable complete pulverization of dried biomass. Samples were placed in ice bath in-between the bursts and zeaxanthin was extracted using organic solvents (Byreddy et al., 2015).

Ultrasonication*::* To the pre-weighed freeze dried algal biomass, 1 ml of aqueous KOH and 9 ml of methanol : dichloromethane were added in 25 ml glass beaker and subjected to sonication with a probe (3mm dia) sonicator (Sonics, Germany) with a frequency of 20 kHz, at different time regimes ranging from 5 to 60 min. In order to prevent over heating due to prolonged sonication, the sample tubes were maintained on ice bath.

Ultrasonication + Liquid nitrogen*:* A combination method of liquid nitrogen and ultrasonication was also tested. The pre-weighed freeze dried algal biomass was ground with liquid nitrogen and subsequently subjected to ultrasonication with 1 ml aqueous KOH and 9 ml of methanol : dichloromethane (3:1) was added in 25 ml glass beaker and subjected to sonication with a probe (3 mm dia) sonicator (Sonics, Germany) at a frequency of 20 kHz for 20 min.

Enzymatic cell disruption: The efficacy of enzymatic cell disruption was evaluated using several combinations and concentrations of enzymes like cellulase, pectinase, hemicellulase, and proteinase ranging from 25% w/v to 80% w/v (Zheng et al., 2016). All the optimization experiments were carried out in darkness and in triplicates.

Statistical optimization of process parameters for zeaxanthin extraction from T. thiebautii

The results of one variable at a time experiments indicated enzyme aided ultrasonication as the suitable method for extraction of zeaxanthin in *T. thiebautii,* because, the use of hemicellulase and ultrasonication marked highest recovery of zeaaxnthin. Hence, enzyme assisted ultrasonication extraction (EAUE) was further investigated using a two-step successive statistical optimization technique involving Plackett-Burman (PB) design and a full factorial Central Composite Rotatable Design (FF-CCRD) of response surface methodology (RSM). The experimental conditions for zeaxanthin extraction were validated using confirmatory trials in large scale experiments.

Plackett Burman experiment

The Plackett Burman experimental design for the optimization of process parameters for zeaxanthin extraction from *T. thiebautii* consisted of six extraction conditions which were tested at two levels (maximum and minimum) in 12 experimental runs as shown in Table 1. The experimental design for PB with zeaxanthin content (mg g^{-1}) as response factor, was constructed using Design Expert 9.0 (DE, Stat-Ease, Inc., Minneapolis, MN, USA) software. The six independent variables of PB were as follows: enzyme (hemicellulase) concentration (%), enzyme exposure time (min), sonication time (min), aqueous potassium hydroxide (alkali) concentration (Molar), solid liquid ratio (CDW/v), ascorbic acid (antioxidant) concentration (%). The Plackett Burman method, to improve zeaxanthin extraction, packed with 12 experimental trials was performed in triplicates in the absence of light.

Table 1. Plackett Burman experimental design for zeaxanthin extraction from *T. thiebautii*

S. No	Enzyme concen-tration (%)	Enzyme exposure time (min)	Sonica -tion time (min)	Alkali concen-tration (in M)	Solid: liquid ratio (w/v)	Ascorbic acid conc-entration (%)	Zeaxanthin content (mg g^{-1})*
1	85 (+1)	4.15 (+1)	5 (-1)	12 (+1)	1:85 (+1)	1 (-1)	3.07 ± 0.15
2	5 (-1)	4.15 (+1)	60 (+1)	1 (-1)	1:85 (+1)	9 (+1)	2.35 ± 0.12
3	85 (+1)	5 (-1)	60 (+1)	12 (+1)	1:85 (+1)	9 (+1)	5.23 ± 0.27
4	5 (-1)	4.15 (+1)	5 (-1)	12 (+1)	1:5 (-1)	9 (+1)	3.60 ± 0.17
5	5 (-1)	5 (-1)	60 (+1)	1 (-1)	1:85 (+1)	1 (-1)	3.94 ± 0.16
6	5 (-1)	5 (-1)	5 (-1)	12 (+1)	1:85 (+1)	9 (+1)	5.01 ± 0.25
7	85 (+1)	5 (-1)	5 (-1)	1 (-1)	1:5 (-1)	9 (+1)	3.79 ± 0.19
8	85 (+1)	4.15 (+1)	5 (-1)	1 (-1)	1:85 (+1)	1(-1)	3.76 ± 0.17
9	85 (+1)	4.15 (+1)	60 (+1)	1 (-1)	1:5 (-1)	9 (+1)	0.67 ± 0.02
10	5 (-1)	4.15 (+1)	60 (+1)	12 (+1)	1:5 (-1)	1 (-1)	0.98 ± 0.05
11	85 (+1)	5 (-1)	60 (+1)	12 (+1)	1:5 (-1)	1(-1)	4.98 ± 0.25
12	5 (-1)	5 (-1)	5 (-1)	1 (-1)	1:5 (-1)	1(-1)	3.16 ± 0.16

*Values in parenthesis denote coded levels

Analysis of variance and regression study was scrutinized to screen the critical variables influencing the extraction efficiency of zeaxanthin. The values were considered significant, if $p < 0.05$ (Plackett and Burman, 1946). The PB experiment which follows the first order model was insufficient to evaluate the interactions between variables, hence the selected factors were further statistically optimized by CCRD of RSM.

Table 2. Response surface methodology for zeaxanthin extraction from *T. thiebautii* (NIOT 152)

S. No.	Factor 1 Solid-liquid ratio (w/v) (g/ml)	Factor 2 Sonication time (min)	Factor 3 Hemicellulase concentration (%)	Zeaxanthin content (mg g^{-1})*	
				Predicted value	Actual value
1	1:65	45	65	4.30	4.73 ± 0.2
2	1:45	30	45	2.98	2.38 ± 0.32
3	1:25	15	25	1.67	1.70 ± 0.19
4	1:45	30	45	2.98	2.63 ± 0.78
5	1:45	30	11.36	3.39	3.42 ± 0.34
6	1:65	15	65	3.21	2.68 ± 0.11
7	1:78.64	30	45	4.69	4.98 ± 0.18
8	1:45	4.77	45	2.07	2.21 ± 0.56
9	1:65	45	25	4.78	5.22 ± 1.4
10	1:25	45	25	2.76	2.61 ± 0.9
11	1:25	45	65	2.27	2.43 ± 0.87
12	1:45	30	45	2.98	3.24 ± 0.67
13	1:45	30	45	2.98	2.80 ± 0.34
14	1:45	55.22	45	3.90	3.27 ± 0.99
15	1:11.36	30	45	1.28	1.25 ± 0.45
16	1:45	30	45	2.98	4.28 ± 0.27
17	1:25	15	25	1.18	1.64 ± 0.77
18	1:45	30	78.64	2.58	2.26 ± 0.43
19	1:45	30	45	2.98	2.63 ± 0.8
20	1:65	15	25	3.69	3.31 ± 1.23

Response surface methodology

The results of PB methodology highlighted three critical parameters, namely hemicellulase concentration, sample solvent ratio and sonication time, which affect the rate of efficiency of zeaxanthin extraction. Hence, these three significant variables were further optimized using a full factorial-central composite rotatable experimental design (FF-CCRD) constructed by Design Expert Stat ease software (version 9.0). The three factor five level RSM design ($-\alpha$, -1, 0, +1, $+\alpha$) along with respective coded values containing 20 experimental runs were displayed in Table 2. The CCRD design, which follows the second order model constituted 2 axial points, 6 center points and 12 random points and each experimental run was executed in triplicates in darkness with other extraction conditions being unchanged (Kalil et al., 2000). The three independent variables were solid-liquid ratio (X1), ultrasonication time (X2) and enzyme concentration (X3). Analysis of variance (ANOVA), Fischer's F test and multiple regression study were performed based on the observed data of RSM experimental trials (Anderson and Whitcomb, 2005) and the results were displayed in Table 5.

The second order quadratic polynomial coefficients were scrutinized to derive the following polynomial equation:

$$Y= \beta_0+ \beta_1A +\beta_2B +\beta_3C + \beta_1\beta_1A^2 + \beta_2\beta_2B^2 + \beta_3\beta_3C^2 + \beta_1\beta_2AB + \beta_1\beta_3AC + \beta_2\beta_3BC$$

Where, Y is the zeaxanthin content in mg g^{-1} (response factor); factors A, B, and C belong to independent variables; whereas β_0 symbolizes intercept, β_1, β_2, and β_3 denotes linear coefficients; while β_1 β_2, β_1 β_3, and β_2 β_3 were the interaction coefficients. Similarly quadratic coefficients were represented by $\beta_1\beta_1$, $\beta_2\beta_2$, and $\beta_3\beta_3$ (Chua et al., 2009)

Statistical analysis

All optimization experiments were repeated thrice unless otherwise specified and the mean value ± S.D was recorded to eradicate any discrepancies in final test report of data analysis. The observed values were considered significant if probability (*p*) value is less than 0.5.

Results

Single factor experiments for cell disruption methods

Different types of cell wall disruption methods inclusive of ultra-sonication, liquid nitrogen, maceration and glass beads were investigated to ascertain their efficacy in promoting zeaxanthin extraction. Liquid nitrogen treatment resulted in freeze fracture and mechanical grinding also improved the zeaxanthin extraction when compared to without cell disruption (Table 3) thus signifying the importance of cell disruption for zeaxanthin extraction. The results (Table 3) showed ultrasonication for a time interval of 60 min resulted in better zeaxanthin extraction yield (1.294 mg g^{-1}) than the group without any cell disruption (control).

Single factor experiments were done to scrutinize the impact of enzymatic cell wall disruption using numerous cellulolytic enzymes like cellulase, proteinase, hemicellulase and pectinase at various combinations and concentrations on the zeaxanthin extraction yield from *T. thiebautii* (Dey and Rathod, 2013). The use of 25% hemicellulase (CDW/v) augmented the zeaxanthin extraction efficiency upto 1.754 mg g^{-1} as evident from Table 4. Hence enzyme assisted ultrasonication (EAU) method was chosen for further optimization using advanced statistical technique.

Table 3. Results of single factor experiments for cell disruption methods

S. No.	Cell disruption methods	Zeaxanthin content (mg g^{-1})
1	Control (no disruption)	0.698 ± 0.17
2	Mechanical (grinding)	1.19 ± 0.29
3	Glass beads	0.716 ± 0.3
4	Liquid nitrogen	1.16 ± 0.32
5	Liquid nitrogen + grinding	0.62 ± 0.07
6	Liquid nitrogen + sonication 20 min	1.115 ± 0.3
7	Sonication 5 min	0.88 ± 0.16
8	Sonication 15 min	1.034 ± 0.04
9	Sonication 30 min	1.128 ± 0.15
10	Sonication 60 min	1.294 ± 0.16

Figure 1. Pareto chart for PB

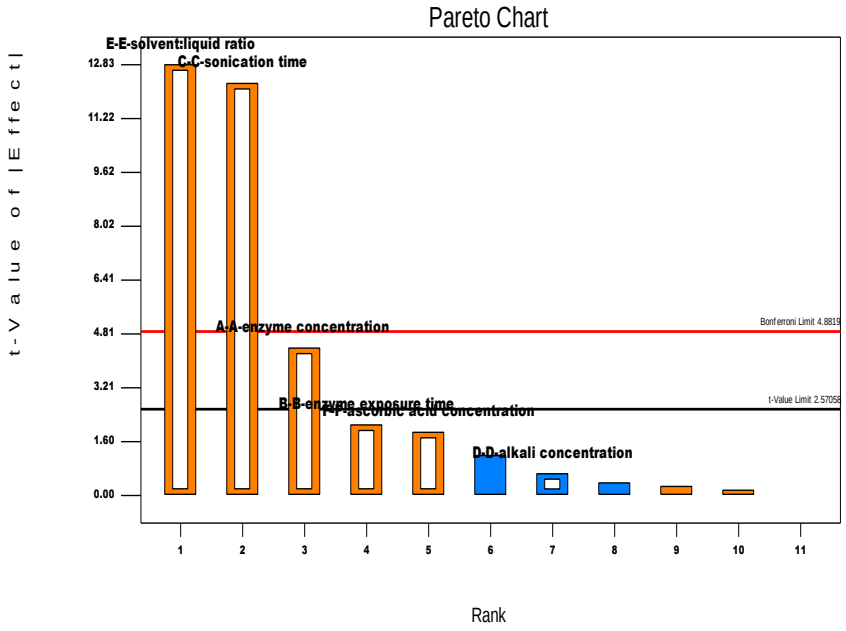


Table 4. Changes in zeaxanthin content under enzyme assisted extraction

S.No.	Enzyme concentration	Zeaxanthin content (mg g^{-1})
1	Control (no enzyme)	0.703 ± 0.32
2	Cellulase 25%	1.116 ± 0.15
3	Hemicellulase 25 %	1.754 ± 0.62
4	Pectinase 25%	0.742 ± 0.03
5	Proteinase 25 %	1.556 ± 0.19
6	Hemicellulase 50%	1.05 ± 0.03
7	Pectinase 50%	0.804 ± 0.16
8	Proteinase 50%	1.038 ± 0.04
9	Hemicellulase 75%	1.025 ± 0.13
10	Pectinase 75%	0.852 ± 0.11
11	Proteinase 75%	0.924 ± 0.01
12	Hemicellulase 100%	0.92 ± 0.05
13	Pectinase 100%	0.998 ± 0.06
14	Proteinase 100%	0.88 ± 0.22
15	Cellulase 25% + pectinase 25 %	1.674 ± 0.25
16	Hemicellulase 50% + pectinase 50 %	1.132 ± 0.16
17	Hemicellulase 100% + pectinase 100 %	1.12 ± 0.25
18	Hemicellulase 50% + proteinase 50 %	0.832 ±0.04
19	Hemicellulase 100% + proteinase 100%	0.934 ± 0.03

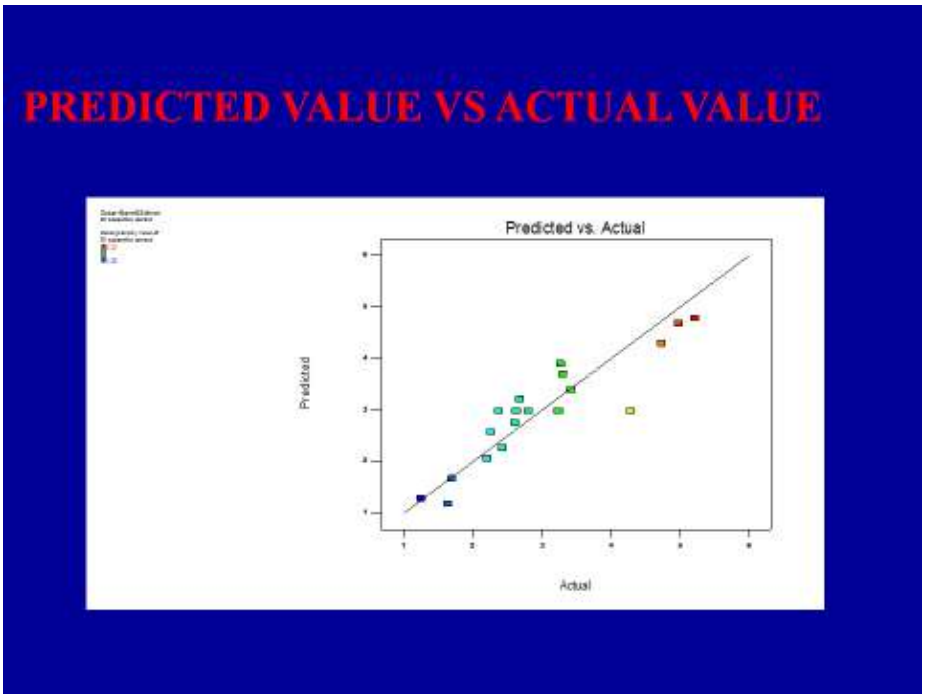
Plackett Burman experiment

In PB method, among six independent factors, three variables namely, hemicellulase concentration, ultrasonication time and sample-solvent ratio were screened as the most crucial parameters affecting zeaxanthin content (response value). In PB experiment, zeaxanthin content varied between 0.67 mg g^{-1} (run 9) and 5.23 mg g^{-1} (run 3), which signifies the effect of statistical optimization. The Pareto chart for PB design was depicted in Figure 1 and the graph for normal probability distribution plot was presented in Figure 2. As noticed from Pareto chart, three screened variables established a positive effect on zeaxanthin extraction as indicated in orange color, while those which were represented in blue color showed a negative effect (Figure 1).

PB results clearly inferred the importance of solid liquid ratio for zeaxanthin extraction. Correspondingly, this factor contributed the highest percentage contribution on zeaxanthin content. Sonication time and hemicellulase concentration were the other two factors which contributed to the zeaxanthin extraction yield (Figure 1). The close proximity

of the predicted and actual values depicts the significance and reliability of the established model (Figure 2).

Figure 2. Probability plot for normal distribution



Optimization of enzyme assisted ultrasonication extraction (EAUE) of zeaxanthin from T. thiebautii* (NIOT 152) *using Response surface methodology

The effects of crucial process parameters of enzyme assisted ultrasonication extraction identified using PB viz. solid-liquid ratio (X1 : A), sonication time (X2 : B) and enzyme concentration (X3 : C) on the zeaxanthin content (yield mg/g) was scrutinized using RSM. These three variables were studied at five levels with 20 experimental runs and construed using Design Expert software (version 11.06, State Ease. Inc. Minneapolis, MN, USA). The zeaxanthin extraction yield obtained at the different design points is depicted in Figure 3. The data obtained was fitted using multivariate regression analysis to the quadratic model (Rao et al., 2000). The analysis of variance (ANOVA) and the regression coefficient for the developed model was calculated and displayed in Table 5. The ANOVA of the data exhibited a high *F* value (59.08) and a very low *p* -value (< 0.0001) which inferred that the predicted model is statistically significant and adequate for predicting the effect of different factors on zeaxanthin content. The lack of fit with a low *F* value of 1.57 and *p* value of 0.3136 indicates that lack of fit is not significant relative to the pure error. For a model to fit, the lack of fit should be non-significant (Dilip Singh et al., 2015). The coefficient of determination (R^2) was 0.9850 and the pre-determined R^2 of 0.8767 was in reasonable agreement with the adjusted R^2 (0.9683) (Table 5) and the difference is only 0.2, which implied that the optimized model is adequate to predict response value. Accordingly there was a high correlation between the observed and predicted data. All the three quadratic variables were significant indicating that they have a negative impact on zeaxanthin extraction yield when their values are high.

Effect of EAUE parameters on zeaxanthin content and analysis of response surfaces

The relationship between the zeaxanthin content and experimental conditions of the independent variables for EAUE of zeaxanthin extraction was visualized using three dimensional surface plots, according to the following quadratic polynomial model equation for coded factors:

$$R1\ \text{zeaxanthin content} = +4.508+1.049A+0.576B-0.219C+0.345AB-0.16AC+0.042BC-0.48A^2-0.506B^2-0.577C^2-0.017ABC$$

Where, A= sample solvent ratio, B = sonication time, C = enzyme concentration. The final equation in terms of actual factors was used to determine predictions about the response value for given levels of each variable.

Maximum zeaxanthin content (5.22 mg g^{-1}) was observed in run 9 (Table 2, Figure 4) with extraction conditions of 25% hemicellulase concentration, solid-liquid ratio of 1:65 and sonication time of 45 min. Whereas, very low zeaxanthin yield (1.25 mg g^{-1}) was witnessed in run 15 with extraction parameters being, 45% hemicellulase, solid liquid ratio of 1:11.36, sonication for 30 min (Table 2, Figure 4). The results implied that very low volume of solvent showed a lower zeaxanthin content as in run 15. This low yield might be attributed to the insufficient solvent concentration for complete extraction of zeaxanthin. Accordingly, the linear parameter, solid –liquid ratio (A) had a significant impact with high *F* value and low (*P*-value) (Table 5). Similarly, zeaxanthin content increased with sonication time reached maximum and then started to decline. Correspondingly, sonication time also depicted a significant impact on zeaxanthin yield with a high *F* value and low *P* value (Table 5). Nevertheless, enzyme concentration did not show a significant impact. All quadratic parameters had a significant impact on the zeaxanthin extraction yield which implied at high level these parameters will have negative impact on the zeaxnthin extraction yield.

Table 5. ANOVA for reduced cubic model (Type III – Partial sum of squares)

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	
Model	31.30	10	3.13	59.08	< 0.0001	significant
A-X1:S/L ratio	15.04	1	15.04	283.92	< 0.0001	
B-X2:Sonication time	4.54	1	4.54	85.64	< 0.0001	
C-X3-enzyme concentration	0.6550	1	0.6550	12.36	0.0066	
AB	0.9522	1	0.9522	17.97	0.0022	
AC	0.2048	1	0.2048	3.87	0.0809	
BC	0.0145	1	0.0145	0.2727	0.6141	
A^2	3.33	1	3.33	62.76	< 0.0001	
B^2	3.70	1	3.70	69.88	< 0.0001	
C^2	4.81	1	4.81	90.74	< 0.0001	
ABC	0.0025	1	0.0025	0.0462	0.8345	
Residual	0.4768	9	0.0530			
Lack of Fit	0.2652	4	0.0663	1.57	0.3136	not significant
Pure Error	0.2116	5	0.0423			
Cor Total	31.78	19				

R^2 = 0.9850, Adjusted R^2= 0.9683, Predicted R^2= 0.8767, C.V%= 6.69, Adeq precision= 22.7515, mean= 3.44, std. dev = 0.2302

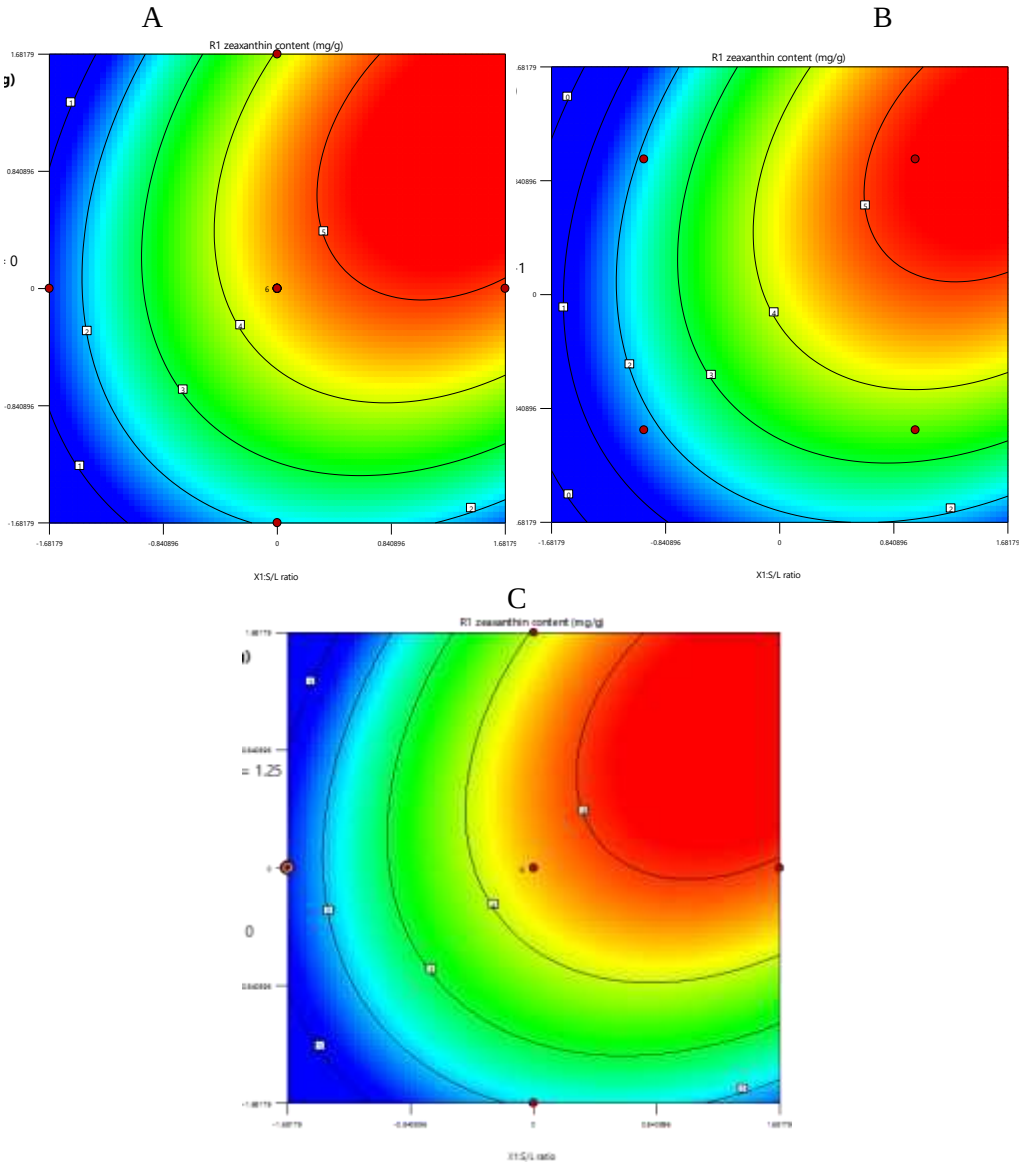


Figure 3. A: Contour plot with solid-liquid ratio and sonication time along two axes and enzyme concentration at zero level showing design points. B: Contour plot for RSM trial 9 (SL ratio: +1 level, sonication time : + 1 level, enzyme concentration: -1 level) and C: Contour plot for RSM trial 15 (SL ratio: -1.68 level, sonication time: 0 level, enzyme concentration: 0 level).

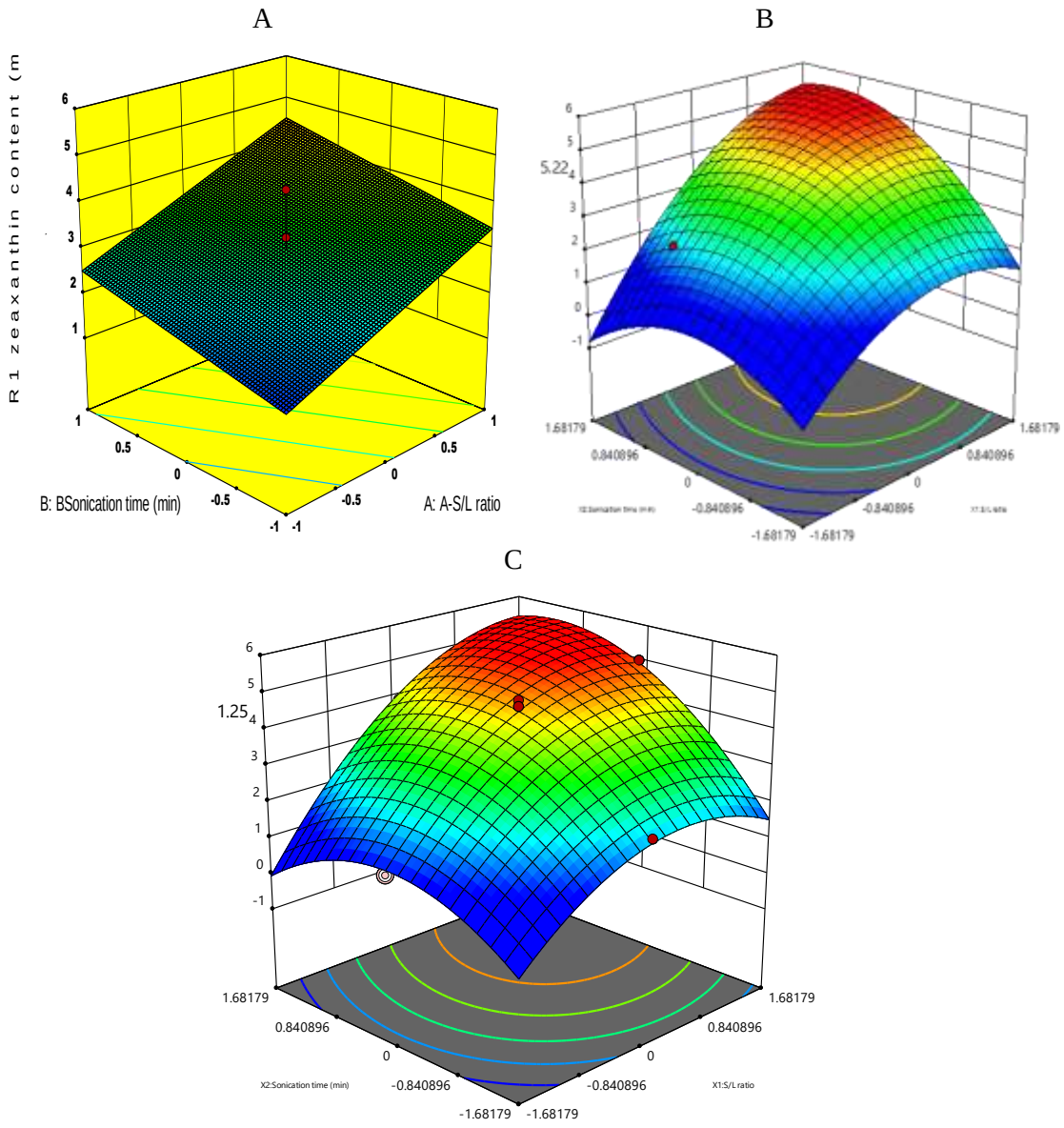


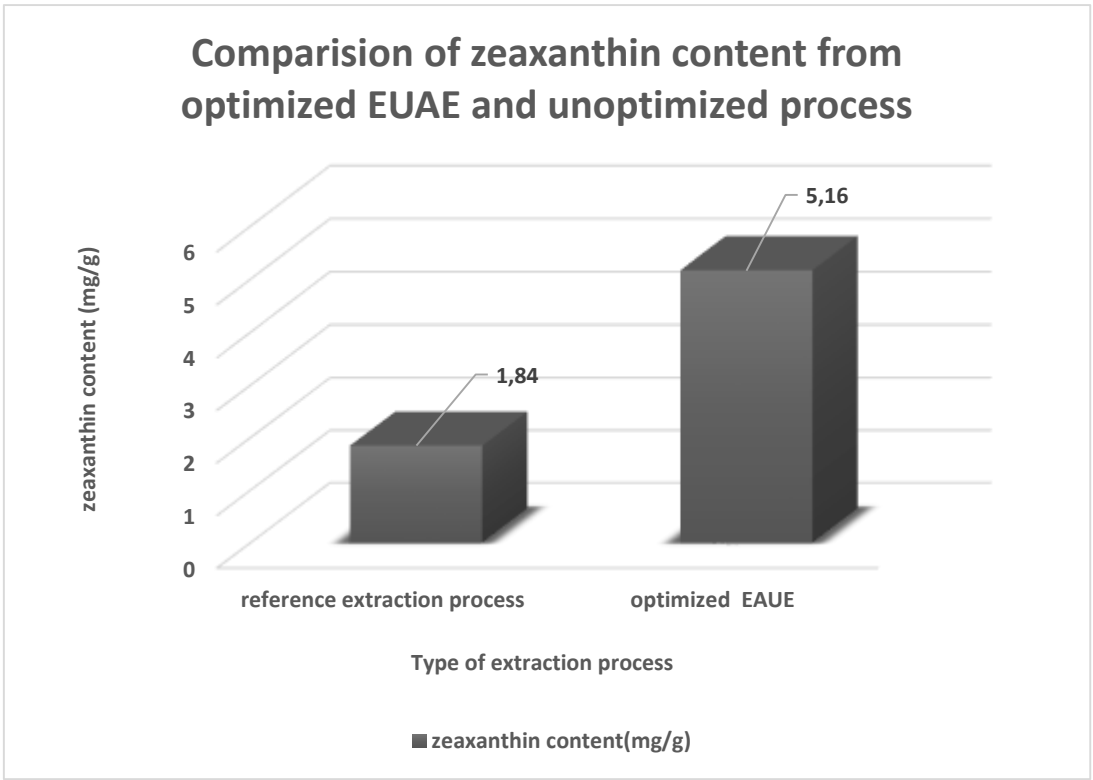
Figure 4. A: Three dimensional surface plot with S/L ratio and sonication time along two axes and enzyme concentration at zero level. B: RSM trial 9 (SL ratio: +1 level, sonication time : +1 level, enzyme concentration: -1 level) and C: RSM trial 15 (SL ratio: -1.68 level, sonication time : 0 level, enzyme concentration: 0 level)

Graphical illustration of quadratic polynomial equation using three-dimensional (3D) response surface plots and contour plots depicted the interaction between significant variables and helped in the determination of optimum values of the process parameters for achieving maximal zeaxanthin extraction yield (Jhample et al., 2015). Figure 3 illustrates the elliptical order of contour plots, which indicates a significant interaction between sonication time and solid liquid ratio. Corroborating to this, the ANOVA of interaction parameter (AB: Solid-liquid ratio x Sonication time) displayed significant impact on zeaxanthin yield. Likewise, the interaction of solid-liquid ratio and enzyme concentration also displayed a significant impact on zeaxanthin content. As visualized from the contour plot, the points lie within the navigated design space which inferred the significance and precision of the model (Bhagwat et al., 2015). Based on the multivariate regression fitting equation the optimized EAUE parameters for zeaxanthin extraction from *Trichodesmium thiebautii* were solid (biomass):

liquid (solvent) ratio of 1:63 (g/ml), sonication time (44.57 min), enzyme concentration (32.66%) with a predicted zeaxanthin content of 5.57 mg g^{-1} (Figures 3 and 4). The results were validated using confirmatory experiments in large scale. The extracted crude zeaxanthin from blue green microalga *T. thiebautii* was depicted in Figure 5.

Figure 5. Extracted zeaxanthin from *T. thiebautii*

Figure 6. Comparison of zeaxanthin extraction using conventional solvent extraction and optimized enzyme enabled ultrasonication extraction



Validation of experiment through large scale extraction and comparison of response values with conventional solvent extraction method

In order to authenticate the appropriateness and reliability of experimental design, confirmatory experiments were performed based on predicted optimized conditions under large scale in triplicates. The optimized EAUE parameters were as follows: sample-solvent ratio of 1:63, sonication time of 44.57 min and enzyme (hemicellulase) concentration of 32.66% and a predicted zeaxanthin content of 5.57 mg g^{-1}. The optimized conditions were experimented in triplicate experiments which augmented the maximum zeaxanthin content to 5.16 ± 0.34 mg g^{-1}, which was 2.8 fold higher than unoptimized control (1.84 ± 0.12 mg g^{-1}) (Figure 6). The obtained experimental value of zeaxanthin content (response) was very close to the predicted response by the regression model, thus substantiating the accuracy and validity of the experiment. Hence, this study established that two step statistical optimization using PB and RSM could be used for efficient optimization of .the process parameters of EAUE of zeaxanthin from *T. thiebautii* (NIOT 152).

Discussion

In the present investigation, the enzyme assisted extraction conditions of zeaxanthin from *T. thiebautii* were optimized using statistical techniques like PB followed by RSM. A comparison of various cell disruption processes for the extraction of zeaxanthin from *T. thiebautii* revealed enzyme assisted ultrasonication was superior to other methods to augment zeaxanthin recovery. Selection of a suitable cell disruption process for the Cyanophycean algae *Trichodesmium,* which has a multilayered peptidoglycan cell wall is vital as it forms the first and foremost step for the extraction of inter cellular metabolites (Andreson et al., 2010). Interestingly, like other diazotrophs, they lack a rigid cell wall which makes the cell disruption process relatively easier when compared to other eukaryotic algae (Kupper et al., 2009). They also contain more proteins, especially glycoproteins and carbohydrates in their cell wall which makes them more susceptible to enzymes (Arthy et al., 2018). The initial step in zeaxanthin extraction is penetration of solvent into the cell wall and recovering the cytoplasmic products like carotenoids. Hence, techniques which can accomplish successful degradation of cell wall facilitate better recovery of intracellular biomolecules like carotenoids (Fleurence 1999). Cooroborating to the above fact major biomolecules of Cyanophycean algae inclusive of zeaxanthin are localized intracellularly and augmented by destructive pretreatment of cellular and subcellular structures (Beveridge 1999). Accordingly, enzyme assisted method gave a better zeaxanthin yield when compared to other cell disruption procedures. Among various enzymes tested, hemicellulase (25% w/v) showed maximum extraction (1.754 mg g^{-1}) when compared to other enzymes, while pectinase gave a poor extraction. Conversely, Wang et al., (2017) have reported higher carotenoid extraction from *Cordyceps militaris* using 0.43% (1:1 w/w) of cellulase and pectinase. Similarly, Strati et al. (2015) have also reported the use of cellulase (70 U/g) and pectinase (122.5 U/g) for achieving a 6-fold and 10-fold higher yield of total carotenoid and lycopene, respectively, from tomato waste. Enzyme concentrations higher than 25% did not improve the zeaxanthin extraction yield. Likewise, the zeaxanthin extraction yield achieved with different combination of enzymes was slightly lower than that obtained with hemicellulase 25% (w/v) (Table 4). These difference in the enzyme effectiveness might be attributed to the variation in the cell wall composition of plant and Cyanophycean cell wall membranes (Anderson et al. 2010). Thus enzyme assisted extraction offered an energy saving process for disrupting *Trichodesmium* cell wall. Among the mechanical methods of disruption ultrasonication for 60 min (1.294 mg g^{-1}) gave a better zeaxanthin yield when compared to other mechanical methods. In agreement with the present study, Singh et al. (2015) have also reported ultrasonication favored cell disruption and improved the astaxanthin yield 6 fold higher in *Thraustochytrium* sp. Furthermore, Araujo et al. (2013) have also reported higher extraction

yield of oils from microalgae *C. vulgaris* (52.5% w/w) using ultrasonication assisted cell disruption. Furthermore ultrasonication has also been reported to facilitate the penetration of the enzymes into the cellwall rapidly and extraction of intracellular biomolecules (Dey and Rathod, 2013). Hence, the study presented herein aimed at the use of enzyme assisted ultrasonication for the extraction of zeaxanthin from *T. thiebautii.*

The three crucial factors (solvent sample ratio, sonication time and enzyme concentration) examined by PB, were deeply analyzed using RSM (three factor, five level) consisting of 20 random experiments. Run 9 of RSM with a solid liquid ratio of 1:65, sonication time of 45 min with an enzyme concentration (hemicellulase) of 25% depicted maximum zeaxanthin content (5.22 mg g^{-1}). Whereas run 15 (45% hemicellulase, solid liquid ratio of 1:11.36, sonication for 30 min) showed a lower response (1.25 mg g^{-1}). Poor zeaxanthin content obtained in run 15 with lower biomass concentration could be attributed to insufficiency of solvent (1:11.36) for diffusion of carotenoid pigment zeaxanthin from microalgal biomass substrate into the solvent. This highlights the significance of optimal sample solvent ratio as foremost significant factor, which determines the rate of extraction efficiency of biomolecules like zeaxanthin. Accordingly, ANOVA of RSM showed a high model *F* value (59.08) and a low probability value (0.0001). The present findings were in agreement with the reports of Yan et al. (2015). They have also reported a higher carotenoid yield of 79.61% with a liquid to material ratio 41.4 ml/g and a sonication time of 48.5 min from rapeseed (*Brassica napus* L). Similarly, the optimized ultrasonication time of 45 min for obtaining high zeaxanthin yield was in agreement to the extraction time of 45 min documented by Wang et al. (2019) for achieving maximum extraction of zeaxanthin and lutein from corn gluten meal. The large scale extraction process under the optimized conditions yielded a 2.8 fold higher extraction of zeaxanthin (5.16 mg g^{-1}) from *T. thiebautii* using enzyme assisted ultrasonication method. Comparison of the zeaxanthin obtained in the optimal operation conditions evidenced that higher zeaxanthin could be obtained through cell wall degradation by enzyme assisted ultrasonication than the control without any enzyme or ultrasonication treatment.

Conclusions

The optimum conditions for the maximum extraction of zeaxanthin was determined by response surface analysis. The following process parameters of enzyme assisted ultrasonication extraction of zeaxanthin : biomass solvent ratio of 1:63, ultrasonication time of 44.57 min and enzyme (hemicellulase) concentration of 32.66% gave maximum zeaxanthin extraction (5.16 mg g^{-1}). The zeaxanthin content obtained at optimized conditions was 2.8 fold higher than the unoptimized control conditions (1.84 ± 0.12 mg g^{-1}). Thus, the study inferred that enzyme assisted ultrasonication method as a prospective method for extracting highly valuable nutraceutical zeaxanthin from Cyanophycean microalga *T. thiebautii* (NIOT 152).

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PRELIMINARY STUDY ON THE ECONOMIC ACTIVITY OF SHEEP CASES OF THE REGION OF TIARET

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ABSTRACT

The sheep meat chain in Algeria is undergoing a series of changes: price differentiation, according to the type of market and demand, structuring of supply according to events (period of high consumption: Aid El-kebir and the month of Ramadhan). Livestock markets are very popular in Algeria. The methodology adopted is based on a weekly monitoring of 2 of the most important livestock markets of a large sheep producing region, which are Sougueur and Hamadia, during the campaign (2018-2019). The choice of these markets was justified by the fact that they are national in scope in terms of transit and marketing of sheep. The data collected related to the following aspects: prices of the various categories of sheep; feed prices; and economic operators of livestock markets. In these markets, transactions are made publicly. In fact, there is a juxtaposition of short and long circuits and sheep meat follows three quite distinct circuits. The marketing system for livestock products and feedstuffs is characterized by the existence of economic agents who are breeders, horse traders, butchers and traders. These operators are in permanent contact with each other. Information on prices and quantities is perfectly accessible at all times and to all operators. No operator by these décisions can alone make the market evolve in a significant way. The objective through this article is to show the sheep chain in the Algerian economy.

Key words : Market price, actor, competition, merchandise, religious festival.

INTRODUCTION

In Algeria, sheep farming plays a major role in the development of agriculture (Sadoud, 2008). It represents the tradition in terms of breeding and constitutes the sole income of a third of the Algerian population (Chellig, 1992). It continues to be the main source of income for the populations of the steppe regions, supporting a sheep herd of nearly 15 million head and a population of nearly 08 million. The majority of the livestock grazing in steppe areas are sheep (about 80% of the national flock) (Kanoun, 2007). The wilaya of Tiaret, considered as a steppe region, occupies the third place in terms of sheep numbers with 4.55% of the national herd after the regions of Djelfa and El Baid (Zoubeidi, 2011).

MATERIAL AND METHODS

The region of Tiaret conceals natural and human characteristics allowing it to be an agricultural pole practicing the breeding of this species having the particularity of being an economic and agricultural source in the production of red meats of high nutritional quality. Despite the richness of this region in terms of the number of sheep (2.1 million heads) and the number of sheep breeders (44,000 breeders) (DSA, 2019), sheep production remains marginal and it is confronted with several constraints which consequently requires the establishment of a development framework

The sample consists of 57 sheep farmers in the Tiaret region, distributed according to the communes All the data are gathered in a spreadsheet-type file. In columns are represented the different farms and in rows the explanatory and explanatory variables represented by the different questions. The collected data were subjected to a statistical analysis using an XLstat evaluation version. This multi-varied statistical treatment proved to be necessary in order to differentiate, clarify and order the types of breeding in the face of the strong heterogeneity observed among existing sheep breeders.

RESULTS AND DISCUSSION

Our results show that the majority of our sample consists of sedentary breeders representing the 1st group, with a rate of 86% of the 57 breeders surveyed, with an average age of about 60 years. They are distributed in the communes of Tousnina, Rechaiga and Feidja, which are considered to be the main areas of concentration of this activity. The breeders have a level of education dominated by Kuttab and are illiterate with more than 40 years of experience in this field, which signifies the solidity of this profession for a long time (Tables 1 and 2). They are landless agropastoralists who practice nomadism . They rent land in fallow land or stubble with a load of 20 heads / hectare in order to feed and/or fatten and make transactions of purchases and sales to ensure their needs.

The second group is that of nomads who are originally from Djelfa, El-Oued, Ain Beida who are nomadic by moving in the family and with their herds, by renting land according to their needs, fallow or stubble at a rate of 20 heads / hectare.

Type of breeding	Nb	%
Sedentary	49	86,00%
Nomad	6	10,50%
Transhumant	2	3,50%
Total	57	100,00%

Table 1: Types of agro-pastoralists

Years of professional experience	Nb	%
Moins de 5 ans	3	5,30%
plus de 5 ans	1	1,80%
plus de 10 ans	19	33,30%
plus de 40 ans	34	59,60%
Total	57	100,00%

Table 2: Professional experience of agropastoralists

CONCLUSION

Sheep are a very important animal resource for the majority of breeders and are well integrated into the agro-pastoral production system of the region. The breeding in its quasi-totality is based on the activity of fattening. Indeed, breeders are integrated towards the market and supply the local, regional and even national market with this product.

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PLANT HEALTH, QUARANTINE AND PEST MANAGEMENT IN TURKEY

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ABSTRACT

Turkey is among the top ten countries, which provide the largest amount of agricultural production in the world, and also ranked as the first within the EU. Legislative framework of the phytosanitary and quarantine are completely identical or harmonized with the EU legislation. Due to the high diversity of Turkey's vegetation patterns and agricultural potential, there is a high export trade with the EU, Russia, the Far East and many neighbouring countries. Agricultural exports of our country have very important economic value, and the statistical data shows advance level of the agricultural production meeting many of the recipient countries' terms, conditions and demands. Turkey has taken the advantage of growth in global agricultural exports in recent years and opportunities to continue search for new markets and it could enter the market of China, South Korea, Taiwan and Seychelles. By gaining good experience during COVID-19, Turkey both provided the food supply of its own people and met the food needs of many countries. Border controls are conducted by **467** inspectors trough **12** agricultural quarantine directorates and provincial directorates and inspection analysis have been made in advanced labs with rapid analysis techniques by specialized staffs. This success both depends on effective border control, and internal plant health measures and pest management strategies. Turkey has taken many innovative steps that can be counted revolution since the 2000s in the field of phytosanitary and quarantine. Also, changes in Turkish agriculture policies go hand in hand with implementations. Biological and biotechnical control practices have been supported by the government since 2010 and 50% of the producer's expenses have been covered. Thus, environmentally friendly control methods are promoted through subsides and producer's usages has enlarged. Total supported area was **529 ha** in 2010, whereas it has increased to **28,560 ha** in 2019. **Integrated Pest Management (IPM)** practices have been begun with a small number of producers and in limited areas in 2012, while it reached over an area of around **400,000 ha** and **50,000** producers until 2019. Through **the pre-harvest pesticide inspections**, plant samples are taken prior to the harvest while the crops are still in the field, orchard or greenhouses and **the post-harvest pesticide inspections** made by taking samples from the wholesalers, grocery stores and other sales points, based on the analyses result, inappropriate use of pesticides are not allowed by performing practices such as harvest delay, product destruction, fines in case of non-conformity. Consciousness level of Turkish producers are improved by practical and theoretical trainings, field schools, and different education activities or by demonstrations about plant health and quarantine activities. Turkey has banned **213** active ingredients covering thousands of pesticides since 2009 that are identified as harmful to the environment, non-target organisms and other living organisms or having toxicology and carcinogenicity findings. These practices have gained public appreciation and reliability on agricultural policies. Considering the amount of pesticides used per unit area in, Turkey uses less amount of pesticides than the EU and many other countries such as China, United States, Netherlands, France, Germany, Israel, Italy, Belgium, South Korea and so on. In order to

control and monitor chemicals, the **QR tracking system** has been implemented by the Ministry of Agriculture and Forestry (MoAF) since 2018, and all pesticide producers and suppliers have to use this QR system. Thus, all processes could be monitored from pesticide production or entrance point to the last users. Due to the COVID-19 pandemic extraordinary situation experienced in 2020 in all countries and it has shown specifically that food supply is more important and even more important than communication, transportation, defence industry and other electronic and technological uses. In addition, it seems that the virus will remain as a top agenda and the circumstances of the COVID-19 shows that even possibility of some new pandemics and other new diseases may arise. It means that sound crop production and food supply systems will be highly importance to the resilience. With this regard, it is a fact that phytosanitary and plant nutrition are integral and essential factors in crop production, and the success in the yield and quality are directly linked with the appropriate practices in terms of these factors. If phytosanitary is managed properly, the health of all living things will be protected. Phytosanitary also refers to human health. That is why 2020 was declared as the **International Year of Plant Health** by the United Nations, although it marks the year of COVID-19 pandemic.

Keywords: IPM, Biological and biotechnical control, subsides, phytosanitary, plant health year, COVID-19, Turkey.

INTRODUCTION

Plant health and quarantine studies are carried out according to law number 5996, which was enforced in 2010, and also including so many regulations under this law. Turkey is a modern agricultural country and a bridge between Europa and Asia continents. It has so many historical and touristic assets, and industrial, defence and technological developments, as well as, in agriculture. Turkey has a remarkable geopolitical location and is a peninsula surrounded by Black Sea, Mediterranean Sea, and Aegean Sea including the Marmara Sea. Therefore, Turkey has so many different climatic conditions from East to West and from North to South. While some places are snowing, it is possible to swim in the sea somewhere else. These differences also provide favourable conditions for producing many different crops, domestic fruits, or vegetables. Therefore, this large production capacity brings many duties to carry out properly in the field of plant health.

There are approximately 660 pest organisms (i.e. pests, diseases, and weeds) available in Turkey and 330 of those are identified as insects in Turkey. Some of them are main pests and listed in international quarantine list as well. Both for domestic needs and international trade, plant health should focus very carefully on pest management to control and sustain plant health and quarantine. In this article only some of good examples of plant health and quarantine studies summarized to give an idea about plant health, quarantine and pest management activities in Turkey.

Plant health, quarantine and pest management studies

Phytosanitary and plant nutrition are integral and essential factors in crop production, and the success in the yield and quality are directly linked with the appropriate practices of these factors.

In this article phytosanitary and quarantine activities, pest management and pesticide management emphasized with some good agricultural examples and statistical data as shown below.

Plant health and pest management activities

Plant health organization in Turkey has so many different applications and management systems such as, IPM, ICPM studies, biological and biotechnical control studies, pre-harvest and post-harvest pesticide inspections, pesticide management program and tracking system, education activities, border control, survey program and so on. Every year plant health program is declared by MoAF and General Directorate of Food and Control is in charge of conducting all activities since it is responsible for plant health activities as the competent authority. Based on the declared program, Phytosanitary activities are carried out by provincial directorates of MoAF across Turkey in 81 provinces, whereas Plant Protection Research Institutes are also another implementing partner for regional activities, identification of pest organisms, education programs, surveys and management programs, lab studies in close cooperation with provincial directorates. On the other hand, Agricultural Quarantine Directorates are responsible for import and export controls. Within this regard, plant health and pest management activities summarized as below.

Biological and biotechnical control activities:

Biological and biotechnical control practices have been supported by the government since 2010 and 50% of the producer's expenses have been covered. Thus, environmentally friendly control methods are promoted through subsides and producer's usages have been enlarged. Total supported area was 529 ha in 2010, whereas it has increased to 28,560 ha in 2019 (Table 1).

Table 1. Subsidies in biological and biotechnical control since 2010 in Turkey (Anonymous, 2020a)

Years	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019*
Area (ha)	529	1,632	6,605	13,350	17,353	16,745	20,859	25,945	31,993	28,560
Amount (Million ₺)	0,5	0,6	3	3,5	9,5	9,2	10,9	13,3	23,1	20,8

*Subsides still continue

Parasitoids are produced by research institutes against main pests such as Sunnpest and within biological control practices they are released in some areas, where Sunnpest is widespread (Figure 1).

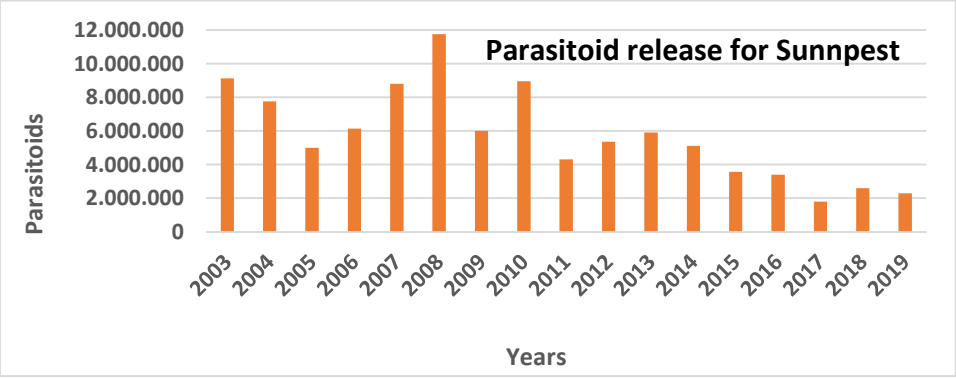

Figure 1. Parasitoid release against Sunnpest since 2003 (Anonymous, 2020a)

Survey teams are established in potentially affected provinces to monitor pest population in an area of approximately 4 million hectares and to warn the producers on time for the pest control. Thanks to these successful implementations, pest is under control by keeping the damage rate on wheat production under 1%, and there is no any yield or quality loss in grain production (Table 2).

Table 2. Sunnpest control studies since 2012 (Anonymous, 2020a)

Years	Survey Area		Pesticide Applied Area		Damage rate
	Provinces	Area (ha)	Provinces	Area (ha)	
2012	53	4,125,000	25	1,035,504	Below %1
2013	50	4,090,349	21	902,557	Below %1
2014	49	4,335,600	25	646,021	Below %1
2015	55	3,922,008	22	410,572	Below %1
2016	59	4,358,378	22	577,934	Below %1
2017	59	3,780,000	24	713,878	Below %1
2018	61	4,100,000	28	885,501	Below %1
2019	62	4,270,000	30	971,967	Below %1

Subsides for biological and biotechnical control of Mediterranean fruit fly (*Ceratitis capitata*), which is an important polyphagous pest, have been increased, and the use of those control methods have been expanded for massive control and monitoring purposes. Thus, the damage of the pest has been decreased by employing more environmentally friendly alternatives, which fosters the access of safe food, while residue and resistance problems have been decreased by using those alternative control methods.

IPM activities:

Projects on **IPM** practices have been implemented since 2012 and expanded to 35 provinces in 2015 and to 50 provinces in 2019. IPM project was carried out with a small number of producers and in small areas in 2012, whereas 50,000 producers have been involved into IPM implementations and it reached over an area of around 400,000 ha in 2019 (Figure 2). The aim of these projects is to increase the awareness of the producers about appropriate control methods by providing knowledge and supporting producers for applying these methods.

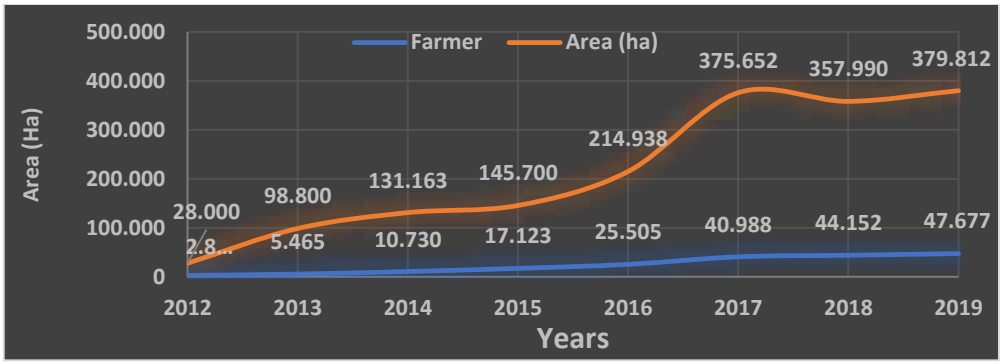

Figure 2. Change in IPM applications since 2012 in Turkey (Anonymous, 2020a)

Integrated and Controlled Product Management (ICPM) Activities:

This project has been carried out since 2015 for establishing a sound system about IPM, by following every step of production including analysis of harvested production. By this activity,

the awareness of producers has been improved about pests, diseases and weeds, including plant nutrition, pesticide use and food security. According to residue analysis of harvested products, if there is no problem, then a flag marking reliability and safety in the production is given to producers by provincial directorates. This flag means that these products have been analysed and controlled officially by the competent authority (Table 3).

Table 3. ICPM application in Turkey (Anonymous, 2020a)

Years	Provinces	Producers	Applied Area (ha)
2015	**35**	**7,172**	**21,128**
2016	**36**	**7,427**	**19,941**
2017	**43**	**6,959**	**20,355**
2018	**50**	**6,492**	**18,765**
2019	**50**	**6,024**	**18,521**

Education activities:

Consciousness and awareness level of Turkish producers are improved by practical and theoretical trainings, field schools, and different education activities or demonstrations about plant health and quarantine activities. Especially, education programs are carried out on highly important main pests or issues such as Sunnpest, *Ceratitis capitate* (Medfly), *Halyomorpha halys, Tuta absoluta,* pre-harvest and post-harvest applications, IPM, ICP or survey activities, biological and biotechnical control methods, or demonstrations. Education programs have been implemented by specialists from different research institutes and technical staff at provincial level. Many technical materials, handbooks, or other informative materials have been distributed to producers for better understanding of pest management (Table 4, 5, 6 and 7). Therefore, Turkish producers' awareness level on phytosanitary and quarantine are quite high, and implementation of environmentally friendly methods, IPM, ICPM, biological and biotechnical applications are getting common practices gradually. Some education activities about pesticide control in pre-harvest (Table 4), Sunnpest (Table 5), *Ceratitis capitata* (Table 6) and *Halyomorpha halys* (Table 7), are shown as below.

Table 4. Number of trainers and trainees involved in Pre-harvest pesticide control trainings

Years	Technical Staffs (Trainers)	Producers (Trainees)
2016	**700**	**13,000**
2017	**700**	**14,000**
2018	**700**	**15,000**
2019	**700**	**16,000**

Table 5. Number of technical staff and producers involved in trainings on Sunnpest activities

Technical Staff					Producers			
Year	**2016**	**2017**	**2018**	**2019**	**2016**	**2017**	**2018**	**2019**
Number	2,000	2,000	2,100	2,200	50,840	57,810	57,777	53,662

Table 6. Number of technical staff and producers involved in trainings on *Ceratitis capitata* and distributed materials for control

Technical Staffs					Producers				Distributed Materials
Year	**2016**	**2017**	**2018**	**2019**	**2016**	**2017**	**2018**	**2019**	**2016-2019**
Number	**344**	**346**	**521**	**523**	**986**	**1,453**	**37,359**	**26,429**	**209-942**

Table 7. Number of technical staff and producers involved in trainings on *Halyomorpha halys* and distributed materials for control

Technical Staffs			Producers		Distributed Materials
Year	**2018**	**2019**	**2018**	**2019**	**2019**
Number	**72**	**82**	**-**	**893**	**16,158**

Chemical control activities:

Turkey has banned **213** active ingredients covering thousands of pesticides since 2009 that are identified as harmful to the environment, non-target organisms and other living organisms or having toxicology and carcinogenicity findings. In addition, considering the domestic need, the use of **14** active ingredients has been restricted. It is allowed to use these restricted pesticides only in some products with extraordinary necessity. These practices have gained public appreciation and have increased reliability in agricultural policies.

Through **the pre-harvest pesticide inspections**, plant samples are taken prior to the harvest while the crops are still in the field, orchard or greenhouses and based on the analyses results, inappropriate use of pesticides are not allowed by performing practices such as harvest delay, product destruction or fines (Figure 3).

By **the post-harvest pesticide inspections**, samples are taken from the wholesalers, grocery stores and other sales points, and possible sanctions could be imposed such as confiscation of products, product destruction and fines in case of non-conformity.

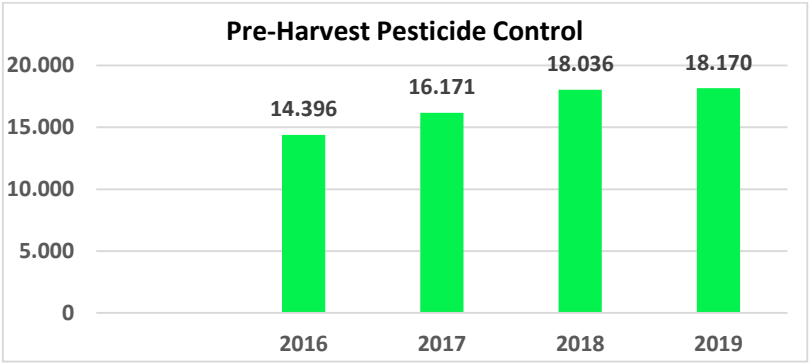

Figure 3. Pre-harvest pesticide Controls (2016-2019) (Anonymous, 2020a)

Comparing the amount of pesticides used per unit area, Turkey uses less amount of pesticides than the EU and many other countries (such as China, United States, Netherlands, France, Germany, Israel, Italy, Belgium, South Korea, and so on) (Figure 4). While some countries have more than 5 folds' pesticide usage such as China, Israel, South Korea, Malta and Japan, the other countries also use much more pesticide than Turkey such as Netherlands, Belgium, Ireland, Italy, Portugal, Slovenia, Germany, France, Spain, Austria, England, Greece and America (Figure 4 and Table 8).

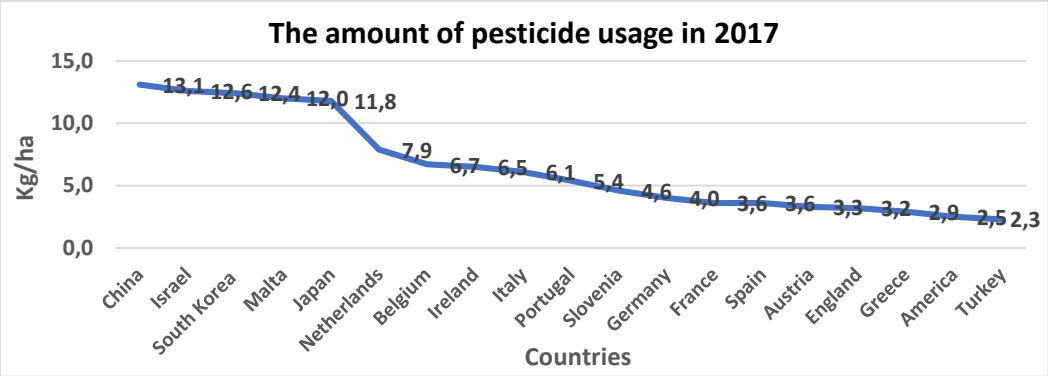

Figure 4. The amount of pesticide usage in 2017 (Kg/ha) (Anonymous, 2020b)

Table 8. Top ten pesticide consuming countries in the World (Anonymous, 2020c)

Rank	Country	Annual Pesticide Consumption (millions of kg)
1	China	1,806
2	United States	386
3	Argentina	265
4	Thailand	87
5	Brazil	76
6	Italy	63
7	France	62
8	Canada	54
9	Japan	52
10	India	40

MoAF still tries to take stricter policies to decrease use of chemical pesticides through effective control activities, and to increase use of environmentally friendly alternative control methods through subsides.

In order to control and monitor chemicals, the "**Plant Protection Products QR Tracking Computer System"** has been implemented by the MoAF since 2018 and all pesticide producers and suppliers have to use this QR system. Through this system all processes could be monitored from pesticide production or entrance point to the last users. Similar QR systems are applied on a voluntary basis and partially in some developed countries, while it is a compulsory system and should be used by all stakeholders in Turkey. This is one of the best practices showing concrete actions in Turkish agricultural policies against chemicals. Meanwhile, by this QR system all stakeholders from producers to consumers could be monitored under unique program. In addition, pesticide formulation plants, storages, wholesalers and retailers are inspected periodically to prevent non-recommended pesticides sale and use.

Border control and quarantine activities

Turkey has very important economic value in terms of amount of agricultural production. Due to the high diversity of Turkey's vegetation patterns and production capacity, there is a high export trade with the EU, Russia, the Far East and many neighbouring countries. The statistical data shows advance level of agricultural production meeting many of the recipient countries' terms and conditions, and demands.

In Turkey, border controls are conducted by 12 agricultural quarantine directorates and provincial directorates of MoAF (Figure 5). Plant health certificates are issued by 467 inspectors. Moreover, Turkey has advanced laboratory infrastructure, rapid analysis techniques and specialists in provinces where the level of import and export are high.

Figure 5. Location of agricultural quarantine directorates

The number of imported and exported items and issued certificate were given on Table 9. The number of export controls of fresh fruits, vegetables and other plant products are much more than import controls as shown on Table 9.

Table 9. Phytosanitary export and import controls (Anonymous, 2020a)

Years	Export Control	Import Control
2017	295.203	61.217
2018	335.960	59.716
2019	313.095	73.268

There are so many reasons for rejection of any products to enter any country. ISPM15 conditions, products not suitable for being carries by passengers, the lack of documents or confirmation, wood pallet harmful organisms, faulty items are some of the common reasons. The most common reason is harmful organisms both for exported and for imported items as seen on Table 10.

Table 10. The number of notifications and reasons for export and import (2017-2019) (Anonymous, 2020a)

The Number of Notifications						
Reason	**Export**			**Import**		
	2017	**2018**	**2019**	**2017**	**2018**	**2019**
ISPM 15	**112**	**108**	**125**	5	22	10
Prohibited Product Accompanying with Passenger	**138**	**52**	**8**	2	0	7
Incorrectly Missing or Incompatible Document	**46**	**32**	**34**	28	24	14
Wooden Pallet-Harmful Organism	**3**	**1**	**2**	0	0	0
Faulty Item (Leafy product that does not comply with special requirements, etc.)	**2**	**5**	**6**	5	3	8
Harmful Organism	**341**	**1.483**	**971**	**72**	**124**	**216**
Totally	**642**	**1.681**	**1.146**	**112**	**173**	**255**

In case of any non-conformity of border control, importer country sends notification and reason to exporter country. However, rejection of products and sending back to the origin country would occur if products are not suitable or dangerous to enter the country. Official controls number of import application, eligibility and refuses was given on Table 11, while the amount of refused plant products was given on Figure 6.

Table 11. Number of import application for official controls, of acceptances and refuses (Anonymous, 2020a)

Years	Total Number of Shipments	The number of Conformities	The number of Nonconformities
2015	**132,889**	**127,858**	**5,031**
2016	**133,711**	**130,140**	**3,571**
2017	**146,913**	**143,404**	**3,509**
2018	**142,676**	**138,781**	**3,895**
2019	**114,099**	**110,958**	**3,141**

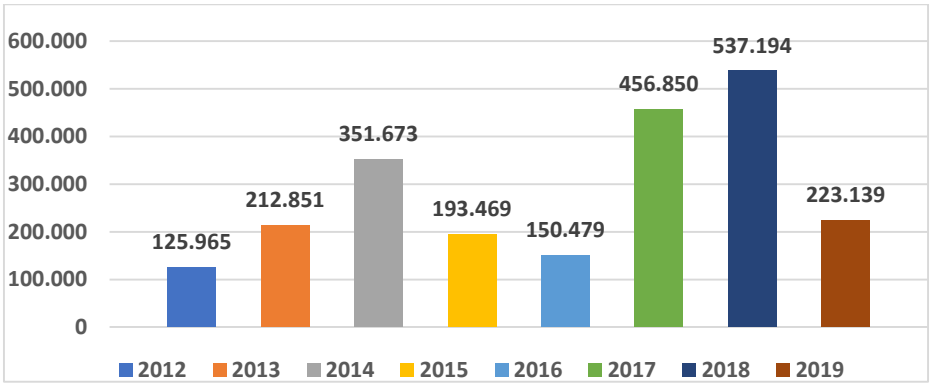

Figure 6. The amount of products that are not allowed to entry into importer countries (in Tons) (Anonymous, 2020a)

DISCUSSION

Although many studies still need to be done on phytosanitary and quarantine areas, Turkey's success on this area coming from strong R&D that was transferred into practices through solid experiences together with producers' high level awareness and efforts, which are indicator of the development of Turkey. Plant health, quarantine, border control, pest and pesticide management activities do not only ensure food safety, but also food security. Turkey has a huge experience about these activities and still developing new strategies for a better management.

Due to COVID-19 pandemic, all countries have been experiencing an extraordinary situation in 2020. It has shown that food supply is more vital than communication, transportation, defence industry and other electronic or technological devices. In addition, it seems that the virus will remain on top of the global agenda. The circumstances of the COVID-19 shows also that even some new pandemics and other new diseases may arise. Therefore, ensuring food supply will be key for all countries.

As a result of its unique geopolitical location to sustain food supply at global level. Turkey has already increased its share in global agriculture trade in recent years. Searching new markets has been always an objective for Turkish agriculture producers. During COVID-19, Turkey had good experience in both managing a smooth food supply in domestic market and meeting export demands of many countries.

It is crucial to highlight that during pandemic, agricultural activities have continued without any disruption so there has been no issue occurred in the production in Turkey in 2020. This is

because of well-managed phytosanitary as explained. Considering all these, Turkey will play even more important role in safe food supply at global level during crisis.

If phytosanitary is managed properly, the health of all living things will be protected. Phytosanitary means also indirectly human health, and food safety and security. That is why 2020 was declared as the **International Year of Plant Health** by the United Nations. It is also so much meaningful and remarkable to announce 2020 as the **"International Year of Plant Health",** when human health, and food safety and security are so much important for the global community.

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THE QUALITY OF DIFFERENT SWEET POTATO FERTILIZER SYSTEMS (IPOMOEAN BATATAS) IN THE CONDITIONS OF FOREST-STEPPE OF UKRAINE

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ABSTRACT

The quality of different sweet potato fertilizer systems is defined for the conditions of Left-bank Forest-steppe of Ukraine. The use of the mineral fertilizer system (N370P370K450) predetermines the intensive growth of plants mass due to the substantial increase of the masses of chums and sheets on a plant. Depending on the dose of mineral fertilizers the productivity of sweet potato tubers grows on 37,7-63,5% in relation to control the productivity of 17,8 t/ha. Also the use of N370P370K450 provides the increase of content in the tubers of dry substance (18,08%), starch (5,39%) and ascorbic acid (5,39 mgs/of 100 g). Bringing the organic fertilizers (20 t/ha) and arboreal ash (1 t/ha) provides the increase of the productivity to the level of 26,3 t/ha. It is set that on typical little humus loamy black earth the plants of sweet potato take from soil about 57% of movable nitrogen forms, 11,6% of movable phosphorus forms and about 61,2% of movable potassium forms. The coefficients of the use of elements of feed from mineral fertilizers fold about 40,7%for nitrogen; 26,1% - phosphorus; 73,5%- to potassium. Without the fertilizers for the first two months of height the plants of sweet potato take about 88,2% of nitrogen, about 60,6% of phosphorus and 80,5% of potassium from the general consumption of the feed elements of plants. At the mineral and organic fertilizer systems of lances of consumption of the feed elements is on 2-3 months of plants growth, especially for forming and growth of the tubers mass.

Keywords: sweet potato, fertilizer system, absorption of the feed elements.

INTRODUCTION

During the last years the demand on a new for Ukrainians culture such as sweet potato, grows substantially. First of all it is related to high curative and nourishing properties of this agricultural plant. The root crops of batatas contain plenty of antioxidants, vitamins, potassium, iron, phosphorus, calcium, magnesium. There are many difficult carbohydrates and cellulose in sweet potato, that is why it is recommended to people with diabetes mellitus and to the sportsmen-athletes. For the second, sweet potato has a high productivity potential.

Despite that the culture of sweet potato is widespread enough in the world, for the ground-climatic terms of Ukraine there are not the prepared technological decisions for growing this culture. The fertilizer system of batata allows to increase the productivity on 30-60% and is the basic effective factor of influence on quality composition of products.

Many researchers marks that the influence of fertilizers on the productivity of batatas substantially depends on a sort, the kind of the soil and the climatic conditions (O'Sullivan et al, 1997; Ali, Costa, Sayed, Basak, 2009; Qwudike, 2010). Dependence of the fertilizers efficiency is also marked on the level of growing technology of batatas(Abd El-Baky et al., 2009).

A culture has high requirements to the nitric feed, but can provide relatively normal marks of the productivity even on soils with subzero fertility (Hill et al, 1990). Often this fact is explained by ability to fix atmospheric nitrogen through the association with associative bacteria. By this way it can be got to 40% nitrogen that sweet potato consumes. (Yoneyama et al, 1998).

It is marked that nitric fertilizers predetermine the increase of the productivity of vegetative plants mass of batatas, while the influence on the productivity of tubers substantially differed at weather conditions and soil. Potassium fertilizers do not almost influence on the increase of vegetative mass of plants, but have substantial influence on the productivity of tubers and their amount in a bush (Bourke, 1977; Hartemink et al, 2000a).

According to data of Dumbuya G., Sarkodie - Addo J., Daramy M. A., Jalloh M. (2017) in the zone of the leafy forests of Ghana the plants of batata react well on the application of potassium fertilizers (de Geus, 1973). In the conditions of Nigeria (Uwah et al, 2013) the use of the norm of potassium fertilizers 160 kg/ha provided the forming of maximal length of vegetative mass, the amount of sheets and branches on plants, while the dry mass of vegetative mass, the diameter and the mass of tubers on plants were statistically identical by using of potassium fertilizers with norms 120 and 160 kg/ha. General productivity of tubers while bringing 120 and 160 kg/ha of potassium fertilizers grew for 7 and 8 times accordingly in relation to control.

Obigbesan et al (1976) point on the fact that the quality of phosphoric fertilizers for batatas growing is already marked on soils with content of movable forms of phosphorus less than 10 мг/кг. According to Marschner (1995), phosphorus is the important component of many organic compounds of plant, that is positively represented on the culture productivity. Phosphoric fertilizers have positive influence both on the level of the general and commodity productivity of tubers and the content of dry substance in the tubers, middle mass and diameter of tuber (Hassan et al., 2005)

Acoording to Floyd et of al (1988) data, the plant of batatas take phosphorus and potassium from organic fertilizers better than from mineral. Brobbey (2015) points on greater quality for growing the sweet potato of organic fertilizers.

Thus, to define the optimal system of fertilizer for sweet potato in the conditions of Forest-steppe of Ukraine is a pressing question

Materials and Methods

The research is undertaken in the Institute of vegetable-growing and water-melon (Ukraine, Kharkiv area) during 2019-2020. The soil of an experience area is presented by black earth typical littlehumus light-loamy on a loesslike loam. Agrochemical description of top-soil (0-25 cm) is following: pH of salt extraction - 5,7; the sum of eaten up bases - 26,0 on 100g of soil; hydrolysis acidity - 2,8 on 100g of soil; the content of humus - 4,3%; nitrogen that hydrolyzes, - 139,0 mg/kg; movable phosphorus - 106 - 119 mg/kg and exchange potassium - 93 mg/kg of soil.

The climate of Left-bank Forest-steppe of Ukraine is milddle continental (average air temperature is 6,8-7,0 0C, the period with higher temperature 10 C - 170-180 twenty-four

hours, the average amount of precipitations is 471 mm, from them in June-July about 75-85 mm).

The chart of experience included the next variants:

1. Without fertilizers (control)

2. $N_{130}P_{130}K_{130}$

3. $N_{370}P_{370}K_{450}$

4. $N_{370}P_{370}K_{450}$+ foliar signups by the complex fertilizers of "Nutrivant plus universal" in 3 terms 5. Humus 20 t/ha + ash of 1 t/ha

6. Humus 20 t/ha + ash of 1 т/ha + treatment of soil to landing by the ground biofertilizer of "Groundfix" (3 l/ha) + at first fertigation the microbal "Azotfit" (1 l/ha) + at second fertigation microbal "Organik balance" (1 l/ha) + foliar signups of "Help - rost for vegetable plants" 2 l/ha in 3 terms.

Foliar signups were conducted in the third ten-day periods of June and July, in the second ten-day period of August.

"Nutrivant plus" is a line of complex fertilizers for the foliar signups, that includes "fertivant", that is laid out for a 30 twenty-four hours. "Nutrivant plus universal" contains N - 18%, P_2O_5 – 18%, K_2O – 18%, MgO – 2%, Mn – 0,02%, Zn – 0,01%, Cu – 0,0025%, Fe – 0,04%, Mo – 0,0025%. Producer - "Agrovant" (Israel).

"Groundfix" is the ground biofertilizer that contains the cages of bacteria of *Bacillus subtilis, Bacillus megaterium var. phosphaticum, Azotobacter chroococcum, Enterobacter, Paenibacillus polymyxa*. Incurrence of viable cages (0,5 - 1,5) $x10^9$ КУО/см^3. A Biofertilizer provides the increase of movability of phosphorus and availability of potassium from soil and mineral fertilizers, prolongs the availability of nourishing elements; improves biological activity of soil and represses the development of phytopathogenes.

"Azotfit" is microbal preparation that contains the cages of natural nitrogen-fixing bacterium Azotobacter chroococcum, biologically active waste of bacteria (amino acids, vitamins, plant hormones, fungicide substances) products. Incurrence of viable microorganisms of producers not less than1×10^{10} КУО/cm^3.

"Organic balance" is microbal preparation for a height and development of agricultural cultures, firmness to stresses, illnesses and balanced feed. Contains the bacterium: nitrogen-fixing; phosfor- and caliumobilising; microorganisms are with fungicide properties; components of nourishing environment (macro, microelements and organic sources of feed). Incurrence of viable microorganisms of producers not less than$1,0x10^9$ КУО/см^3.

The technology of batatas growing included the use of tiny irrigation, mulching of soil with a straw, a chart of growing is (50+90) x25 cm.

During the research the row of concomitant supervisions and accounts were conducted. The mass of chums, the mass of stems and sheets were determined during the vegetation of plants of sweet potato. The account of harvest was determined by area by a gravimetric method with determination of commodity and uncommodity part.

The chemical analysis of tubers was conducted in the phase of technical ripeness. The content of dry substance was determined by a thermogravitational method, the content of general sugar by a cianometrical method, the content of starch was determined colorimetrically

with an iodine, the content of ascorbic acid by titrometrical method by Murri, the content of nitrates by an ion selective method.

The contents of nitrogen, phosphorus and potassium is plants by V.V. Pinevich by the method of wet ashing with further determination of nitrogen, by the volume method by microyeldal, the phosphorus – colorimetrically, the potassium on a flame photometer (Bulygin, Balyuk, 1999).

The calculation of economic and biological bearing-out of elements of sweet potato feed and coefficients of the use of nourishing elements plants from soil and fertilizers was conducted in obedience to methodology, worked out by Z.I. Zhurbytskiy. The coefficients determinations of the plants use of nitrogen, phosphorus and potassium from fertilizers was conducted in comparison of bearing-outs of these elements of feed on areas with top-dressing and on control (without fertilizers) with a further calculation by formula:

$$K = \frac{(Bд - Bк) \cdot 100}{Д},$$

where K is a coefficient of the use of fertilizers %;

Вд is a general bearing-out of nutritives at top-dressing, kg/ha;

Вк is a general bearing-out on a control variant, kg/ha;

Д is a dose of the brought in mineral fertilizers, kg/ha (Bondarenko, Yakovenko, 2001).

Results and Discussion

Dependences of biometrical parameters of sweet potato plants are analysed at the different fertilizer systems. It is marked that beginning from the early stages of sweet potato plants growth and development (June) the most general mass of plants is formed by using the mineral system of fertilizer (pic. 1). Thus middle mass of plants presented 294 g/plant, by using the organic fertilizers - 244 g, on control is 151 g/plant. This tendency is marked at a further growth and development of sweet potato plants.

It was also marked that at the organic system of fertilizer the accumulation of middle mass of plants goes slowly to the initial periods of growth and accelerated on the end of vegetation, that, in our opinion, is related to slow mineralization of organic substance of humus and forming the optimal nourishing mode of soil in later period of sweet potato plants development.

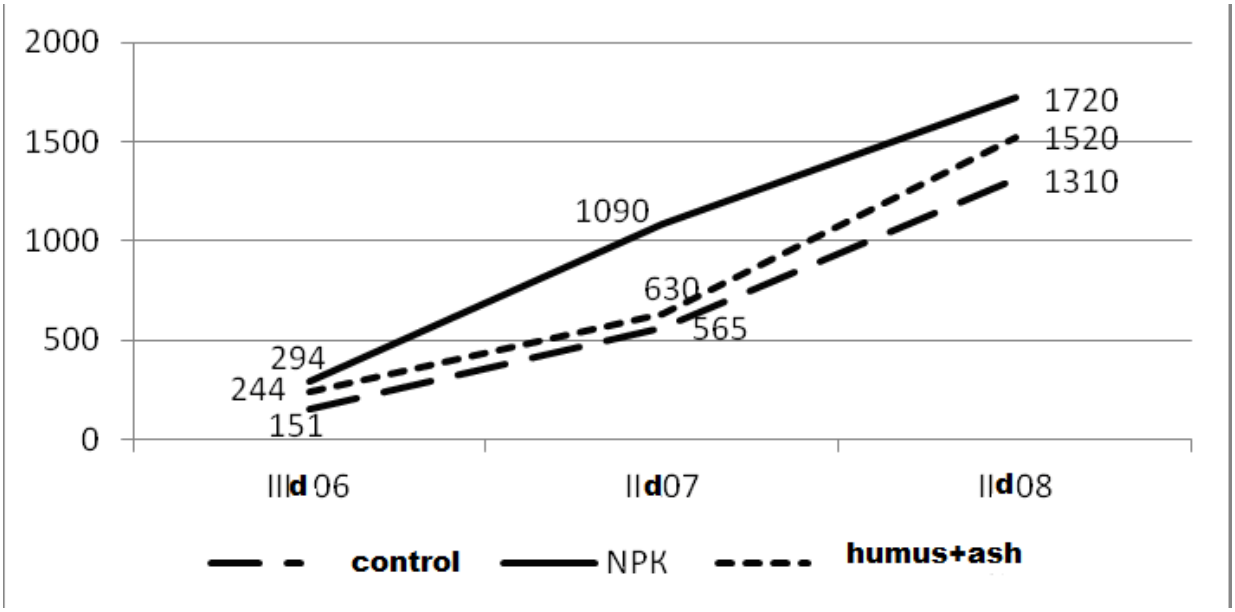


Figure 1. The influence of different fertilizer systems on the dynamics of forming the general sweet potato plants mass

It is set at the analysis of dynamics of forming the mass of sweet potato chums (pic. 2), that at the beginning of vegetation (June) the mass of chums at the different systems of fertilizer was at one level (18 gs/of plant), at a value given to the index on control 12 g/ plant. In future this conformity is kept, the substantial increase of chums mass is marked at mineral, and some less level - at the organic system of fertilizer. In that time till the end of vegetation the difference of the chums mass between mineral and organic fertilizer systems diminishes, that can point to the improvement of terms of feed of plants of sweet potato on the end of vegetation for the use of organic fertilizers that have the prolonged action.

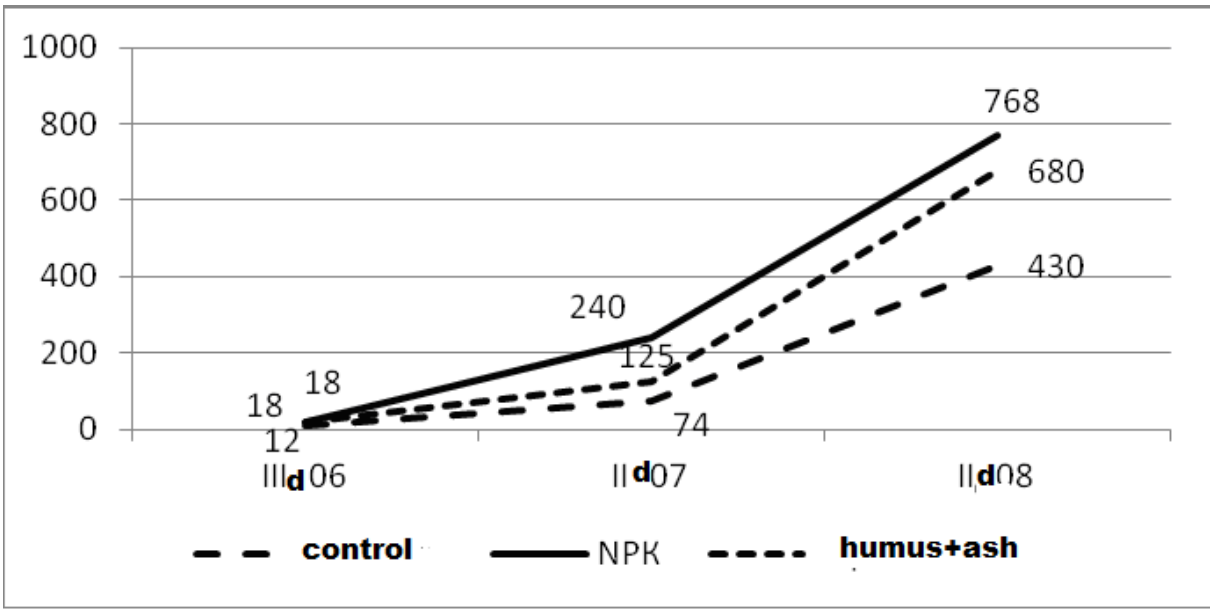
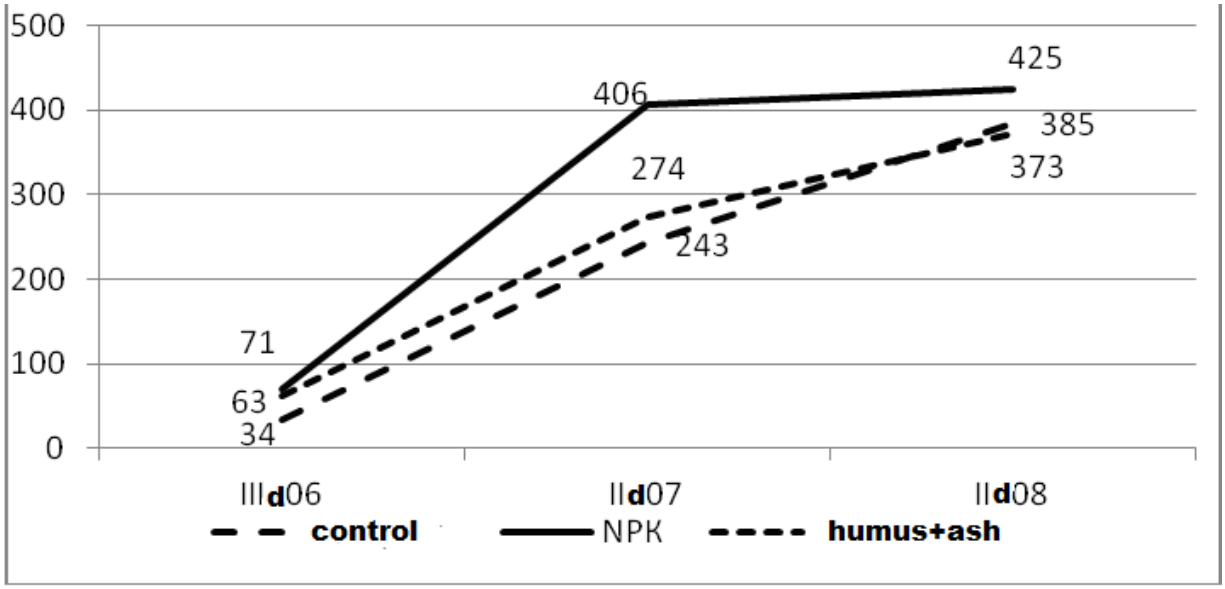


Figure 2. The influence of different fertilizer systems on the dynamics of forming the chums mass of sweet potato plants

Beginning from the early stages of growth and development of sweet potato plants (June) pedicellate mass of sweet potato plants at the mineral system of fertilizer presented a 71 g/plant, at the organic system of fertilizer there are 63 g/ plant that considerably differs from control with an index 34 g/ plant (pic. 3). In a middle of the period of growth and development of plant the dynamic increase of pedicellate mass at the mineral system of fertilizer 406 gs/of plant, while at the organic system pedicellate mass presented 274 g/ plant is marked, control is 243 g/plant. By the end of vegetation the growth of pedicellate mass is slowed by bringing of organic and mineral fertilizers, while the height of stems on control lasts and in August exceeds the indexes of the organic system of fertilizer.



Pic. 3 The influence of different fertilizer systems on the dynamics of forming pedicellate mass of batatas plants

It is set, that beginning from the early stages of growth and development of batatas plants (June) the biggest sheet mass of plants is formed by using the mineral system of fertilizer (pic. 4). Thus the average sheet mass of plants was 164 g/plant, by using the organic fertilizers - 207 g, on control was 151 g/plant. It is also marked, that at further development of plant this tendency is kept. Beginning from July mass of sheets grows on control (248 g/plant) that exceeds even an index at the organic system of fertilizer (231 g/plant).

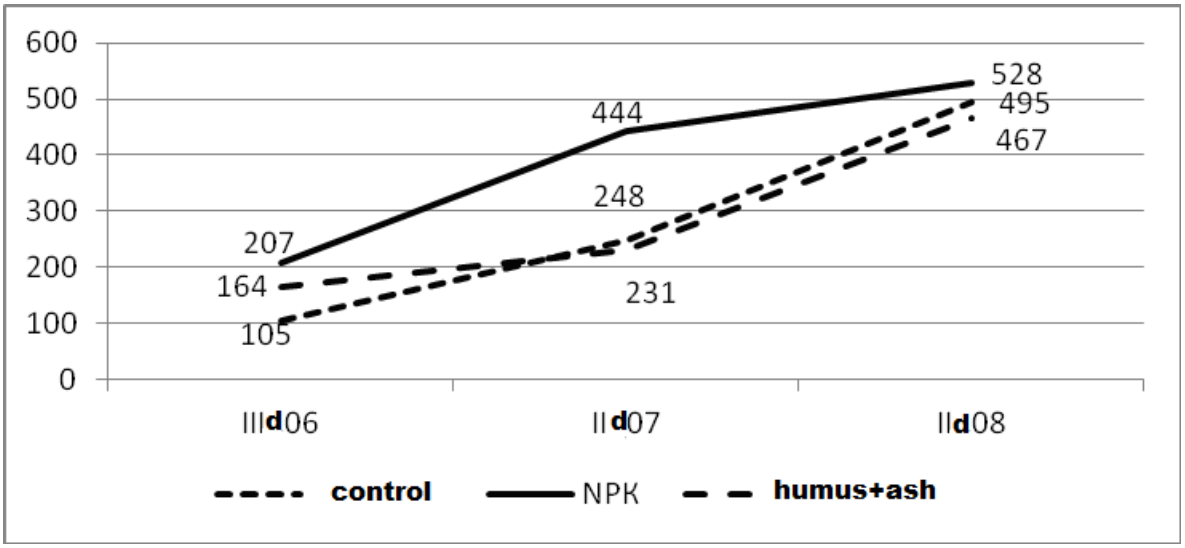


Figure 3. The influence of different fertilizer systems on the dynamics of forming the sheet mass of plants

The improvement of the nourishing mode of soil and improvement of biometrical parameters by using the fertilizers assisted the increase of the productivity of batatas tubers on 6,7-11,27 t/ha or 37,7-63,5% (table. 1). The greatest quality is provided by the use of mineral fertilizers of $N_{370}P_{370}K_{450}$ with foliar signups by complex fertilizers. Thus the productivity arrives at 29,01 t/ha, that exceeds the control on 63,5%. The substantial increase of the productivity of batata provides realization of additional signup complex fertilizers.

Table 1. The influence of different fertilizer systems on the batatas tubers productivity

	Fertilizer system	General productivity t/ha	The growth to control	
			t/ha	%
1	Without fertilizer (control)	17,75	-	-
2	$N_{130}P_{130}K_{130}$	24,45	6,7	37,7
3	$N_{370}P_{370}K_{450}$	24,46	6,71	37,8
4	$N_{370}P_{370}K_{450}$+ foliar feeding by complex fertilizer «Nutrivant plus universal» in 3 terms	29,02	11,27	63,5
5	Humus 20 t/ha +ash 1 t/ha	26,33	8,58	48,3
6	Humus 20 t/ha + ash 1 t/ha + microbal preparations by fertigation and by the leaf+ foliar feeding "Help-rost for vegetable plants" 2l/ha in 3 terms	26,99	9,24	52,1
	$HIP_{0,95}$		3,6	20,3

From the organic systems of fertilizer the bringing of humus of 20 t/ha and ash of 1 t/ha is effective, that provides the receipt of the general productivity at the level of 26,3 t/ha. The additional use of microbal preparations does not assist the substantial increase of the productivity of culture.

Positive influence of the systems of fertilizer is marked on an increase in the tubers of dry substance (17,58-18,9%) at a value given to the index on control of 16,12% (table. 2). The decline of content of general sugar is marked to the level of 3,63-3,83% in relation to control (4,41%) by using mineral fertilizers with different to the fertilizers. By using the fertilizers a tendency is marked to the increase in the tubers of maintenance of vitamin C, but substantially this index rises by using the dose of $N_{370}P_{370}K_{450}$ without signups by micro fertilizers and for bringing of organic fertilizers, ash and microbal preparations (5,39-5,47 mg/ 100 g). At these variants and bringing of $N_{130}P_{130}K_{130}$ with micro fertilizers a substantial increase of starch is marked in the sweet potato tubers (7,61-8,65%). All systems of fertilizer predetermine the increase in the tubers of content of nitrates (28,1-78,5 mg/kg), at a value given to the index on control 15,8 mg/kg of a raw mass.

The elements of feed from fertilizers and soil are expected for the correction of doses of mineral fertilizers for sweet potato for the conditions of Ukraine. It is set that on typical little humus loamy black earth the plants of sweet potato take about 57% of movable forms of nitrogen, 11,6% of movable forms of phosphorus and about 61,2% of movable forms of potassium from soil.

The coefficients of using the elements of feed from mineral fertilizers fold for nitrogen about 40,7%; for phosphorus - 26,1%, for potassium - 73,5%.

Table 2. The influence of different fertilizer systems on the sweet potato tubers quality

	Fertilizer system	The contain in the tubers, %				
		Dry substance	General sugar	Vitamin C mg/100g	Starch	Nitrates, mg/kg
1	Without fertilizer (control)	16,12	4,41	4,71	6,26	15,80
2	$N_{130}P_{130}K_{130}$	16,66	3,83	5,09	8,75	31,20
3	$N_{370}P_{370}K_{450}$	17,68	3,69	5,24	6,67	58,50
4	$N_{370}P_{370}K_{450}$+ foliar feeding by complex fertilizer «Nutrivant plus universal» in 3 terms	18,08	3,63	5,39	7,61	78,50
5	Humus 20 t/ha +ash 1 t/ha	17,58	4,61	4,94	5,89	50,60
6	Humus 20 t/ha + ash 1 t/ha + microbal preparations by fertigation and by the leaf+ foliar feeding "Help-rost for vegetable plants" 2l/ha in 3 terms	18,90	4,32	5,47	8,09	28,10
	$HIP_{0,95}$	1,34	0,43	0,55	0,76	6,4

The rates of absorption the elements of feed of sweet potato plants at the different fertilizer systems were also expected by us (table. 3). Without the fertilizers the plants of sweet potato for the first two months of growth take about 88,2% of nitrogen, about 60,6% of phosphorus and about 80,5% of potassium from the general consumption of elements of feed plants. By using the mineral and organic systems of fertilizer the lances of consumption of elements of feed is on 2-3 months of plants growth, during the active forming and growth of tubers mass.

Table 3. The influence of fertilizer systems on the percent of consumption the feeding elements by the plants of sweet potato

Fertilizer system	% the consumption of the feeding elements to general bearing-out					
	N		P		K	
	II decade of June	II decade of July	II decade of August	II decade July	II decade of June	II decade July
Control	24,2	88,2	2,3	60,6	13,3	80,5
$N_{370}P_{370}K_{450}$	9,4	47,8	1,4	47,3	6,6	36,3
Humus 20 t/ha+ash 1 t/ha	15,4	58,0	2,1	39,0	6,3	30,5

At the mineral fertilizer system the rates of consumption of basic elements of feed are higher (pic. 4-6). By the end of active vegetation at the mineral fertilizer system the plant of sweet potato consume 246,3 kg/ha of nitrogen, 201,1 kg/ha of phosphorus and 683,2 kg/ha of potassium.

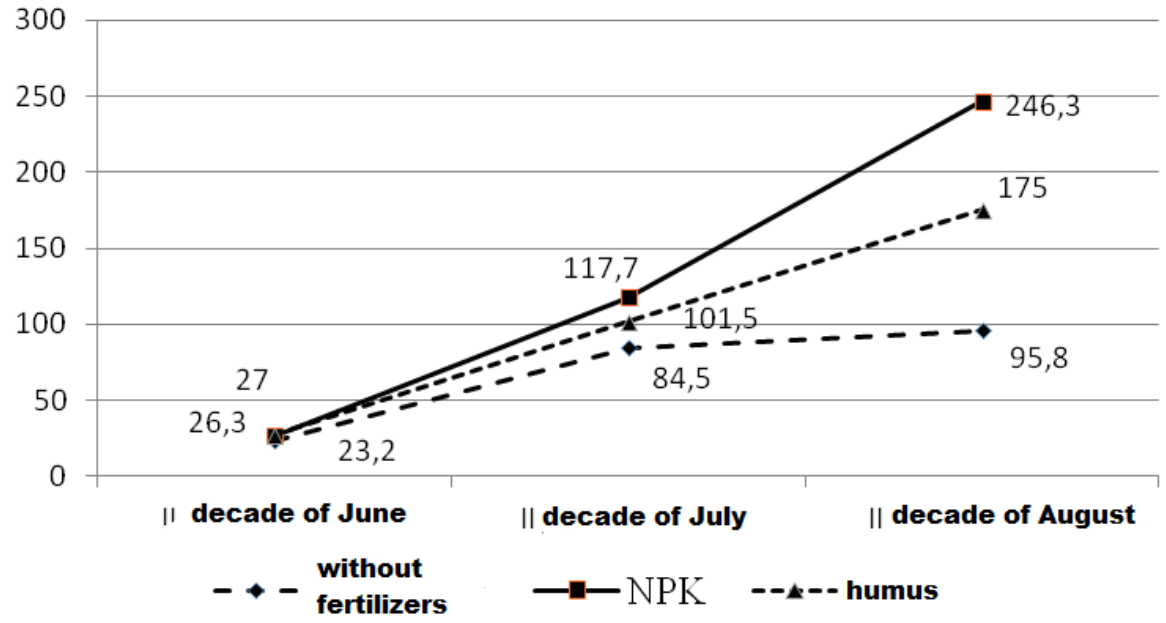


Figure 4. The rates of the nitrogen consumption by the batatas plats while using different fertilizer systems

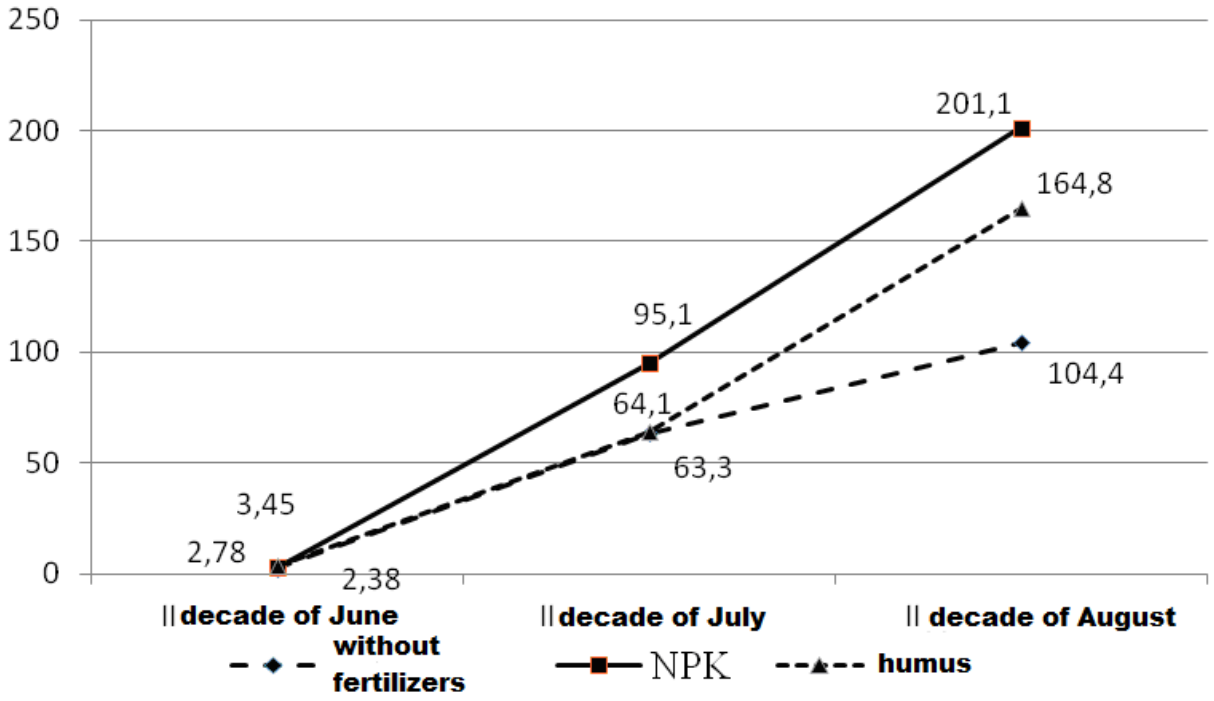


Figure 5. The rates of the phosforus consumption by the sweet potato plats while using different fertilizer systems

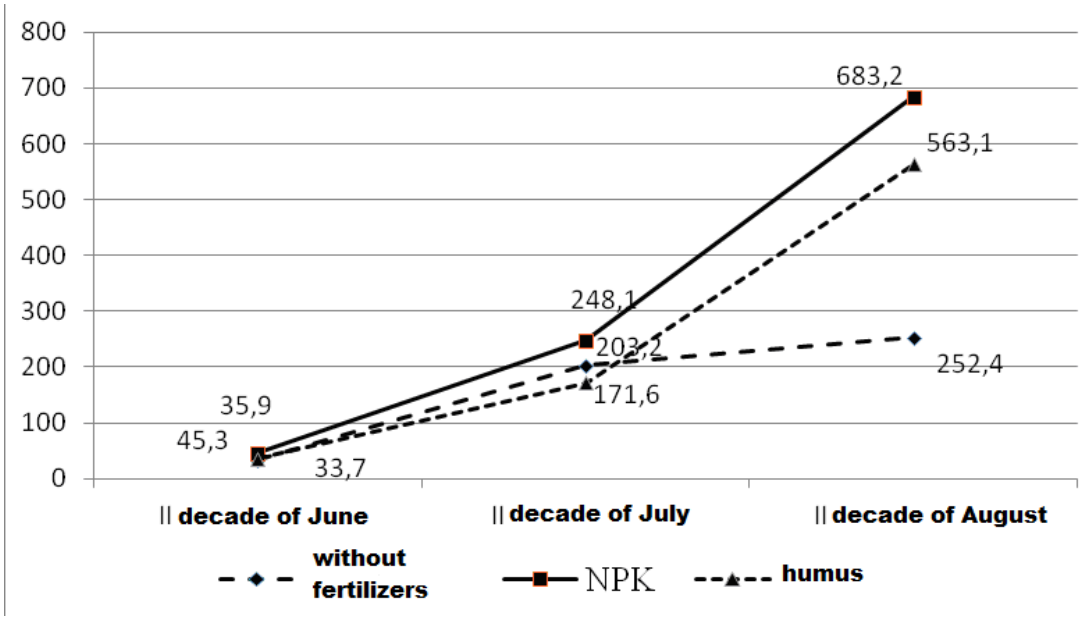


Figure 6. The rates of the potassium consumption by the sweet potato plats while using different fertilizer systems

Without application of fertilizers by the end of vegetation the absorption of phosphorus (104,4 kg/ha) and potassium (252,4 kg/ha) goes down substantially, that, in our opinion influences on reduction of level to the productivity of sweet potato. It should be noted that

absorption of potassium by the plants of sweet potato in the conditions of Forest-steppe of Ukraine is higher, than in soils of south regions. Thus, By Degras (2003) data the bearing-out of potassium with the plants of sweet potato presents 110 kg/ha.

It is marked that depending on the system of fertilizer of 56,9-76,9% of nitrogen is accumulated in sheets, 10,2-13,6% in stems, 12,9-29,5% in roots and tubers, phosphorus about 31,2-49,9% accumulated in sheets, 13,4-20% in stems, 30,1-55,4% in roots and tubers; potassium about 41,6-51,9% in sheets, 13,2-21,2% in stems, 26,9-45,2% in tubers and roots.

Conclusions

Thus, the use of mineral fertilizers predetermines active growth of vegetative and root mass of sweet potato plants during all vegetation. By using the organic fertilizers intensive development of the plants chums is marked in the second half of vegetation and braking of processes of forming the leaf-stem mass. Due to mineral and organic fertilizers the productivity of sweet potato tubers grows to 37,7-63,5% in relation to control. Bringing of $N_{370}P_{370}K_{450}$ provides the increase of content of dry substance (18,08%), starch (5,39%) and ascorbic acid (5,39 mg/ 100 g) in the tubers.

On the typical little-humus loamy black earth the plants of sweet potato take in about 57% of movable forms of nitrogen, 11,6% of movable forms of phosphorus and about 61,2% of movable forms of potassium from soil. The coefficients of using the elements of feed from mineral fertilizers are: for nitrogen about 40,7%; for phosphorus - 26,1%, for potassium - 73,5%. Without the fertilizers the plants of sweet potato for the first two months of growth take in 88,2% of nitrogen, about 60,6% of phosphorus and 80,5% of potassium from the general consumption of elements of feed plants. By using the mineral and organic fertilizer systems the lances of the feeding elements consumption is on 2-3 months of plants growth, especially while forming and growth the mass of tubers.

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CHANGES IN BIOCHEMICAL BLOOD PARAMETERS IN ARBIA GOATS OF NORTH-EASTERN ALGERIA DURING LACTATION AND DRY PERIOD

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ABSTRACT

Farmers in Algeria are more interested in goats rearing and breeding, as a result of the high demand of their products by consumers. The nutritional importance of goat's milk is well known and there is also importance in their kid's meat. The Arbia goat is one of the most popular breeds for milk and meat production. Therefore it's important to conserve, and control the health of goats for increasing animal production. However, in the last few years, many researchers had shown that blood parameters of small ruminants were influenced by many factors such as age, different climate, breed and physiological stages of production. The aim of this study was to investigate the variations in some biochemical blood parameters during lactation and dry period in Arbia goats in semi-arid zones of North-Eastern Algeria. Ten healthy Arbia goats (12±0 months old) were chosen from one farm in Touzeline (20 km from Oum El Bouaghi province). Blood was collected once from each doe in the 3rd (early lactation), 8th (mid lactation) week of lactation and during 2nd week of dry period. Plasma glucose (Glu), cholesterol (Chol), triglycerides (TG), urea, creatinine (Creat) were measured. Plasma levels of Chol and urea were not significantly affected by different stages of lactation and dry period of the Arbia goats. During mid lactation, Glu recorded the lowest level compared to early lactation and dry period. Compared to early lactation, TG increased ($p<0.05$) and Creat decreased ($p<0.05$) in dry period. In conclusion, the obtained results would certainly help breeders and clinical veterinarians in order to control health and nutritional status of Arbia goats in semi-arid zones of Algeria to increase the animal production.

Key words: *Biochemical parameters, Arbia goats, lactation, dry period, Algeria.*

INTRODUCTION

In Algeria, the number of goats has increased to reach 4.9 million out of 35 million heads of all types of ruminants in Algeria, (Faostat, 2017). The Arbia goat is one of the most popular breeds for milk and meat production. Therefore it is important to conserve and control the health of the goats for increasing animal production. It is evident that the blood levels of various blood parameters can be used as criteria for diagnosis and prognosis of metabolic diseases, as well as for assessment of nutritional status. However, many researchers have shown that the blood parameters of small ruminants are influenced by many factors such as age, geographical locations with different climates, season, breed (Piccione et al., 2012; Bagnicka et al., 2014; Ribeiro et al., 2016), and the physiological stages of production (Sadjadian et al., 2013; Donia et al., 2014; Antunović et al., 2017). Several studies showed great variations in the levels of

blood parameters between breeds of goats during the periods of pregnancy, parturition and lactation (Waziri et al., 2010; Darwesh et al., 2013; Soares et al., 2018).These three physiological stages of production are known to cause metabolic stress.

The aim of this study was to investigate the variations in some biochemical blood parameters during lactation and dry period in Arbia goats in semi-arid zones of North-Eastern Algeria.

Material and Methods

Experimental site, animals and rearing system

The experiment started in the Maameri farm in Touzeline, 20 km from the Oum El Bouaghi province (35◦52'39″N, 7◦6'49″E) at an altitude of 902 m. This area of Algeria is considered as a semi-arid zone, and its climate is characterized by hot summers and cold dry winters. Ten clinically healthy Arbia does, with a mean body weight of 23.74 ± 1.28 kg and a mean age of 12 ± 0 months, were chosen from a herd of 45 animals. During the trial, all the animals were reared in a traditional production system and kept under natural photoperiod and temperatures. The animals were fed by natural grazing for the whole day, while in the evening they were fed straw or hay according to what was available on the farm. In winter and during extreme weather conditions, the animals were kept indoors, and once a day were given a mixture (500-1000 g/goat/day) of barley either as grain or crushed (35%) and wheat bran (65%); barley was replaced by flour in the grain (500 g/goat/day) when it was not available. In addition to the food mixture, 8-9 kg/day/ ten goats high quality hay was also given, salt licks and water ad *libitum*. The duration lactation in these animals was 122.38 ± 8.61 days, and lactation in all goats began during the winter season (in December). The goats were in their first lactation. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and were approved by the Ethics Committee of our Institution.

Blood sampling and analyses

Blood was collected once from each doe in the 3rd (early lactation), 8th (mid lactation) week of lactation, and during the 2nd week of the dry period. Blood samples were collected into10 mL heparinized tubes from the jugular vein, always in the morning (7a.m.) before feeding. They were immediately centrifuged for 15 min at speeds of 3000 rpm. Plasma was harvested and stored at -20 °C until analysis of glucose, cholesterol, triglycerides, urea and creatinine by using a Siemens, ADVIA 1800 Chemistry System (Germany, 2007), and corresponding commercially available kits for each plasma parameter studied.

Statistical analysis

All data are presented as means ± SD (standard deviation).The results were analysed by ANOVA (StatisticaVersion 5.1, StatSoft France, 1997). Multiple comparisons were made using the test of LSD for post- ANOVA multiple comparisons ($P<0.05$).

Results

The results show that the productive stage of Arbia goats did not have a significant effect on plasma levels of cholesterol, but the levels of the remaining blood parameters showed different significant variations (Table 1).

The mean plasma concentrations of glucose decreased significantly during the mid (8th week) compared to the early (3rd week) lactation ($p<0.003$), and the 2nd week of dry period ($p<0.002$).

Differences in the cholesterol plasma levels were not significant during any of the experimental periods of reproduction studied. During the dry period, triglyceride levels were also significantly higher than those determined during the early lactation ($p<0.02$). The lowest triglyceride concentrations were observed at 3 weeks of lactation (0.16 mmol/L ± 0.05). There are no significant variations of triglyceride concentrations between early and mid lactation.
Urea did not show any significant changes between the three studied periods.
A significant increase in creatinine was seen at early lactation compared to the 2nd week of dry period ($p<0.00002$). However, mean plasma concentration of creatinine did not significantly change at early compared to mid lactation.

Table1. Mean (±SD, $n = 10$) peripheral plasma concentration of some biochemical in Arbia does during lactation and dry period

Blood metabolites	Stage of production		
	Early lactation	Mid lactation	Dry period
Glucose (mmol/L)	3.54 ± 0.26^{a}	2.92 ± 0.56^{b}	3.84 ± 0.78^{a}
Cholesterol (mmol/L)	1.87 ± 0.22	1.91 ± 0.32	2.03 ± 0.32
Triglycerides (mmol/L)	0.16 ± 0.05^{a}	0.24 ± 0.06^{ab}	0.32 ± 0.12^{b}
Urea (mmol/L)	5.59 ± 1.5	5.43 ± 1.16	5.20 ± 2.14
Creatinine (mg/L)	6.22 ± 0.66^{a}	5.85 ± 1.42^{a}	4.11 ± 0.66^{b}

SD- standard deviation
Means in the same row with different superscript letters are significantly different ($P< 0.05$).

Discussion

The results of the present research on Arbia goats reared traditionally in semi-arid zones of North-Eastern Algeria indicated that glucose decreased significantly at mid lactation compared to early lactation. Similarly, Bedo et al. (1997) reported a decrease in glucose during the same period in ewes. In contrast, Darwesh et al. (2013) found an increase in glucose at 6 weeks of lactation (early lactation) as compared to mid and late stages of lactation in Black does. In this study, the decrease in glucose levels at mid lactation compared to the dry period appeared to be related to high energy demands due to lactation. Meanwhile, there were non-significant changes at 3 weeks postpartum as compared to the dry period.

In contrast, a study on Tuj ewes showed that serum glucose concentrations in ewes on days 1 and 30 of lactation were higher than those at 3 weeks after drying (Karapehlivan et al., 2007). The glucose values obtained in this study were similar to the reference range reported by Kaneko et al. (2008), which indicated that the animals were in a positive energy balance.

In this study, the total cholesterol plasma values were not significantly influenced by the physiological status of Arbia goats as was also found by Antunović et al. (2011a; 2011b) in ewes. The decrease in triglyceride during early lactation in the present study was also found in Surti goats from 14 to 45 days of lactation (Manat et al., 2016). Our finding showed no significant changes in triglyceride levels between early and mid of lactation. Similar results were detected in black goat by Darwesh et al. (2013), and in Valfortorina goats by Casamassima et al. (2007), whereas triglyceride tended to increase as Montefalcone goats advanced in stages

of lactation (Casamassima et al., 2007). The decrease in triglyceride levels could be the result of the use of fat reserve mobilization for milk production (Wangsheng et al., 2014).

Urea plasma levels showed non-significant changes between all studied periods. In contrast, urea content increased significantly at the end of lactation, compared with the dry period (Balicki et al., 2007). However, urea levels at the 30th day of lactation were higher than those in the dry period in sheep (Karapehlivan et al., 2007). The results indicate that Arbia goats possess high potential for conservation of nitrogen during the early and mid-lactation periods.

Creatinine plasma levels were also significantly affected by the physiological stage of production, and they reached the lowest values during the dry period. Contrary to our results, creatinine levels were higher in the dry period (Piccione et al., 2009). During the dry period, the decrease in creatinine was due to replenishment of body reserves, and regeneration of mammary tissue.

Conclusions

In conclusion, physiological status of Arbia goats did not have a significant effect on plasma levels of cholesterol and urea but the levels of glucose, triglyceride and creatinine showed different significant variations. The obtained results would certainly help breeders and clinical veterinarians in order to control health and nutritional status of Arbia goats in semi-arid zones of Algeria to increase the animal production. Moreover, some parameters showed specificity for this breed when compared with results of other breeds of goats.

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GROWTH PERFORMANCE OF SMALL RED BEAN (*Phaseolus vulgaris* L.) VARIETY GROWN UNDER FIELD AND GREENHOUSE CONDITIONS IN MERSIN PROVINCE

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ABSTRACT

Bean is one of the most cultivated edible legume plants in the world. Although it is seen as a dry bean national food in Turkey, it is seen that there has been a decrease in the production of bean in our country in the last 30 years compared to world production. The study aims to investigate the effect of environmental conditions on the growth of small red bean. For this purpose, the seeds of bean were obtained from the breeders living in Niğde province. Small red bean plants were grown simultaneously in the greenhouse and the field between March and July 2020 in Mersin province. Morphological observations such as leaf and seed structure were made, and the number of seed was determined. According to the results, although the greenhouse provides more uniform conditions than the field, plants found in nature showed the best growth performance than the plants grown under greenhouse conditions.

Keywords: *Field, greenhouse, growth performance, Mersin, small red bean*

INTRODUCTION

Legume crops are essential for the human diet. In many low-income families in developing countries, protein availability is less than one-third of the required intake (Paul et al. 2011). Legumes like *Phaseolus vulgaris L.* (common bean) were preferred to grow due to their high iron, zinc, folic acid, and thiamin content (Pennington et al., 1990; Broughton et al., 2003; Petry et al., 2015). In the food chain, legume crops need low inputs and more seed protein than animal protein in a unit area (Saxena et al., 2013). Small red bean (*Phaseolus vulgaris* L.) is a type of vegetable that can be evaluated in different ways, including fresh broad beans, pods with unripe seeds and dry beans. Dry and green beans have 127.328 ha of cultivation area and 800.949 tons of production in Turkey according to 2018 data. In this production amount, small red beans have a rate of 10.9% with a production of 88.024 tons (FAOSTAT, 2018). When the production amount is analyzed by provinces, it is seen that the most important production center is Isparta (15.384 tons), followed by Muğla (15.284 tons) and Izmir (11.341 tons) in Turkey, respectively (DrDataStats, 2019). It has been reported that bean cultivation and the quality of the product obtained were affected by ecological conditions, especially air temperature and humidity, and

this effect varied starting from seed planting (Vural and Eşiyok, 2000). Sepetoğlu (1994) stated that beans are sensitive to frost damage, so the planting time should be paid attention to the last frost date and temperature should be above 15°C for uniform germination. It is known from the previous study that high temperatures during the flowering period cause blooming and decrease the yield (Yaman, 1997).

In the province of Mersin in the Eastern-Mediterranean Coast of Turkey, small red bean planting is carried out in the spring season in free farming. However, in this period, depending on the climatic conditions and the producers, there is no distinctly uniform planting date in terms of sowing time, and therefore seed sowing is carried out from the beginning of March to the end of April. The study was, therefore, aimed to determine the effects of Mersin ecological conditions and greenhouse conditions on productivity and quality traits of small red bean variety when grown as fresh fruit.

MATERIAL AND METHODS

Plant material and growth conditions

In the research, the snap-type small red bean variety was used, and seeds (Figure 1) were obtained from Niğde. Seed planting was carried out simultaneously in the greenhouse of Mersin University, Mersin, Turkey, and in the field in Mersin in March 2020. 10 plants were used in each experiment. The seeds were sown in a viol filled with coconut shell and turf (1:1) in the greenhouse environment. The pot was placed in a plastic basin and regularly watered. After germination, the seeds were transferred to cultivation pots containing garden soil. At the same time, seeds were sown in the field and were maintained weekly.

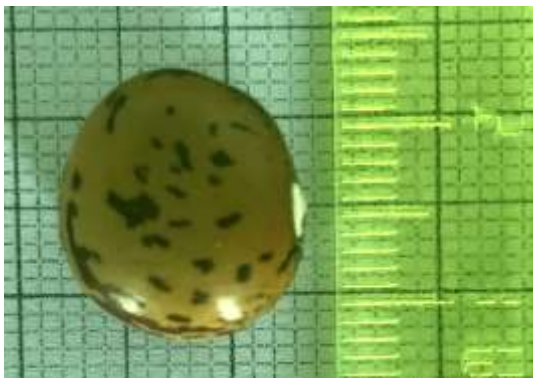

Figure 1. The view of the seed used in the present study

Description of the study site

This research was carried out from March to July 2020 in the field and the greenhouse at Mersin University. The latitude and longitude of Mersin are 36° 47' 30" N, 34° 38' 00" E (TÜİK, 2013). During March-July, when the plants were cultivated, the temperature of the cultivation greenhouse ranged between 30-40°C. During this time, the mean ambient temperature was 34°C.

Measurement of parameters

Morphological observations such as leaf color and flower bud view were carried out, and the size of leaf and flower bud were determined using a ruler. For the pod harvest, it waited until the seeds started to develop and were harvested in the shell. The seeds in the pods were removed and counted.

Sampling and chlorophyll analysis

Fresh leaves were used for chlorophyll analysis. Chlorophyll extraction from fresh leaves was conducted with 80% acetone (buffered to pH 7.8 with phosphate buffer). The chlorophyll a, chlorophyll b, and total chlorophyll measurements were carried out using a Cecil 5000 spectrophotometer. Chlorophyll contents were calculated according to Porra et al. (1989).

RESULTS AND DISCUSSION

Seed germination and plant growth

The seeds of small red bean were simultaneously sown in the greenhouse at Mersin University and in the field in Mersin. A week later, seed germination was observed in both experiments, and plantlets were obtained (Figure 2). After a month of germination, plants were obtained (Figure 3).

Figure 2. Seed germination of the samples at 7th day of sowing in the greenhouse (A) and the field (B)

Figure 3. The situation of the plant growth in the greenhouse (A-B) and the field (C-D). A and B indicate the development status of the plants in the 2nd week, while C and D indicate the development status in the 1st month.

Morphological observations, biochemical analysis, and seed calculation

Leaf, flower bud, and pod samples were collected from the plants grown in the greenhouse and in the field between April and July 2020 and evaluated. Morphological observations were conducted on one-month-old plants grown in both under greenhouse and natural conditions.

In view of the morphological observations in our study, no morphological difference was observed in the leaf colors of the plants growing in the greenhouse and the field. Leaf samples in the first 3-leaf stage developing were examined (Figure 4). The average petiole length was measured as 45 mm, leaf blade length was 46 mm, and the leaf blade width was 30 mm.

Figure 4. Analyzed leaf structure

The chlorophyll content of young and mature leaves was investigated. Plants were harvested after three weeks and two months of growth for chlorophyll analysis. While 943.6 chlorophyll a and 919.6 chlorophyll b ratios were detected in young leaves, 943.6 μg g^{-1} FW chlorophyll a and 564.6 μg g^{-1} FW chlorophyll b were measured in mature leaves, respectively. Chlorophyll b values were high in young leaves and decreased with leafage. Senescence destroys chlorophyll (Munne-Bosch and Alegre, 2002). According to the results of chlorophyll analysis in this study, chlorophyll b is more rapidly destroyed than chlorophyll a. Chlorophyll may have been enzymatically destroyed in older leaves, and chlorophyll b reductase may have been involved.

The flower structure (Figure 5) of red mullet was examined, and the petal length was measured as 14.5 mm, petal width was 8 mm, flower stem length was 12 mm, and flower length was 17 mm.

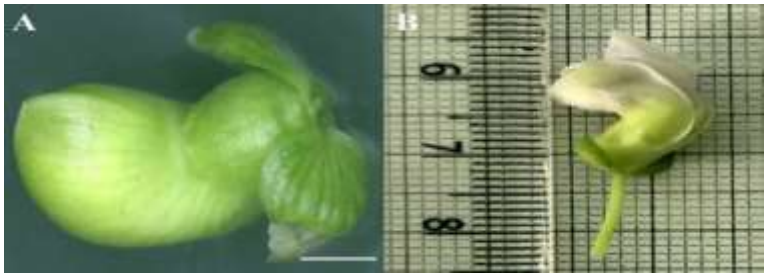

Figure 5. Flower bud and flower morphology of small red bean grown in the greenhouse

Mature seeds were obtained after nearly two months of sowing the seeds (Figure 6). A total of 96 pods were collected from the field, and 417 seeds were obtained. A total of 60 pods were obtained from the plants in the greenhouse, and a total of 175 seeds were obtained. In this case, we can say that the plants in the field give almost 3 times more seeds than the plants in the greenhouse. Pod length was measured as 95 mm, and the fruit width was 13 mm. The average number of seeds was determined as 6, and the seed length was measured as 7 mm, and the width was 5 mm.

Figure 6. Pod and seed structure of small red bean grown in the field

The ripe fruit forming dates of the plants growing in the greenhouse and the field have been at different times according to planting times. The harvest time of the plants grown in the greenhouse is earlier than grown in the field. This can be explained by the fact that the greenhouse conditions are more sheltered than the field. Besides, low temperatures and high adhesion in natural conditions in the first months of the experiment extended the harvest period. However, with the warming of the weather, the maturation period of the plants in the field has shortened. There was no significant difference between harvested fruit sizes and thicknesses. The pods of the plants are spotted, and the speckle colors are determined as red.

One of the essential criteria in terms of marketing is uniform and the well-shaped pod structure without curling. Non-curled pods were obtained from the greenhouse and field-grown plants.

It has been determined that there is a significant difference in pod productivity between the plants planted in the greenhouse and the field. This difference is due to both climatic conditions and soil structure. Plants grown in the field produced many more pods than plants in the greenhouse. It has been determined that the number of seeds per pod is very high in the plants grown in the field in the varieties harvested as the inner pod. It has been observed that the natural conditions have positive effects in terms of the number of seeds in the pod. We can say that the plant root system growing in the field develops better due to the fight against soil stones and particles, and this situation has a positive effect on plant nutrition.

Ecological conditions in crop production are useful not only in terms of plant planting time but also throughout the entire plant vegetation (Ceylan and Sepetoğlu, 1983). Temperature not only affects plant growth, development, and metabolism but also affects plant development status and timing (Baytorun et al., 1993). Various researchers have reported that the stick-shaped beans require 14-15°C temperature, the maximum growth is at 30°C, and at higher temperatures, growth slows down, flower fall increases, and seed formation is prevented (Akçin 1974; Şehirali, 1988). Therefore, we can say that March is suitable for the cultivation of small red beans in terms of Mersin climate conditions.

CONCLUSION

From the data obtained from this study, two main conclusions were drawn. First of all, seed sowing time is an essential parameter for product yield in open bean cultivation. In this study, small red beans seeds were sown in March and found that this season was suitable for Mersin province climate conditions. Even though the greenhouse offers a sheltered environment, natural conditions are more effective in product efficiency in small red beans.

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DETERMINATION OF YIELD AND FEATURES AFFECTING YIELD IN CORN-COWPEA MIXED PLANTING SYSTEMS

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ABSTRACT

The profitability of livestock can be accomplished by providing sufficient feed with high quality and reasonable prices. The decreasing trend of our existing agricultural lands requires the methods to increase the yield from the unit area. This study was carried out in four replications according to the randomized blocks trial design to determine the yield, some characters that affect the yield and some quality characteristics in different corn+cowpea mixed cultivation systems in Samsun province conditions. Six different applications, 1M + 1B, 1M + 2B, 2M + B and 2M + 2B, were covered in the research, together with the plain cultivation of corn (M) and cowpea (B). Plant height, the number of cobs, leaf weight, stem weight, cob weight, cowpea coiling rate, diameter, plant height, leaf crossing-over rate and green and grass yields were estimated in the plots.
As a result, while the green forage yield of 1M + 2B is 8770 kg/da, this is 8227 kg/da with alone corn, 2M + 2B 5659 kg/da, alone cowpea 5601 kg/da M + B 5187 kg/da, 2M + B 3284 kg/dafollowed. While alone corn yield is 2873 kg/da in hay yield, it is superior to plain cowpea and other mixtures with 2714 kg/da in 1M + 2B application. The mixture with the highest LER (area equivalence ratio) value is 1M + 2B with a value of 1.11. In terms of protein yield, the protein yields of alone M, alone B and M + 2B have been found statistically similar to each other and are higher than other systems. It has been resolved that the 1M + 2B application is more suitable for the coastal part of Samsun province in terms of hay production.

Keywords: corn, cowpea, mixture, yield, quality.

INTRODUCTION

Samsun province, located in the middle of the Black Sea coastline between the deltas where Yeşilırmak and Kızılırmak rivers flow into the Black Sea, has a surface area of 9 083 km². As a geographical location, it is between 40 ° 50 '- 41 ° 51' north latitudes, 37 ° 08 'and 34 ° 25' east longitudes. The province has significant agricultural potential due to its geographical location, natural and human resources. The total area of the province is 957 900 ha, of which 40.3% is agricultural areas.

While there is a notable decrease in the agricultural land assets and processed agricultural lands in Samsun Province, there is an increase in the unprocessed agricultural lands. Herbal products planted in the province are cereals, fruits, other oilseeds, corn, rice, sunflower, tobacco, soybean, dried legumes and sugar beet, respectively. There is a decrease in cereals, corn, dried legumes, sugar beet, soybean and tobacco, and an increase in the production of paddy, sunflower and other oilseeds. Significant increases have been accomplished in

cultivation areas and production of corn, vetch, alfalfa and sainfoin, especially for silage, thanks to the support provided to forage crops (Anonymous, 2018).

The total roughage need of Samsun province is approximately 1.582.033 tons, and the roughage produced from the cultivated areas is approximately 450.523 tons. When it is calculated that 21,000 tons of hay are obtained from 154 635 da of pasture, the roughage produced throughout the province is 471 523 tons. 30% of the need for roughage is met by planting forage crops. Most of the production (54%) is made of maize for silage (TUIK, 2019).

Decreasing arable agricultural lands and the continuous increasing in the population have led those engaged in crop production to seek the maximum use of existing agricultural areas. One of these ways is the mixed cultivation system (Acar et al., 2006). In mixed planting, plants with various root systems create a more productive plant than alone planting since plants with varying leaf anatomy of the water and nutrients in the soil and light better (Koç et al., 2004) and legume-wheatgrass mixtures give excellent results in this regard (Gökkuş et al., 1999). Corn-legume co-cultivation has been traditionally practised in the Middle and Eastern Black Sea for many years (Şehirali and Öztürk, 1983).

This research was carried out to determine the most suitable growing system of corn and cowpea in Samsun conditions and to study the yield and quality characteristics.

MATERIAL AND METHODS

This research was carried on the Çarşamba trial plots of the Black Sea Agricultural Research Institute during the 2019 growing season. The soils of the trial area are clayey loam, slightly alkaline, very insufficient in terms of phosphorus, insufficient in potassium, low in organic matter, moderately calcareous and salt-free. In Samsun, where the winter and spring months are warm and rainy, and the summer months are hot and partially dry, the distribution of precipitation throughout the year is relatively regular. Annual precipitation total is 730 mm, and the average temperature is around 14 ^{o}C.

The field trial, which was arranged in 4 replications according to the random blocks trial pattern, consisted of 6x4 = 24 plots in total. The planting was carried out on a total area of 480.2 m² with 40 cm X 10 cm intervals, the length of the parcel 4 m, the width according to the number of rows and 1.20 m between the parcels. The seeds were chosen by taking into account the ripening day lengths of the country cowpea diversity and FAO 650 group TK 6063 silage corn variety with a period of 90-95 days to harvest maturity. Along with the plain cultivation of corn and cowpea, alternate rows of 1M + 1B, 1M + 2B, 2M + B and 2M + 2B, were planted on 20.05.2019. Corn parcels were given 16, mixed parcels 12, and cowpea parcels 4 kg da^{1} nitrogen. 8 kg da^{-1} phosphorus and potassium were applied to all parcels. Irrigation was carried out twice through sprinkler irrigation when needed by observing the development of the plant. No plant protection drugs were applied. In maize, hand harvest was made when the bottom cobs reached the dough maturation period or when the seeds of the lower pods became fully visible in cowpea. The harvest area was determined by throwing the first and last rows of each parcel as well as 50 cm parts from the beginning and the end as the edge effect. Plants were harvested from the bottom, and green forage yield was determined by weighing them as wet. Later, after drying at 60 ° C for two days, hay yield was calculated by weighing (Uzun et al., 2018). The data of the experiment were obtained by measuring on ten randomly selected plants for cowpea and corn, and the values were calculated by taking the average of the data. After shaping the samples were dried in the oven at 60 ° C for two days, the hay yield was calculated by weighing, and the suitability of the mixtures was evaluated by the LER method. ADF, NDF, K, Ca, P, and

Mg contents in the ground samples were determined using Near-Infrared Reflectance Spectroscopy (NIRS) (Foss 6500) device and IC-0904FE package program.

The assumption of normality within the group of the data received was analyzed with the Shapiro-Wilk test, and it was determined that the data were distributed normally (P> 0.05). Consequently, the data were analyzed according to the random blocks trial design. SPSS 17.0 V statistical package program was applied to analyze the data (Açıkgöz, 1993; Smileer et al., 2013).

FINDINGS AND DISCUSSION

Morphological Properties

Table 1. Morphological property values determined in maize for silage according to the processes.

Mixing Systems	Plant Height (cm)	Cob Height (cm)	Cob Number (Item)	Node Number (Item)	Cob weight (g)
Plain Maize	187.4 ± 13.16	81.00 ± 9.37	1.17 ± 0.17	10.92 ± 0.08	143.7 ± 15.65
M+B	176.7 ± 7.24	77.25 ± 8.09	1.17 ± 0.10	10.00 ± 0.49	161.5 ± 30.47
M + 2B	167.0 ± 4.77	77.75 ± 4.15	1.08 ± 0.08	10.58 ± 0.25	194,3 ± 33,40
2M + B	170.4 ± 7.94	88.92 ± 1.53	1.17 ± 0.10	9.92 ± 0.55	187.0 ± 21.62
2M + 2B	187.9 ± 19.20	74.00 ± 6.80	1.00 ± 0.01	10.10 ± 0.50	157.9 ± 33.10
P	0.621	0.446	0.763	0.461	0.662

According to the results of variance analysis conducted to examine the effect of various mixing systems on the morphological properties of corn, the effect of the mixture systems on plant height, cob height, cob number, node number and bare weight of the cob was insignificant (P> 0.05).

Table 2. Morphological property values determined in the feed cowpea according to the processes *

Mixing Systems	Plant Height *	Cowpea Diameter
Plain Cowpea	56.42 ± 2.39^{a}	9.09 ± 0.29
M+B	41.42 ± 1.68^{c}	8.23 ± 0.26
M + 2B	44.25 ± 2.34^{bc}	8.42 ± 0.35
2M + B	45.67 ± 6.20^{abc}	8.18 ± 0.44
2M + 2B	56.00 ± 4.30^{ab}	8.60 ± 0.03
p	0.036	0.418

* There is no difference at p≤0.05 level within the values given with the same letter in the same column.

According to the results of variance analysis conducted to examine the effect of different mixing systems on the morphological properties of cowpea, the effect of the mixture systems on the plant height was found to be significant (P <0.05). In contrast, the effect on the stem diameter was found to be insignificant (P> 0.05). When the plant height of the cowpea was examined, it was found that the plant height was the highest in plain cowpea, 2M + B and 2M + 2B treatments (Table 2). In mixing systems, since plants compete in terms of light, water and

nutrients at a certain level, differences may occur between processes in terms of morphological features (Portes et al. 1984; Tansı, 1987, Etebari and Tansı, 1994, Geren et al. 2007 and Erdoğdu et al. 2013). In a study conducted by Pekşen and Gülümser (1999) in the conditions of Çarşamba Plain in 1995-1996, they determined the highest average LER in terms of grain and stem yield in different system of corn and bean cultivation, 2M: 1B planting system and the solely planting of plants. Researchers have advised that 2M: 1B planting arrangement would be the most appropriate mixed planting method, since sowing, maintenance and harvesting processes are easier to apply in farmer conditions and large areas. In this study, the most advantageous cultivation for the region in terms of LER value and hay yield was concluded as M + 2B.

Yield

Green forage, hay and crude protein yield values collected from the experiment are given in Table 3. There were significant differences between transactions in terms of all three properties. While the highest green forage and hay yields were obtained from M + 2B and plain corn systems, it was discovered that the crude protein yields obtained from alone corn, cowpea and M + 2B system were higher.

Table 3. Green Forage, Hay and Crude Protein Yields (kg da^{-1}) *

Mixtures	Green Grass Yield	Dry Grass Yield	Protein Yield
	Mean ± SH	Mean ± SH	Mean ± SH
M	8226.6 ± 744.3^a	2873.4 ± 332.4^a	221.2 ± 18.8^a
B	5601 ± 598.5^b	1487.3 ± 208.5^b	220.7 ± 27.4^a
M+B	5186.5 ± 817.0^b	1732.8 ± 291.7^b	132.7 ± 11.5^b
M + 2B	8770 ± 808.0^a	2713.8 ± 444.5^a	204.4 ± 9.1^a
2M + B	3283.5 ± 124.0^c	985.7 ± 10.00^b	63.0 ± 2.9^c
2M + 2B	5658.8 ± 219.0^b	1688.6 ± 66.3^b	130.3 ± 15^b
p	<0.001	0.001	<0.001

* Difference within averages given with the same letter in the same column (p≤0.01)

ADF, NDF and Mineral Substances

Table 4. ADF, NDF, Protein, Ca, Mg, K and P values determined for maize according to the processes (%) *

Mixing Systems	ADF	**K**	P	NDF	Ca	Mg	Protein
Plain Maize	32.65 ± 1.58	0.763 ± 0.139	0.248 ± 0.014	59.82 ± 3.68	0,340 ±0,092	0,340 ±0,092	7.81 ± 0.39
M+B	33.38 ± 2.24	1.025 ± 0.068	0.248 ± 0.011	60.26 ± 3.57	0,303±0,154	0,303±0,154	6.37 ± 0.95
M + 2B	31.1 ± 2.03	0.905 ± 0.26	0.263 ± 0.013	56.26 ± 3.28	0,270±0,097	0,270±0,097	7.10 ± 0.93
2M + B	35.9 ± 1.49	0.453 ± 0.185	0.228 ± 0.005	62.88 ± 2.83	0,278±0,093	0,278±0,093	5.10 ± 0.13
2M + 2B	35.2 ± 1.3	0.835 ± 0.175	0.235 ± 0.005	61.72 ± 2.54	0,260±0,116	0,260±0,116	6.20 ± 0.63
P	0.166	0.234	0.194	0.398	0,234	0,234	0.144

* Difference within averages given with the same letter in the same column (p≤0.01)

When the NIRS properties for corn were examined in terms of ADF, K, P, NDF, Ca, Mg and Protein properties, there was no significant difference between the applications (P> 0.05).

Table 5. ADF, NDF, Protein, Ca, Mg, K and P values determined for cowpea according to the procedures (%) *

Mixing Systems	ADF	K	P	NDF	Ca	Mg	Protein
Plain Cowpea	35.81 ± 1.33	1.49 ± 0.282	0.308 ± 0.008	42.46 ± 2.12	1,308±0,083	0,428±0,014	14.99 ± 0.63
M+B	32.79 ± 0.93	2.103 ± 0.221	0.325 ± 0.014	40.98 ± 1.38	1,095±0,151	0,383±0,030	16.05 ± 1.26
M + 2B	36.79 ± 1.12	1.545 ± 0.093	0.29 ± 0.007	44.77 ± 1.74	1,203±0,058	0,420±0,023	13.12 ± 0.6
2M + B	35.61 ± 1.39	1.938 ± 0.223	0.325 ± 0.017	41.8 ± 1.01	1,265±0,131	0,418±0,028	15.68 ± 1.66
2M + 2B	36.13 ± 1.86	1.568 ± 0.249	0.305 ± 0.009	41.34 ± 1.73	1,450±0,130	0,468±0,026	14.88 ± 0.65
P	0.401	0.275	0.124	0.552	0,311	0,336	0.264

When the NIRS properties of cowpea were examined in terms of ADF, K, P, Ca, Mg, NDF and Protein properties, there was no significant difference between the applications (P> 0.05).

3.4 Land Equivalence Ratio (LER)

The yields from fodder cowpea and silage maize in the mixture plots and the LER values calculated on these yields (Kızılşimşek and Erol, 2000) are given in Table 6.

Table 6.Land Equivalence Ratios (LER) *

Breeding Systems	Dry grass average yield of corn (kg/da)	Cowpea average dry grass yield (kg/da)	Avg. LER value *
Plain Maize	2873.4 ± 332.4^{a}	-	-
Plain Cowpea	-	1487.2 ± 208.5^{a}	-
M+B	1433.8 ± 301.0^{b}	298.9 ± 42.2^{b}	0.71 ± 0.07^{b}
M + 2B	2314.6 ± 443.8^{a}	399.2 ± 23.8^{b}	1.11 ± 0.18^{a}
2M + B	869.0 ± 9.7^{c}	116.7 ± 15.7^{c}	0.40 ± 0.04^{c}
2M + 2B	1390.9 ± 72.4^{b}	297.7 ± 33.4^{b}	0.72 ± 0.06^{b}
p	0.001	<0.001	0.004

* Difference within averages given with the same letter in the same column (p≤0.01)

LER values of lean systems in the chart are calculated as 1.00. Of the mixing systems, M+ B (1434/2873 + 299/1487) = 0.71. In this case, the field use efficiency decreased by 29% (0.71-1 = -0.29) by the growing mixture. In other words, the green forage yield collected from 1 decare of the mixture was obtained from the area of 0.71 da by growing the plants pure. Mix cultivation does not provide an advantage in this case. The situation is the same in other 2M + B and 2M + 2B. Still, LER was found to be 1.11 in the M + 2B system where mixed cultivation is superior to plain cultivation. In other words, ecological resources; therefore, the area was

used 11% (1.11-1 = 0.11) more effectively. In other words, we could obtain the herb in this composition, which we obtained from 1 decare area thanks to the mixture cultivation, from the field only in 1.11 by growing the plants separately. In this system, the total green forage yield is 2315 + 399 = 2714 kg / or lower than plain corn, the total yield of the mixture is higher than the pure cowpea yield. Besides, LER values were calculated on the hay yield of the species and mixture. Yet, in animal production, digestible crude protein ratio is taken into account as much as the amount of forage (Kızıl Şimşek, 2000).

CONCLUSION

In our province, intervention to infrastructural deficiencies of forage crops is minimal due to the land structure and irrigation difficulties. Still, the insufficiency in feeding the local livestock in forage crops necessitates the methods of improving the yield per unit area. In this study, the most advantageous cultivation for the region in terms of LER value and hay yield was concluded as M + 2B.

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SEEDLING PROPERTIES AND FIRE BLIGHT RESISTANCE ON OPEN-POLLINATED *CHAENOMELES JAPONICA* HYBRIDS IN TURKEY

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ABSTRACT

Chaenomeles japonica (Thunb.) Lindl. (Japanese quince) is an important fruit species that widely used for food industry, medicinal purposes and also decorative ornamental. It contains high amount of L-ascorbic acid, pectin, dietary fiber, polyphenols, flavonols, proanthocyanidins, quercetin, catechin, epicatechin, procyanidin, chlorogenic acid, B1, B2. *C. japonica* is also known a new host of *Erwinia amylovora* and there is limited study in worldwide. In this study, seedling properties and fire blight resistance of open-pollinated *C. japonica* hybrids were determined. Germination started at 18 days after sowing and ended 31 days later with 96.42% germination rate. Nearly 6 weeks after seed sowing, seedling deaths were occurred at 23.80% level due to fungal diseases during the development period. Coefficient of variation (CV) was obtained for seedling parameters (total shoot length, stem diameter) and fire blight susceptibility on 3 months old seedlings. CV of total shoot length, stem diameter, and SI were calculated as 19.31, 14.02, and 118.58 %, respectively. It is understood that *C. japonica* has a high germination rate and homogenously seedling reproduction, with seedling parameters that had CV values less than 20 %. Randomly selected forty-one healthy hybrids in active growing period were selected for fire blight inoculation. Fire blight susceptibility index (SI) were ranged 3.90-100 % and hybrids were grouped as highly resistant (15 hybrids), resistant (18 hybrids), medium susceptible (3 hybrids), susceptible (2 hybrids), and highly susceptible (3 hybrids) according to the Gardner scale. As a result of the correlation analysis, significant negative correlation was observed between SI and total shoot length ($R= -0.634$, $P\leq0.0001$), stem diameter $R= -0,5073$, $P\leq0.0001$). According to these results, it was determined that susceptibility of fire blight was high in hybrids which had thinner stem diameter and shorter shoot length. In the light of the results obtained from this study, we suggest that resistant individuals could be used as a parent for registered cultivars or further studies.

Keywords: Seed germination, stem diameter, Japanese quince, *Erwinia amylovora*, disease resistance.

INTRODUCTION

The genus *Chaenomeles* consists four species: *Chaenomeles japonica*, *Chaenomeles speciosa*, *Chaenomeles cathayensis* and *Chaenomeles thibetica*, which are widely cultivated in Japan, Korea, China and Baltic Sea countries (Tics, 1992; Antoniewska et al., 2017). Among them, *Chaenomeles japonica* (Japanese quince), is a dwarf shrub belonging to Rosaceae family, especially can be used as a multipurpose fruit for fruit juice, fibre, flavor, medicinal and also ornamental purposes all over the world. Flower clusters of *C. japonica* have up to 6 flower buds and locate on two years old or older branches (Rumpunen, 2010). Flowering starts on May and

takes up 15 to 22 days depending on air temperature and genotype (Andersone and Kaufmane, 2003). Fruits are small, apple-shaped and nearly 4 cm with yellow green to dark yellow with red spots (Rumpunen, 2002; Dimkova and Mihova, 2019).

Japanese quince can be propagated by tissue culture method, seed, hardwood and softwood cuttings (Kauppinen, 2001; Andersone and Kaufmane, 2003; Çavuşoğlu et al., 2013; Kaufmane and Ruisa, 2018). In our country, it is used only for decorative purposes but in Lithuania and Latvia big orchards have been established and used for fruit production and/or medical purposes. It contains high amount of L-ascorbic acid, pectin, dietary fiber, phenols, polyphenols, flavonols, proanthocyanidins, quercetin, catechin, epicatechin, procyanidin, chlorogenic acid, B1, B2 and minerals Fe, Mg, P, Zn, Mo, Cu, Ca, and also have antibacterial activity (Seglina et al., 2009; Du et al., 2013; Nahorska et al., 2014; Antoniewska et al., 2017; Baranowska-Bosiacka et al., 2017; Urbanavičiūtė et al., 2020). Fruits and seeds of *C. japonica* are used as a raw material with high industrial potential (Antoniewska et al., 2017) and especially fruits are important for using as chemical preservatives in the food and cosmetic industry (Urbanavičiūtė et al., 2020). In addition, fruits can be processed into marmalade, pure, jam, candied fruit, and also be added to jelly, ice cream, yogurt, teas.

Studies on *C. japonica* that can be used as a new fruit crop for our country are limited and also it is only cultivated for as an ornamental plant like in China and many other countries. On the other hand, it is cultivated as a fruit crop in Latvia, Lithuania, Russia, Poland, Belarus, Sweden, and Finland (Panteev et al., 1995; Kauppinen and Weckman, 2002; Rumpunen, 2002; Fedulova et al., 2009; Mierina et al., 2011; Nahorska et al., 2014). Breeding of *C. japonica* was initiated by S. A Ruisa in Latvia nearly 1980's (Panteev et al., 1995) but resistance of abiotic and biotic factors has not been considered although it is reported that this species is important host for lots of diseases and pests (Norin and Rumpunen, 2003; Salmane and Ozoliņa-Pole, 2019). *Erwinia amylovora*, causes fire blight disease, is one of the most destructive disease in Rosaceae family. In addition, *C. japonica* is known a new host of *Erwinia amylovora* (Gavrilovic and Arsenijevic, 1998; Balaž and Smiljanić, 2004) and there is limited study in worldwide. Using fire blight resistant varieties is the key solution of fighting against disease and testing the susceptibility levels of genotypes is necessary to create breeding population (Şahin et al., 2020a).

When the studies are examined, seedling properties and the susceptibility levels of *C. japonica* varieties or genotypes to fire blight have not been found. It was aimed to determine seed germination rate, seedling growth characteristics and the fire blight resistance levels of *C. japonica* hybrids obtained by open-pollination. The results of the study on this subject, which has a limited number of studies in the world, will shed light on the future projects to be carried out for the resistance breeding studies.

Material and Methods

Plant material and seedling properties

Mature *Chaenomeles japonica* fruits were harvested from AARI garden that used for decorative purposes in December 2019. Seeds were prepared for sowing with procedures as seeds keeping in moist perlite for 1 month at +4^{o}C according to Şahin et al. (2020). Seeds were planted in viols and cultural practices were carried out. Germination time and germination rates of Japanese quince seeds were determined. In addition, the rate of loss due to fungal diseases was observed.

Fire blight susceptibility of hybrids

Inoculation was carried out on three months old seedlings with mixture of three *Erwinia amylovora* strains that known high virulent (Şahin et al., 2020a). Inoculation was done out by cutting the plant growth tips with scissors dipped inoculum in the fully automatic cooling system gothic tunnel greenhouse.

After inoculation, plant tips were covered with plastic bags for 72 hours, in order to provide 95-100% relative humidity conditions (Şahin et al., 2020a,b). Subsequently, the susceptibility indexes of hybrids were calculated at 28th day after inoculation by proportioning the total shoot length to the infected shoot length. SI classes of hybrids obtained by using Gardner scale (Gardner et al., 1980) (Table 1).

Table 1. Fire blight susceptibility index (SI) and susceptibility classes of *Chaenomeles japonica* hybrids according to Gardner et al., (1980)

Susceptibility index (SI %)	**Susceptibility class**
0–10	Highly resistant (HR)
10.1–30	Resistant (R)
30.1–50	Moderately susceptible (MS
50.1–90	Susceptible (S)
90.1–100	Highly susceptible (HS)

Statistical analyses

Data on the homogeneity of seedling growth were obtained by calculating the coefficients of variation (CV) in the seedling characteristics (total shoot length, stem diameter) and susceptibility to fire blight parameters. The correlation coefficients and statistical significance between the fire blight susceptibility and total shoot length and stem diameter were evaluated.

Results and Discussion

Germination of *Chaenomeles japonica* seeds were started at 18th day after seed sowing with 19.05 % germination rate and ended at 31st day with 96.43% (Figure 1). Like our study, Rumpunen and Kviklys (1996) suggested that *Chaenomeles* spp. can be propagated by seeds with almost 100% germination rate. Effects of cold stratification times were studied for germination of *C. sinensis* seeds and stratification 60 days at 4ºC on humidified cotton was found the best treatment (Entelmann et al., 2009; Pio et al., 2010). The procedure "keeping in moist perlite for 1 month at +4ºC" that was used for *Cydonia oblonga* (Şahin et al., 2020b), tested in this study and it is thought to be suitable for *C. japonica* too.

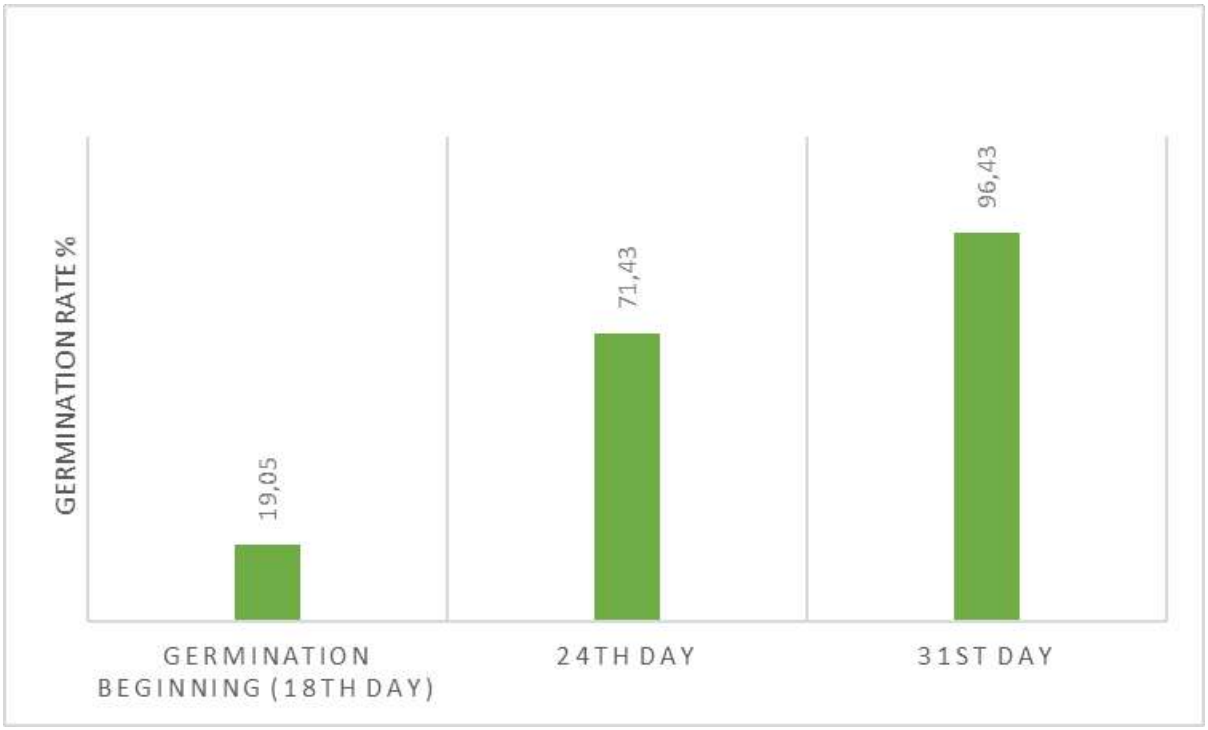

Figure 1. Germination rates of *Chaenomeles japonica* seeds

In the development period of seedlings, approximately 6 weeks after sowing, seedling deaths were occurred 23.80% due to fungal diseases as shown in Figure 2. Fighting against diseases should not be ignored in the cultivation of seedling. When the studies are examined it is observed that fungal diseases which causes fruit spots, fruit rot, and die-back of shoots come to the forefront (Norin and Rumpunen, 2003; Grigaliūnaitė et al., 2012; Jakobija and Bankina, 2018). It is thought that if the production areas increase, some of the fungi that common in other Rosaceae family members will also effect *C. japonica* (Jakobija and Bankina, 2018).

Figure 2. Seedling deaths due to fungal diseases

Standard deviation, coefficient of variation, mean, max., and min. values of total shoot length (cm), infected shoot length (cm), stem diameter (mm), and SI (%) were calculated (Table 2). When the seedling properties were evaluated, total shoot length was changed between 5.00 to 14.50 in hybrid population with 19.31% CV and stem diameter was changed between 1.12 to 2.21 with 14.02% CV. In studies conducted on different plant species, if the coefficient of variation is less than 20%, it is assumed that there is a homogeneous distribution among the examined individuals (Liu et al., 2020). It has been determined that *C. japonica* have a high potential for use as a seedling rootstock, since CV is lower than 20% in total shoot length and stem diameter parameters. Molecular and morphological diversity were studied in populations of *Chaenomeles* spp. and it was found that *C. japonica* and *C. speciosa* were showing wide variation than populations of *C. cathayensis* and *C. thibetica* (Rumpunen et al., 2003).

Table 2. Descriptive statistics for seedling properties and SI traits

Traits	**Mean**	**SD***	**Min.**	**Max.**	**CV****
Total shoot length (cm)	10.33	1.99	5.00	14.50	19.31
Stem diameter (mm)	1.67	0.23	1.12	2.21	14.02
Infected shoot length (cm)	1.98	1.85	0.50	8.60	93.26
SI (%)	22.32	26.47	3.90	100.00	118.58

*SD: Standard deviation, ** CV: Coefficient of variation %.

Wide range of variation was observed on infected shoot length (cm) and SI (%) values as 93.26% and 118.58% CV, respectively (Table 2). Using populations with high variation is necessary in resistance breeding studies. Our findings indicate that open-pollinated *C. japonica* hybrids are suitable for resistance breeding purposes. Previous studies about other Rosaceae family members had showed similar results on importance of open pollination (Evrenosoğlu et al., 2011; Mertoğlu and Evrenosoğlu, 2017; Kaufmane and Ruisa, 2018; Şahin et al., 2020b).

Erwinia amylovora, that causes fire blight in Rosaceae family, is one of the riskiest pathogen of pome fruit production. It has been determined that *Chaenomeles japonica* is one of the new hosts of *Erwinia amylovora* (Gavrilovic and Arsenijevic 1998; Balaž and Smiljanić 2004) but to date there is no report about fire blight susceptibility levels of *Chaenomeles* spp. including *C. japonica* on the worldwide. This study is the first study about fire blight susceptibility on *C. japonica*.

Fire blight susceptibility index (SI %) of *C. japonica* hybrids were found between 3.91 to 100%. When the distribution of hybrids to susceptibility classes is examined, 36.58 % of the hybrids were found in highly resistant (HR) resistant class, 43.93% in resistant (R) class, 7.31% moderately susceptible (MS) class, 4.87% susceptible (S) class, 7.31% highly susceptible (HS) class. Hybrids in HR and R classes can be used as a registered variety or parent after examining the fruit characteristics. In addition, these hybrids could be used for interspecific hybridizations.

Correlation analysis were made on SI between total shoot length and stem diameter parameters (Table 3). As a result of correlation analysis, significant negative correlation was observed between SI & total shoot length (R= -0.634, P≤0.0001), and SI & stem diameter (R= -0,5073, P≤0.0001). In addition, positive correlation was determined between stem diameter and total shoot length at P≤0.0001 significance level. According to these results, it was found that susceptibility of fire blight was high in hybrids which had thinner stem diameter and shorter

shoot length. Similarly, significant and positive correlation was found between shoot length and susceptibility to fire blight in apple, pear and quince genotypes (Özrenk et al., 2012).

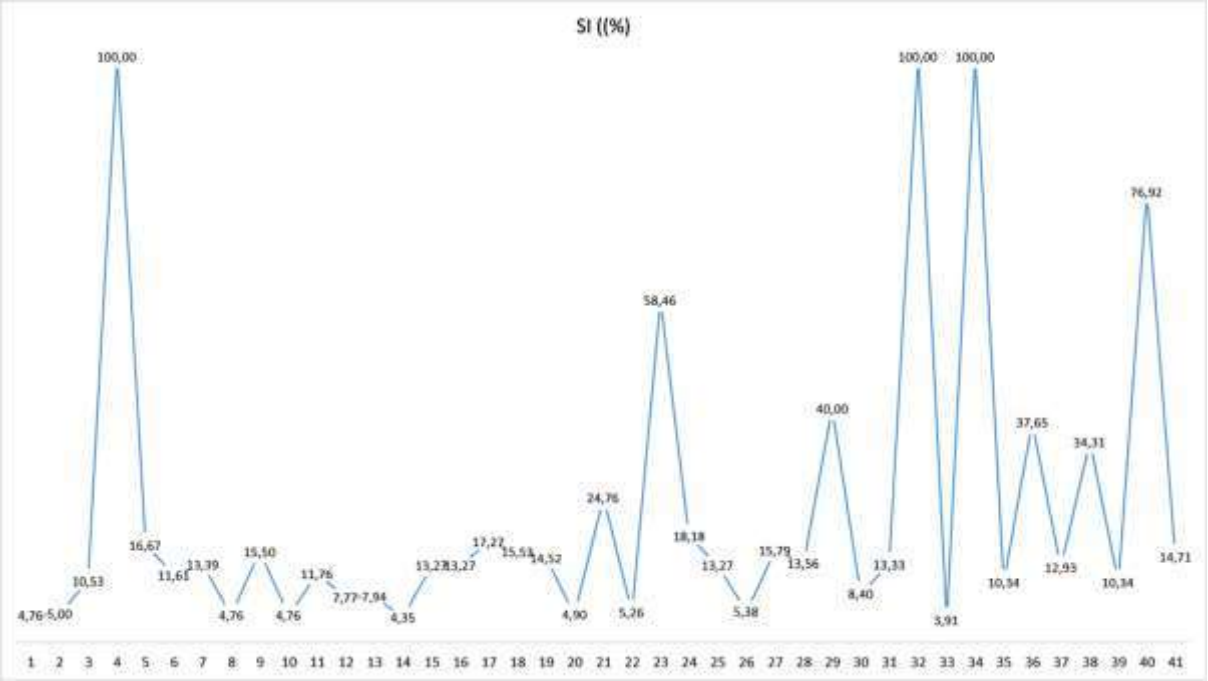

Figure 3. Fire blight susceptibility index (SI %) of *C. japonica* hybrids

Table 3. Descriptive statistics for seedling properties and SI traits

Variable	**by Variable**	**Correlation**	**Signif. Prob**
SI(%)	Total shoot length	-0.6346	<.0001
SI(%)	Stem diameter	-0.5073	0.0007
Stem diameter	Total shoot length	0.6146	<.0001

CONCLUSIONS

As a result of the findings of this study, it is expected that the production potential of *Chaenomeles japonica* will develop as a not only decorative purposes but also fruit crop. Turkey will guide to researchers with this study conducted in terms of resistance to fire blight in this species, which has not been studied on the breeding of resistance to diseases and pests in the world. Also parameters whose correlation is statistically significant can be used as early selection criteria, thus may be allowed the shortening of the breeding period. In the light of the results obtained from this study, we suggest that resistant hybrids could be used as registered cultivars or parent for further breeding studies.

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EFFECTS OF SOYBEANS POWDER ON SENSORY PROPERTIES OF A FISH SHAPED BREAD

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ABSTRACT

Life is the most precious God given asset, which everybody globally cares the most. Daily breakfast consumption is a routine way of keeping our body healthy. Having said this, bread particularly made from wheat flour is daily used over breakfast globally. Therefore, new technologies enchancing nutritional values of bread is required in order to support the measures of malnutrition prevention in every moities of the World. It is in this regard, this study aimed at determining the effect of soybeans powder on enhancing nutritional values of the fish shaped bread, which is a Korean unique product. The study was conducted at food science and technology laboratory of University of Rwanda and designed in triplicates following completely randomized design. The treatments used for this study were: 0% of soybean powder, 10g of red bean paste and 100% wheat flour (Treatment 1), 5% of soybean powder, 10g of red bean paste and 95% wheat flour (Treatment 2), 10% of soybean powder, 10g of red bean paste and 90% wheat flour (Treatment 3) and 15% of soybean powder, 10g of red bean paste and 85% wheat flour (Treatment 4). Results of the study indicated that while fat content experienced a downward trends, soybean powder incorporated into the fish shaped bread substantially enhanced nutritional values of the fish shaped bread (Dry matter, Calcium, Iron, Magnesium, Phosphorous, crude protein, total soluble sugar content and fat content). We concluded that soybean powder deserves to be incorporated into fish shaped bread for improving nutritional values in order to take care of our body by consuming a breakfast using nutrient loaded fish shaped bread.

Keywords: soybean powder, fish shaped bread and nutritional value

INTRODUCTION

Bread is an important staple food for several countries. Wheat flour (*Triticum aestivum*) is more popular than other cereal grains for bread making. Its popularity has stemmed from the gluten and its mild, nutty flavor. Gluten is an essential structure-forming protein which contributes to the elastic characteristics of dough and good appearance of bread (Ibrahim and Krishnan, 2011). Wheat is able to provide you an immense energy source due in all parts of the grain kernel, including the bran, germ, and endosperm. Until recently, the nutrient value of the wheat bran, although long understood, has been ignored and the bran discarded and used as animal feed. However, its rich nutrient composition (Antoine et al., 2004), and its dietary fiber content has motivated numerous campaigns for increasing the consumption of whole wheat products. Whole grain products are perceived as more nutritionally balanced, healthy and natural, being bread the most consumed product (Claupein et al., 2007). The nutrient value of wheat is retained even after processing into flour. However, if you wish to get the maximum benefit out of wheat

products, it is advisable to choose wheat products that are made from whole wheat flour rather than the refined varieties.

Unfortunately, wheat is no longer a healthy food for anyone. Even organic, whole wheat is greatly hybridized and is not healthful. Wheat tends to inflame the intestines due to its high glutamine content today, and it is also much lower in protein and in many essential minerals than it was in the past, say 100 years ago. This fact is well-documented by the US Department of Agriculture and by many scientists as well. It is truly a shame, because wheat was a fine food, and it is used in thousands of prepared food items as a thickener, in flours, and in other ways as well. Wheat bread, especially made of white flour, is one of the worst foods on planet earth because the best part of the wheat flour is removed and fed to the pigs and other farm animals. Whole wheat is not healthful, but at least it is rich in various vitamins, minerals and fiber. To make white bread, however, the germ and the bran of the wheat are stripped away.

This leaves a product that has almost no fiber, almost no vitamins and minerals, and is mostly just starch. It is sometimes called 'empty calories'. It is basically just calories that put on weight, without providing any micronutrients such as minerals at all. It is truly an empty food. One of the worst features of enriched white bread is the addition of iron to the bread. Not only is too much iron put back into the bread. The form of the iron is also not very available to the body, and this is another insult. The iron can build up in the body in a toxic form that is extremely irritating and inflammatory. It can contribute to every possible disease from diabetes and arthritis to cancer.

The fish shaped bread is basically made from wheat flour and red beans. Dried red beans are nutrient-dense and the amount of nutrients provided per calorie is particularly high. Dry beans have been used as a staple of the diet, and the health benefits derived from them have been well recognized, the popularity of bakery products has contributed to increased demand for ready-to-eat, convenience food products, such as bread, biscuits and other pastry products (David, 2006). Therefore, soybean powder was used in this study with an eye of enhancing nutritional values of the fish shaped bread. The outcome of this study may provide nutritional benefits to the diet, and help to reduce disease risk and enhance longevity.

MATERIAL AND METHODS

This study of evaluating the effects of soybean on enhancing nutritional values of the fish shaped bread was conducted at UR-CAVM Laboratory in triplicates following a completely randomized design. Wheat flour, sugar (300g), milk (1000ml), baking powder (50g), chipsy (135g), water (300-400g), salt (3-5g), red bean paste and soybeans powder are the materials used in this study for producing fish shaped bread. Four bread samples were prepared from Samples made of 0% of soybeans powder, 10g of red bean paste and 100% wheat flour [S1], Samples made of 5% of soy beans powder, 10g of red bean paste and 95%wheat flour [S2], Samples made of 10% of soy beans powder, 10g of red bean paste and 90%wheat flour [S3] and Samples made of 15% of soy beans powder, 10g of red bean paste and 85% wheat flour [S4].

LABORATORY ANALYSIS

After producing the fish shaped bread, the following chemical properties were analyzed:

Determination of dry matter

The dry matter of food would include carbohydrates, fats, proteins, vitamins, minerals and antioxidants (e.g., thiocyanate, anthocyanin, and quercetin). 5g for each type of fish shaped bread flour were weighed and put in dried crucibles and then put in the oven. The oven should be maintained at $103+2^0$C until getting a constant weight. It was then cooled and weighed. This process was done until the decrease in mass between successive weighing does not exceed 0.05mg per gram of sample loss in weight (AOAC, 1990)

The percentage of dry matter is calculated by:

Weight of the crucible= w_0

Weight of the crucible + wet sample = W_1

Weight of the crucible + dry sample = W_2

Moisture content of the sample (%) = $\frac{W1-W2}{W1-W0} \times 100$

Determination of crudes ashes

Ash content was determined by ignition of the samples resulting from the determination of moisture content in a muffle furnace at 550℃ for 8 hours and the percentage of ash is calculated as follow:

Ash content of the fresh sample (%) =$\frac{W2-W0}{W1-W0} \times 100$

Where:

Wo is the weight of clean dry crucible

W2 is the weight of the clean dry crucible + ash content

W1= weight of clean dry crucible + dry sample

Proteins determination

The proteins were determined with Kjeldhal method

$$N\% = \frac{(T-Bl) \text{ x } 0.2 \text{ x } FC6.25 \text{ x } FD\ (10) \text{ x } 100}{\text{Sample weight x } 1000}$$

The amount of protein in most samples is obtained by multiplying the percent nitrogen with the appropriate conversion factor, the conversion factor is 6.25.

% proteins = % Nitrogen × 6.25

Total Fats Determination

Total fats determination was determined in line with (Pomeranz and Meloan, 1977).

Determination of minerals contents

Minerals contents (Ca and Mg) were determined using EDTA Method (Kilyobo, 2009). Determination of iron was realized by using nitro-perchloric method as well as phosphorus content was determined from the samples.

Extraction of soluble sugars

Approximately 0.1g dried sample was weighed, finely ground and then put it in a plastic bottle. 25 ml of the ethanol 80% was added and the mixture was agitated for 2 hours using electric agitator. The extract of soluble sugars was carefully transferred in a centrifugation tube and centrifuged at 3000 rpm for 15 minutes. The aliquot of 1ml starting from a supernatant was added into pipette. 1ml of phenol 5% and 5ml of the concentrated sulphuric acid were added this aliquot, and then the mixture was vigorously mixed to homogenize the color. The absorbance was determined at 490 nm.

$$\% \text{ SS} = \frac{\text{AbscxFCxFDx100x10}-6}{\text{Sample weight}}$$

FC= calibration factor

FD= dilution factor = 7x25

Sample weight= 0.1gr

Determination of starch

The residue remaining after extraction of soluble sugars was used to determine starch content as they contain starch (insoluble complex sugars). This starch underwent the acid hydrolysis to obtain dextrose.

% of Starch = Weight of dextrose + weight of CO_2

$$= \frac{[\text{AbscxFCx7x5x100+ (nber moles dextrose} - 1) \text{ x } 44]\ 10^{-6}}{0.1} \text{ x } 100$$

Statistical analysis

Data were subjected to One way ANOVA and Minitab software was used for determining significant difference among properties at 5% significant level.

RESULT AND DISCUSSIONS

The parameters analyzed in this study were dry matter, ash content, proteins, fats, soluble sugars and minerals for four fish shaped bread samples. The following nutritional properties of the fish shaped bread samples were analyzed and discussed by mean of charts.

Dry* matter *(DM)

As shown in the Figure 1, S4 showed the higher amount of DM compare to the other samples but the effect seemed not really significant.

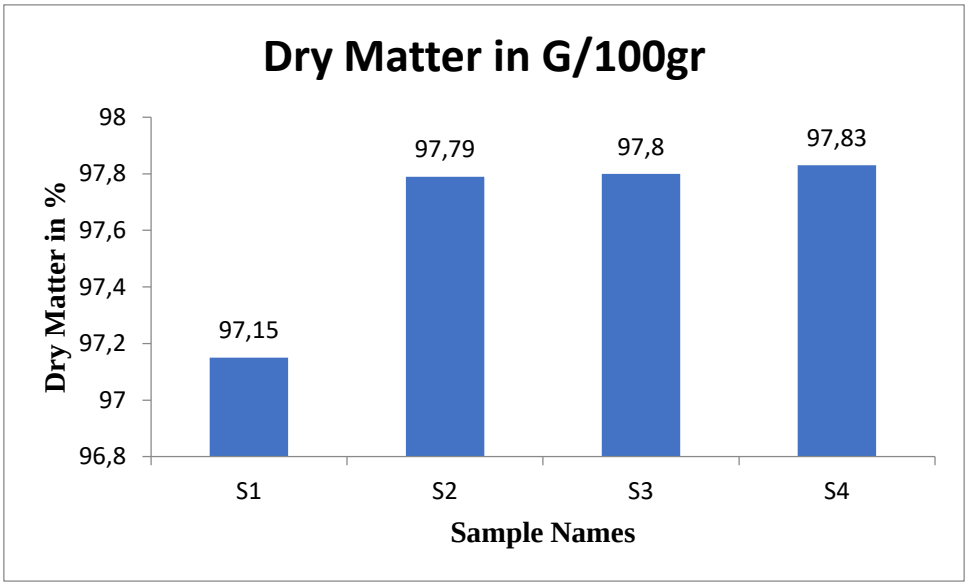

Figure 1: Effects of soybean on dry matter content of the fish shaped bread

Ash content

As shown in the figure 2, the ash content of S4 was lower compare to other samples (7.47) but the difference was not really significant, this may due to the increased amount of soybeans powder and decreased amount of wheat flour.

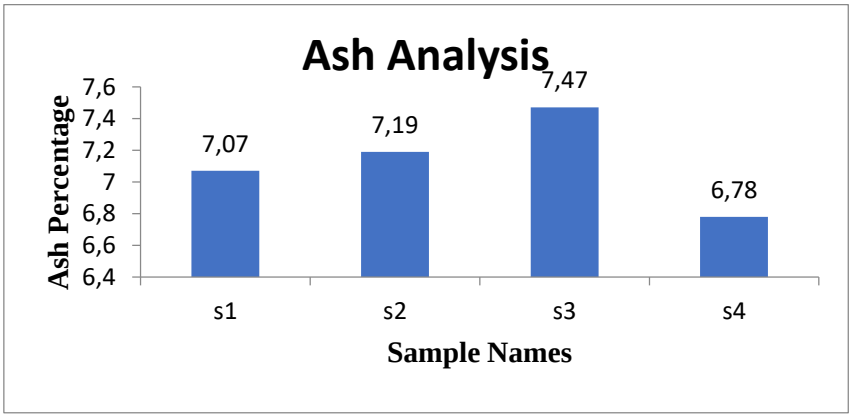

Figure 2: Effects of soybean on ash content contents of the fish shaped bread

Minerals (calcium: Ca, Phosphorus: P, Magnesium: Mg and Iron: Fe)

The recommended dietary allowance for calcium is 1,000 milligrams per day. Women over age 50 need 1,200 milligrams, while men don't need this higher amount until after age 70.The mineral compositions of the fish shaped bread is generally higher in S4 than other samples (Figure 3) and also this minerals are highly concentrated compared to their concentration in wheat and red beans, indicating the effectiveness of soybeans in enhancing nutritional values of the fish shaped bread.

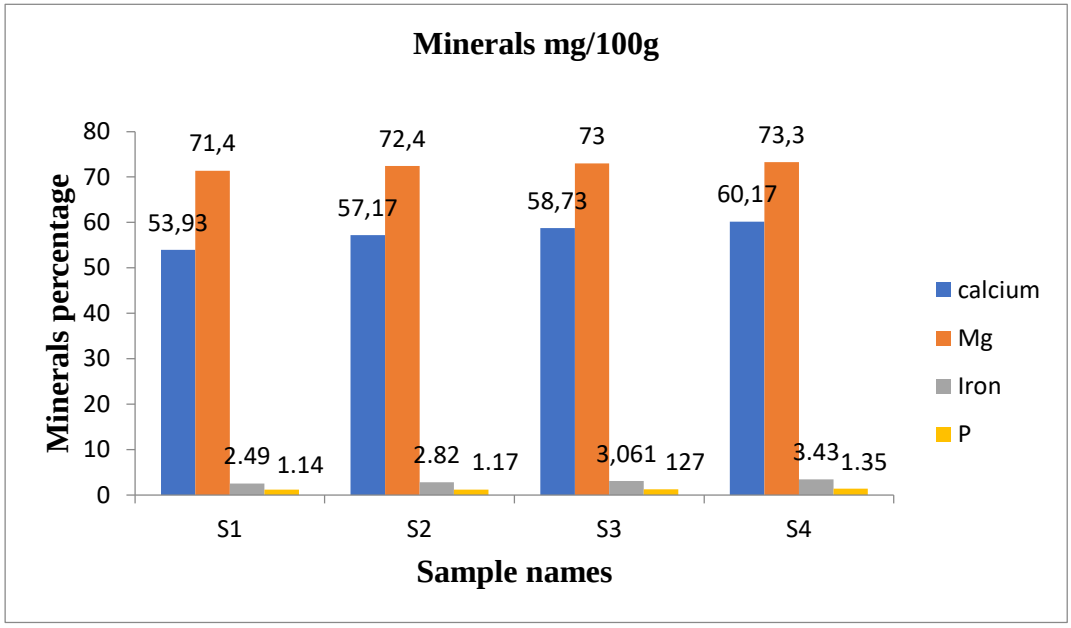

Figure 3: Effects of soybean on mineral contents of the fish shaped bread

Soluble sugars and starch

Our body uses carbohydrates to make glucose which is the fuel that gives the body energy and helps keep everything going. Our body can use glucose immediately or store it in your liver and muscles for when it is needed. Healthier foods higher in carbohydrates include ones that provide dietary fiber and whole grains as well as those without added sugars. "Good" carbohydrates are used to describe foods that have more fiber and complex carbohydrates. Complex carbohydrates are carbohydrates that take longer to break down into glucose; such as vegetables, fruits, whole grains and beans. Examples include white bread, Fish bread etc.

As shown in the Figure 4, the highest soluble sugars were observed in S4 compared to other samples, indicating the powerful effect of soybean on the fish shaped bread. However, starch content decreased as the amount of soy beans powder increased (Figure 5).

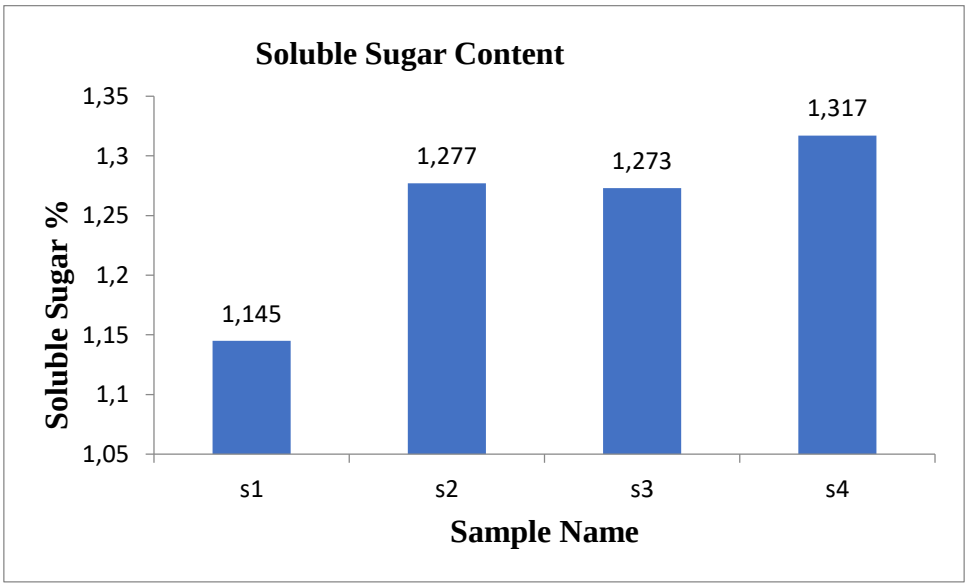

Figure 4: Effects of soybean on soluble sugar content of the fish shaped bread

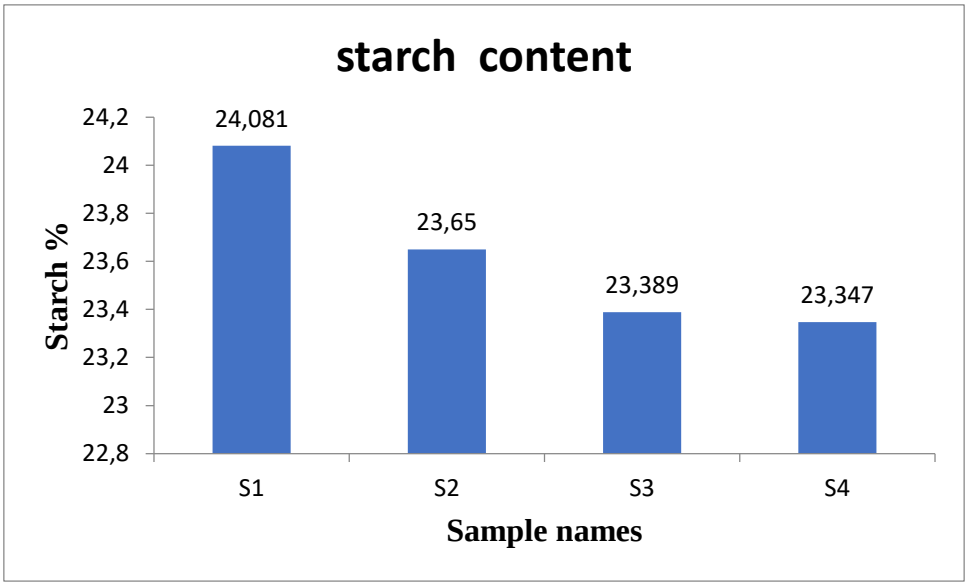

Figure 5: Effects of soybean on starch content of the fish shaped bread

Protein

Protein is the main structural component of all cells in human body. Enzymes and some hormones are also proteins. Food proteins break down into amino acids during digestion, and human body rebuilds them into whatever it needs, most people get more or less protein to meet their needs. It's rare for someone who is healthy and eating a varied diet to not get enough protein. Wheat contains low amounts of certain essential amino acids; however, these same essential amino acids are found in greater amounts in dry beans. Similarly, dry beans contain lower amounts of other essential amino acids that can be found in larger amounts in wheat. Together, these two foods can provide adequate amounts of all the essential amino acids the body needs (Source: Acceptable Macronutrient Distribution Range (AMDR) Protein analysis show that S4 has higher protein of 20.36% and S1 shown the lower percentage of 18.62 with the mean value of 19.16 (35% of DRA) (Figure 6), thus there are impacts soybeans powder to the whole protein content of fish bread compared to protein content its raw materials.

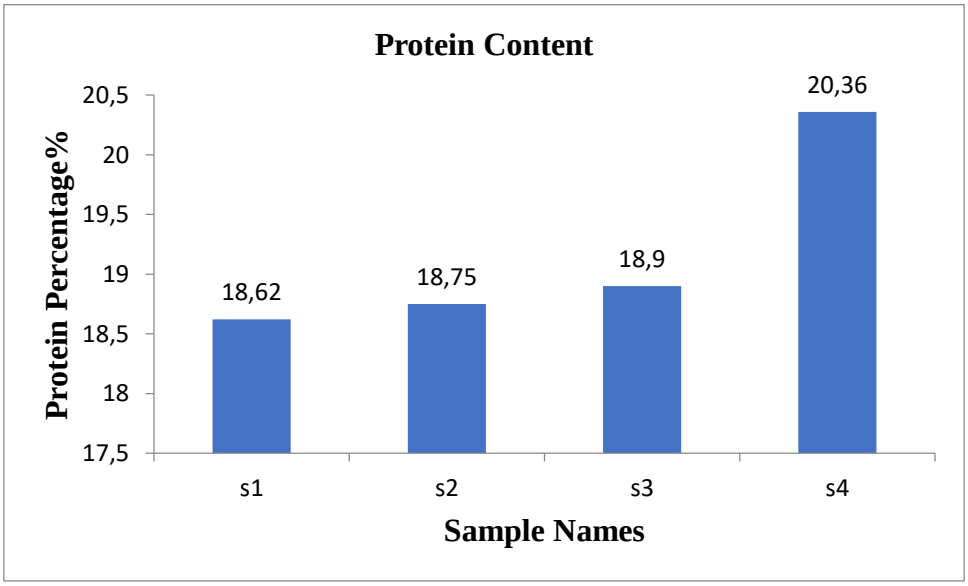

Figure 6: Effects of soybean on protein content of the fish shaped bread

Fats content

As shown in figure 7, the S4 showed higher amount of fat contents compared to other samples because of higher amount of fats found in soybeans powder and the mean value is 14.29% (20% of DRA of an adult). Fat contributes to energy intake and helps the body absorb vital vitamins; therefore a healthy diet should always contain a certain amount of fat. The two main forms of fat are saturated, predominately from animal sources, and unsaturated, predominately from vegetable sources. Fats and oils are necessary in a healthy diet. Some are called "essential" because the body cannot make them, and they are required for life. Because fat is a rich source of energy, people should try and eat no more than recommended intake. The reference value for fat for an average adult is 70 grams (Source: Acceptable Macronutrient Distribution Range (AMDR). It's best to keep the body total fat intake between 20 and 35% of the body total calories each day, a healthy eating pattern includes between 20% to 35% of your day's calories from fat. For women, this works out to 45 to 75 grams of fat a day, and for men, 60 to 105 grams of fat a day. (For example, 15 ml (1 tbsp) of oil equals about 14 grams)

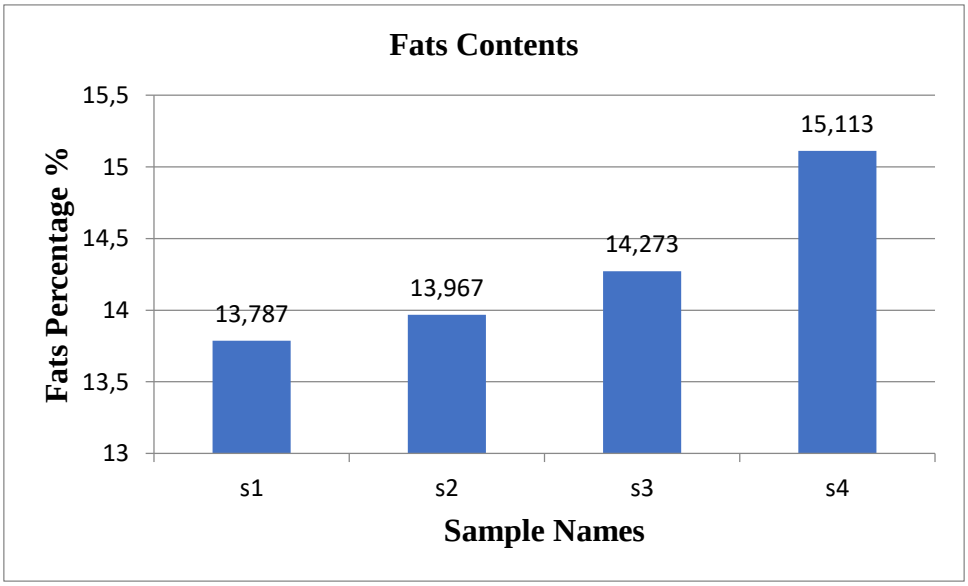

Figure7: Effects of soybean on fat content of the fish shaped bread

Table 1: Biochemical &Chemical properties of fish shaped bread (Dry basis)

Sample	DM %	Protein %	Fat %	Ashes %	Calcium mg/100gr	Magnesium mg/100gr	Soluble sugars %	Starch %	Iron mg/100gr	Phosphorus mg/100gr
S1	97.15	18.62	13.787	7.07	5.393	7.140	1.145	24.081	0.2485	0.1004
S2	97.79	18.75	13.967	7.19	5.717	7.240	1.277	23.650	0.2822	0.1173
S3	97.80	18.90	14.273	7.47	5.873	7.300	1.273	23.389	0.3061	0.1273
S4	97.83	20.36	15.113	6.78	6.017	7.330	1.317	23.347	0.4284	0.1483

S1: Samples made of 0% of soy beans powder, 10g of red bean paste and 100% wheat flour.

S2: Samples made of 5% of soy beans powder, 10g of red bean paste and 95%wheat flour.

S3: Samples made of 10% of soy beans powder, 10g of red bean paste and 90%wheat flour.

S4: Samples made of 15% of soy beans powder, 10g of red bean paste and 85% wheat flour.

CONCLUSION

This study indicated that soybean incorporation into the production of the fish shaped bread had a significant impacts in enhancing nutritional qualities of the fish shaped bread. Therefore, based on the results from this study, we concluded that soybean powder deserves to be incorporated into fish shaped bread production for improving nutritional values in order to take care of our body by consuming a breakfast using nutrient loaded fish shaped bread, thereby contributing to the reduction of malnutrition as consumption of bread increase in Rwanda.

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THE FIRST CHARACTERIZATION OF POMEGRANATE SEEDLINGS OF HICAZNAR AND SILIFKE AŞISI CULTIVARS

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ABSTRACT

Turkey is one of the major pomegranate producing countries in the world and it has made considerable progress in pomegranate production, processing, and marketing. The total pomegranate production of Turkey reached approximately 560 thousand tonnes in 2019. Turkey is also an important pomegranate exporter country in the world, pomegranate export of Turkey increased at 203 thousand tons in 2018. Although Hicaznar cv. has been mainly growing and exporting in Turkey, new varieties are still needed for pomegranate cultivation. The aim of this study was to obtain new pomegranate varieties with soft seeds, red fruit peels, and arils, and some other superior properties. In the study, 300 pomegranate seedlings were obtained from open-pollinated seeds of Hicaznar and Silifke Aşısı cultivars. The pomegranate seedlings obtained were planted at a distance of 5x1.5 m. in the field at Alata Horticultural Research Institute. The fruits were obtained from 134 pomegranate seedlings. After, pomological characters belonging to all seedlings have been examined. It has determined that 24 seedlings have soft seed, 27 seedlings have medium-soft seed and 83 pomegranate seedlings have hard seeded. The fruit peel and aril colors of the seedlings showed a wide distribution.
Keywords: *Pomegranate, Breeding, Seedling, Variety*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a traditional and widely grown fruit species in Turkey. In recent years, pomegranate fruits have been in demand in worldwide markets because of their superior pharmacological and therapeutic properties (Özgüven et al., 2015). The total pomegranate production of Turkey was 560 thousand tonnes in 2019. The biggest production in 2019 was grown in Antalya as 130 031 tons, which Antalya is a coastal city in the Mediterranean region, and has a lot of regular pomegranate orchards. The second and third provinces in its production in 2019 are Mersin and Muğla provinces which have 87 239 and 86 608 tons respectively. 71 066 tons of pomegranates were produced in Adana province in 2019 (Anonymous, 2020).

There are different types and forms because Turkey takes place in native spreading areas of the pomegranate. In this respect, many selections are found the result of the several breeding works, and some of these were registered in Turkey (Özgüven and Yılmaz, 2000, Özgüven et al., 2015). In the light of the research done in Turkey, there are a lot of pomegranate varieties. Among these varieties, in respect of fruit taste, there are sour, sour-sweet, and sweet varieties. Also, in terms of seed toughness, there are different types such as soft seed, intermediate, and hard seed. Hicaznar, Çekirdeksiz VI, Silifke Aşısı, Katırbaşı, Lefan are some of the most important varieties are grown in Turkey (Özgüven and Yılmaz, 2000, Özgüven et al., 2015).

Turkey is among the countries exporting pomegranates in the world. In our country, Hicaznar variety is mostly exported due to its attractiveness. However, exports of seedless varieties have been started in recent years. Most of the seedless varieties grown in our country have a very low appeal and they have some quality problems. There is a big problem, especially in coloration. In this respect, new pomegranate varieties are required in our country to increase the consumption of table pomegranate and export of pomegranate.

The aim of this study was to obtain new pomegranate varieties with soft seeds, red fruit peels, and arils, and some other superior properties.

Material and Methods

In the study, 300 pomegranate seedlings were obtained from open-pollinated seeds of Hicaznar and Silifke Aşısı cultivars. Hicaznar is the main variety in pomegranate production in Turkey. The tree is moderately strong growth rate and high yielded. The peel color is dark red, aril and juice color are dark red. It has a sweet-sour taste and intermediated seed hardness. Silifke aşısı is strong growth rate and high yielded. The peel color is attractive yellow-pink, aril and juice color are pink-red. It is a sweet-sour and hard seeded variety.

The pomegranate seedlings obtained were planted at a distance of 5x1.5 m. in the field at Alata Horticultural Research Institute Erdemli, Mersin. The experimental orchard was fertigated by drip irrigation and trained on multi-trunk.

Pomological analyses were done after harvest in ten fruits for each genotype. Fruit weight, fruit diameter, fruit length, aril rate, juice rate, peel thickness, total soluble solid (TSS) content, acidity, peel color, aril color, taste, seed hardness were determined at fruits in pomological analyses

Results and Discussion

The pomological traits of 15 pomegranate genotypes (Figure 1) were shown in Tables 1, 2, and 3. It has been shown that the pomological attributes of the seedlings showed a wide distribution.

When fruit characteristics were investigated, the highest fruit weight was determined in “3-50” and (593,8 g) and “3-22” (582,8 g), the lowest in “1-14” (218,5 g). The fruits of “3-50” have the largest diameter (106,52 mm), while the fruits of “1-14” had the smallest diameter (76,45 mm). While 3-22 has the longest fruits (97,29 mm), “1-14” has the shortest fruits (66,37 mm).

It was determined that “3-22” (66.2 %) has the highest aril ratio, “2-27” has the lowest aril ratio (42,9 %). “3-22” (55.3 %) has the highest juice ratio, while “2-27” (34,7 %) have the lowest juice rates. “1-30” (4,43 mm), “3-50” (431 mm), and “3-62” (4,28 mm) have the thicker fruit peel, while 1-25 (2,24 mm) has the thinnest fruit peel. “3-22” genotype has the biggest (52,8 g) 100 aril weight, although “1-10” was the smallest (22,7 g). Sepal number of seedlings varied 5-6. Sepal diameters were between 15,67 mm and 26,87 mm, sepal lengths were between 14,09 mm and 28,87 mm.

Peel and aril colors are very important characters in pomegranate production and trade in the world. Dark red colored cultivars are more preferred. In the experiment, it was found that most of seedlings have red or dark red peel and aril colors. But some of these are both dark red and soft seeded. It was determined that “P153” (18.1 %) and “1-30” (17.2 %) have the highest TSS rates, “2-31” (14.0 %), and “1-13” (14.6 %) have the lowest TSS rates.

Table 1. The pomological traits of 15 pomegranate seedlings in Mersin ecological conditions.

Genotypes	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Fruit shape Index	Peel thickness (mm)	100 aril weight (g)
1-10	425,2	100,00	76,60	0,77	2,98	22,7
1-13	376,9	94,66	83,07	0,88	3,00	31,9
1-14	218,5	76,45	66,37	0,87	3,93	32,4
1-25	413,3	95,69	81,07	0,85	2,24	33,7
1-30	366,5	92,53	76,69	0,83	4,43	41,3
2-6	467,5	94,62	91,09	0,96	3,22	38,9
2-27	496,7	98,53	83,32	0,85	4,05	30,2
2-31	421,7	99,33	81,21	0,82	3,52	33,5
2-49	301,2	90,28	74,24	0,82	3,74	24,8
2-52	385,0	89,69	81,41	0,91	2,56	36,4
3-22	582,8	100,61	97,30	0,60	2,49	52,8
3-27	420,0	94,73	79,87	0,84	4,03	26,0
3-50	593,8	106,52	90,98	0,85	4,31	35,6
3-62	283,6	81,21	73,49	0,90	4,28	30,8
P153	255,0	82,70	69,08	0,97	2,50	26,4

Table 2. The pomological traits of 15 pomegranate seedlings in Mersin ecological conditions.

Genotypes	Sepals (No.)	Sepal length (mm)	Sepal diameter (mm)	Peel color	Peel background-color	Aril color
1-10	6	17,16	19,95	Dark red	-	Dark Red
1-13	5	15,34	18,57	60 % Pink	55% Yellow	Light Pink
1-14	6	15,54	18,42	Red	-	Red
1-25	5	15,90	17,29	Red	10% Yellow	Dark Red
1-30	6	26,66	18,20	Pink	-	Red
2-6	6	18,37	18,89	Pink	-	Drk Red
2-27	5	19,73	19,44	20% Pink	80% Yellow	Light Pink
2-31	6	22,15	18,57	Dark Pink	-	Pink
2-49	5	16,60	17,39	Dark Pink	-	Claret Red
2-52	6	17,49	15,80	Pink	40% Yellow	Dark Red
3-22	6	14,09	15,67	Pink	-	Pink
3-27	6	18,62	26,87	Dark Pink	-	Dark Red
3-50	6	18,85	25,93	Red	50% Yellow	Red
3-62	5	28,87	22,45	80% Red	20% Yellow	Red
P153	6	15,90	19,57	Claret Red	-	Claret Red

Table 3. The pomological traits of 15 pomegranate seedlings in Mersin ecological conditions.

Cultivars	**Aril ratio (%)**	**Juice ratio (%)**	**TSS (%)**	**Taste**	**Seed hardness**
1-10	51,2	38,5	15,0	Sour-sweet	Intermediate
1-13	55,4	43,8	14,6	Sour-sweet	Intermediate
1-14	50,5	42,1	16,0	Sour-sweet	Soft
1-25	65,1	53,4	15,9	Sweet	Intermediate
1-30	57,4	41,9	17,2	Sour-sweet	Intermediate
2-6	55,9	46,1	15,2	Sour-sweet	Intermediate
2-27	42,9	34,7	15,2	Sour-sweet	Soft
2-31	54,2	39,5	14,0	Sour-sweet	Soft
2-49	56,1	45,0	16,0	Sweet	Intermediate
2-52	51,4	41,9	16,3	Sweet	Intermediate
3-22	66,2	55,3	14,9	Sour-sweet	Soft
3-27	46,2	36,7	15,8	Sour-sweet	Soft
3-50	52,5	42,6	15,6	Sour-sweet	Intermediate
3-62	44,1	36,5	15,0	Sour-sweet	Intermediate
P153	54,9	40,7	18,1	Sweet	Hard

Figure 1. Some of successful pomegranate genotypes in the experiment

Conclusions

In the study, 300 pomegranate seedlings were obtained from open-pollinated seeds of Hicaznar and Silifke Aşısı cultivars. The pomegranate seedlings obtained were planted at a distance of 5x1.5 m. in the field at Alata Horticultural Research Institute. The fruits were obtained from 134 pomegranate seedlings. After, pomological characters belonging to all seedlings have been examined. It has determined that 24 seedlings have soft seed, 27 seedlings have medium-soft seed and 83 pomegranate seedlings have hard seeded. The fruit peel and aril colors of the seedlings showed a wide distribution. The evaluation process of the pomegranate seedlings obtained continues. When all seedlings are evaluated, the "1-14" genotype can be considered as a candidate for a new variety with soft seed and dark red color and sour sweet taste.

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THE STATUS OF PEEL MINERAL CONTENTS AND POMOLOGICAL PROPERTIES RELATION TO FRUIT CRACKING IN POMEGRANATE

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Abstract

There are a lot of problems in pomegranate growing. The fruit splitting is the main quality problem in pomegranate growing. Fruit splitting is a physiological disorder that occurs because of the different growth rates between peel and flesh of fruit. There are a lot of causes for fruit cracking in pomegranate growing. The aim of this experiment was examination of the effects of peel mineral content and pomological properties on fruit cracking in pomegranate (Punica granatum L.). The experiment was carried out on İzmir 8, İzmir 10, İzmir 16, İzmir 1264, İzmir 1513, Wonderful and Türkmen cvs. as 5 replicates at the pomegranate orchard of Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. The fruit peel minerals and water contents and pomological characters have been examined in cracked and intact fruits belonging to all cultivars. It was determined that the cracked fruits have lower fruit weight, size, peel thickness, peel water ratio, and potassium contents.

Keywords: *Pomegranate, Fruit cracking, Mineral, Pomology.*

INTRODUCTION

There are a lot of problems in pomegranate growing in Turkey. Fruit cracking is a very common physiological disorder in pomegranate cultivation. Fruit cracking is a problem seen in many species of horticultural crops like apple, pear, plum, peach, apricot, cherry, sour cherry, orange, mandarin, grape, persimmon, pomegranate, pineapple, lychee, tomato, pepper, melon, and watermelon (Yılmaz and Özgüven, 2003). Fruit splitting in pomegranate is a physiological disorder that occurs because of the different growth rates between peel and flesh of fruit. There are a lot of causes for fruit cracking in pomegranate growing (Yılmaz and Özgüven, 2006). It usually occurs at the maturity stage and the amount of cracked fruit increases as maturity progresses. Since the pomegranate fruit consists of many watery arils, there is an internal pressure created on the peel. This pressure increases even more as a result of increasing maturity and increasing water content of the arils. Fruit cracking is caused by the sudden rupture of the peel that cannot withstand the internal growth pressure. There are many factors that encourage fruit cracking. Fruit cracking in pomegranates is more important than other physiological problem types. Due to this problem, there may be a high amount of yield loss. The main reasons of fruit cracking are genetic factors, delay of harvest, and fluctuating soil moisture levels. The other factors are deficiencies of some plant nutrients, sunburn on fruit peel, the injures on fruit peel, some pathogenic factors, and some insect injuries (Yılmaz 2005; Yılmaz, 2007).

The aim of this experiment was examination of the effects of peel mineral content and pomological properties on fruit cracking in pomegranate (*Punica granatum* L.).

Material and Methods

The experiment was carried out with 7 pomegranate cultivars ('Wonderful', 'Türkmen', 'İzmir 8', 'İzmir 10', 'İzmir 16', 'İzmir 1264', and 'İzmir 1513') at 7 years old trees in 4 replicates of randomized complete blocks in the Alata Horticultural Research Institute, Erdemli, Mersin. The pomegranate trees were pruned as the multi trunk. The trees were irrigated by drip irrigation and fertilized.

In this experiment, cracked and healthy fruits were sampled at harvest time. After harvest, pomological and mineral analyses were done. Fruit weight, diameter, and length, aril rates, peel thickness, moisture contents of peel, and aril were determined. Nitrogen content was determined by Kjheldahl method. Barton method was used for phosphorus content. Other mineral contents (K, Ca, Mg, Fe, Zn, Mn, Cu) in peels were analyzed by ICP. In the experiment, the data were analyzed using the SAS statistical program.

Results and Discussion

Pomological attributes

The pomological attributes of cracked and healthy fruits of the 7 pomegranate cultivars are shown in Table 1 and 2. It was determined that there were no significant differences between fruit weight, fruit diameter, fruit index, aril percentage, peel thickness, and aril's moisture content of cracked and healthy fruits. However, it was found that the differences between fruit length values and peel's moisture contents of cracked and healthy fruits were statistically significant. Cracked fruits were found to be shorter in fruit length and flattened. Considine and Brown (1981) and SaeiAhagh et al. (2015) demonstrated that fruit shape had an influential effect on fruit cracking. Considine and Brown (1981) reported that in spheroid fruits, pressure on the containing membrane increases when the shape deviates from a perfect spheroid toward oblate or prolate. This shape deviation implied the inner fruit pressure this made more sensitive to cracking.

The difference between the mean values of the peel's moisture contents of the cracked and healthy fruits was statistically significant. It was found that the peel's moisture contents of the cracked fruits have less moisture levels than those of healthy fruits. Erickson (1957), in his research on the Washington navel orange variety, determined that the peels of cracked fruits contain less water than the peels of intact fruits and stated that this difference is due to the more water loss of the cracked fruits. As the fruit cracks, more water loss will occur in the cracked areas of the peels. Therefore, the water rate in the peel of cracked fruits will be lower.

Table 1. The effects of fruit weight, diameter, and length on the fruit cracking in seven pomegranate cultivars

Cultivars	Fruit weight (g)		Fruit diameter (mm)		Fruit length (mm)		Fruit index (length/diameter)	
	Split	Healthy	Split	Healthy	Split	Healthy	Split	Healthy
Won	445,2	420,7	94,1	92,7	81,8	83,2	0,88	0,88
Türkmen	346,8	340,2	87,1	87,8	81,2	82,2	0,92	0,94
İ-8	294,5	344,3	85,3	88,1	79,3	75,9	0,90	0,89
İ-10	314,3	316,8	87,0	85,5	78,6	81,6	0,92	0,94
İ-16	292,7	225,2	85,3	79,4	71,0	78,4	0,89	0,92
İ-1264	468,2	460,2	95,8	95,3	84,0	89,2	0,88	0,93
İ-1513	413,0	370,8	91,7	89,1	80,0	83,1	0,90	0,91
Mean	367,8	354,0	89,5	88,3	79,4b	81,9a	0,90	0,92
$LSD_{0,05}$	NS		NS		2.21		NS	

Table 2. The effects of fruit index, aril ratio, and peel thickness on the fruit cracking in seven pomegranate cultivars

Cultivars	Aril percentage (%)		Peel thickness (mm)		Moisture content of peel (%)		Moisture content of arils (%)	
	Split	Healthy	Split	Healthy	Split	Healthy	Split	Healthy
Won	58,5	51,9	3,3	3,9	69,1	72,6	77,1	77,4
Türkmen	61,9	60,7	3,1	3,0	68,3	71,7	77,4	77,2
İ-8	56,6	55,6	4,3	4,4	67,2	70,4	75,0	73,6
İ-10	58,6	57,4	3,7	3,9	67,1	71,1	73,8	73,4
İ-16	52,6	56,1	3,3	4,1	66,9	70,6	72,2	75,5
İ-1264	51,7	49,9	4,2	4,1	71,1	73,0	76,5	76,1
İ-1513	53,4	53,2	4,4	4,5	70,1	70,9	73,8	74,2
Mean	56,2	55,0	3,8	4,0	68,6b	71,5a	75,1	75,3
$LSD_{0,05}$	NS		NS		1,61		NS	

Mineral Contents

The contents of N, P, K, Ca, Mg, Fe, Zn, Mn, Cu in cracked and healthy fruit peels of the 7 pomegranate cultivars are shown in Table 3, 4, and 5. The rates of N/K, K/Ca are shown in Table 5.

The difference between the average fruit peel nitrogen and phosphor values of cracked and healthy fruits was nonsignificant. Hovewer, the potassium levels in cracked and healthy fruit peels were found as significant by statistically. When the potassium contents of the peels of cracked and healthy fruits were compared, it was determined that the peels of the cracked fruits contained lower levels of potassium than the healthy ones. The reports of Bar-Akiva (1975), Hasan and Chattopadhyay (1996), Koo (1961), and Yılmaz and Özgüven (2019) supported the results of the experiment.

The difference between the mean fruit peel calcium values of cracked and healthy fruits was statistically significant It has been determined that the peels of the cracked fruits contain lower calcium than the healthy fruits. There was no statistically significant effect of other elements (Mg, Fe, Zn, Mn, and Cu) on fruit cracking. It was determined that the peels of the cracked

fruits contain N/K values higher than those of the healthy fruits. The K/Ca ratio was found to be lower in cracked fruit peels than in the healthy fruit.

Table 3. The effects of N, P, K, and Ca contents of the peel on the fruit cracking in seven pomegranate cultivars

Cultivars	N (%)		P (%)		K (%)		Ca (%)	
	Split	Healthy	Split	Healthy	Split	Healthy	Split	Healthy
Won	0,49	0,48	0,09	0,09	1,21	1,27	0,27	0,25
Türkmen	0,44	0,48	0,10	0,10	1,18	1,42	0,15	0,19
İ-8	0,37	0,31	0,08	0,09	1,13	1,27	0,12	0,29
İ-10	0,40	0,45	0,07	0,07	1,16	1,20	0,25	0,23
İ-16	0,61	0,41	0,09	0,10	1,16	1,21	0,17	0,23
İ-1264	0,44	0,44	0,09	0,08	1,05	1,26	0,15	0,19
İ-1513	0,31	0,32	0,10	0,11	1,04	1,21	0,12	0,22
Mean	0,44	0,41	0,09	0,09	1,13b	1,26a	0,18b	0,23a
$LSD_{0,05}$	NS		NS		0,053		0.029	

Table 4. The effects of Mg, Fe, Zn, and Mn contents of the peel on the fruit cracking in seven pomegranate cultivars

Cultivars	Mg (%)		Fe (ppm)		Zn (ppm)		Mn (ppm)	
	Split	Healthy	Split	Healthy	Split	Healthy	Split	Healthy
Won	0,06	0,06	18,93	18,77	7,42	7,84	5,45	5,91
Türkmen	0,05	0,05	18,59	13,95	7,70	7,99	4,67	5,10
İ-8	0,04	0,07	17,51	17,12	8,48	7,85	5,10	7,45
İ-10	0,05	0,05	16,23	14,39	7,69	6,81	4,57	4,03
İ-16	0,05	0,05	18,29	15,46	7,71	7,65	6,20	5,97
İ-1264	0,05	0,06	22,36	19,71	8,31	8,55	7,12	6,91
İ-1513	0,04	0,05	14,19	17,13	8,85	8,84	4,69	5,50
Mean	0,05	0,06	18,01	16,65	8,02	7,93	5,40	5,84
$LSD_{0,05}$	NS		NS		NS		NS	

Table 5. The effects of Cu content and, N/K, and K/Ca ratios of the peel on the fruit cracking in seven pomegranate cultivars

Cultivars	Cu (ppm)		N/K		K/Ca	
	Split	Healthy	Split	Healthy	Split	Healthy
Won	8,46	9,36	0,40	0,38	4,47	4,97
Türkmen	7,75	9,42	0,37	0,34	7,82	7,47
İ-8	10,17	9,68	0,33	0,24	9,09	4,34
İ-10	11,00	9,08	0,34	0,37	4,74	5,34
İ-16	9,04	10,25	0,52	0,34	6,65	5,32
İ-1264	10,28	8,47	0,42	0,35	7,01	6,76
İ-1513	10,48	10,06	0,30	0,27	8,76	5,56
Mean	9,60	9,47	0,38a	0,33b	6,94a	5,68b
$LSD_{0,05}$	NS		0,032		0,921	

CONCLUSIONS

It was determined that the cracked fruit peel contained less mositure than that of healthy fruits. The cracked fruits was shorter than healthy fruits as fruit length. The peels of cracked fruits have lower level of potassium, and calcium, higher rates of N/K ratio than that in healthy fruits.

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AREAS OF USE OF BIOMASS ENERGY AND ITS INTEGRATION INTO BUILDING ENVELOPE

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ABSTRACT

Today, the rapid depletion of natural energy resources and the effects of global climate change have accelerated the orientation to renewable energy sources. Renewable and clean energy sources such as solar, wind, biomass, etc. are used to meet energy needs. Renewable energy sources are very important because they are environmentally friendly and can provide a continuous flow. Biomass energy, one of the sources of renewable energy, is a species of plant and animal-derived energy that has been used for a long time. Biomass energy is used for purposes such as heating, electricity generation, fuel, and is used both as the main energy source and as auxiliary energy to the system in buildings where energy needs are high. In this context, biomass energy is often used in heating and electricity generation systems of buildings and is involved in mechanisms such as pellet stove, biomass boiler and biomass cogeneration. This process continues with studies on the use of biomass energy in the building envelope. The integration of biomass into the building envelope is achieved by photobioreactor systems, which can be found as open and closed systems in many forms such as flat, panel, tubular, vertical column. Although the integration of these systems into the building envelope is with various living things, the situations such as the visual effect and the energy generated by this visual effect are considered to be positive; Also, the factors such as how the biomass source used in the systems is obtained, which systems are used, and whether the places, where the structure is located, receive the solar rays are decisive in regard to cost. In this study, it is aimed to investigate the integration of renewable clean energy sources such as biomass into the building envelope, where energy consumption and efficiency in buildings are very important today. For this purpose, sample buildings using biomass energy were studied. Although biomass energy is used to meet energy needs in these sample buildings, it was found that studies on the integration of biomass energy into building envelopes were more limited. In this context, it is thought that more detailed research should be carried out and applications related to the use of biomass energy in building envelopes should be increased.

Keywords: *Energy, Renewable Energy, Biomass Energy, Building Envelope*

INTRODUCTION

Although energy is important in almost all processes from the past to the present, as a result of increasing demand for energy for reasons such as industrialization and population growth, this need has been tried to be supplied with fossil fuels. As the amount of fossil fuels decreases over time and the need for energy increases despite this, situations such as irreversible damage to nature as a result of the use of fossil fuels have led people to renewable energy sources such

as solar, wind, and biomass. Renewable energy is important because it is environmentally friendly, clean, and green energy (Koç & Şenel, 2013).

As in all areas, renewable energy has been tried to be used in the construction sector, which needs energy at every stage from its construction to its destruction. Considering that the structure consumes the most energy during the use phase in its life cycle (Yüksek & Esin, 2009), various renewable energy systems have been tried to be integrated into the structures to meet the energy required for the use of the structure. The use of renewable energy sources in buildings was attempted to be solved by integrating them into the building envelope with facade systems in Figure 1 and into the technical volumes in Figure 2.

Figure 1. Integration of renewable energy sources into the building envelope (URL1-URL4).

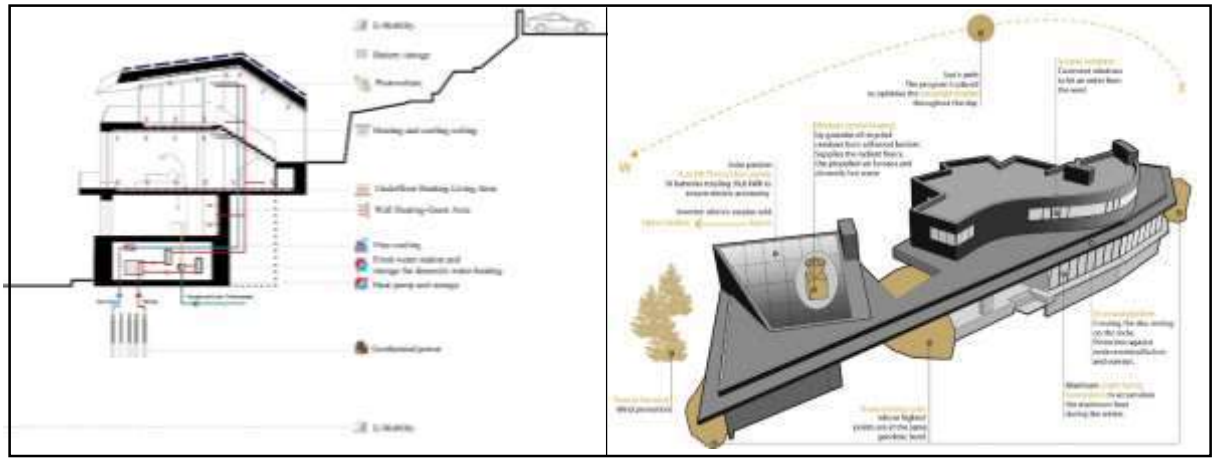


Figure 2. Integration of renewable energy into the technical volume of buildings (URL5, URL6)

Biomass energy, one of the renewable energy sources, is a type of energy of plant and animal origin that can also produce its own resources while using existing resources that have been used for a long time for reasons such as heating and cooking (Figure 3). Biomass energy is used extensively for purposes such as heating and cooking, as well as for electricity generation and heating through biofuels and bioenergy in some systems today (Ersoy, 1997).

Figure 3. Biomass resources (URL7-URL12).

Biomass energy contributes to the building with pellet stove, biomass boiler, cogeneration systems, and heating and electrical energy, in addition, many research and projects on how biomass energy can be integrated into building envelope systems to contribute to energy production. In this context, different systems have emerged.

Integration of biomass energy into the building is important for reasons such as releasing the amount of carbon when it is burning, also the reasons such as how the biomass source is obtained, whether existing sources are used, and the proximity of the building to the source make the situation determinant in terms of cost (Bay, 2006).

The integration of biomass energy into the building envelope can be carried out with various living beings, but also by using systems called photobioreactors. These applications are regarded as a positive development due to visual effect and the combination of energy produced by this effect, while the characteristics of how to obtain and use the biomass source used in the systems, the sunbathing time, and humidity of the location of the building are determining factors in terms of cost.

As part of this study, the necessity and importance of using renewable energy sources were addressed and the integration of biomass energy from renewable energy sources into buildings was studied through examples. It is recommended to increase its usage areas due to the characteristics of biomass energy such as meeting the oxygen need which is a necessity of life, low damage to the environment, and the used materials creating a visual element for the building.

MATERIAL AND METHODS

The study is based on the research on the use of biomass energy from renewable energy sources and studies on the application of biomass to the building envelope.

It is known that resources containing building material, furniture (wood materials), insulation material (wood fiber plates, mushrooms, sheepwool, thatch linen and hemp fibers, cellulose, cotton wool), and biomass, cleaner energy than fossil fuels, are used for heating purposes (URL13).

Biomass energy is currently used as a direct or auxiliary source in buildings using different methods and systems for purposes such as electricity generation, heating, and water boiling. In buildings, biogas obtained by airless decay from biomass source is used in electricity production, ethanol obtained by pyrolysis is used for heating purposes, and hydrogen obtained by direct combustion is used for water heating purposes (Sohrabi, 2015).

It is observed that biomass energy is used during the production of building materials in different functional structures such as houses, offices, schools, swimming pools, and renovation projects, for the purposes of heating and electricity generation in flooring systems, pellet stove, biomass boiler, and biomass cogeneration.

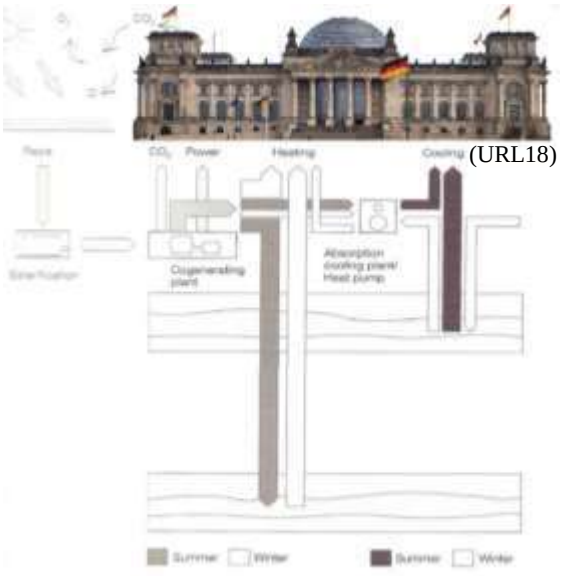
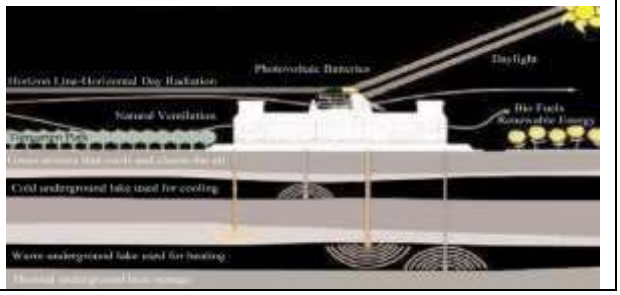
The cycles applied by several of the research on the use of biomass waste in buildings are shown below. The first example displays a prototype of the hotel room of the future, while the second one describes a cycle where it argues that nothing is wasted (URL14, URL15).

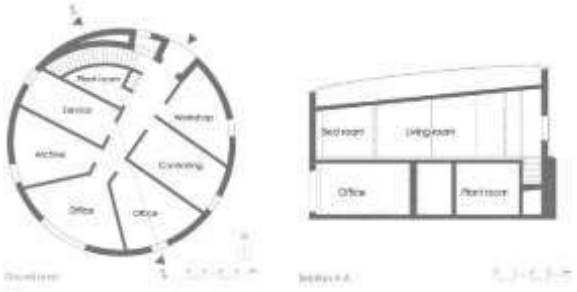
Figure 4. Examples of studies on the use of biomass waste

It is possible to see biomass energy not only in the structure and as a building material, but also in the production process of building materials. It was used in 2019 in the production of bricks of a structure, which was renovated to be used as a coffee shop and concert venue designed by Aulets Architecture and Carles Oliver in Spain (URL16). Another example of this issue is on a study called carbon negative cement produced by creating a low-temperature production process that works with biomass fuels with low energy and carbon density (URL 17).

Table 1 also displays examples of different biomass systems being used in buildings of different functions.

Table 1. Examples of the buildings using biomass energy

Location/Name of the Building	**Year of Construction / Purpose of Use / Type of Fuel / Usage System**
Berlin / Germany **Reichstag German Parliament Building** (Özçiftçi, 2010)  (URL18)	In the building, which was renovated by Norman Foster and his team in 1995, plant fuels were used to reduce CO_2 emissions, which were seen to be produced 7000 tons, and to generate electricity; Thus it was produced 94% less CO_2 emissions than fossil fuels, which were considered as a radical decision at that time; It was used with co-generator systems in mechanical volumes and as a result, it was seen that the amount of CO_2 emission was reduced to 440 tons per year. 

Les Olives / Spain **b- Empordà Residence** (URL19) 	The residence that was built in 2013 is heated by the pellet stove located in the building using pellet fuel; The chimney of the stove is opened to the roof garden for reasons such as providing thermal insulation. 
Medebach /Germany **Villa F Residence (**URL20)  (URL21) 	The building, produced in 2017, is important for its proximity to the biomass energy generation plant and for its user to be a pioneer of biogas technology. In order to provide electricity and heat energy, it produces biogas produced with biomass obtained from forestry, agriculture, and waste in the technical volume. Oval bath warms up with the energy generated by the biogas plant. 
Empedrado / Chile **San Ignacio de Empedrado high school** (URL22) 	The high school building, established in 2005, uses biomass in its heating system. The building, which uses a biomass boiler in mechanical volume as a system, uses sawdust and organic waste materials with high-power and low-cost features as energy sources, since wood is an abundant resource in this area. 

Covasna/ Romania

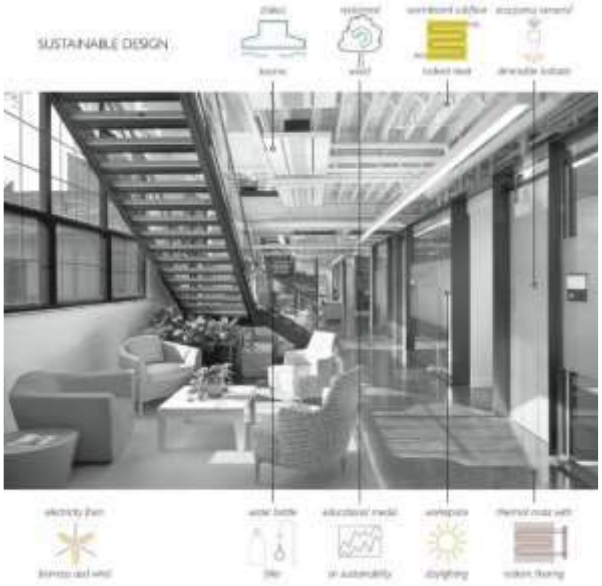
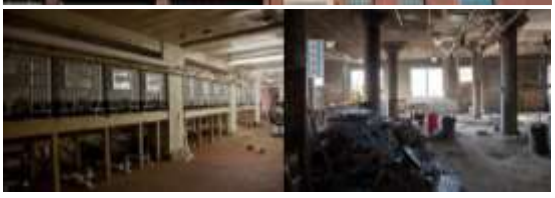
CLT Multi Confort Office Building (URL23)

The building, which was built in 2016, uses biomass energy as an aid to geothermal energy for heating and electrical energy. Using mixed wood construction as a building material, the structure provides energy with a high-efficiency biomass cogeneration plant inside the building.

Granada / Spain

Swimming pool at the "Bola de Oro" Sports Center (URL24)

In 2009, in order to strengthen the relationship between the buildings of the Sports Complex, which includes buildings of different typologies and functions, the part that includes a swimming pool was closed with a lattice facade system that can control solar energy. In addition to solar energy in the heating system, hot water production for dressing rooms and swimming pool is provided by biomass energy. For this, the building uses the biomass boiler in mechanical volume.

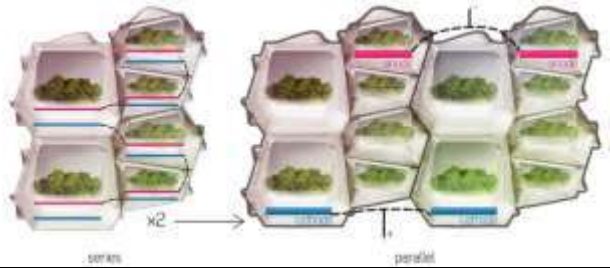
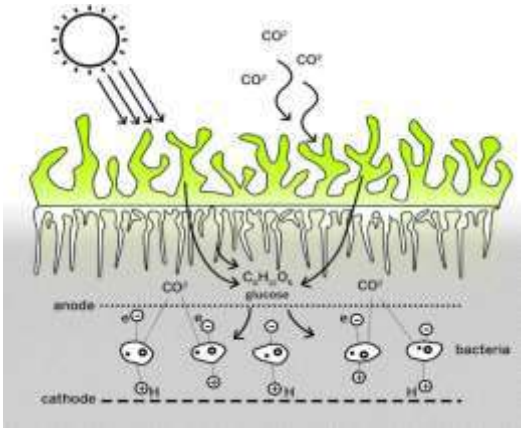
Granada /Spain **Westborough Elementary School Renovation Project** (URL25) 	The renovation project, a 2012 project aimed at demonstrating how schools can be renovated in a sustainable, affordable, and low-carbon way, uses photovoltaics for heating and electricity. A biomass boiler is used inside the building for the processing of biomass energy. 
Minnesota /USA **Minnesota Morris Welcome Center Renovation Project** (URL26)   	The building, which was renovated in 2009, uses wind energy and biomass energy for electricity generation in the mechanical volume with the biomass power plant in the building. 
Chicago /USA **Old Chicago Brewery building intended to be converted into a wasteless food factory** (URL27)  	To make a net-zero energy system, the building aims to make the transformation and obtain energy by using biomass resources of plant and animal origin including aquaponic breeding systems, fish breeding, and digestion systems.    

Photobioreactor systems containing living beings such as algae and moss are of great importance in the integration of biomass energy into the building envelope.

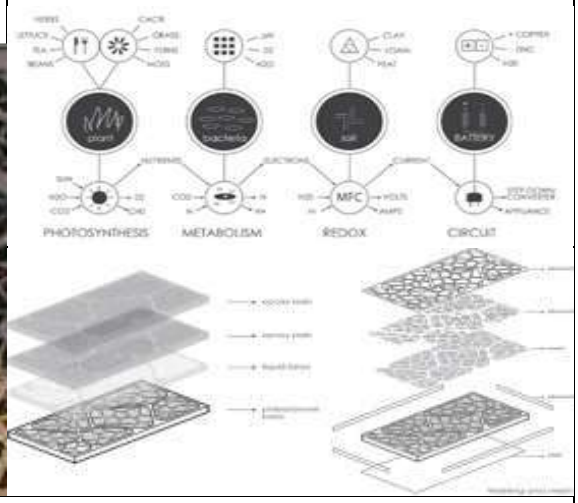
Photobioreactors are systems that function with the logic of transforming solar energy into bioenergy by consuming carbon dioxide from creatures such as algae and moss, which have been present since the first existence of life. It's on the basis of photosynthesis. Photobioreactors can be in the form of open systems that are in contact with the external environment and closed systems that cut off contact with the outside air to reduce the risk of contamination, or they can be in different forms, such as flat panel, tubular, and vertical column (Kükdamar, 2017).

The urban furniture elements, pavilion, competition projects and examples of their use on the buildings were determined during the investigation of the use of biomass energy in the building envelope. These are shown in Table 2 below.

Table 2. Examples of the studies based on the use of biomass energy in the building envelope

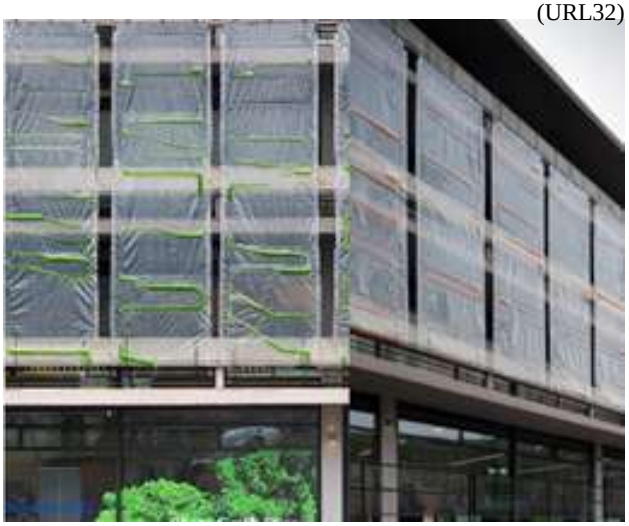
Application Name	**The functioning of the System- How to Make Photosynthesis**
Moss Voltaics-Moss Batteries-Green Wall System That Can Produce Electricity - (URL28)   	  (URL29)  (URL30)
It was able to produce 3 volts with a system consisting of 16 modules, it is believed that devices whose efficiency increases with the developed systems will consume less energy, and therefore the generated energy may be sufficient in the future.	

Bio-Photovoltaic Panel System (URL31)



The system, which has the form of Voronoi, has sensors that show its status so that it becomes self-sufficient, it can also provide information about how it varies according to different parameters.

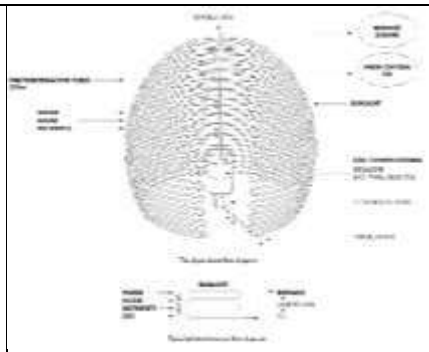
Photo.Synth.Etica- Biodigital Urban Curtain – "Exterior Curtains That Clean The Air" (URL32)



With this practice in Dublin, one kg of CO_2 is collected per day, which is equivalent to the performance of almost 20 large trees. With this study, it is assumed that buildings can be alive and that they can produce energy and consume air pollutants and also act as biosensors of the city.

Pavilions Made Using Photobioreactors- Urban Elements

(URL33)



(URL38)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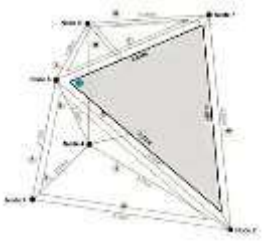
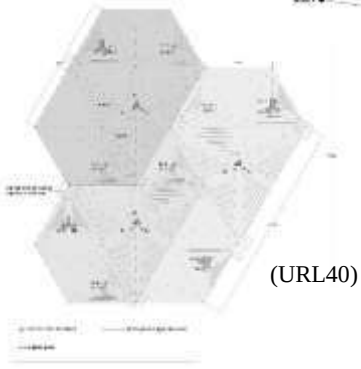

(URL39)


(URL40)

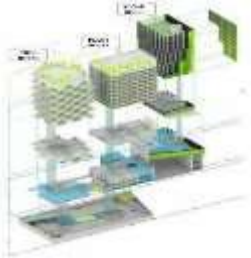
There are many urban elements, pavilions based on photobioreactor systems made at different times and for different purposes. Their examples include 'Algae Dome', 'Urban Algae Canopy', 'Urban Algae Folly' temporary installations.

Competition Projects using Photobioreactors -2011 (URL41)

The southern facade is covered with a frontispiece created with moss in different winding tubes, depending on the amount of sunlight that will use carbon-rich air from the highway. Algae are connected to a bio-reactor to create more lipids than those stored and burned in a combined cycle generator.

Most of the building's wastewater will be used in this process and will finally be filtered through pools adjacent to the office (Kükdamar, 2017).

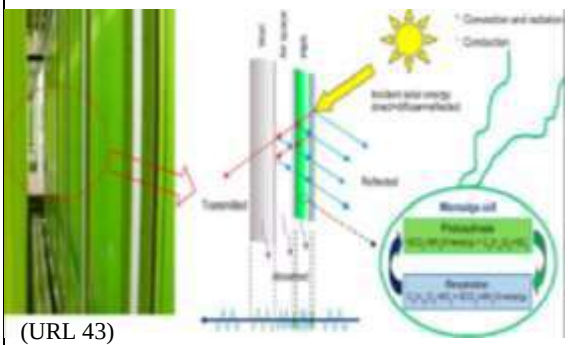
Competition Projects In Which Photo bioreactors Are Used - "In Vivo" Green Project (Kürkdamar, 2017)



Photobioreactor facades aim to ensure the hot water and warming of the house. Annual energy consumption caused by hot water and heating in the building will be reduced thanks to the photobioreactor system.

Solar Leaf -BIQ House

(URL 42)



(URL 43)

(URL44)

(URL 45)

(Kürkdamar, 2017)

Solar Leaf is a special kind of photobioreactor designed for being applied to the building facade. This system was applied to the BIQ house at the IBA exhibition in Hamburg in 2013 with a mechanical infrastructure (URL 42).

CONCLUSIONS

Energy has become more important with industrialization, population growth, and the gradual decline of non-renewable energy sources, it has not decreased from the past to the present, and even the need for energy, which will continue to increase in the future, has led mankind to search for different energy sources. In this context, in fact, the demand for energy types that have existed for centuries and are constantly renewing themselves has increased and the work on this issue has started to gain momentum.

Biomass energy is also one of these energy sources and is seen as the energy source of a future that is considered and tried to be developed over time, due to reasons such as the fact that it will not run out as long as the sun is present, it does not release carcinogenic substances and sulfur in its production and consumption, it releases as much carbon as it uses, and it is found in organic materials called waste.

In this context, it is used in mechanical and flooring systems in buildings and studies are continuing for its integration into the building envelope. In the integration into the building envelope; photobioreactor systems using various living beings such as algae and moss are used, but it was found to be positive from some perspectives such as visual effect and the combination of energy produced by this visual effect. With which systems the biomass resource used in the systems is obtained and used, the features of the location of the building such as the sunshine duration and humidity are important, which can result in high costs in terms of the establishment of the mechanism.

Many studies have been carried out on this subject and only one of them has been implemented within the building. In this context, it was concluded that the materials used on the buildings should be used more and studies should be done to create a visual element for the building because it meets the need for oxygen, which is the necessity of life, and reduces air pollution; the waste it creates when burned can be decomposed in nature; the damage it does to the environment is minimal, thus foods can be obtained.

It is important to consider how this system can be integrated into the building with simple systems by minimizing existing disadvantages such as cost, mechanics, and how to use it more efficiently in the building envelope or in a different element of the building, and how its advantages can be used with high efficiency from the point of view of Turkey, which has high potential as a country of agricultural origin.

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TRANSGENIC AND CLONE ANIMALS FROM PAST TO PRESENT

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ABSTRACT

The first studies to establish transgenic animals were recorded on mice to produced human disease models in the early 1980s. The first applied gene transfer method began as an injection of a cloned DNA fragment into the pronucleus of the single-cell embryo. The biggest problem with this method is that the integration of DNA is random and sometimes the individual being formed is chimeric. In order to solve this problem, a new method was developed so that the genetic material of the embryonic stem cells was altered in a targeted manner by homologous recombination using a template DNA. These modified stem cells were then injected into the embryos of the mice. Both microinjection and gene transfer to stem cells have produced transgenic model laboratory mice on the face and these have been widely used both in basic scientific studies and in the development of treatment strategies of diseases. Both methods were limited to use in livestock. With the discovery of nuclear transfer technology, gene transfer to somatic cells after 1997 allowed targeted genetic changes in livestock. Nuclear transfer can be used for both reproductive and therapeutic cloning. In reproductive cloning, a reconstituted embryo derived from the fusion of an enucleated oocyte and somatic cell is allowed to go to term that results in a cloned animal similar to the nuclear donor. In therapeutic cloning, the reconstituted embryo is allowed to grow to the blastocyst stage and cultured under appropriate conditions to develop stem cells lines. Generation of transgenic animals by somatic cell nuclear transfer adds another variable..In nuclear transfer, genetic modifications are introduced into somatic cells. The genetically modified cells are selected and fused with an oocyte devoid of its genetic material. The reconstituted oocyte is activated, and introduced into the recipient animal where it grows to term like a normal embryo. This method can be used to produce genetically modified animals in all species where nuclear transfer is successful. Despite all these improvements, the success of transgenic animal production is low. Over time, newer techniques, such as site-specific recombinases, ZFNs, and TALENS, have increased the sensitivity of regulation of specific genomic targets in animals. The introduction of CRISPR-Cas9 technology in 2012 gave new impetus to genetic engineering. Since CRISPR enables targeted genome regulation in a simple, efficient and economical way, the process of creating transgenic animals has become easier than ever before. Today, the aim of transgenic animal production has been far beyond the transgenic model mice, which were originally produced to understand human diseases and develop treatment strategies thanks to evolving genetic engineering methods.

Key word: Transgenic animal, embryonic stem cell, nuclear transfer, CRISPR-Cas9

INTRODUCTION

Cloning means the creation of living organisms whose genetic structure is identical with asexual reproduction method. The first cloning studies started in the early 1980s with the division of the embryo and the creation of more than one living thing from one embryo, but gained great momentum in 1997 with the production of a genetic copy of an adult sheep (Wilmut et al. 1997). In recent years, many studies have been conducted on the cloning of farm animals. It is the consensus of both scientists and the private sector that cloning technology will find a huge application area in agriculture and medicine.

This technology has various application areas. A farm animal that has a superior genetic structure but cannot produce offspring for any reason or is about to die can be cloned and reproduced.(Arat et al. 2011). Another application area is the reproduction of animals, which are endangered and cannot be produced due to their limited number, using this technology (Wildt and Wemmer 1999, Ryder et al. 2002, Andrabi and Maxwell 2007, Leon-Quinto et al. 2009, Arat et al. 2011, Selokar et al. 2018). In addition, it can be produced in genetically modified clones. In this way, it is thought that genetic improvement, especially in animal husbandry, will be completed in a very short time, the lost genetic resources can be protected and many drugs used for treatment can be obtained in large quantities from transgenic clone animals.

The technique first started with the division of embryos to create more than one embryo from one embryo, but the first successful cloning in farm animals with nucleus transfer (NT) took place in 1986 through embryonic cells (Wolf 1998). Later, the first sheep cloning was made from embryonic cells obtained from the embryo in 1996 (Campbell 1996), followed by the cloning of a mature animal by transfer of a differentiated cell (Wilmut 1997). Following the first successful use of cells for cloning (Wilmut et al. 1997), cattle (Cibelli et al. 1998,), mouse (Wakayama et al. 1998), goat (Baguisi et al. 1999), pig (Onishi et al.2000), rabbit (Chesne´ et al.2002), cat (Shin et al.2002), rat (Zhou et al.2003), horse (Galli 2003), mule (Woods et al.2003), dog (Lee et al.2005), ferret (Li et al.2006), buffalo (Shi et al.2007), camel (Wani et al.2010), and Most recently, many mammalian species, including monkey (Liu et al.2018), have been cloned using fetal or adult somatic cells. The first clone sheep produced in our country were born healthy in 2007, the first clone cattle in 2009 and 2010 (Arat et al. 2011). Cattle cloned in our country are still alive (Tüten et al.2017) and they gave third generation healty offspring.

Transgenic animals are defined as animals carrying a gene that does not belong to them in their genome and have found wide application in life sciences such as medicine, biology and animal husbandry. Gene transfer applications to produce transgenic animals were first performed in mice in 1980. These transgenic mice have proven powerful tools in elucidating complex biological processes in fundamental research at the molecular level. Transgenic animals are produced using recombinant DNA technology and usually contain elements of two or more different genes. It may contain the regulatory elements (enhancer element and promoter) of a transgene gene and the protein-producing DNA base sequence of the target gene. Regulatory elements are regions that enable the expression of the target gene in the desired development period and in the desired tissue. Depending on the method of gene transfer, the transgene may also contain some marker genes in addition to these elements to detect expression and success of gene transfer.

PRODUCTION OF CLONE ANIMALS

The nuclear transfer technique for reproductive purposes alone (nuclear transfer) can be used to replicate selected qualified animals for the improvement of livestock. In addition, individuals who have developed natural immunity to diseases can be detected and increased. Another application area is to increase the number of endangered animals.

In today's sense, cloning is defined as putting a body cell obtained from an adult living creature into an egg cell whose genetic material has been extracted (enucleated) from that species. The basic steps of cloning are listed as follows.

1. Polar body and metaphase chromosomes of mature egg (Metaphase II oocyte) are removed (Enucleation).

2. The cultured G0-G1 phase (silent) donor cell is placed in the perivitelline space of the enucleated MII oocyte.

3. The oocyte and donor cell are fused.

4. Cell division is activated by chemicals or electric current.

5. Successfully developing embryos are transferred to carrier females.

6. Clone offspring that will be born have exactly the same nuclear genes as the donor cell.

The first step in nuclear transfer is the removal of the nucleus of the egg cell in the MII period. For this, the zona is pierced with a micropipette, entered into the oocyte and the nucleus is drawn.The second step is the transfer of the cell nucleus of the creature to be cloned to the oocyte. There are three different applications for this. 1: A single cell is dropped under the zone of the nucleated oocyte with a pipette and the cell is combined with the oocyte by applying an electric fulse 2: The nucleus of the cell is removed by puncturing the membrane and injected into the egg cell where the nucleus has been removed. 3: The cell is adhered to the egg whose zone is melted and the nucleus is removed, and they are combined by giving an electric pulse. Regardless of the method by which the somatic cell nucleus is transferred, the oocyte is subjected to artificial activation and then it is cultured in vitro until the blastocyst stage. Developing clone bastocysts are transferred to recipient mothers. Clone offspring come to the world at the end of the species-specific gestation period.

TRANSGENIC ANIMAL PRODUCTION

While the transgene is designed in various ways according to the targeted purpose, the method of gene transfer and the animal to be transferred are selected in line with the targeted purpose.

Pronuclear DNA injection;

This method is commonly used mostly in small laboratory animals such as mice. It is used less frequently in animals with economic value, such as farm animals, as the success rate is low. In this method, the gene to be transferred first is designed properly. The target gene may belong to that animal or may belong to another animal or human. For the expression of the target gene, in whichever tissue it is desired to be expressed, regulatory regions specific to that tissue are added to the transgene. The designed transgene is injected into a pronucleus of the unicellular

embryo, resulting in a random integration and the transgene will settle in any region of any chromosome. However, it is expressed in the desired tissue with the promoter selected.

Embryonic stem cell technology;

EK cells were first obtained from mouse embryos by Evans and Kaufman (1981). After a short while, the first chimeric animals were obtained by transferring these cells to the embryo and then the embryos to the recipients, and it was shown that these cells enter different tissues in the chimera and form reproductive cells (Bradley 1984). The first study on genetic manipulation of EK cells was conducted by Robertson et al. (1986). Until today, more than 100 genes have been modified by homologous recombination and transferred to progeny. Mouse embryonic stem cells are obtained from the inner cell mass of mouse embryos in the blastocyst stage. The pluripotency of these cells, which have the ability to transform into pluripotent tissue, can be maintained by adding leukemia inhibitor factor (LIF) to prevent differentiation into the medium and culturing with mitotically inactivated fibroblast cells. When these cells are transferred to the embryonal environment, they continue their normal development and participate in the formation of all tissues, including the germline, causing the formation of a chimeric animal. They can be genetically modified in vitro and allow the generation of mutant mice. One of the advantages of this system is that the results of genetic manipulation can be monitored both in undifferentiated cells and during differentiation in vitro before the cells are introduced into the embryo. Giving these cells to the embryo allows the results to be evaluated in vitro. Another important advantage is the shaping of homologous recombination by targeting the desired gene. Thus, mutant mice can be developed by replacing the mouse's own gene with another gene that is its homologue. These mutant animal models allow the study of the functions of target genes in the mixed development system in vivo and are used as models of genetic human diseases due to gene mutations for diagnosis and development of new treatment strategies.

Nuclear transfer (cloning);

When cloning is combined with genetic modification technologies, much more effective biotechnological applications may come to the fore. The combination of nuclear transfer with genetic modification has created a powerful technology with many potential biotechnological applications. The donor cells used in nuclear transfer can be obtained from an appropriate genetically modified transgenic animal (Bondioli 2001) or the cultured donor cells can be genetically modified before nuclear transfer (Schnieke et al. 1997, Cibelli et al. 1998, Arat et. 2001, Keefer et al. 2001, Arat et al. 2002, 2003, Lai et al. 2002). In addition, unlike DNA microinjection, targeted recombinations of genes are possible and therefore genetic modification will occur both precisely and as desired. The donor cell can be controlled to ensure that proper modification has occurred to minimize loss of time, energy and money. Thus, it can be ensured that surrogate mothers only carry genetically modified embryos. Biotechnological applications for obtaining genetically modified clones can be listed as: the production of pharmaceuticals, the production of organs or tissues / animals that can be transplanted into humans (xenotransplantation), destruction of diseases and breeding of livestock.

Sperm-mediated gene transfer

Another method for introducing exogenous DNA into animals for the purpose of producing transgenic animals is sperm-mediated gene transfer (SMGT). Sperm of many species have been shown to bind naked DNA as well as DNA-Liposome complexes. The transformed sperm may be used for in vitro fertilization systems or artificial insemination ; however, the majority of studies have focused on in vitro fertilization systems. In order to use sperm cells as vectors for gene transfer, transportation of exogenous DNA into the oocyte at fertilization must occur. SMGT has been reported in the mouse (Maione et al.1998), rabbit (Brackett et al. 1971), pig (Sperandio), chicken, cow and other species (Smith el al 2005).

Despite all these improvements gene transfer techniques, the success of transgenic animal production is low. Over time, newer techniques, such as site-specific recombinases, ZFNs, and TALENS, have increased the sensitivity of regulation of specific genomic targets in animals. The introduction of CRISPR-Cas9 technology in 2012 gave new impetus to genetic engineering. Since CRISPR enables targeted genome regulation in a simple, efficient and economical way, the process of creating transgenic animals has become easier than ever before. CRISPRs are the most recently discovered genome engineering technology. This system was discovered in a microbial adaptive immune system and has allowed great advancements because it is easy to design, does not take long to construct, is low cost and effective. Since the CRISPR/Cas9 technology is so new, it was initially largely used in mice and only recently has there been success in large animals (Ran et al. 2013, Zhou et al. 2013, Zhou et al. 2016).

CONCLUSION

Cloning is an elite biotechnological method in the reproduction of animals with superior genetic efficiency in animal husbandry, endangered creatures or transgenic animals produced by genetic manipulations, as well as treatment methods called therapeutic cloning. The technology has been commercialized in many areas, although application transfer is not allowed in some countries because it is still not considered reliable. Today, narcotic dogs, race and polo horses are cloned and used, drug raw materials obtained from the milk of clone transgenic farm animals are used in treatment, and in some countries clones are included in the food chain.

In agricultural applications, the ultimate utility and value of transgenic technology will be limited by our ability to identify genes, incorporate these desired genes in an appropriately expressed and regulated manner into our domestic livestock. The establishment of ES/EG cells, and nuclear transfer methods in livestock will be useful for the production of transgenic livestock as well as for studies of cell differentiation, development, and gene regulation in farm animals. Finally, there are a number of developing technologies such as ZFNs, TALENs, and CRISPRs that will have profound impact on genetic improvements to livestock. The rate at which these technologies are incorporated into production schemes will determine the speed of achieving the goal of more efficiently producing livestock to meet consumer and market demand.

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THE EFFECTS OF GAMMA IRRADIATION ON SUNFLOWER POLLEN VIABILITY AND MORPHOLOGY

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ABSTRACT

Haploid plants can be induction of maternal haploids embryos by pollination with irradiated pollen has been an effective method in different plants. Penetration of different types of irradiation (uv, gamma, X-rays) varies in species, depending on the size and shape of the pollen grain, and the thickness of the pollen wall. Commonly pollen is irradiated with gamma rays because of their simple application, good penetration, reproducibility, high mutation frequency, and low disposal (lethal) problems. Gamma-irradiated pollen can germinate on the stigma, grow though the style, and reach the embryo sac. Despite, being unable to fertilize the egg cell and the polar nuclei, it stimulates the development of haploid embryos. This method requires immature embryo rescue under in vitro conditions. Among the different factors influencing the efficiency of the progress, the irradiation dose, the developmental stage of the embryo during in vitro culture, the culture media composition and the culture conditions are especially important. The general objective of the present study was to evaluate the effect of 60Co gamma radiation and illuminate pseudo-pollination during the haploid induction with irradiated pollen in sunflower. Female flowers of sunflower were pollinated using pollen collected from different genotypes and mixed with three doses of gamma rays, 500, 750 and 1000 Gy γ-ray doses, which have been applied for parthenogenetic induction in sunflower. In order to examine the effect of irradiation doses on pollen viability, the pollen of the control (no irradiated) group and irradiated groups were stained with aniline blue (cotton blue) prepared in lactophenol. While the pollens of the control group showed 92% viability, the viability of the irradiated pollen decreased by more than 50% compared to the control group. Polar and equatorial axis lengths of 50 pollen were measured for each group to examine whether irradiation doses caused a change in the morphological structure of the pollen. In irradiated pollen, polar and equatorial axis lengths were found to be higher than those of the control group. Based on the results obtained showed that the effects of radiation depend on the dose applied and interaction with the pollen donor genotypes and mother genotypes used and the doses used in the study can be used to obtain haploid plants in sunflower.

Key words: Gamma Radiation, Pollen Viability and Morphology, Double Haploid

INTRODUCTION

The quality of pollen is assessed based on viability and vigor of the pollen grain. Determination of the functional quality of pollen helps in monitoring pollen vigor during storage, genetics and pollen-stigma interaction studies, crop improvement and breeding (Dafni, 1982; Ottaviano and

Mulcahy, 1989). The irradiated pollen technique has been a frequently used method to induce haploid plants in situ. Pollens are treated with high-dose irradiation (UV, gamma rays, and X-rays). Irradiated pollen is genetically inefficient but physiologically active. It can germinate on the stigma but not fertilize egg and polar nuclei (Save 2009). Haploidy induction success in higher plants with irradiated pollen depends on several factors, such as genotype, physiological status of parents, irradiation source and dose (time of exposure), and the in vitro culture conditions (Sestili, 1996).

Pollen viability after irradiation can be determined depending on the in vitro germination rate and the length of pollen tubes after a specific duration of time. If the pollen tube exceeds the cell wall, the pollen is considered germinated (Brewbaker and Emery, 1962).

Apart from pollen, anthers, flower buds, whole flowers and inflorescences can be irradiated and used for haploid induction. Fresh pollen was preferred for this application but storing the pollen under proper conditions do not negatively affect the application.

Different irradiation types can create various changes on pollen morphology depending on the size, shape, and thickness of the pollen wall (Savaskan, 2002). Diameter is also affecting the radio resistance of the pollen grain. Various methods are used to determine the morphology of pollen. These are especially made by proportioning the axis of the pollen body which named the polar diameter (P) and the equatorial diameter (E). Pollen grains are classified according to the ratio expressed as P / E as follows perprolate, prolate, subpurolate, sphaeroidea, suboblata and oblata.

In this study, the general objective of the present study was to examine the effect of irradiation doses on morphological structure and viability of the pollen of the control (no irradiated) and irradiated groups with the aim of producing haploid plants in sunflower.

Materials and Method

The pollen samples were collected from grown plants in land of the Thrace Agricultural Research Institute at around 7-8 am to avoid thermal shocks during the day. Collected pollen from different sunflower plant were mixed before irradiation for haploid plant production and store at suitable temperature. Pollen was provided by the Thrace Agricultural Research Institute. As used in the haploid induction study, pollens were collected from different flowers and made into a mixture. Irradiation process was made by Gamma Pak A.Ş. and Co60 was used as radiation source. Pollen were irradiated at 500, 750 and 1000 Gray doses. Control group and irradiated sunflower pollen groups were stored at -20 ° C until the time to be examined.

Pollen viability was estimated using staining technique. The control and irradiated pollens were stained with aniline blue (cotton blue) prepared in lactophenol, then 20 g of phenol crystal was dissolved in 20 ml of distilled water, and 20 ml of lactic acid and 20 ml of glycerol were added to examine the effect of irradiation doses on pollen viability. This solution was mixed with 0.1% aniline blue and diluted in equal amount of water (Mclean and Cook 1941) and kept at a temperature below 30° C and away from bright light. Add one, or at most two drops of the cotton blue were dropped on the pollens which placed into 2ml volume tubes and then the tubes were gently shaken. After five minutes of incubation, a few drops of cotton blue-pollen mixture were dropped on the slide and covered with a coverslip.

The preparations were examined with the Olympus BX-51 light microscope and photographed with the KAMERAM digital camera and the KAMERAM app. The photos were analyzed in Image J program, 250 pollens were counted for each group and vitality percentages of pollen were calculated. The polar and equatorial axis lengths of 50 pollens for each group were

measured under the Olympus BX-51 light microscope with the KAMERAM digital camera and KAMERAM app in order to examine whether the irradiation doses caused a change in the morphological structure of the pollens. Calculations were made with Excel 2016. To examine the pollen germination on the stigma, crushed preparations were prepared by adding 1 drop of aniline blue on the stigmas pollinated with irradiated pollen. The preparations were examined with Olympus BX-51 fluorescent microscope and photographed with KAMERAM color digital camera and KAMERAM app.

Simultaneously with determining the pollen viability was performed also the measuring of the diameter of sunflower pollen grains, by using the ocular micrometer and objective micrometer.

RESULTS

Anilin blue stained fresh pollen bright blue and successfully distinguished between fresh and inanimate pollen. In the fluorochromatic test, both fresh and dead pollen showed fluorescence and the variation between individual grains was too great to allow fresh and dead pollen to be distinguished reliably (Figure 1).

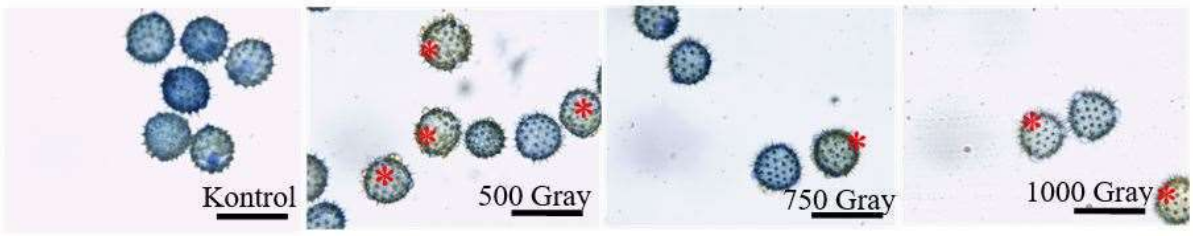

Figure 1. Effect of irradiation doses on pollen viability (* inanimate pollens). Bar: 50 µm.

It was determined that pollen vitality was affected by irradiation. While the pollens of the control group showed a vitality of 84%, it was determined that the vitality of the irradiated pollens decreased by more than 50% compared to the control group (Figure 1). However, there was no evidence that increasing irradiation doses linearly decreased pollen viability (Figure 2).

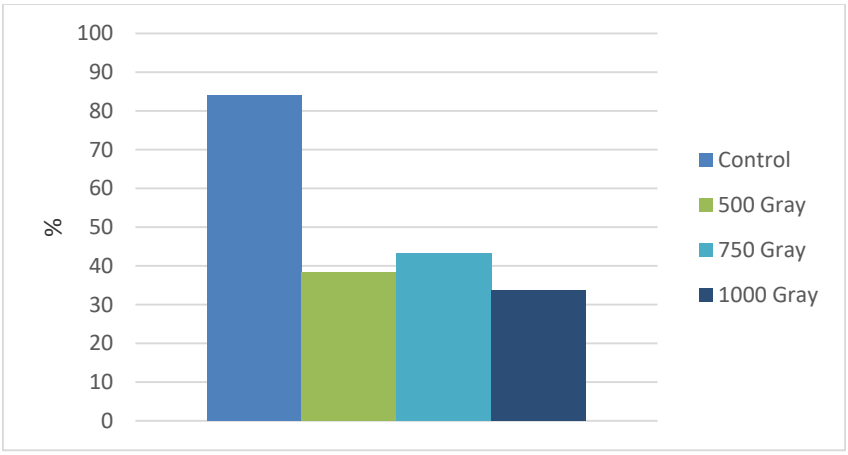

Figure 2. The effect of irradiation doses on pollen viability.

It was determined that the irradiation doses did not cause a significant change on the polar and equatorial axis length of the pollen. However, it was observed that the polar and equatorial axis lengths of the irradiated pollens were longer compared to the pollens of the control group.

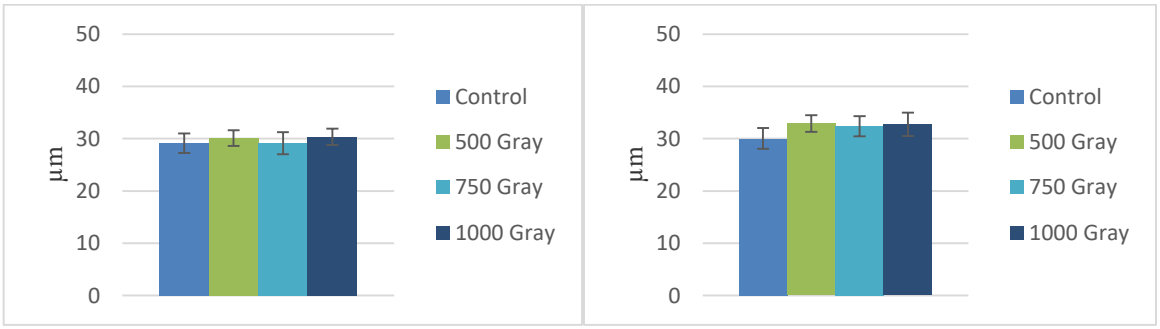

Figure 3. Measurement of the polar (a) and equatorial (b) axis lengths of pollen (µm).

It was thought that this situation was caused by the explosion of many of the irradiated pollens (especially 750 and 1000 Gray) and the pollen showing various abnormalities (Figure 4).

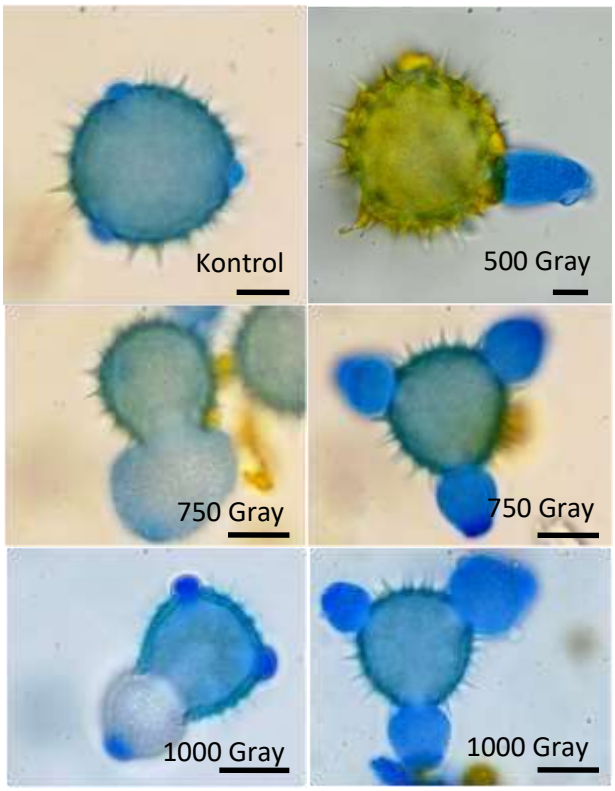

Figure 4. The effect of irradiation doses on pollen morphology. Bar: 10 µm.

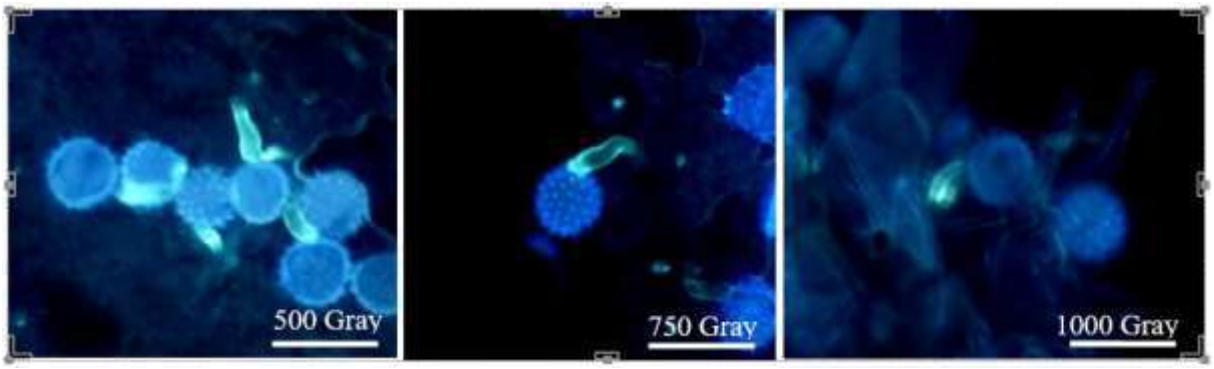

Figure 5. Pollen germination on stigma. Bar: 50 µm.

In this study, we tested pollen viability using staining technique applied to sunflower pollen from the same source plants and importantly used both fresh control and irradiated pollen in the viability test to assure accuracy of the obtained results. Our experiments showed that fresh and dead pollen could be differentiated by staining with anilin blue and can be relied upon to determine pollen viability in sunflower. The results obtained showed that the effects of radiation depend on the dose applied and interaction with the pollen donor genotypes and mother genotypes used in sunflower. We demonstrated that Co60 can be effectively used as alternative sources to for pollen irradiation and it provide sufficient percentages of pollen viability for production haploid sunflower plants. The development of parthenogenetic embryos are also investigated besides the viability of irradiated pollens.

ACKNOWLEDGEMENT

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PREPARATION, CHARACTERIZATION AND IN-VITRO EVALUATION OF SUSTAINED RELEASE 6-MERCAPTOPURINE-LOADED SOLID LIPID NANOPARTICLES

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ABSTRACT

6-Mercaptopurine (6-MP) is widely used cytotoxic and immunosuppressant drug and is used in human leukemia and many other diseases such as inflammation of colon and small intestine e.g. Crohn's disease and ulcerative colitis, systemic autoimmune disease and rheumatoid arthritis. The use of this drug is restricted due to its poor bioavailability and short plasma half-life. In order to reduce these drawbacks, 6-mercaptopurine loaded solid lipid nanoparticles (6-MP-SLNs) were prepared and characterized. For characterization studies; particle size, polydispersity index (PDI), zeta potential (ZP), encapsulation efficiency (EE %), recovery % were determined and in vitro dissolution test studies were conducted. Release profiles shown that nanoparticles prolonged short half life of 6-MCP and obtained sustained release. These results demonstrate that 6-MP-loaded SLNs are good candidates for enhancement bioavailibility and sustain release systems.

Key words : 6-mercaptopurine, nanoparticle, drug delivery, sustained release, solid lipid nanoparticle

INTRODUCTION

The 6-mercaptopurine (6-MP) is an effective immunosuppressant and anti-cancer agent. The agent is increasingly prescribed in human and veterinary medicine to treat inflammatory (Crohn's syndrome, ulcerative colitis, rheumatologic disorders, etc.) and leukemia (acute lymphoblastic leukemia and acute myelocytic leukemia) conditions. Because of the undergoing a complex biotransformation in liver, incomplete and variable bioavailability (about 16–50%) was obtained. This insufficient bioavailibility is stem from poor solubility (0.734 mg/mL), short plasma half-life (0.5–1.5 h), moderate plasma protein binding (19–30%) and narrows therapeutic index which in turn results in plummeting chemotherapeutic effect and hazardous toxicity, mainly in the form of myelosuppression (Kumar et al. 2015).

In this study, it is aimed to sustained release of 6-MP and increase the absorption by encapsulating 6-MP solid lipid nanoparticles.

Material and Methods

Materials

6-mercaptopurine was purchased from Sigma–Aldrich Co. (St. Louis, Missouri) and polyvinyl alcohol was purchased from Zag Chemistry(Turkey). Precirol ATO5 was kindly gifted Gattefose (France) and Ultrapure water purified by Milli-Q Plus system (Millipore Corp.,

Molsheim, France) was used in all experiments. All other chemicals and reagents were of analytical grade.

Preparation of solid lipid nanoparticles

Double emulsion method (w/o/w) and solvent evaporation techniques was used. Briefly, 0.5 mL 6-MP solution (40.0 mg/mL in 1 N NaOH) (aqueous phase) was emulsified in 2.0 mL organic solvent (dicholoromethan) containing 50 mg Precirol ATO 5 lipid (oily phase). This mixture was stirred 5 min vigorously to produce a w/o emulsion. Then, this emulsion was added 10 mL a 0.1% (w/w) PVA solution with insulin syringe which was being mixed at 700 rpm on a magnetic stirrer at room temperature. After addition w/o emulsion, w/o/w emulsion was sonicated for 5 min in the ultrasonic bath. The organic solvent was evaporated under the fumed hood while stirring. After evaporation, nanoparticle dispersion were recovered by centrifugation at 13 500 rpm, for 20 min at ambient temperature.

Characterization of nanoparticles

Particle size, size distribution and polydispersity indexes of lipid nanoparticles were determined by dynamic light scattering (DLS) method using zetasizer (NanoSeries, Nano-ZS, Malvern Instruments, UK). Analyses were performed at 25˚C with He-Ne laser (633 nm) with a scattering angle of 173˚. Zeta potential of nanoparticles was measured to determine the surface charge of the nanoparticles on the zetasizer (NanoSeries, Nano-ZS, Malvern Instruments, UK). Disposable zetasizer cuvettes were used for the measurement of nanoparticle suspensions and 6 measurements were made for each sample.

Indirect method was used for the quantification of encapsulation efficiency (EE%) of 6-MP in lipid nanoparticles. After ultracentrifugation of the nanoparticles, the amount of unloaded 6-MP in the supernatant was determined by UV spectrophotometer using the calibration equation for 6-MP in methanol (n = 3) .

Release studies

In vitro release tests were performed using static methods. The medium was 50 mL of phosphate buffer solution (PBS) pH 7.4 and the temperature was kept at 37±0.5°C. Pure 6-MP and SLNs introduced into the dissolution medium. While stirring the release medium using the magnetic stirrer at 300 rpm, 1.5 mL of dissolution medium was withdrawn at the predetermined period (10 min, 20 min, 40 min, 60 min, 120 min, 240 min, 360 min). After each sampling, 1.5 mL of fresh medium was added to the incubation medium. The amount of released drug in the medium was filtered by 0.45 μm membran filter, determined by UV spectrophotometer using the calibration equation for 6-MP in PBS (n = 3) and cumulative release percentage of 6-MP was calculated.

Results and Discussion

Preparation of solid lipid nanoparticle

Solid lipid nanoparticles were prepared by double emulsion method (w/o/w) and solvent evaporation techniques. The characterization results of the formulation are given in Table 1.

Table 1. Characterization results of solid lipid nanoparticle.

	Size ± SE(nm)	PDI	Zeta Potantial ±SE (mV)	EE%±SE	Process Yield%
SLN	460,7± 23,25	0,650± 0,02	-15.4±1.89	46.54±2.48	50.29

[1] S.E. standard error

6-MP dissolution rate was lowered because of the encapsulation in the lipid matrix carrier (Figure 1). One of the bioavailibility limitation of 6-MP is short half life and encapsulation approach is done successfully. In the evaluation particle size graph, bimodal distribution was shown (Figure 1).

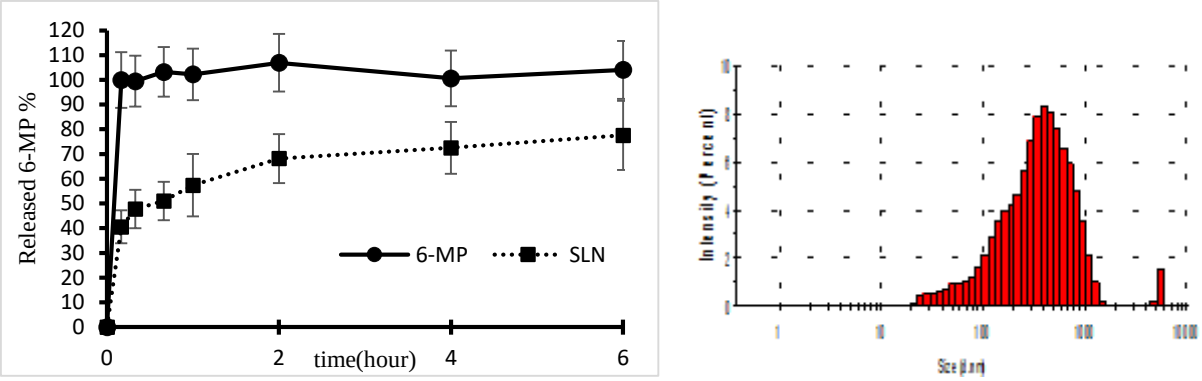

Figure1. Release profiles and particle size distribution

CONCLUSIONS

This study revealed that solid lipid nanoparticles are suitable for the encapsulation of 6-MP. Further studies will be carried out to evaluate the in vivo pharmacokinetic properties of the nanoparticle formulation.

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MORTALITY AND SUPPRESSION OF DEVELOPMENT AND REPRODUCTION OF SOYBEAN ARMYWORM *SPODOPTERA LITURA* TREATED WITH NEEM OIL FORMULATION

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ABSTRACT

This study was conducted to evaluate the effect of a neem oil formulation (NOF) on mortality, development, and reproduction of the soybean armyworm *Spodoptera litura*. A carbamate insecticide, methomyl, was included in the study for comparison. In leaf-residue feeding tests, LC_{50} and LC_{95} of NOF at 9 d after treatment (DAT) were 15.1 and 28.5 ml neem oil/l, respectively, whereas those of methomyl at 4 DAT were 0.13 and 0.26 g a.i./l, respectively. Sublethal treatments of the third-instar larvae *S. litura* with NOF at LC_{10} and LC_{25} completely suppressed their pupation, whereas the percentage of pupation in the treatment with methomyl at LC_{10} and LC_{25} was 72.0% and 72.7%, respectively, and that in the control was 93.8%. Moreover, sublethal treatments with NOF at $LC_{0.25}$ to LC_5 reduced normal adult emergence by 88.4% to 97.1% compared with control. Those treatments also reduced egg production by the emerging females by 79.2% to 98.3% relative to control. Thus, the treatment with neem will not stimulate the increase in *S. litura* reproduction and as such, it is not expected to induce resurgence in *S. litura* population. On the other hand, sublethal treatments with methomyl at LC_{10} and LC_{25} increased the fecundity of the emerging females by 30% to 92% compared with control. This suggests the risk of resurgence effect of this synthetic insecticide on *S. litura* through the increase in insect reproduction. Thus, neem is a promising botanical insecticide alternative to synthetic insecticides for the control of *S. litura*.

Keywords: *Neem, Resurgence, Soybean armyworm, Survival supression*.

INTRODUCTION

Soybean plants in Indonesia can harbour around 15 major insect and mite pests, including the soybean armyworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) (Marwoto et al., 2013). Despite integrated pest management (IPM) implementation, some farmers still use broad-spectrum and toxic, older insecticides to control this pest due to limited knowledge of farmers on effective non-chemical methods (Marwoto and Suharsono, 2008; Marwoto and Indiati, 2017). Regular use of synthetic insecticides may cause some unwanted side effects such pest resistance and resurgence, reduction in biodiversity of non-target organisms including pest natural enemies, contamination of food web and environment in general, risks of pesticide residues, and poisoning of pesticide applicators (Aktar et al., 2009; Gilden et al., 2010; Yu,

2014; Wyckhuys et al., 2020). Thus, safer alternatives to synthetic insecticides need to be sought.

Neem is now considered as one of the feasible botanical insecticide alternatives to synthetic insecticides (Benelli et al., 2016; Chaudhary et al., 2017). Today, neem-based products have become widely used botanical insecticides in the world, second only to pyrethrum (Isman 2020). Neem active substances have a multitude of effects on insects including feeding inhibition, moulting inhibition, developmental delay and defects, increased mortality, oviposition deterrent, and reduced fecundity (Koul, 1996; Mordue and Nisbet, 2000).

Neem-based insecticides have been reported to affect more than 400 insect pest species (Schmutterer and Singh, 1995; Nicoletti et al., 2011) including the soybean armyworm, *Spodoptera litura*. The effectiveness of neem-based products against this pest, however, varied with source and nature of the test materials (Razak et al., 2014; Ramadhan et al. 2016; Paramita et al., 2018; Kristiawan et al., 2019). Thus, efficacy of locally available neem products against a particular pest should be properly known.

This study was conducted to evaluate the effect of a neem oil formulation on mortality, development, and reproduction of the soybean armyworm *Spodoptera litura* in comparison with that of a synthetic carbamate insecticide, methomyl. The latter is a neurotoxicant which acts as an inhibitor of acetylcholinesterase enzymes at nerve synapses.

Materials and Methods

Insect rearing

Soybean armyworm, *S. litura*, initially collected from taro farmer's fields in Bogor, West Java, Indonesia, were reared in the laboratory. The larvae were kept in plastic boxes (35 cm x 26 cm x 6 cm) and fed with pesticide-free soybean leaves obtained from soybean plants cultivated at a farmer field. Approaching pupation, *S. litura* larvae were transferred to other, similar plastic boxes provided with oven-sterilized sawdust for pupation. One week after pupation, the pupae were transferred to cylindric plastic cages with gauze top (60 cm in diameter, 30 cm high) and the emerging adults were fed 10% honey solution in cotton swab. Soybean leaves were placed inside the adult cages as oviposition sites. Eggs deposited on the soybean leaves were collected daily and placed in plastic boxes as before. The emerging larvae were fed with pesticide-free soybean leaves as above. Third-instar larvae of *S. litura* were used for bioassays.

Test materials

Neem seeds were acquired from East Java, Indonesia, and neem oil (azadirachtin content: 343.82 ppm) was obtained by pressing the seeds using a screw press as described by Nisya et al. (2017). Neem oil formulation (NOF) containing neem oil 92%, diethanolamide B14 5% and glycerol 3% was also prepared following Nisya et al. (2017). A commercial formulation of methomyl (Metindo 40 SP, 40% a.i.), purchased from a local pesticide kiosk, was used for comparison.

Bioassays

This study consisted of three experiments. The first experiment was the toxicity test to determine the relationship between NOF concentration and *S. litura* larval mortality. The second experiment was sublethal tests to assess the effect of NOF at sublethal concentrations on *S. litura* development until pupation and adult emergence. The third experiment was the sublethal test to evaluate the effect of NOF on reproduction of *S. litura* adult females that developed from the larvae treated as third instars. Similar tests were also done using methomyl.

Toxicity test. NOF and methomyl were tested separately at six concentration levels that were expected to give 15%-95% larval mortality as determined in preliminary tests. Bioassays were done using a leaf-dip feeding method. Test materials were diluted separately in distilled water containing a pesticide sticker Agristick 400 L (alkyl aryl polyglycol ether 400 g/l) 0.5 ml/l. Distilled water containing Agristick 400 L 0.5 ml/l was used as a control solution.

Trifoliate soybean leaves were dipped in NOF or methomyl suspension at a particular concentration until complete wetness. Control leaves were dipped in the control solution. Treated and control leaves were put separately in glass petri dishes (diameter 9 cm) placed upside-down and lined with tissue paper which extended up to the space between the top and bottom of each petri dish (Nailufar and Prijono, 2017). Ten newly-moulted third-instar larvae of *S. litura* were put in each petri dish containing either a treated or control soybean trifoliate leaf. Each concentration treatment was done in six replicates. Test larvae were fed with NOF-treated leaves for 6 days, then were fed with untreated leaves for the next 3 days. Methomyl-treated leaves were given for 2 days, then were fed with untreated leaves for the next 2 days. The number of dead larvae was counted daily up to day 9 for NOF treatment dan day 4 for methomyl treatment. Larval mortality data were analysed with probit method using PoloPlus (Robertson et al., 2002-2003). The whole toxicity bioassays with NOF and methomyl were done twice.

***Sublethal effect on development tests*.** These tests consisted of two parts: (1) effect of NOF and methomyl at LC_{10} and LC_{25} on development of *S. litura* from the third instar to pupal stage, (2) effect of NOF at $LC_{0.25}$ $LC_{0.5}$, LC_{15}, and LC_{25} on development of *S. litura* from the third instar to adult emergence. In the first part, 150 and 250 third-instar larvae were used for the treatment with NOF at LC_{10} and $LC_{25,}$ respectively, and 100 and 150 larvae for the treatment with methomyl at LC_{10} and LC_{25}. In the control, 80 larvae were used. In the second part, 250 third-instar larvae were used for each treatment with NOF at the above-mentioned concentrations and 100 larvae were used for the control.

Dilution of test materials and treatment procedures were the same as in the toxicity test. Test larvae were kept in petri dishes, in groups of 10, until day 6 and then the larvae were kept individually in clear plastic pots (2.5 cm in diameter, 4.5 cm high) until pupation to avoid cannibalism. In the first test, the percentage of pupation was recorded after all larvae pupated. In the second test, records were also kept with regard to the number of deformed pupae in additon to normal pupae. The surviving pupae were kept until adult emergence and the percentage of emerging adults was recorded.

***Sublethal effect on reproduction test*.** In this test, NOF was tested at concentrations equivalent to $LC_{0.25}$ $LC_{0.5}$, LC_{15} and LC_{25} while methomyl was tested at LC_{10} and LC_{25}. The

number of test larvae for each treatment and procedures of treatment were the same as in the sublethal effect test on development. After adult emergence, 15 pairs of adults were confined separately in cylindric plastic cages with gauze top (7 cm in diameter, 25 cm high) and fed 10% honey solution in cotton swab. A soybean leaf was placed inside each cage for oviposition. Eggs deposited on the soybean leaf were collected and the used soybean leaf was replaced with another fresh leaf. The number of eggs deposited in each treatment were counted and analysed with *t*-test using SAS statistical program package (SAS Institute, 2002-2003).

Results

Toxicity

The results of leaf-residue feeding tests showed that the average LC_{50} and LC_{95} of NOF at 9 d after treatment (DAT) were 15.1 and 28.5 ml/l, respectively, whereas those of methomyl at 4 DAT were 0.13 and 0.26 g a.i./l (Table 1). The content of azadirachtin, the main active ingredient, in NOF used in this study was 343.82 ppm (Nisya et al., 2017). In term of azadirachtin concentration, LC_{95} of NOF was about 0.1 g/l (azadirachtin equivalent) which was lower than that of methomyl. The recommended field rate of methomyl as shown on its product label was equivalent to 0.25-0.5 g a.i./l. Thus, methomyl can be regarded still effective against *S. litura.*

Table 1. Results of probit analysis of mortality of *S. litura* larvae as affected by neem oil formulation and methomyl[a]

Test material[b]	Slope ± SE[c]	LC_{50} (95% CI)[d]	LC_{95} (95% CI)
Neem (ml/l)			
Test 1	5.27 ± 0.61	17.1 (15.3-19.3)	35.0 (29.0-48.8)
Test 2	7.28 ± 0.78	13.1 (11.3-14.5)	22.0 (19.1-29.6)
	Average	15.1	28.5
Methomyl (g a.i./l)			
Test 1	5.14 ± 0.51	0.10 (0.09-0.12)	0.22 (0.18-0.30)
Test 2	6.20 ± 0.60	0.16 (0.14-0.19)	0.30 (0.25-0.46)
	Average	0.13	0.26

[a]Insect mortality was assessed at 9 and 4 days after treatment for neem and methomyl, respectively. [b]n = 420 third-instar larvae in each test. [c]SE: standard error. [d]CI: confidence interval.

Development derangement

Sublethal treatments with NOF at LC_{10} and LC_{25} on the third-instar larvae of *S. litura* completely suppressed development of the test larvae to the pupal stage, whereas the pupation in the treatment with methomyl at LC_{10} and LC_{25} was 72.0% and 72.7%, respectively, and that in the control was 93.8% (Table 2). The feeding treatment with NOF was given for 6 days and thereafter untreated leaves were given until pupation time. Most test larvae, however, died after 6 days (data not shown) and no larvae succesfully developed to the pupal stage. This data indicates that NOF inhibited the development of *S. litura* larvae even after the treatment was removed. Thus, NOF has an insect growth regulating (IGR) activity with extended interference on *S. litura* development. On the other hand, most larvae surviving the 2-d feeding treatment with methomyl could develop successfully from the third instar to the pupal stage. Methomyl

is a neurotoxic carbamate insecticide (Yu 2015) which causes a more immediate effect without any apparent IGR activity.

In further tests, the treatments with NOF at $LC_{0.25}$ to LC_5 reduced normal pupation by 12.9% to 81.7%, compared with control, and the percentage of deformed pupae tended to increase with the increase in NOF concentration (Table 3). In addition, the same NOF treatments decreased normal adult emergence by 88.4% to 97.1%, relative to control. These data also indicates that NOF caused extended interference on *S. litura* development including morphological defects. This is consistent with the fact that azadirachtin, the main active ingredient in NOF, interferes with the function of hormones that control insect growth and development (Mordue and Nisbet, 2000).

Table 2. Sublethal effect of neem oil formulation and methomyl on development of *S. litura* larvae to pupation

Test material	Concentration[a]	Number of larvae	Mean % pupation (SD)[b]
Control	-	80	93.8a (9.2)
Neem	LC_{10} (9.24)	150	0c (0)
Neem	LC_{25} (11.65)	250	0c (0)
Methomyl	LC_{10} (0.08)	100	72.0b (19.3)
Methomyl	LC_{25} (0.10)	150	72.7b (16.7)

[a]LC_{10} and LC_{25} of neem and methomyl were based on mortality assessment at 9 and 4 days after treatment, respectively (*see* Table 1); concentration of neem formulation was expressed as ml neem oil/l and that of methomyl as g a.i./l. [b]SD: standard devviation.

Table 3. Sublethal effect of neem oil formulation on development of *S. litura* (further tests)

Concentration[a] (ml/l)	Number of larvae	% Pupation		% Emerging adults	
		Normal	Malformed	Normal	Malformed
Test 1					
Control	100	83.0	9.0	55.0	12.0
LC_1 (6.22)	250	34.4	12.8	6.4	1.6
LC_5 (8.05)	250	15.2	27.2	1.6	0
Test 2					
Control	100	90.0	5.0	60.0	6.0
$LC_{0.25}$ (5.20)	250	69.2	9.2	5.6	3.6
$LC_{0.50}$ (5.67)	250	78.4	16.4	2.8	2.4

[a]LC_x's of neem were based on mortality assessment at 9 days after treatment (*see* Table 1).

Fecundity reduction

The treatments with NOF at $LC_{0.25}$ to LC_5 on the third-instar larvae of *S. litura* decreased egg production by the emerging females by 79.2% to 98.3%, compared with control (Table 4). In additon to growth and development, insect reproduction in Lepidoptera is also controlled by two insect growth hormones, i.e. juvenile hormone and ecdysteroids (Telfer, 2009). Any inhibition on the function of either of these hormones may result in decreased egg production. On the other hand, sublethal treatments with methomyl at LC_{10} and LC_{25} increased the fecundity of the emerging females by 30% and 92% relative to control (Table 5).

Table 4. Sublethal effect of neem oil formulation on reproduction of *S. litura*

Concentration[a] (ml neem oil/l)	Mean number of eggs/female (SD)[b]	$\|t\|$[c]	$P > \|t\|$
Further test 1			
Control	875.9 (511.0)		
LC_1 (6.22)	60.9 (93.2)	5.99	< 0.0001
LC_5 (8.05)	44.0 (62.2)	5.98	< 0.0001
Further test 2			
Control	938.9 (581.2)		
$LC_{0.25}$ (5.20)	16.4 (30.1)	6.13	< 0.0001
$LC_{0.50}$ (5.67)	195.3 (156.7)	4.61	0.0002

[a]LC_x's of neem were based on mortality assessment at 9 days after treatment (*see* Table 1). [b]SD: standard deviation. [c]For comparison between each neem treatment and its respective control.

Table 5. Sublethal effect of methomyl on reproduction of *S. Litura*

Concentration[a] (g a.i/l)	Mean number of eggs/female (SD)[b]	$\|t\|$[c]	$P > \|t\|$
Control	694.4 (367.2)		
LC_{10} (0.08)	1333.5 (736.7)	3.01	0.007
LC_{25} (0.10)	899.4 (489.2)	1.30	0.205

[a]LC_{10} and LC_{25} were based on mortality assessment at 4 days after treatment (*see* Table 1). [b]SD: standard deviation. [c]For comparison between each methomyl treatment and control.

DISCUSSION

The results of this study with regard to increased mortality, deranged development, and reduced reproductive capacity of the soybean armyworm, *S. litura*, as affected by NOF are accordant with known multiple effects of neem derivatives (Mordue and Nesbit, 2000). The main active ingredient of neem, i.e. azadirachtin, interferes with the function of insect growth hormones so that neem-derived insecticides can interfere with insect development and reproduction (Rembold, 1989; Koul, 1996). Owing to these various effects of neem along with its antifeedant property (Schmutterer, 1990), application of neem products in the field in the long run can result in pronounced decreases in target pest population and crop damage (Shah et al., 2019; Lengai et al., 2020).

Unlike neem which acts as a slow-acting insect growth regulator, a synthetic carbamate insecticide methomyl included in this study is a fast-acting nerve poison which acts by inhibiting acetylcholinesterase enzyme in synapses of the insect nervous system (Yu, 2015). Moreover, sublethal treatments with NOF on the third-instar larvae of *S. litura* significantly decreased egg production by the emerging adult females, whereas methomyl treatment increased the fecundity of *S. litura* females. Thus, sublethal treatments with NOF did not stimulate the increase in *S. litura* reproduction and as such, it is not expected to induce resurgence in *S. litura* population. On the other hand, sublethal treatments with methomyl increased the fecundity of the emerging *S. litura* females. This suggests the risk of resurgence

in *S. litura* population associated with methomyl through stimulation of insect reproductive capacity. The increase in reproductive potential of the diamondback moth, *Plutella xylostella*, induced by methomyl has been reported by Nemoto et al. (1993). Concerning the mechanism of reproductive potential increase, Khaeso et al. (2014) reported that increased reproduction in the rice brown planthopper, *Nilaparvata lugens*, could be caused by enhancement of vitellogenin synthesis.

Neem-derived insecticides are relatively safe to pest natural enemies and other non-target organisms (Schmutterer 1997; Boeke et al., 2004). In addtion, neem products are biodegradable (Schmutterer, 1990; Widayani et al., 2018) so that they are not expected to cause pronounced environmental contamination and residue hazards. Thus, environmentally friendly neem products can be compatibly incorporated into many pest management programs (Campos et al., 2019). Since neem products are relatively safe to natural enemies, if neem application cannot reduce the target pest population to the level below its control threshold, the residual pest population is expected to be suppressed further by its natural enemies. In the field, the short persistence of neem derivatives may require repeated application. Thus, it is necessary to observe proper timing of neem application in the field such as when target pests are in their most vulnerable stage of development.

Neem is native to Indian subcontinent and can grow easily and rapidly in a wide range of environmemtal conditions. At present, neem has spread to many tropical and subtropical parts of Asia, Africa, Australia, as well as Central and South America (Mondal and Chakraborty, 2016). Efficacious neem-based insecticides can be prepared by simple extraction with water (Anjarwala et al., 2016) and this simple preparation method may benefit many resource-limited farmers in neem growing regions. Neem is the most widely recommended botanical insecticide in agricultural extension materials in developing countries of Africa and Asia (Dougoud et al., 2019). Availability of neem sources, the ease of neem-based insecticidal preparation, and various advantageous properties of neem have made neem the most promising botanical insecticide in the world today (Benelli et al., 2016; Chaudhary et al., 2017; Isman, 2020).

CONCLUSIONS

The treatment with neem oil formulation (NOF) increased mortality, inhibited development, and decreased reproductive potential of the soybean armyworm, *S. litura*. The treatment with sublethal concentrations of NOF did not stimulate *S. litura* reproduction and as such, it is not expected to induce resurgence in the pest population. Sublethal treatments with methomyl increased the fecundity of the emerging *S. litura* females suggesting the risk of resurgence of the pest population through stimulation of the pest reproductive potential. Thus, neem is a promising botanical insecticide alternative to synthetic insecticides for the control of the soybean armyworm *S. litura*.

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THEILERIOSIS IN COW - A CASE REPORT

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Abstract

Theileriosis is a tick-borne disease of cattle caused by protozoan parasite from genus Theileria. These parasites destroy red blood cells, causing anemia and, in some cases, jaundice or hemoglobinuria. Incubation are usually 10-12 days and the first symptoms are fever, jaundice, anemia and hemoglobinuria. The animals lie down, have a fast, weak pulse, depression, edema of the lymph nodes that are enlarged and painful, diarrhea is possible. There is a complete cessation of secretion of milk. In our paper we give an overview of the case of cow theileriosis recorded during 2020.

Key words: Cow, *Theileriosis*

INTRODUCTION

Theileriosis is a tick-borne disease of cattle caused by protozoan parasite *T. parva* and *T. annulata* (Demessie and Derso,2015). Parasites are obligate intracellular parasites from phylum Apicomplexa, order Piroplasmida, family Theileriidae and genus Theileria. In Europe most usually infection are caused by *T. annulata* transmitted by ticks of the genus *Hyalomma*. In Serbia and West Balcan most usually found *Hyalomma marginatum marginatum* (*Hy. savignyi*) and *Hy.detritium* (Milutinović et al., 1996; Pavlović et al.2016; Pavlović et al. 2020).

A spread of infection are in direct corelation with population dinamic of ixodid ticks. *T. annulata* only mature after an infectedtick attaches to a host, and thetick must be attached for a few days before these organisms are transmitted Bishop et al.,2004).. Sporozoites we occurred in salivary glands of infected ticks. Schizonts occur in spleen, lymph nodes, liver and whole blood (mononuclear cells) from infected animals, transmission by inoculated schizonts occurs readily to cattle. Theileriosis can affect cattle of all ages, however heavily pregnant, lactating, and stressed cows tend to be at greatest risk, due to resultant reduction in immunity (Morrison,2015).

The incubation period varies from 4 to 14 days after attachment of the infected ticks to the host. Because the parasite is living inside the red blood cells, the body attacks its own infected red blood cells (haemolysis) to destroy the parasite induced anemia of animals.

The first and only data on theileriosis in cattle in Serbia come from 1924 by Džunkovski and Urođević and after that there is no data about this disease. For these reasons, here we give an overview of the first case of theileriosis in cattle recorded during 2020, almost one hundred years later than the first and only case recorded until now in Serbia.

History

In one cow aged 3 years, which is 8 months pregnant, we occurred signs of fever (rectal temperature > 39ºC), anaemia and jaundice. The cow gave birth soon after that and later drop in milk production. Soon after these symptoms appeared difficulty breathing, with rapid and shallow breaths, depression, weakness and inappetence. It also was noted edema of the lymph nodes that are enlarged and painful.

Laboratoryl finding

Based on the clinical symptoms, a suspicion of infection with blood parasites was established and blood was taken for analysis. The blood smears were air-dried, fixed in absolute methanol for 1 minute and stained in 10% Giemsa stain for 20-30 minutes (Asanin et al.,2006, Mans et al.,2015). After that smears were examined by light microscope and theileria were identified by morphological characteristic (Sharma and Gautam,1977).

Diagnosis

Theileriosis caused by *T. annulata* (fig.1,2)

Figure 2. *T. annulata* in blood smear

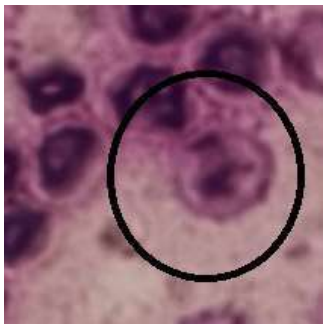

Figure 2. *T. annulata* in blood smear

Therapy

The cow was given an antipyretic and then a depot of tetracycline. In addition, she received dexamethasone and vitamin preparations. After three days, a new blood test was performed and a significant drop in the number of parasites was found. the therapy was repeated and no parasites were found in the blood at the next examination. The cow started giving milk again and achieved a milk yield of 16 liters per day.

Conclusions

Theileriosis rarely occurs in cows in Serbia and in about last fifty years only few cases have been reported. Climate change, which in recent decades has led to a drastic microclimatic disturbance of living conditions in certain areas, has led to a change in the epidemiological situation and to the introduction of many diseases characteristic of tropical and subtropical areas

into Europe. Thus, we increasingly have the appearance of spirocercosis, anaplasmosis, bluetongue virus, leishmaniasis, etc. Theileriosis is another disease that is added to this list and it is to be expected that in the coming period it will appear more and more frequently and in wider areas of both the Western Balkans and Europe.

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THE INFLUENCE OF DIFFERENT PRESOWING TREATMENTS ON SEEDLINGS BEHAVIOURS OF *ILEX AQUIFOLIUM* L.

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ABSTRACT

Plants from the group of evergreen deciduous shrubs used in landscaping practice in our country are not many. *Ilex aquifolium* L. is one of those plants. With its high decorative qualities and unpretentiousness to the environmental conditions, the plant deserves wider use. Problem in the production of seedlings is the deep peace of seeds and consequent low germination. This study was conducted to explore some possibilities for pre-sowing treatments of seeds of *Ilex aquifolium* L. and for increasing of their germination. They were used variations of soaking in tap, hot water and a solution of GA_3 at concentrations of 0.5 or 1,0 gl^{-1}. The seeds were stratified after different treatments. Were used seeds with or without pericarp. Stratification was done in pure washed river sand at a temperature of 4^0 C for 90 days. All treated seeds showed a higher rate of germination in comparison with the control. With the highest germination rate - 86.7 - were seeds treated with hot water and then stratified for 90 days. Treatment with GA_3 without endocarp seeds showed a higher germination rate - 74.5% and the necessity of shortening the period of stratification in comparison with seeds with endocarp. It was established that soaking in a hot or tap water and the subsequent 90-days stratification are applicable treatments that would result in practice of ornamental nurseries in our country.

Keywords: *Ilex, pre-sowing treatment, soaking, gibberellic acid*

INTRODUCTION

Ilex aquifolium L. is a species widespread in the Mediterranean region. There are many species that differ in habitus, shape and color of the leaves, flowering time and timing of seed maturation. The plant is suitable for use as ornamental in our parks and gardens, but it is not yet as widespread as it deserves. One of the reasons is the difficulties in the propagation and production of planting material. The traditional way of propagating this species is by sowing pre-stratified seeds. Studies on the germination of seeds of *Ilex aquifolium* L. are not many, and the information is limited to general conclusions and recommendations (Alcántara et al., 2000; Norman, 1993; Young and Young, 1992). Many different methods have been used by specialists to overcome seed dormancy. Gibberellic acid has been used for this purpose and to increase seed germination in some species (Andersson et al., 1998; Hartmann et al., 1975; Riley, 1987). Soaking in water, lukewarm or hot, also stimulates seed germination, allows gas exchange and water penetration through the seed coat, as well as softening of the seed coat itself and facilitating germination (Irvani et al., 2012; Nikolaeva et al., 1985; Murdoch et al.,1992). It is especially important to establish the influence of stratification, treatment with growth regulators and soaking in lukewarm (30^0C) or hot water (70^0C) on seed germination in order to use this knowledge in the practical work of ornamental nurseries for production of planting

material or in the selection to obtain new cultivars and varieties. Therefore, the aim of the present study was to establish the influence of the above factors on the germination of seeds of *Ilex aquifolium* L. and to make some practical recommendations.

Material and Methods.

Ripe fruits of *Ilex aquifolium* L. were collected in October 2017 and 2018 from plants on the territory of the Dendrological Park of the Agricultural University - Plovdiv. The fruits were picked by hand and stored in the refrigerator until use. Germination research began in October of that year. The viability test was performed on three representative samples, each containing 20 seeds of the 2, 3, 5, - tri-phenyl-tetrazolium method of ISIA (1996). In this study, the seeds of Ilex aquifolium L. showed 75% viability. The seeds were then surface sterilized with 1% Ca $(ClO)_2$ solution for 5 minutes and then rinsed three times with distilled water. The following pre-sowing treatment options were tested:

1. Control - seeds were stratified for 60, 90, 120 and 150 days and sown without pre-sowing treatment.
2. Variant 1 - soaking the seeds with endocarp in lukewarm water (30 ° C) for 5 days and stratification for 60, 90, 120 and 150 days

3. Variant 2 - soaking the seeds with endocarp in hot water (70 ° C) for 3 minutes, cooled immediately and then stratification for 60, 90, 120 and 150 days.

4. Variants - 3 and 4 - soaking seeds with and without endocarp in a solution of 0.5 gl^{-1} GA_3 for 24 hours and then stratification for 60, 90, 120 and 150 days.

5. Variants 5 and 6 - soaking the seeds with and without endocarp in a solution of 1 gl^{-1} GA_3 for 24 hours and then stratification for 60, 90, 120 and 150 days.

The seeds were placed in washed river moistened sand and stratified in plastic bags at 4 ° C in a refrigerator. At the end of the stratification period, the seeds were removed from the sand and sown in substrate in boxes. The temperature was maintained at 25 ° C and 16/8 hours photoperiod. Germination was checked every four days.

The whole experiment lasted 190 days and ended when it was found that the seeds had stopped germinating. Seeds with at least a 2 mm long root were reported as germinated (ISTA, 1996).

Results

The data in Table 1 show that the germination of *Ilex aquifolium L*. seeds is influenced by both the pre-sowing treatment and the length of the stratification period. In all experimental variants, the germination values exceeded those of the control untreated variant. In it, the germination values are in the range between 17.13% for 60-day stratification and 58.67% for 90-day stratification. With the extension of the stratification period, the percentage of germinated seeds decreases, but remains relatively high - 52.76% for 120-day stratification and 50.36% for 150-day stratification. Soaking in lukewarm water (30 ° C) for 5 days leads to a significant increase in the percentage of germinated seeds, in the variant with 60 days stratification -58.67 %, while at 90; 120 and 150 daily differences are insignificant – respectively 8.20 %, 5.14 % и 4.86 %. Soaking in hot water (70 ° C) for 3 days and 90 days of subsequent stratification leads to the highest germination of seeds of *Ilex aquifolium L*. -

93.33%. In this variant maintains the tendency to reduce the percentage of germinated seeds with increasing duration of stratification period. The differences compared to the 90-day stratification are 32.40 % for the 120-day stratification and 28.07 % for the 150-day stratification, respectively. Pre-sowing treatment of seeds with 0.5 gl^{-1} GA_3 has an extremely positive effect on germination, as the values of the different variants are in the range of 73.21% - soaking in 0.5 gl^{-1} GA_3 with pericarp at 150 days of subsequent stratification, up to 91.33% - when soaked in 0.5 $gl^{-1}GA_3$ without pericarp at 90 days stratification. Exceptions are seeds with 60-day stratification, in which the variant with pericarp germination is 21.67%, and without pericarp - 50.08%. Treatment of *Ilex aquifolium L.* seeds with 1 gl^{-1} GA_3 leads to a significant increase in germination compared to untreated seeds. The seeds with 90-day stratification have the highest values - 81.67% for seeds with pericarp and 85.07% for seeds without pericarp. Again, there is a decrease in the germination rate with increasing duration of stratification period. Germination values at 60 days stratification were 38.48% for seeds with pericarp and 41.67% for seeds without pericarp. It is noteworthy that doubling the concentration for seed treatment with GA_3 from 0.5 gl^{-1} to 1 gl^{-1} does not lead to a significant increase in the germination rate, namely the average values for the variant treated with 0.5 gl^{-1} GA_3 with pericarp is 63.42%, and in that with 1 gl^{-1} GA_3 also with pericarp - 71.82%, i.e. the difference is only 13.24 %, and the treatment with a concentration of 1 gl^{-1} GA_3 in the seeds without pericarp even demonstrates a slight negative effect - when treated with 0.5 gl^{-1} GA_3 without pericarp, the germination of the seeds is 77.06% in 69, 79% for treatment with 1 gl^{-1} GA_3 without pericarp.

Table 1. Influence of pre-sowing treatment of cold stratification on seed germination of *Ilex aquifolium* L.(%)

Variants	Duration of cold stratification (days)			
	60	90	120	150
Control	17,13	58,67	52,76	50,36
Soaking in lukewarm water (30°C) for 5 days	27,18***	63,48**	55,47*	52,81*
Soaking in hot water (70°C) for 3 days	45,18***	93,33***	70,49***	72,87***
Soaking in 0,5 gl^{-1} GA_3 with pericarp	21,67**	80,35***	78,43***	73,21***
Soaking in 0,5 gl^{-1} GA_3 without pericarp	50,08***	91,33***	86,47***	80,37***
Soaking in 1 gl^{-1} GA_3 with pericarp	38,48***	81,67***	85,57***	81,57***
Soaking in 1 gl^{-1} GA_3 without pericarp	41,67***	85,07***	78,61***	73,81***

(P≤0.05), ** (P≤0.01), *** (P≤0.001), and the unproven difference – ns

With regard to germination energy (Table 2), the data show that for untreated seeds it is quite low - from 4.28% for the stratified 150 days seeds, to 7.18% for the stratified 90 days seeds.

Table 2. Influence of pre-sowing treatment and cold stratification on germination energy of *Ilex aquifolium* L. seeds (%)

Variants	Duration of cold stratification (days)			
	60	90	120	150
Control	6,65	7,18	6,94	4,28
Soaking in lukewarm water (30 ° C) for 5 days	14,81**	9,54**	10,90*	10,74**
Soak in hot water (70 ° C) for 3 days	13,68**	19,30**	10,80**	10,21**
Soaking in 0.5 gl^{-1} GA_3 with pericarp	13,34**	12,18**	10,17*	9,89**
Soaking in 0.5 gl^{-1} GA_3 without pericarp	12,74***	9,95**	8,11**	8,27***
Soaking in 1 gl^{-1} GA_3 with pericarp	9,54***	8,07***	7,88***	7,74***
Soaking in 1 gl^{-1} GA_3 without pericarp	8,43***	7,57***	7,15***	7,98***

(P≤0.05), ** (P≤0.01), *** (P≤0.001), and the unproven difference – ns

In the experimental variants with different treatments, the germination energy is highest in seeds stratified for 60 days and varies from 8.43% when treated with 1 gl^{-1} GA_3 without pericarp, to 14.81% when soaked in lukewarm water (30 ° C) for 5 days. An exception is the variant with soaking in hot water (70 ° C) for 3 days - 19.80%, which value is actually the highest for all treatment variants and all durations of stratification period. In the case of germinating energy, there is a clear tendency to decrease its values with increasing duration of stratification period.

Discussion

The results show that the seeds of *Ilex aquifolium L*. definitely have exogenous and endogenous dormancy. The results of a number of studies by other authors on the embryoculture in *Ilex aquifolium L*. also support the claim that the seeds have a dormant period (Arrieta et al., 2004; Thompson et al.,2003; Vleeshouwers et al.,1995). Regarding the average values of germination with the highest levels is the variant treated with GA_3 in a concentration of 0.5 gl^{-1} - 77.06%, and 1.0 gl^{-1} concentration has a negative effect, but the values are significantly higher than those in the control variant. Treatment with GA_3 at concentrations of 0.5 or 1.0 gl^{-1} before cold stratification is a successful practice and results in seed disruption with or without pericarp. The results obtained are in line with the results of other researchers that combining cold stratification with GA_3 treatment leads to increased germination in a number of tree and shrub species (Baskin et al., 2001; Pickett et al., 1989; Pipinis et al., 2012). Removal of the endocarp from the seeds increases the effect of GA_3 treatment and germination increases significantly for 60 and 90 days of stratified seeds. However, for mass propagation and production of propagating material, removal of the endocarp without damaging the embryo is a rather difficult practice, and the use of GA_3 is relatively expensive. The combination of soaking in lukewarm and hot water with cold stratification increases the percentage of germinated seeds, with the highest percentage of germinated plants (93.33%) observed when treated with hot water after 90 days

of stratification. Interruption of dormancy of seeds is a very important condition for shortening the germination period (Meyer et al., 2000; Yu 2004). If germination is delayed, the seeds become more sensitive and are attacked by fungal diseases (Bewley et al., 1994; Hacker et al., 1989; Rahnama-Ghahfarokhi et al. 2007). The results show that there are significant differences in germination and depending on the duration of the cold stratification. The 60-day stratification showed the lowest germination rate in all pre-sowing treatment variants, but the values significantly exceeded those in the control variant. The germination rate increases with increasing period to 90 days and then decreases at 120 and 150 days stratification, which is probably due to damage to these seeds during the stratification period due to removal of the endocarp and soaking in water.

Conclusions

The present study creates an effective system for interrupting dormancy in the seeds of *Ilex aquifolium L.* and increase their germination. Soaking the seeds without pericarp in 0.5 gl^{-1} GA_3 for 24 hours and subsequent cold stratification for 90 days increases the percentage of germinated seeds and germinating energy. Treatment with lukewarm and hot water, followed by 90 days of cold stratification, also increases germination. These results can be successfully adapted and used in contemporary nursery practice.

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APPLICATION OF FOLIAR FERTILIZER AGROARGENTUM FORTE IN CULTIVATION OF SEEDLINGS OF THREE SPECIES *TILIA*

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ABSTRACT

Species of the genus *Tilia* are some of the most common plants in our landscaping practice. One of the key problems in the production of seedlings of these species is the slow growth of seedlings in the first years of their cultivating. The application of foliar fertilizers is one way to solve this problem. This study examines the impact of foliar fertilizer AgroArgentum Forte on growth processes and biometric characteristics of annual seedlings of three species of linden: *T. cordata Miller*; *T. platyphyllos* Scop. and *T. tomentosa* Moench. Foliar fertilizer AgroArgentum Forte was used in 5 different concentrations: 0.3%; 0.6%; 0.9%; 1.2% and 1.5%. Treatment began with the formation of the first leaf at 90% of the plants and was applied every 14 days throughout the growing season to the end of September. It was found that the growth characteristics of the treated plants exceed substantially those of the untreated control plants. With the highest and thickest stems of seedlings are the three types of linden treated with 0.9% Agro Argentum Forte. Treatment with 1.2% concentration positively influences the number and size of leaves. Impact on biometric characteristics of the root system was not established. No difference was observed in biometric characteristics between the three types of linden treated with different concentrations of foliar fertilizers. We suddest the use of AgroArgentum Forte in nursery practice in concentration 1.2%.

Keywords: *Tilia, seedlings, foliar fertilizer, AgroArgentum Forte*

INTRODUCTION

The ecological requirements for protection of the natural environment and human health require us to comply with the type of fertilizers and preparations used, the norms and terms of their application in order for the residues in the production and soil to be in the permissible quantities required by international standards (Atanasova, 2012; Malinova, 2007). A large number of ecologically friendly fertilizers are mass-produced and offered on the market, which have not been studied in all agricultural crops (Sengalevich, 2007; Valchovski et al., 2007). To ensure a balanced nutrition, flowers require a rational fertilization system for their development (Ivanova et al., 2005; Ivanova and Kadum 1996; Sapundjieva et al., 2001). In our country, scientific research on biological testing of ecologically friendly fertilizers in flowers and ornamental trees and shrubs is less than in other crops. In Bulgaria such experiments were carried out on a number of flower species (asters, gypsophila, calendula, chrysanthemum, lily, mini carnation, etc.) to establish the effect of complex mineral fertilizers – HortiGrow, Lactofol, Kristalon and Vege (Atanasova et al., 1999; Atanasova et al. 2000;; Atanassova, 2013; Ivanova and et al., 1995; Kotopanova et al., 1999; Kotopanova and Atanasova, 2008;), as well as the effect of fertilization with organic fertilizers - Baikal, Biostim, Humustim and Lumbricol, for ornamental crops for cut flowers (gypsophila, chrysanthemum and mini carnation), for potted

flowering species (petunia, impatiens, mini rose, carnation and chrysanthemum) and for other ornamental crops (Atanasova, 2011, Atanasova, 2012; Atanassova et al., 2007; Kotopanova and Nencheva, 2008; Atanassova and Nencheva, 2012; Zapryanova and Atanassova, 2013). The aim of the present study was to investigate the effect of the biomineral fertilizer AgroArgentum Forte on the growth and development of linden seedlings and to determine the optimal concentration for plant treatment.

MATERIAL AND METHODS

During the period 2017-2019 in the Agricultural University - Plovdiv a pot experiment was carried out with 3 species of linden *Tilia cordata* Mill., *Tilia platyphyllos* Scop., *Tilia tomentosa* Moench. to study the influence of the biomineral fertilizer AgroArgentum Forte in foliar treatment of plants in the following concentrations 0.3%, 0.6%, 0.9%, 1.2% and 1.5%. AgroArgentum Forte works by accelerating the growth of the components of the classic fertilizers, by improving the absorption of light, thanks to the silver particles and by a strong systemic effect, stimulating the production of phytoalexins in high concentrations. The manufacturer of the fertilizer is ECE Technology group Ltd. To carry out the experiment, seeds were collected from the available trees in the Dendrological Park of the Agricultural University - Plovdiv, sown on September 10 in a polyethylene greenhouse, in pots № 12 with substrate of soil, peat and perlite, in a ratio of 2: 2: 0.5. The experiment was set in 5 variants with different concentrations, with 10 plants in each variant, and untreated plants were used for control. The treatment began the following spring, after the germinating seeds formed the first true leaf. A total of 19 foliar treatments were carried out in 14 days, the last being in the second half of September. Measurements of the height, diameter of the plant stem, number and size of leaves, as well as the indicators of the root system were performed at the end of the experiment. Statistical data processing was performed by ANOVA test. The significant difference between the control and the variants is represented by the sign * ($P \leq 0.05$), ** ($P \leq 0.01$), *** ($P \leq 0.001$), and the unproven difference - ns.

Results

The results of the influence of the biomineral fertilizer AgroArgentum Forte on the biometric characteristics of the root system of plants are presented in Table 1. At all tested concentrations of foliar treatment with AgroArgentum Forte, a positive effect was found on the number of roots, the length of the root system and its volume in all three spesies of linden, but the differences compared to the control are small and statistically unproven. Regarding the biometric characteristics of the stem (Table 2.) the increase in height at all concentrations (variants) exceeds that of the control and varies from 7.7% - 18.5% for small-leaved linden, from 6.3% - 8.6% for large-leaved and from 0.5% - 4.8% for silver-leaved linden.

Table 1. Influence of Agro Argentum Forte foliar fertilizer on the biometric characteristics of the root system in linden seedlings

Variants	*Tilia cordata*			*Tilia platyphyllos*			*Tilia tomentosa*		
	Number of roots	**Length of roots**	**Volume of roots**	**Number of roots**	**Length of roots**	**Volume of roots**	**Number of roots**	**Length of roots**	**Volume of roots**
Control	2,48	3,81	1,38	4,51	4,18	2,38	7,84	4,32	3,01
0,3%	2,34^{ns}	4,01*	1,54*	4,23	4,21*	2,44*	7,91^{ns}	4,51*	3,21*
0,6%	2,57^{ns}	3,93^{ns}	1,48*	4,81	4,38*	2,48*	7,98*	4,48*	3,11*
0,9%	2,38^{ns}	4,21*	1,49*	4,54	4,59*	2,58*	7,78^{ns}	4,78**	3,17*
1,2%	2,74^{ns}	4,11*	1,53*	4,48	4,66*	2,44*	7,70^{ns}	4,59*	3,28**
1,5%	2,51^{ns}	4,17*	1,68**	4,73	4,53*	2,47*	7,54^{ns}	4,63*	3,33**

*(P≤0.05), ** (P≤0.01), *** (P≤0.001), non - significant– ns

Table 2. Influence of AgroArgentum Forte foliar fertilizer on the biometric characteristics of a stem in linden seedlings

Variants	*Tilia cordata*		*Tilia platyphyllos*		*Tilia tomentosa*	
	stem height	**stem diameter**	**stem height**	**stem diameter**	**stem height**	**stem diameter**
Control	7,18	0,18	9,47	0,21^{ns}	12,51	0,24^{ns}
0,3%	7,73*	0,18^{ns}	10,07*	0,22^{ns}	12,73*	0,25^{ns}
0,6%	8,18**	0,19^{ns}	10,15**	0,24^{ns}	13,04**	0,25^{ns}
0,9%	8,48**	0,18^{ns}	10,28**	0,28^{ns}	13,57***	0,27^{ns}
1,2%	8,51***	0,19^{ns}	10,17**	0,27^{ns}	13,07**	0,25^{ns}
1,5%	8,40**	0,19^{ns}	10,08*	0,26^{ns}	13,11**	0,23^{ns}

*(P≤0.05), ** (P≤0.01), *** (P≤0.001),), non - significant – ns

The differences with the control are very well proven at P≤0.001. The highest plants in all three types of lindens were reported when treated with 0.9% solution of AgroArgentum Forte. The indicator of stem diameter also showed a positive effect of the biomineral fertilizer, but less pronounced than that of height. The number of leaves (Table 3) of the plants treated with different concentrations exceeds those in the control variant by 1.9% - 147.4% for small-leaved linden, from 2.2% - 13.6% for large-leaved linden and from 8.4% - 57.9% for silver-leaved linden. The following trend was observed - with increasing concentration the number of leaves increases. In all three species, the best growth results were reported with 1.2% consentration of AgroArgentum Forte. The results have a high level of evidence, with the exception of 0.3% and 0.6% for large-leaved linden. The obtained results for the influence of the biomineral fertilizer

AgroArgentum Forte on the size of one leaf in all three tested species linden confirm the trend observed in the number of leaves - with increasing concentration, the size of the leaves increases. There is evidence of differences in all variants of the three species.

Table 3. Influence of foliar fertilizer AgroArgentum Forte on the biometric characteristics of the leaf in linden seedlings

Variants	*Tilia cordata*		*Tilia platyphyllos*		*Tilia tomentosa*	
	Number of leaves	**leaf size**	**Number of leaves**	**leaf size**	**Number of leaves**	**leaf size**
Control	1,54	2,45	2,71	4,38	1,78	2,38
0,3%	1,58^{ns}	2,81*	2,57 ^{ns}	4,54*	1,93*	2,54*
0,6%	1,57 ^{ns}	2,90*	2,63 ^{ns}	4,38 ^{ns}	1,99*	2,68*
0,9%	2,34***	2,93*	2,77*	4,75*	2,24**	2,75*
1,2%	3,81***	3,07***	3,08**	4,98*	2,78***	2,98**
1,5%	3,47***	2,98**	3,00**	4,58*	2,81***	2,78**

*(P≤0.05), ** (P≤0.01), *** (P≤0.001),), non - significant – ns

Discussion

The positive results obtained in the foliar treatment of linden seedlings with AgroArgentum Forte are due to the included in a completely new formula of silver particles, which interact with chlorophyll molecules and thus significantly increase photosynthesis. This process causes more intensive absorption of carbohydrates, which leads to an increase in the size of the individual vegetative parts of plants and makes them healthier. The overall functional action of the microbial complex on plant development, which stimulates the root system, improves the overall habit of plants and increases the productivity of photosynthesis. The good results found in the use of new ecologically clean organic and mineral fertilizers are due on the one hand to the balanced formulas rich in organic matter, macro- and micro-elements, vitamins, humic acids and hormones and on the other - to the easily digestible form of nutrients. Our research with the biomineral fertilizer AgroArgentum Forte once again confirms the advantages of modern organic and mineral fertilizers. When treated with organic fertilizers - Humustim and Lumbricol, tested in gypsophila, potted species (carnation and chrysanthemum) and annual flowers (petunia and impatience), a positive effect on plant growth and development was also observed (Atanassova and Nencheva, 2012; Atanassova and Zapryanova, 2013; Zapryanova and Atanassova, 2013). A positive impact was also reported in the study of the new complex mineral fertilizer HortiGrow on the overall habitus and the individual phases of the development of mini carnation, cyclamen and gypsophila (Atanasova, 2012; Sapundzhieva et al., 2001; Atanassova, 2013). The positive impact of ecologically friendly fertilizers on ornamental crops is indisputable proof not only for improving the growth and development of plants, but also for protecting human health and the environment.

CONCLUSIONS

From the obtained results for the influence of the biomineral fertilizer AgroArgentum Forte in three species of linden *Tilia cordata* Mill., *Tilia platyphyllos* Scop., *Tilia tomentosa* Moench. the following conclusions can be drawn:- Leaf treatment of plants with AgroArgentum Forte has a positive effect on the number and length of roots, the volume of the root system, the height and diameter of the stem, the number and size of leaves in linden.- The optimal concentration for foliar treatment of plants with the biomineral fertilizer is 0.9% solution.- Species specificity was observed with regard to the influence of the biomineral fertilizer AgroArgentum Forte, and it was found that a better effect of foliar treatment was reported in *Tilia cordata* Mill. and *Tilia tomentosa* Moench.

For nursery practice, a concentration of 1.2% solution of AgroArgentum Forte for foliar treatment of linden can be recommended, which also gives good results close to those of the optimal concentration (0.9%).

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INFLUENCE OF RAPE SEEDS TREATMENT WITH BIOACTIVE PRODUCTS OBTAINED FROM COLLAGEN EXTRACT ON GERMINATION, PLANT DEVELOPMENT AND YIELDS

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ABSTRACT

The paper aims to present the results of the Coll-Rape project started three years ago, in which a series of bioactive composites obtained from collagen and keratin extracts, by-products left over from the processing of cattle and sheep skins have been tested. Not having a practical applicability, until now these products were discarded. Following the laboratory tests, it has been found that the new products are stimulating the germination of rape seeds, an effect that also extends during the first stages of vegetation in the field. Several formulas of treating seeds have been tested, on different varieties, with various amounts of bioactive additive and mixed with classic fungicide. In the agricultural year 2018-2019, 84 test plots have been sown in southern Romania, noting that the use of sprayed (coating) seeds with bioactive composites led to accelerated plant development, but also to protect them in the early stages of development. The lots have been carefully monitored and periodic measurements have been made, from emergence to harvest. After harvesting, all data have been statistically processed by the Anova program, highlighting the fact that treatment with 2 l product/t seed leads to yields increases of 200-500 kg, depending on the amount of fungicide it combines with. The use of bioactive extracts in agriculture is an innovation and an additional help, especially for organic farming. One of the tested products is already in the process of approval as a biostimulator of rape seeds.
Keywords: *Biostimulator, Collagen, Rape, Coating, Yield.*

INTRODUCTION

Collagen and keratin-based biostimulators whose effects we test in agriculture are obtained from by-products, respectively residues left over from the tanning process of cow and sheep skins (Gaidau et al., 2013; Niculescu et al., 2019). The benefits of this biotechnology include the fact that are used by-products that otherwise would have been classified as waste, being particularly polluting for the environment (Mihalache et al., 2014).

Protein hydrolysate products, similar to those tested by us, have been shown to be effective for optimal plant growth and for a better yielding (Balian and Bowes, 1977; Maini,

2006) in cereal crops (Epure et al., 2018), corn (Ertani et al., 2013), tomatoes (Koukounararas et al., 2013), carrots (Grabowska et al., 2012), spinach (Kunicki et al., 2010), cucumbers (Boehme et al., 2008; Wilson et al., 2015; Hiromi et al., 2018), broccoli (Amirkhani et al. 2016) and many others.

In recent years, increasing the tolerance of plants to the many abiotic factors they face is increasingly supported by biostimulating products, as an ecological alternative to chemical fertilizers (Wezel et al., 2014; Le Mire et al., 2016). As a result of laboratory tests, it has been found that the new products, applied as film-coating, have an effect of stimulating the germination of rapeseed (Niculescu et al., 2017; Gidea et al., 2018), an effect that extends throughout the first stages of vegetation in the field, so that in the end it is reflected in the realized yields.

MATERIAL AND METHODS

Considering the structure of the agricultural year, the sowing of the research lots within the field from Calarasi county (Romania) took place in autumn 2018, more precisely on September 7, 2018.

The sowing has been done with a seed drill with the preparation of the land, having a working width of 4 meters. Sowing rate: 60 germinating grains / m2.

The experiment was a three-factorial one, and for a greater accuracy of the results, 3 repetitions have been used, in a randomized system depending on the variety (factor A).

Each plot had an area of 500 sqm, ie 20 x 25 m. Thus, in each of the rehearsals were placed 27 plots, plus a control one (untreated).

The total number of sown plots was 28 x 3 = 84 plots.

Total sown area: 84 plots x 500 sqm = 42,000 sqm = 4.2 ha.

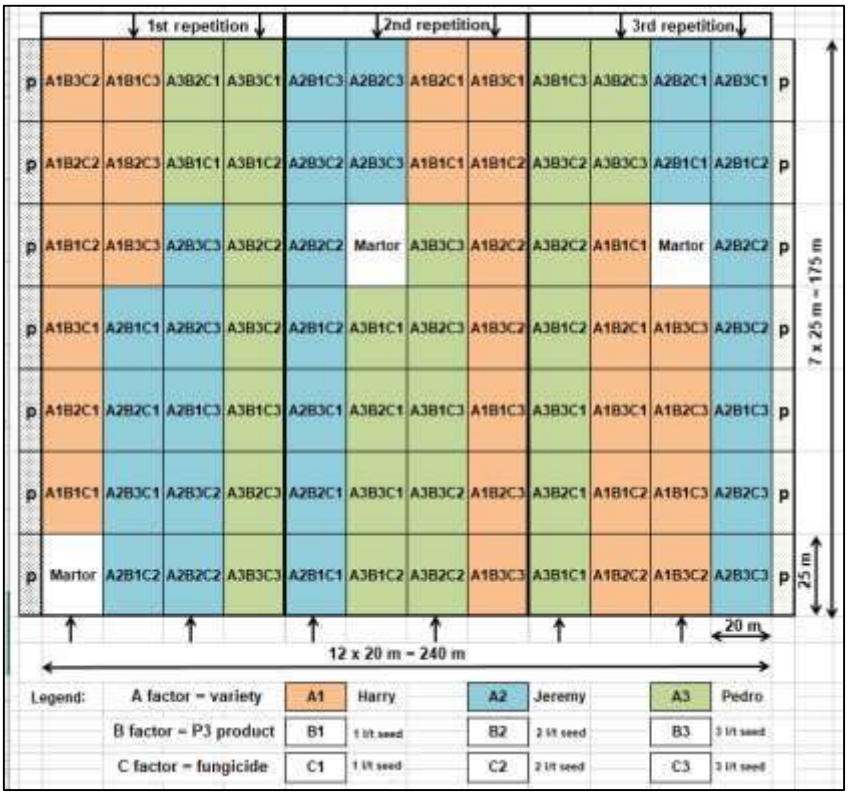

Figure 1. Technological scheme of experimental rapeseed lots located in Calarasi (Romania) in autumn 2018

The factors used in the experimental field, according to the technological scheme in Figure 1 are:

1) Factor A – Variety
   - → A1 – Harry;
   - → A2 – Jeremy;
   - → A3 – Pedro.
2) Factorul B – Test treatment (product P3 was chosen for this series of experiments, in different doses)
   - → B1 – 1 liter / ton of seed;
   - → B2 – 2 liters / ton of seed;
   - → B3 – 3 liters / ton of seed.
3) Factorul C – Fungicide treatment (Tiram 480 g / l) in different doses
   - → C1 – 1 liter / ton of seed;
   - → C2 – 2 liters / ton of seed;
   - → C3 – 3 liters / ton of seed.

For the treatments applied to the seed, various combinations have been made between factors B and C, the obtained solutions being then applied, by spraying, to the rapeseed before being sown.

From a technological point of view, all plots have benefited from the same technological scheme from establishment, maintenance during the vegetation until harvesting.

DISCUSSION

The evolution of the experimental rape crop (agricultural year 2018-2019)

The autumn of 2018 provided favourable climatic conditions for rape cultivation, which is why the plants had a very good emergence, managing to enter the winter in the optimal stage of vigour (Figure 2), so as not to be affected by extreme frosts.

Figure 2. Rapeseed experimental field – visit on October 22, 2018

In the spring of 2019 it has been found that the plants came out very well after winter, as a result the crop resumed its development at the intensity necessary for flowering and then for

the formation of silicates (Figure 3) and obtaining optimal yields. At the same time, the plants from the experimental plots showed a good natural resistance to diseases and pests.

Figure 3. Rape crop in different stages of vegetation – April and June 2019

Calculation of yields obtained from experimental rape plots (2018-2019)

The harvesting of the 84 experimental plots was carried out on 20.06.2019. According to the data in Table 1, there were several treatment variants that brought production increases constantly, regardless of the test variant - it is especially noteworthy the treatment with 2 l / t of bioactive product (P3), which each time led to significant increases in yield, compared to the control variants. It seems that this dose of bioactive product caused production increases regardless of the amount of fungicide (Tiram 480 g / l) with which it was mixed, but the highest values are those of C2 (+525.33 kg / ha), followed by C1 (+405.67 kg / ha).

Table 1. Yield data obtained following the unifactorial analysis according to the treatment applied to rape before sowing – average values for the 3 varieties

Variants	Average yield (kg/ha)	Control ratio (%)	Control difference	Signification
Control	3374.67	100.00	–	Control
B1C1	3484.67	103.26	110.00	
B2C1	3780.33	112.02	405.67	* * *
B3C1	3616.00	107.15	241.33	* * *
B1C2	3555.00	105.34	180.33	* *
B2C2	3900.00	115.57	525.33	* * *
B3C2	3388.67	100.41	14.00	
B1C3	3458.33	102.48	83.67	
B2C3	3600.00	106.68	225.33	* * *
B3C3	3261.33	96.64	-113.33	
		Limit differences	DL5% = 124.10	
			DL1% = 164.81	
			DL0.1% = 213.79	

Based on the information in the table above, it was possible to elaborate the graphical representation in Figure 4, from which it is also observed that at the maximum treatment doses (B3C3) the production has a sudden decline, falling visibly below the value of the untreated control. In these conditions, we can consider, for the next stage, the exclusion of this variant of treatment.

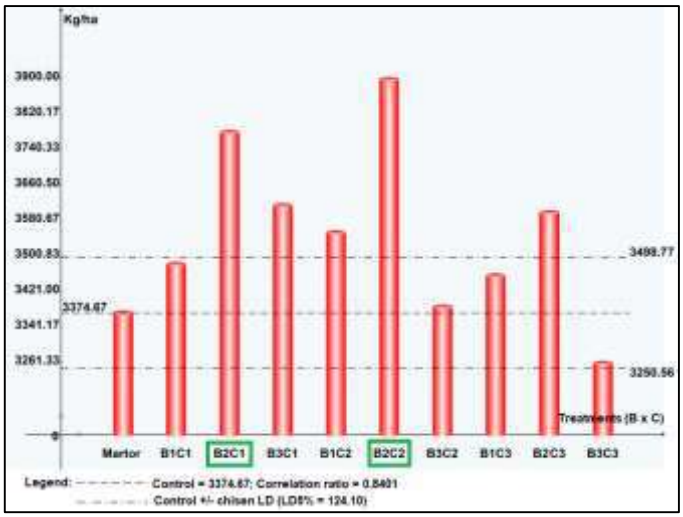

Figure 4. Yield graph obtained in the test plots according to the applied treatments

After the unifactorial analysis, in order to highlight and influence the variety on the background of the various treatments performed, we also represented graphically the productions of each research plot (Figure 5). As a basic observation, we must start with the difference between the varieties tested, namely:

→ Jeremy variety has significantly lower yields than Harry and Pedro, most likely due to genetic characteristics, proving to be unadapted to the pedo-climatic conditions in our country;

→ the reaction of the Jeremy variety to the various treatments is in the same trend as in the case of the other varieties, but for the future stages of testing it is considered that it can be excluded;

→ Harry and Pedro proved a very high ecoplasticity, but also similar productions, as a result it is enough to keep testing one of the varieties (Harry was chosen for the continuation, in this case);

→ we can say that the variety fails to change the influence of the applied treatment, another argument in order to keep in testing a single rapeseed variety for the next agricultural year.

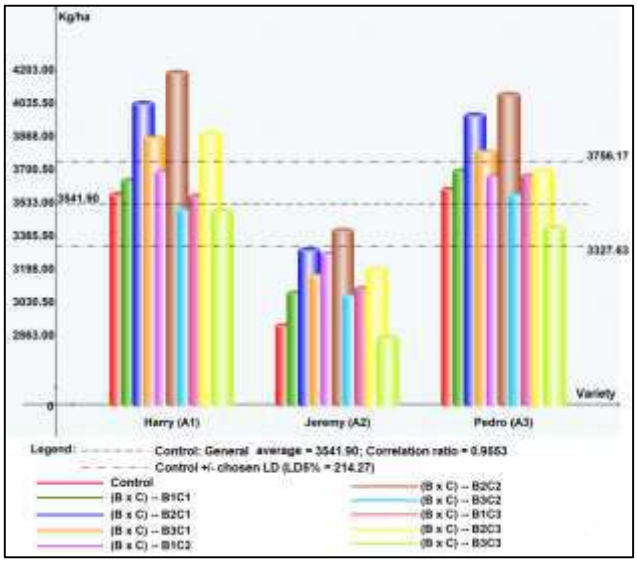

Figure 5. Yield results for each tested variant – bifactorial analysis, depending on the rape variety and the treatment applied to the seed

CONCLUSIONS

The tested bioactive product (P3), applied to rapeseed as a film-coating before sowing, provided the most relevant results at the amount of 2 liters of biostimulator / ton of seeds. Production decreases with increasing dose of fungicide with which it was mixed in the testing process. The biostimulator proves to be economically efficient, and for organic farming it is an innovative product, which can be introduced in practice immediately after approval.

ACKNOWLEDGEMENTS

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PRELIMINARY MORPHOLOGICAL AND BIOCHEMICAL EVALUATION OF SOME ANNUAL AND PERENNIAL WILD *HELIANTHUS* SPECIES

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ABSTRACT

Helianthus is a diverse genus comprised of 51 species and 19 subspecies with 14 annual and 34 perennial species. Wild sunflower relatives constitute a genetic resource of useful traits for crop improvement *via* transfer and infusion of genes into the cultivated *Helianthus annuus* genome, broadening the narrow genetic base of cultivated sunflower. Wild sunflower species are phenotypically and genetically much more diverse than cultivated *H. annuus* and their genetic potential has not been fully exploited. In the current study, an evaluation of morphological and biochemical traits on annuals *Helianthus argophyllus* (5 populations), *H. porteri*, *H. agrestis*, *H. paradoxus* (2 populations), diploid perennials *H. nuttallii* (6 populations), *H. salicifolius* (2 populations), *H. winteri*, and hexaploid *H. laetiflorus* was performed. Relative water content (%), the total antioxidant capacity (DPPH) and FRAP (Ferric reducing antioxidant power), total flavonoid content, total protein content and peroxidase activity were estimated. There was a highly significant variation among the all genotypes for all the characters studied. Further screening of wild species, potential donors of specific traits that will contribute to enhance the agronomic qualities of cultivated sunflower is needed.

INTRODUCTION

Objectives of the present work were: (1) to determine the differences in some physiological and biochemical parameters between wild annual *Helianthus* species; (2) to determine the differences in some physiological and biochemical parameters between wild perennial *Helianthus* species; (3) to identify species suitable as donors in future targeted breeding programs.

MATERIALS AND METHODS

The sunflower collection of Institute of Plant Physiology and Genetics, Sofia, Bulgaria (42˚ 50′N, 23˚00′E, 595 m above the see level) contains annual and perennial wild sunflower genotypes represented by a large number of different populations as well as lines in an advanced selection cycle. The predominant part of wild annual and perennial populations were grown

from seeds originally obtained from the Trakya University, Edirne, Turkey. Of all genotypes in the collection, twenty-one representatives were analyzed for morpho-biochemical traits. The cultivated sunflower *H. annuus* cultivar 1114 developed in IPPG, Sofia was used as a control. To evaluate and compare the biochemical parameters of the individual wild species, the following characteristics were measured: relative water content, % (RWC), the total antioxidant capacity (DPPH) and FRAP (Ferric reducing antioxidant power), total flavonoid content, total protein content and peroxidases activity were estimated. Data were subjected to one-way ANOVA analyses of variance for comparison of means, and significant differences were calculated according to Fisher LSD-test as P=0.05 using a statistical software package (StatGraphics Plus, version 5.1 for Windows).

RESULTS AND DISCUSSION

Leaf relative water content (RWC) is an indicator of physiological water status of plants, which reflects the balance between water supply to the leaf tissue and transpiration rate. RWC can control plant response to water stress (Bradford and Hsiao, 1982; Sinclair and Ludlow, 1985) It was reported that RWC is highly heritable genetically determined trait and as physiological definition of drought tolerance, it might be used for screening tool for yield improvement (Lugojan and Ciulca, 2011). Among the all investigated wild genotypes, one population of annual *Helianthus paradoxus* (2n=2n=34) revealed the highest value of RWC thus allow to stipulate that *H. paradoxus* could be involved in sunflower breeding programs as donor of drought tolerance genes (Fig. 1 a, b).

The total antioxidant activity (free radical scavenging activity) was measured by DPPH (α, α-

diphenyl-β-picrylhydrazyl) method. The recognized high antioxidant potential of sunflowers makes it a promising source of antioxidants (De Leonardis et al., 2005; Vassilevska-Ivanova et al. 2014; Zoumpulakis et al., 2017). The antioxidant activity of sunflower leaves extracts was determined as the ability to scavenge free radicals ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$) and to reduce ferric ions (FRAP). The results of these determinations are presented in Fig. 2 (a, b). High variability for DPPH was observed both in the group of compared annual species and in the group of perennial species. The lowest level of DPPH among annuals revealed one population of *H. argophyllus* 19 followed by *H. paradoxus* B1 population. Much of the variability among investigated annual species was associated with *H. argophyllus* populations. A similar trend was found in the diploid perennial *H. nuttallii* populations evaluated in the current study.

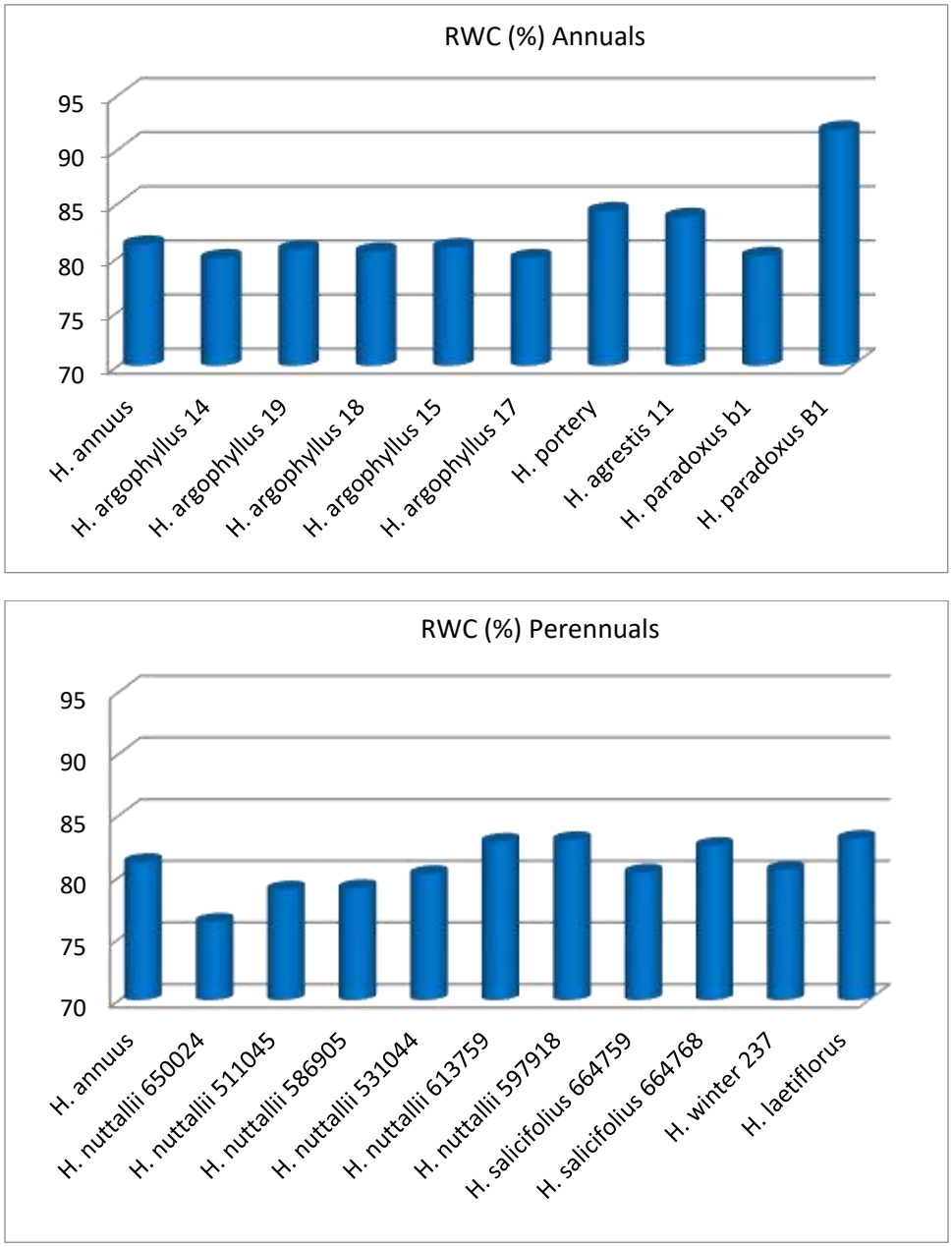

Fig. 1. Relative water content (RWC, %) of leaves measured at flowering stage in annual (a) and perennial (b) wild *Helianthus* species.

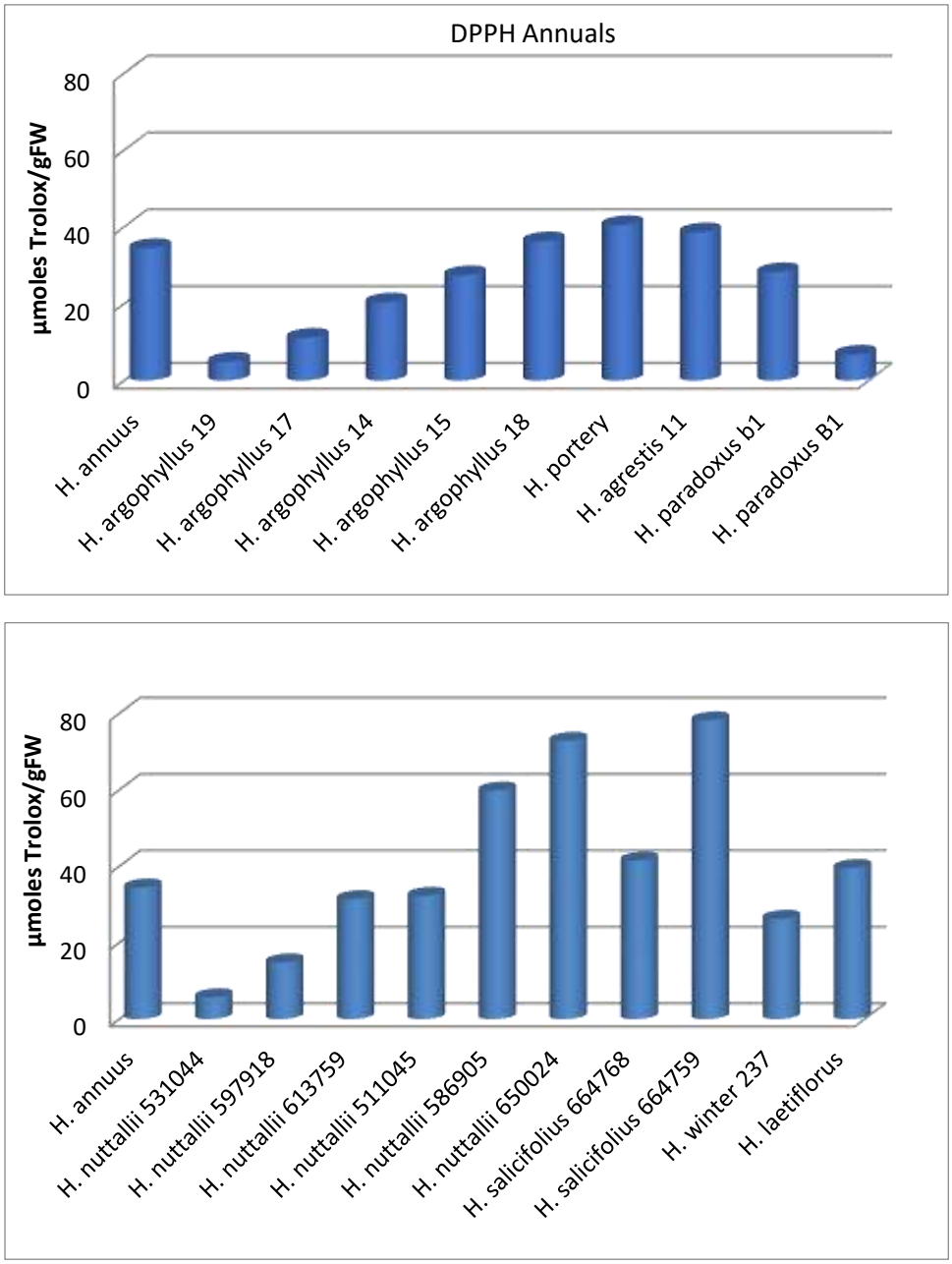

Fig. 2. The total antioxidant activity measured by DPPH method in fresh leaves of wild annual (a) and perennial (b) *Helianthus* species.

FRAP

Ferric Reducing Antioxidant Power (FRAP) values of the studied plants varied similarly to the way it varied in the DPPH evaluation. The strongest antioxidant properties, measured by FRAP assay were in two populations of perennial *H. nuttallii* 650024 and *H. nuttallii* 586905.

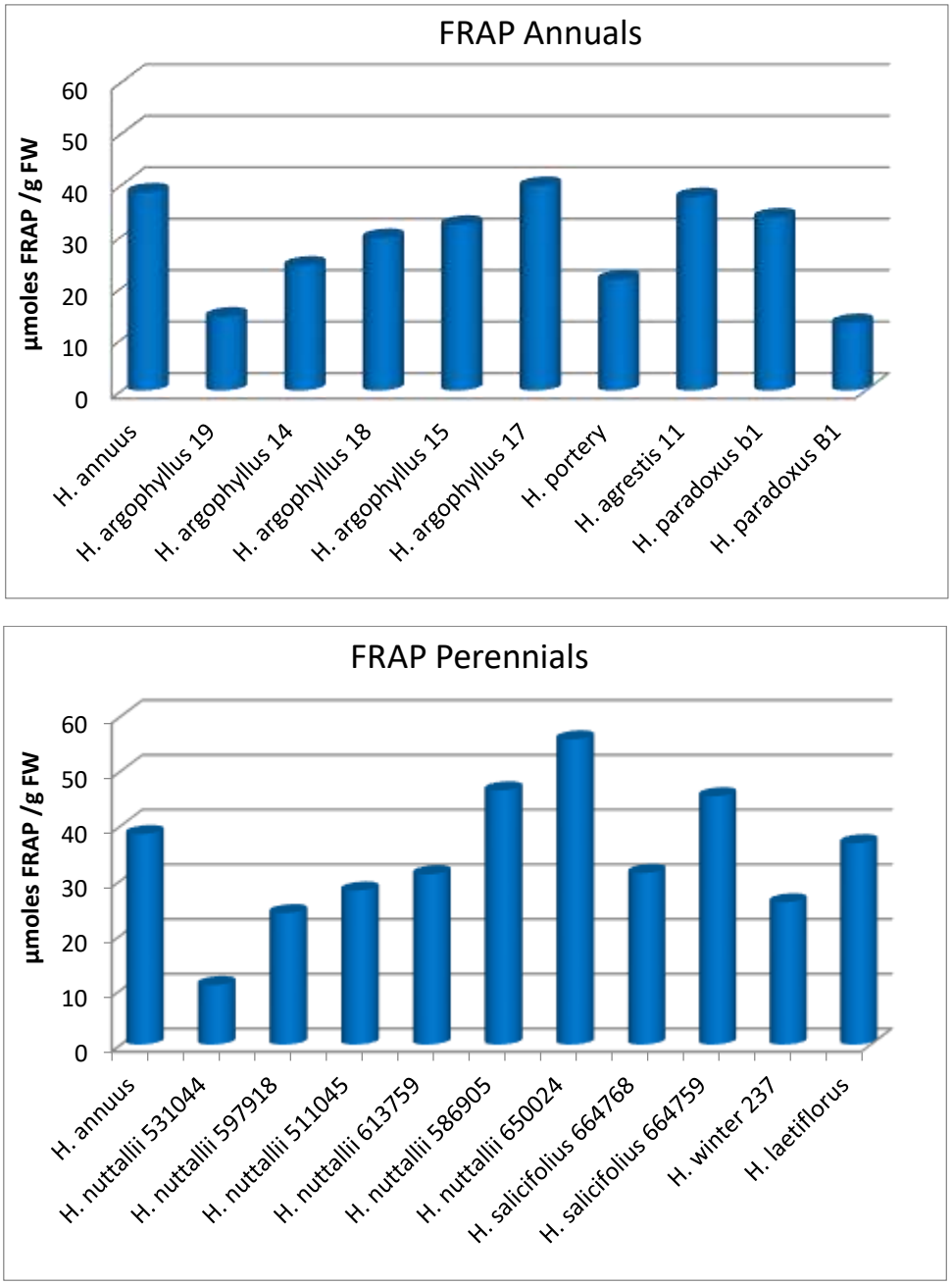

Fig. 3. The antioxidant potential of different annual (a) and perennial (b) wild *Helianthus* species determined by FRAP method

Flavonoid content

Flavonoids are phenolic substances, which exhibit a wide range of biological benefits, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory. Flavonoids are the important metabolites found in the sunflower family (Guo et al., 2017), which belong to the group of natural antioxidants such as tocopherols and phenolic acids, peptides (reduced glutathione), carotenoids etc. In the current study, among annual species, the highest level of flavonoids was found in one population of *H. paradoxus* followed by cultivated sunflower *H. annuus* (cv. 1114). It was interesting to note that the total flavonoid content did not correlate positively with the results from the DPPH assay. Among perennial species

investigated in this study, *H. nuttallii* 650024 and *H. salicifolius* 664759 showed high mean antioxidant capacity.

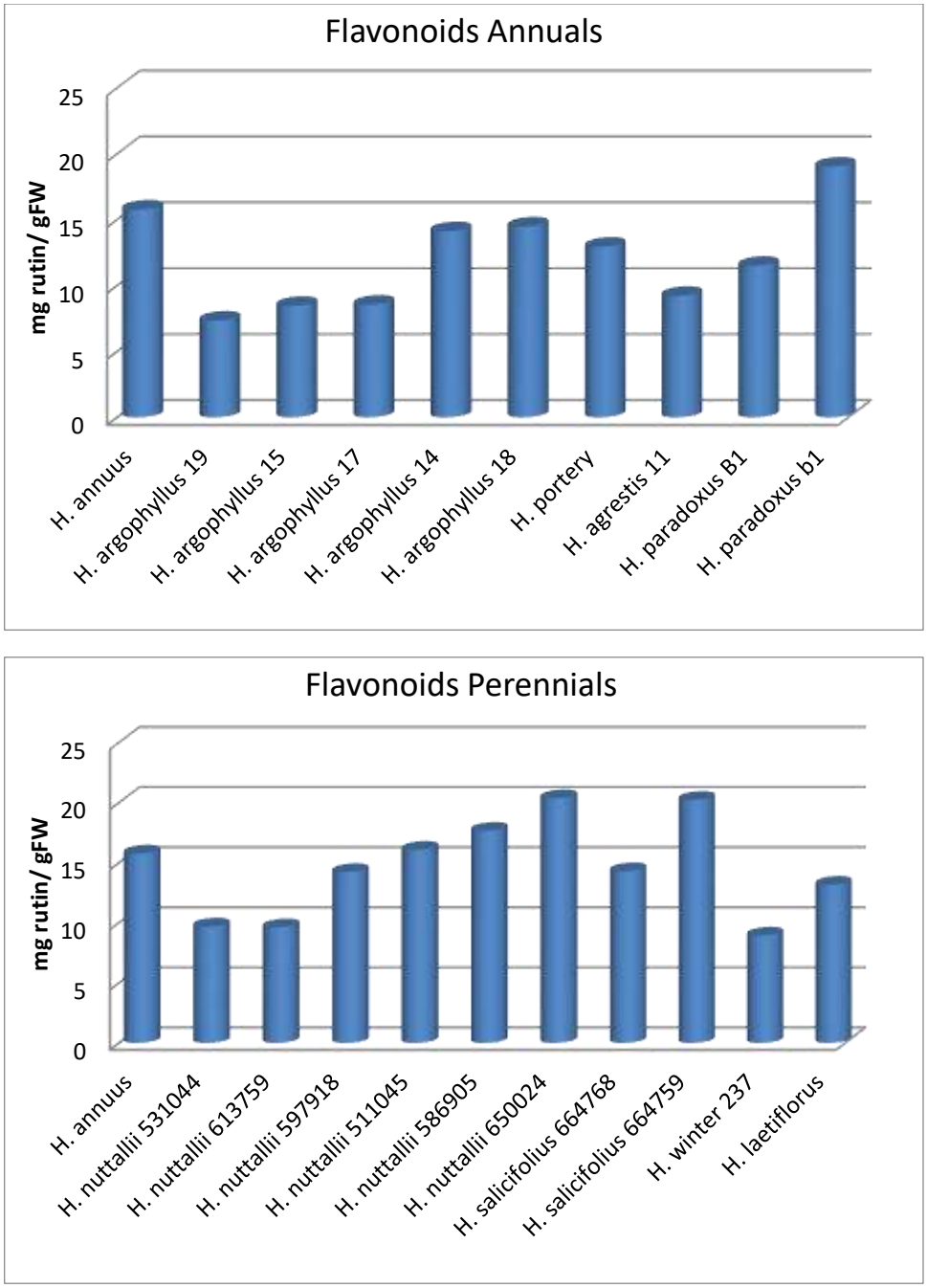

Fig 4. The total flavonoid content in different annual (a) and perennial (b) wild *Helianthus* species

Peroxidase activity

The peroxidase enzymes belong to the important reactive oxygen species (ROS) scavenging mechanisms in plants removing active oxygen species (Vranova et al., 2002; Apel and Hirt, 2004). They are part of antioxidative defense systems available to prevent the toxic effect of ROS. They occur because of oxidative stress caused by the biotic and abiotic stress conditions.

Therefore, the effectiveness of antioxidative defense system is related to peroxidase activity. Among annual species, the highest peroxidases activity revealed *H. portery* followed by one population of *H. paradoxus*. The lowest amount of peroxidases activity belonged to cultivated sunflower *H. annuus* (cv. 1114). The high activity level of antioxidant enzymes such as peroxidases is associated with defense related pathways in plants and stress tolerance (drought or salinity) and response to wide range of pathogens.

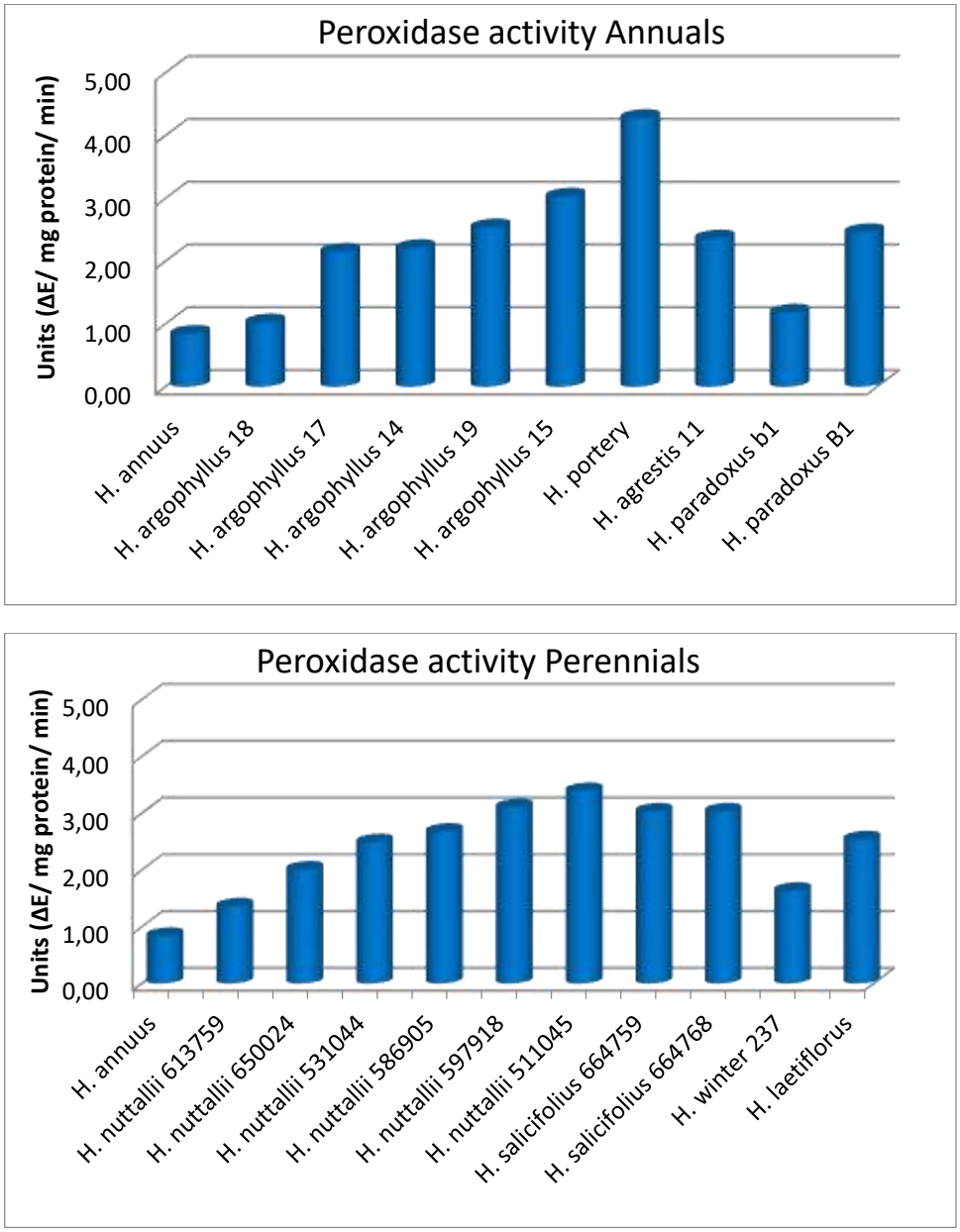

Fig 5. Peroxidases activity in different annual (a) and perennial (b) wild *Helianthus* species.

Protein content

Among annual species, the highest level of protein content showed cultivated sunflower *H. annuus* followed by *H. paradoxus* b1 and *H. portery*. Diploid perennial *H. nuttallii* 650024 revealed the highest amount of protein followed by two *H. salicifolius* populations.

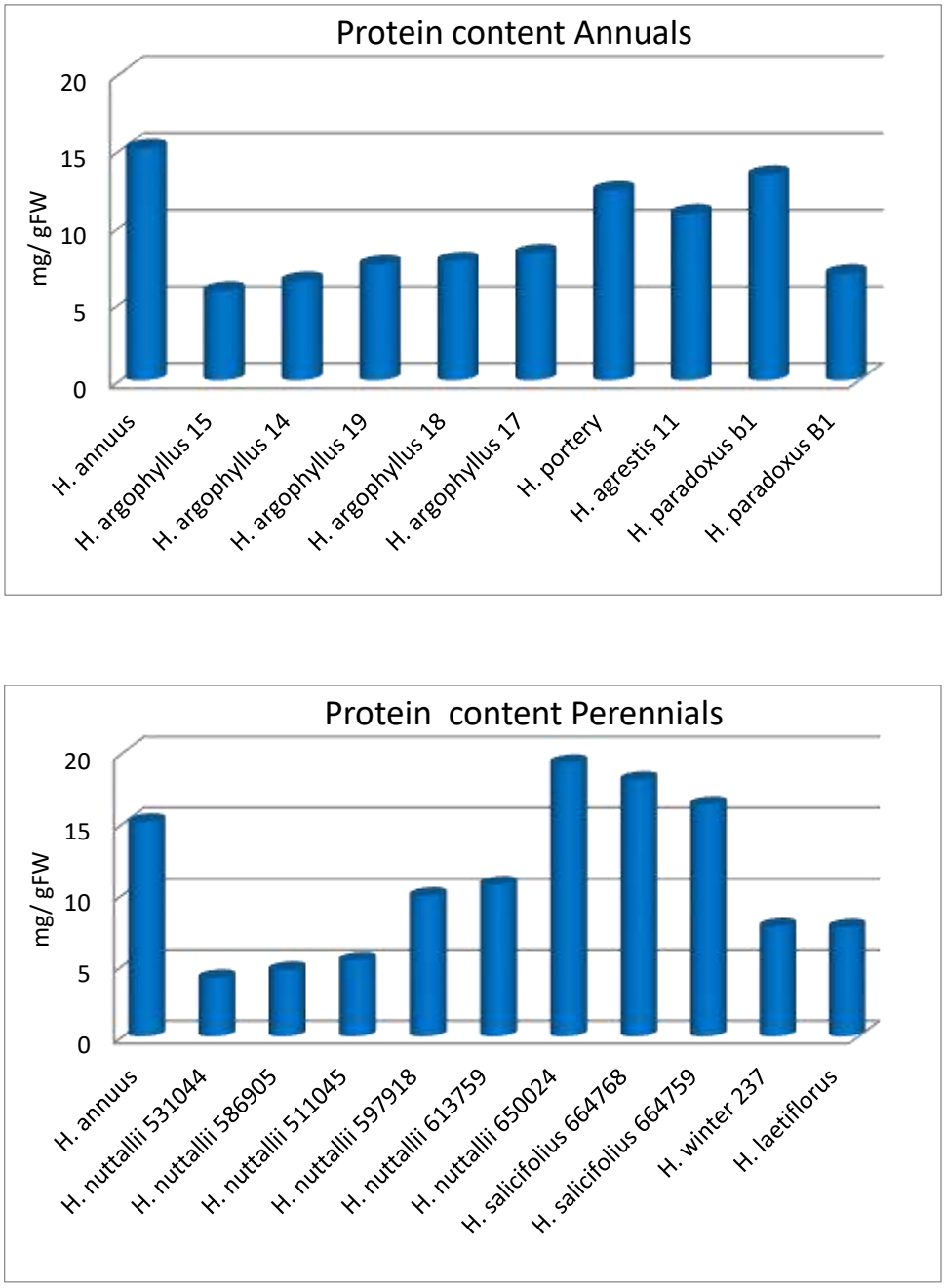

Fig 6. Protein content in different annual (a) and perennial (b) wild *Helianthus* species

CONCLUSION

According with our data, biochemical characteristics of studied sunflower genotypes varied considerably both within populations and between individual wild species. Depending on the objectives of the selection programs, suitable donors of genetic material could be selected.

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YIELD DETERMINATION OF SOME BROOMRAPE RESISTANT SUNFLOWER HYBRIDS IN DROUGHT CONDITIONS IN TRAKYA REGION, TURKEY

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Abstract

Sunflower (*Helianthus annuus* L.) is the most important oil crops in Turkey. Hybrids exists totally in sunflower production both in Turkey and also in other main sunflower producer countries in the world due to higher yielding, homogeneity and adaption capability. Downy mildew and broomrape parasite is two main factors reducing seed and oil yield as well as abiotic stress such as drought, higher temperatures during growing period and lower soil fertility in sunflower production. The study conducted to determine their performances of some sunflower candidate hybrids in conducted yield trials in Tekirdag and Edirne existing in Trakya Region which is European part of Turkey which has more than 40 % of Turkish sunflower production. Furthermore, Trakya region exist in Black sea region which has over 60% of world sunflower planted areas so these results represent base performances of sunflower planted areas in the world. The candidate hybrids were existed in yield trials and all important yield traits were measured and compared with control hybrids which are most selling commercial hybrids in the market in 2017. Some candidate hybrids had higher performances than control hybrids in the study.

Keywords: Sunflower, Sustainable production, Hybrid, Seed yield, Yield traits, Yield performance,

INTRODUCTION

Sunflower is one of the most important oil crop in the world. Black Sea countries as Ukraine, Russia, Romania, Bulgaria, Turkey and Moldova has 60% of world sunflower production and planting areas in the world. Sunflower oil is the most preferable and consuming vegetable oil in Turkey. Sunflower grows widely in Turkey due to higher adaption capability, higher mechanization use, easy marketing. Trakya Region which is European part of Turkey has most planted areas (50%) and Middle Anatolia and Cukurova regions are following this region (Kaya, 2016, 2020). Sunflower grows mostly without irrigation in rainfeed areas in spring and summer so it influences more from climatic conditions such as drought and hot temperatures especially during the grain filling period (Chigeza et al., 2012 Çiçek et al., 2019). Therefore, sunflower yields affect from these changeable environmental conditions in some years in Turkey and also in other growing countries in the world. On the other hand, biotic stresses such as downy mildew, broomrape parasite and weeds are another main limiting factors of in sunflower production (Kaya, 2020; Akpınar et al., 2019).

Broomrape is *(Orobanche cumana* Wallr.) holoparasitic parasite as one of the top most limiting factors and reduces sunflower yield severely until 100%. It mostly could observe in many parts of the world except America but it found mostly in Eastern Europe and Black Sea Region with developing new aggressive races historically (Skoric and Pacureanu, 2010; Kaya, 2014; Sezer et al., 2014, Jocic et al., 2015). Downy mildew is another devastating problem in sunflower production so sunflower breeders are trying to develop broomrape downy mildew as

well as IMI herbicide resistant sunflower hybrids in their programs (Jocic et al., 2015; Arslan and Kaya, 2019, Kaya and Beser, 2020). The study covers conducted yield trials in 2017 for determining the performance of sunflower hybrids in Tekirdag and Edirne provinces in Trakya Region which has half part of sunflower production areas in Turkey.

MATERIAL AND METHOD

The yield trials were conducted in Edirne and Tekirdag location in 2017 to determine yield performances of candidate sunflower hybrids. There were 23 hybrids including 4 controls from commercial hybrids (ITALICA, SY GIBRALTAR, P 64 LL 62, LG 5582) in the market. The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,50-m long with the 70 x 35 cm plant spacing. Total plot area at planting was 7,5*2,8 as 21 m^2. The middle two rows were harvested and the border rows were discarded, and plot size was 9.66 m^2 at harvest. The compose fertilizers (20-20-0, Zn) were applied 200 kg/ha dose at planting. Statistical analysis was performed with JMP statistical program.

Tekirdag location was conducted in Beyazkoy village fields, Saray County and the trials were planted by hand in 15 April 2017. Emergence date of sunflower plants was in 22 April 2017 and left only one plant each as mentioned plant density above. The trials were harvested by hand in 25 August 2017 as middle two rows except one plant at the beginning of the middle rows. Edirne location was conducted in Sarayakpinar village fields and the trials were planted by hand in 28 April 2017. Emergence date of sunflower plants was in 5 May 2017 and the trials were harvested by hand in 5 September 2017.

Seed yield (SY), 1000 seed weight (TSW), flowering and physiological maturity period, plant height (PH), head diameter (HD), husk (HC) and oil content (OC) were measured in the study. The flowering period (FP) of hybrids were measured at stage which 50% of plants in the plot with first open ligule petals. The physiological maturity period (PM) of hybrids were counted at the stage which the back of heads was yellow and 10 % were brown color. The plant height and head diameter of hybrids were measured from 3 plants at mid rows of the plots in each replication at PM stage. Oil content of the hybrids were determined utilizing Nuclear Magnetic Resonance (NMR) analysis.

RESULTS AND DISCUSSION

Based on the study results, some candidate sunflower hybrids exhibited higher performance than control hybrids in Beyazkoy, Tekirdag location (Table 1). Highest seed and oil yield was obtained from SY GIBRALTAR control sunflower hybrid. The candidate hybrid 1628, control hybrid P 64 LL 62 and 9718x RHA64 DMR hybrid followed it respectively for seed yield. This hybrid was 2nd the highest oil content hybrid among hybrids existed in the study. The highest oil content was obtained from 9718x RHA64 DMR candidate hybrid as 45,18% in the research.

Some candidate sunflower hybrids exhibited higher performance like control hybrids in Edirne location based on the study results like Tekirdag location (Table 2). However, the highest seed yield was obtained from 1623 candidate hybrid then followed by LG5582 and SY GIBRALTAR sunflower commercial hybrids. The highest oil yield obtained from SY GIBRALTAR, P 64 LL 62 and LG5582 sunflower commercial hybrid. The highest oil content was obtained from P 64 LL 62 control hybrid as 50,8 % in the research.

Based on the average results in the study, some candidate sunflower hybrids exhibited higher performance than control hybrids in average values for both two locations (Table 3). Highest seed yield was obtained from 1623 sunflower candidate hybrid for average values of the locations and SY GIBRALTAR and 1624 candidate hybrid followed it respectively. For oil yield, SY GIBRALTAR existed in the top and P 64 LL 62 and 9718x RHA64 DMR had higher

performances in the study. The highest oil content was obtained from SY GIBRALTAR commercial hybrid as 47,00% in the research and 9718x RHA64 DMR candidate hybrid and P 64 LL 62 control one followed it respectively in the research.

Table 1. Yield trial results of sunflower hybrids in Beyazkoy, Saray, Tekirdag locations

#	Cultivars	Flower Days	Phy. Mat Days	Plant Hght (cm)	Head Diam (cm)	Hectlt Weght (g/lt)	Oil contnt (%)	Oil Yield (kg/da)	Group	Seed Yield (kg/da)	Group
1	ITALICA	62	100	176	20	38,7	42,77	76,8	B-D	180	B-G
2	SY GIBRALTAR	60	95	182	19	37,8	43,19	98,9	A	229	A
3	P 64 LL 62	60	94	184	19	33,8	42,74	84,7	B	198	A-C
4	LG 5582	60	97	184	17	41,0	40,34	61,5	F-I	153	F-I
5	OS -900	60	99	178	18	36,4	36,6	58,9	F-I	161	E-I
6	9718x RHA64 DMR	59	95	199	16	38,6	45,18	86,7	A-B	192	B-E
7	161	62	100	193	17	36,1	37,71	52,9	H-I	140	H-I
8	163	62	97	205	18	38,3	36,75	55,6	G-I	151	G-I
9	1619	61	97	169	18	37,1	40,03	74,3	B-E	186	B-F
10	1623	61	95	199	18	35,0	37,73	65,9	D-G	175	B-G
11	1624	61	97	185	19	38,7	38,87	75,7	B-D	195	B-D
12	1625	63	100	199	21	39,7	37,37	61,5	F-I	165	D-I
13	1628	62	94	192	20	35,7	34,91	69,7	D-F	200	A-B
14	1643	61	95	195	20	33,5	38,07	67,1	D-G	176	B-G
15	1644	62	95	202	21	34,6	34,73	58,1	F-I	167	B-H
16	1649	60	95	201	20	33,5	34,76	58,1	F-I	167	B-H
17	1652	61	95	205	17	36,2	37,24	64,5	D-H	173	B-H
18	1654	59	95	219	20	35,5	36,35	59,5	F-I	164	D-I
19	1656	61	95	185	18	35,6	36,99	51,5	I	139	I
20	1522	61	96	189	21	32,3	36,58	54,7	G-I	149	G-I
21	1557	60	94	184	18	37,0	36,7	60,6	F-I	165	B-I
22	1563	60	94	169	18	37,0	49,37	82,7	B-C	168	B-H
23	1434	63	98	204	20	39,1	39,75	70,0	C-F	176	B-G
24	1451	64	98	191	19	41,2	42,3	76,2	B-D	180	B-G
25	1462	64	99	201	20	39,7	37,01	60,8	F-I	164	D-I
26	AKT-17011	62	96	203	22	35,3	35,38	55,9	G-I	158	F-I
27	AKT-17012	61	95	206	22	33,7	33,73	59,1	F-I	175	B-G
28	AKT-17013	62	96	191	21	33,0	32,28	55,7	G-I	172	B-H
29	AKT-17014	63	96	196	21	34,9	32,13	58,5	F-I	182	B-G
30	AKT-17015	63	98	218	18	38,4	40,15	61,6	E-I	153	F-I

Seed Yield CV (%): 11,5 kg/da Oil Yield CV (%) 11,8 kg/da

Table 2. Yield trial results of sunflower hybrids in Edirne location

#	Cultivars	Flowering days	Hectoliter Weight (g/lt)	Oil content (%)	Oil Yield (kg/da)	Group	Seed Yield (kg/da)	Group
1	ITALICA	61	40,9	48,79	81,8	C-F	168	C-F
2	SY GIBRALTAR	62	40,6	50,78	109,2	A	215	A-C
3	P 64 LL 62	62	36,7	50,8	102,9	A	203	A-F
4	LG 5582	61	40,0	44,76	101,9	A	228	A
5	OS -900	63	37,0	44,96	60,9	G-H	135	G-H
6	9718xRHA64DMR	59	41,4	48,57	95,8	A-C	197	A-G
7	161	59	37,7	42,72	73,5	F-G	172	F-I
8	163	59	38,4	37,74	72,6	F-H	192	B-H
9	1619	58	40,5	41,84	73,0	F-H	174	F-I
10	1623	63	39,6	37,26	85,5	B-H	230	A
11	1624	59	39,0	38,67	82,0	C-F	212	A-D
12	1625	58	40,8	40,11	86,7	B-F	216	A-C
13	1628	58	38,8	43,94	79,8	D-F	182	D-H
14	1643	59	36,0	44,35	78,5	E-F	177	E-I
15	1644	57	41,7	43,19	79,0	D-F	183	C-H
16	1649	59	37,0	38,51	80,8	D-F	210	A-F
17	1652	57	39,7	41,1	82,2	C-F	200	A-G
18	1654	58	38,6	41,66	78,9	D-F	189	B-H
19	1656	60	39,7	42,4	74,2	F-G	175	F-I
20	1522	59	36,2	40,98	82,7	C-F	202	A-F
21	1557	59	37,6	42,68	93,6	A-D	219	A-B
22	1563	58	38,4	45,38	99,8	A-B	220	A-B
23	1434	60	40,8	42,79	81,6	D-F	191	B-H
24	1451	59	44,5	43,3	92,3	A-E	213	A-D
25	1462	59	43,2	43,5	92,1	A-E	212	A-D
26	1655	58	39,6	36,03	58,1	H	161	H-J
27	1676	59	41,2	41,68	76,8	F	184	C-H
28	1431	63	39,1	49,36	79,6	D-F	161	H-J
29	1673	62	43,0	42,92	73,4	F-G	171	F-I
30	1680	60	34,1	46,35	83,5	C-F	180	D-H

Seed Yield CV (%): 11,0 kg/da Oil Yield CV % 11,2 kg/da

Table 3. Yield trial results of sunflower hybrids in the average of Edirne and Tekirdag locations

#	Cultivars	Hectoliter Weight (g/lt)	Oil content (%)	Oil Yield (kg/da)	Group	Seed Yield (kg/da)	Group
1	ITALICA	39,8	45,8	79,3	B-F	174,0	A-E
2	SY GIBRALTAR	39,2	47,0	98,2	A	209,0	A
3	P 64 LL 62	35,3	46,8	93,8	A	200,5	A-B
4	LG 5582	40,5	42,6	81,7	A-E	190,5	A-B
5	OS -900	36,7	40,8	59,9	I	148,0	E
6	9718xRHA64DMR	40,0	46,9	91,3	A-B	194,5	A-B
7	161	36,9	40,2	63,3	H-I	156,0	D-E
8	163	38,4	37,2	64,1	G-I	171,5	B-E
9	1619	38,8	40,9	73,6	D-H	180,0	A-D
10	1623	37,3	37,5	79,7	C-H	213,0	A
11	1624	38,9	38,8	78,9	C-H	203,5	AB
12	1625	40,3	38,7	74,1	C-H	190,5	A-B
13	1628	37,3	39,4	74,8	C-H	191,0	A-B
14	1643	34,8	41,2	72,8	D-H	176,5	A-E
15	1644	38,2	39,0	68,6	F-I	175,0	A-E
16	1649	35,3	36,6	69,5	F-I	188,5	A-C
17	1652	38,0	39,2	73,4	D-H	186,5	A-D
18	1654	37,1	39,0	69,2	F-I	176,5	A-E
19	1656	37,7	39,7	62,9	H-I	157,0	C-E
20	1522	34,3	38,8	68,7	F-I	175,5	A-E
21	1557	37,3	39,7	77,2	C-F	192,0	A-B
22	1563	37,7	47,4	91,3	A-B	194,0	A-B
23	1434	40,0	41,3	75,8	C-G	183,5	A-D
24	1451	42,9	42,8	84,3	A-D	196,5	A-B
25	1462	41,5	40,3	76,5	C-G	188,0	A-D

Seed Yield CV (%): 15,0 kg/da Oil Yield CV % 14,4 kg/da

CONCLUSIONS

Based on the study, some candidate hybrids resistant to broomrape parasite exhibit over performance than control hybrids. 9718xRHA64DMR, 1623, 1624 and 1628 candidate hybrids exhibited higher seed yield in the study. However, other than 9718xRHA64DMR has lower oil contents then lower oil yield too. Therefore, 9718xRHA64DMR has promising results but it will be evaluated in this year then if still keep higher performances, then it will send the registration to commercialize and sell to farmers for further years.

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THE EVALUATION OF SOME DROUGHT INDICES IN SUNFLOWER HYBRIDS IN DRY CONDITIONS

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ABSTRACT

Sunflower (*Helianthus annuus* L.) which is the most important oil crops grows mainly drylands in the world. Therefore, environmental conditions mostly influence severely sunflower yields especially in some seasons. In addition to biotic stresses such downy mildew, broomrape parasite, weeds, some other diseases, etc. biotic stress such as drought is the most limiting factor of sunflower production in different parts of the world. Due to global warming, sunflower will affect more these conditions so new developing hybrids need to drought tolerant. The study was conducted to determine their drought tolerance performances and to evaluate their drought indices of some sunflower candidate hybrids in conducted yield trials in Tekirdag and Edirne existing in Trakya Region which is European part of Turkey which has more than 40 % of Turkish sunflower production. Based on the study results, some candidate hybrids had higher drought tolerant indices such as total chlorophyll content, hairiness, dry root weight at stem than control hybrids in the study.

Keywords: Sunflower, Sustainable production, Drought tolerance, Hybrid, Yield traits, Yield performance,

INTRODUCTION

Sunflower grows widely in not only in Turkey both also in Blacksea region countries which has over 60% of the world sunflower planted areas as well as other parts of the world due to higher adaption capability, higher mechanization use, easy marketing (Kaya, 2016, 2020). Hybrids are preferred and used totally in sunflower production both in Turkey and also in other producer countries in the world due to higher yielding, homogeneity and adaption capability (Arslan and Kaya, 2019).

Biotic stresses such as downy mildew and broomrape parasite are the most limiting factors reducing seed and oil yield in sunflower production areas (Sezer et al., 2014, Jocic et al., 2015). However, abiotic stress such as drought, higher temperatures in lower fertility soils especially during grain filling period because as a summer crop sunflower grows mostly in rainfeed areas (Ahmed et al., 2009; Ghaffari et al., 2012; et al., 2012; Pekcan et al., 2012; Andrade et al., 2013). Total chlorophyll measurement which is a novel and easy technique and widely used to measure the plant's response to drought stresses (Cicek et al., 2019; Arslan et al., 2020). Therefore, these changeable environmental conditions with increasing global warming recently lead that sunflower breeders should consider more and develop drought tolerant hybrids to solve these problems and to produce enough for humanity in the future (Rauf, 2008; Jocic et al., 2015; Kaya and Beser, 2020).

The study was conducted to determine their drought tolerance performances and to evaluate their drought indices of some sunflower candidate hybrids in conducted yield trials in the most planted provinces as Tekirdag and Edirne existing in Trakya Region in 2017.

MATERIAL AND METHOD

The yield trials were conducted in Edirne and Tekirdag location in 2017 to determine yield performances of candidate sunflower hybrids. There were 23 hybrids including 4 controls from commercial hybrids (ITALICA, SY GIBRALTAR, P 64 LL 62, LG 5582) in the market. The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,50-m long with the 70 x 35 cm plant spacing. Total plot area at planting was 7,5*2,8 as 21 m^2. The middle two rows were harvested and the border rows were discarded, and plot size was 9.66 m^2 at harvest. The compose fertilizers (20-20-0, Zn) were applied 200 kg/ha dose at planting. Statistical analysis was performed with JMP statistical program.

Tekirdag location was conducted in Beyazkoy village fields, Saray County and the trials were planted by hand in 15 April 2017. Emergence date of sunflower plants was in 22 April 2017 and left only one plant each as mentioned plant density above. The trials were harvested by hand in 25 August 2017 as middle two rows except one plant at the beginning of the middle rows. Edirne location was conducted in Sarayakpinar village fields and the trials were planted by hand in 28 April 2017. Emergence date of sunflower plants was in 5 May 2017 and the trials were harvested by hand in 5 September 2017. The plant height and head diameter of hybrids were measured from 3 plants at mid rows of the plots in each replication at PM stage. Oil content of the hybrids were determined utilizing Nuclear Magnetic Resonance (NMR) analysis.

Some sunflower hybrids both from classical and IMI types were planted in the pots to measure responses to drought conditions with measuring of dry and wet root weight as well as total chlorophyll content as the most known drought indices in sunflower drought tests (Figure 1, 2 and 3). The ratio of Chlorophyll content of sunflower hybrids was recorded by Portable Florescence Device (HandyPEA, Hansatech Ltd.) at R5-1 vegetative stages (Figure 4). Furthermore, plant height, plant number per area, leaf number per plant, leaf area, anthocyanin existence, head inclination, hairiness at stem, total chlorophyll content, leaf width and leaf length were measured at the yield trials conducted in the field to determine their responses to drought stress.

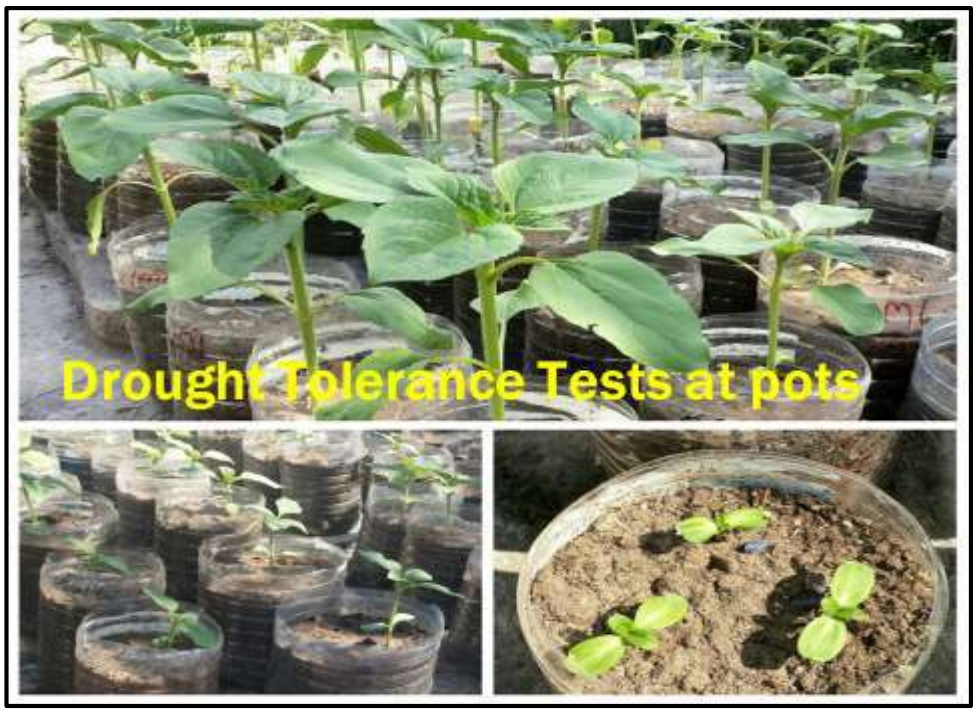

Figure 1. Some classical type sunflower hybrids at the pot study

Figure 2. The roots of some sunflower hybrids at the pot study

Figure 3. The weighing of the roots of some sunflower hybrids at the pot study

Figure 4. The measuring total chlorophyll content some sunflower hybrids at the field.

RESULTS AND DISCUSSION

Based on the study results, the classical sunflower hybrids exhibited higher performance both for seed and oil yield as SY GIBRALTAR control sunflower hybrid, the candidate hybrid 1628, control hybrid P 64 LL 62 and 9718x RHA64 DMR in Edirne location in the research. On the other hand, in Tekirdag location, the highest seed yield was obtained from 1623 candidate hybrid followed by LG5582 and SY GIBRALTAR sunflower commercial hybrids and the highest oil yield obtained from SY GIBRALTAR, P 64 LL 62 and LG5582 sunflower commercial hybrid. Based on the average results in the study, we could include 1624 candidate hybrid in addition to above ones. In the IMI type hybrids, DT5234 CLP, NS--H-7801, LG5565CL, P-LC108 and IMIO44AXIMI-NI hybrids seems promising ones for seed and oil yield in the study.

Based on study results at the pot study; In IMI herbicide resistant hybrids, Surimi CL hybrid had highest wet and dry root weight as 30,33 g and 6,66 g. LG5565 CL hybrid followed that hybrid and the lowest values were obtained from PUNTASOL CL and SUNFLORA hybrids (Table 1). In classical hybrids, LG5582 had the highest performance had 26,3 g wet and 5,4 g dry root weight respectively. P64LL62 had the lowest value among these hybrids in the study (Figure5 and 6).

The total chlorophyll contents of sunflower hybrids were changed between 7,4 and 15,7 in classical hybrids in Edirne location (Table 2) and between 8,7-15,4 in IMI hybrids in Edirne location (Table 3). The total chlorophyll contents of sunflower hybrids were changed between 6,4 and 12,9 in classical hybrids in Tekirdag location (Table 4) and between 7,8-15,2 in IMI hybrids in Tekirdag location (Table 5). SY Bento, 1644, 1652 and 1654 in classical hybrids; PARAISO 102 CL, 1448 IMI, NS--H-7806, NS--H-7812, NS--H-7854, NS--H-7851, CARRERA CLP, DT5234 CLP and IMIO44A X IMI-NI hybrids in IMI types.

Figure 5. The roots of some classical type sunflower hybrids at the pot study

Figure 6. The roots of some IMI type sunflower hybrids at the pot study

Table 1. IMI and Classical sunflower hybrids root weights at the pot trial

IMI hybrids				Classic Type Hybrids			
#	IMI Type Cultivars	Avg Wet Root Weight (g)	Avg Dry Root Weight (g)	#	Classic Type Hybrids	Avg Wet Root Weight (g)	Avg Dry Root Weight (g)
1	P-LC108 (C)	17,53	4,96	1	ITALICA (C)	11,2	2,3
2	SY BENTO (C)	13,66	3,53	2	GIBRALTAR(C)	8,3	2,7
3	METEOR (C)	13,8	3,36	3	P 64 LL 62 (C)	5,13	1,7
4	LG5565 CL (C)	**21,16**	**5,66**	4	LG 5582 (C)	**26,3**	**5,4**
5	SUNFLORA	11,33	2,43	5	161	13,7	3,3
6	PARAISO102CL	17,33	4,6	6	163	14,3	3,2
7	SURIMI CL	**30,33**	**6,66**	7	1619	9	1,8
8	PUNTASOL CL	9,36	3,1	8	1643	10,4	2,7
9	CARRERA CLP	16,76	4,73	9	1644	**18,6**	**3,4**
10	DT5234 CLP	15,83	3,23	10	1563	16,8	3,0

Table 2. Classical sunflower hybrids total chlorophyll content at the yield trial in Edirne location

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
9,1	**15,7**	8,4	9,3	10,8	8,2	10,4	8,7	14	11,1	10,4	10,7
13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	
9,6	8,8	8,1	6,9	6,5	9,7	9,8	8,8	7,4	9,3	8,1	

Table 3. IMI sunflower hybrids total chlorophyll content at the yield trial in Edirne location

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
8,06	9,6	10,2	10,6	11	**15,4**	10,6	10,9	11,8	13	11,8	**14,1**	13,4
14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	
12,5	**14,3**	**15,6**	**13,8**	**14,2**	11,1	9,8	9,5	13,5	9,9	8,9	8,7	

Table 4. Classical sunflower hybrids total chlorophyll content at the yield trial in Tekirdag location

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
6,4	8,3	9,4	8,5	7,1	10	7,3	8,7	9,0	11,2	9,4	11,5	10,9
14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	
11,1	**12,9**	11,4	**12,1**	**12,4**	9,5	9,7	11,8	11,9	8,4	9,0	8,4	

Table 5. IMI sunflower hybrids total chlorophyll content at the yield trial in Tekirdag location

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
10,3	**15,2**	8,3	10,9	11,7	8,7	9,4	9,5	**12,6**	**12,0**	7,8	10,5
13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	
11,8	9,1	9,6	8,8	9,4	11,4	11,01	8,8	8,5	9,7	**13,7**	

For drought tolerance in sunflower hybrids, larger leaves ones have more photosynthesis capacity and preferable ones. For larger leaf areas, 1624 1625, LG5565CL SUNFLORA, NS--H-7850, 163, 1619, 1624, 1625, 1434 and 1462 sunflower hybrids seem promising ones (Table 6, 7, 8 and 9). Similarly, hairiness in the stem also preferable ones for drought tolerances. Among hybrids, NS--H-7812 and IMIO44A X IMI-NI in IMI types and 1522 candidate hybrid in classical ones seems having higher values.

Table 6. Some drought indices of sunflower IMI hybrids in Beyazkoy, Tekirdag

#	HYBRIDS	Plant Height (cm)	Plant Number	Leaf Number per plant	Hairiness in the stem (1-9)
1	P-LC108	171	82	30	3
2	SY BENTO	178	103	30	3
3	METEOR	178	98	28	3
4	LG5565CL	174	95	29	3
5	SUNFLORA	146	42	30	5
6	PARAISO 102 CL	150	115	26	3
7	SURIMI CL	190	90	26	1
8	PUNTASOL CL	169	111	28	5
9	CARRERA CLP	174	98	29	5
10	DT5234 CLP	175	101	29	5
11	162 IMI	156	94	26	3
12	1448 IMI	192	80	29	5
13	NS--H-7800	187	97	30	3
14	NS--H-7801	171	100	32	3
15	NS--H-7806	193	95	31	5
16	NS--H-7812	194	107	31	7
17	NS--H-7854	196	101	31	1
18	NS--H-7851	200	93	28	3
19	NS--H-7850	214	92	29	5
20	NS--H-7856	190	101	29	1
21	NS--H-7863	159	80	28	5
22	NS--H-7859	192	103	28	3
23	IMIO44A X IMI-NI	172	101	28	7

Table 7. Some drought indices of sunflower IMI hybrids in Beyazkoy, Tekirdag

#.	Hybrids	Anthocyanin Existence (1-9)	Head Inclination (1-9)	Leaf Shape (1-9)	Branching (1-5)	Leaf Width	Leaf length	Leaf Area
1	P-LC108	1	4	8	5	13	25	325
2	SY BENTO	1	4	8	-	11	21	231
3	METEOR	1	6	8	-	14	23	322
4	LG5565CL	1	5	8		13	**26**	**338**
5	SUNFLORA	1	5	8	-	**15**	**27**	**405**
6	PARAISO102CL	1	4	8	-	11	21	231
7	SURIMI CL	1	4	8	-	10	19	190
8	PUNTASOL CL	1	5	8	-	12	20	240
9	CARRERA CLP	1	5	8	-	10	21	210
10	DT5234 CLP	1	6	8	-	12	23	276
11	162 IMI	1	6	8	2	14	24	336
12	1448 IMI	7	4	8	4	13	23	299
13	NS--H-7800	1	5	8	5	13	21	273
14	NS--H-7801	1	5	8	5	12	22	264
15	NS--H-7806	1	5	8	1	12	25	300
16	NS--H-7812	7	5	8	-	11	23	253
17	NS--H-7854	1	6	8	-	13	21	273
18	NS--H-7851	1	7	8	-	11	21	231
19	NS--H-7850	1	5	8	-	**15**	**24**	**360**
20	NS--H-7856	1	5	9	-	11	23	253
21	NS--H-7863	1	5	9	-	11	21	231
22	NS--H-7859	1	5	8	-	12	23	276
23	IMIO44A X IMI-NI	1	5	8	-	11	22	242

Table 8. Some drought indices of sunflower classical hybrids in Beyazkoy, Tekirdag

#	HYBRIDS	Plant Height (cm)	Plant Number	Leaf Number per plant	Hairiness in the stem (1-9)	Anthocyanin existence
1	ITALICA	176	72	27	5	1
2	SY GIBRALTAR	182	85	29	6	1
3	P 64 LL 62	184	79	28	5	1
4	LG 5582	184	85	27	2	1
5	OS -900	178	88	27	4	1
6	9718xRHA64-DMR	199	93	29	4	1
7	161	193	93	27	4	1
8	163	205	80	30	6	9
9	1619	169	72	24	4	1
10	1623	199	94	30	3	1
11	1624	185	82	27	6	1
12	1625	199	75	29	5	9
13	1628	192	79	29	4	1
14	1643	195	77	30	4	1
15	1644	202	79	31	6	1
16	1649	201	88	31	6	1
17	1652	205	94	32	6	1
18	1654	219	85	34	6	1
19	1656	185	88	27	6	1
20	1522	189	60	28	**8**	1
21	1557	184	78	26	6	1
22	1563	169	82	25	5	1
23	1434	204	73	31	4	1
24	1451	191	79	28	5	9

Table 9. Some drought indices of sunflower classical hybrids in Beyazkoy, Tekirdag

#	HYBRIDS	Head Inclination (1-9)	Leaf Shape (1-9)	Branching (1-5)	Leaf Width	Leaf length	Leaf Area
1	ITALICA	7	6	-	11	20	220
2	SY GIBRALTAR	5	6	-	13	23	299
3	P 64 LL 62	7	6	-	9	20	180
4	LG 5582	5	6	-	13	25	325
5	OS -900	7	6	-	10	21	210
6	9718xRHA64-DMR	7	6	-	13	22	286
7	161	4	6	-	11	21	231
8	163	7	6	-	13	25	**325**
9	1619	8	6	-	14	27	**378**
10	1623	8	6	-	11	21	231
11	1624	7	6	-	14	24	**336**
12	1625	6	6	-	14	24	**336**
13	1628	5	6	-	13	25	325
14	1643	5	6	-	12	22	264
15	1644	4	6	-	12	20	240
16	1649	6	6	-	13	24	312
17	1652	5	6	-	12	22	264
18	1654	5	6	-	11	22	242
19	1656	4	6	5	11	22	242
20	1522	4	6	-	12	25	300
21	1557	5	6	-	11	21	231
22	1563	5	6	-	10	21	210
23	1434	5	6	-	13	27	**351**
24	1451	3	6	-	12	21	252
25	1462	5	6	-	14	28	**392**

CONCLUSIONS

Based on the study, some hybrids exhibit different performances on measured drought indices. Some of the hybrids seems promising ones for drought tolerance. However, there is no specific and concrete results on evaluated data mainly could be used to determine for drought tolerance connected with seed and oil yield together. Maybe the study was not performed in controlled environments or it could need to conduct longer years or many trials in different locations studies.

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THE RESPONSES OF WILD SUNFLOWER CROSSES TO DROUGHT STRESS

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the most planted oil crop in Turkey and it grows mainly in rain fed regions especially in Trakya Region which is European part of Turkey has about 50% of Turkish sunflower production areas. Therefore, sunflower encounters frequently in some seasons with severe droughts affecting yield highly then sunflower breeders look for resistance genes especially from wild sunflowers which adapted dry areas. *Helianthus mollis* is known and exists in one of the most tolerant wild sunflower species in *Helianthus* genus. The study was conducted to determine drought tolerances of the crosses of common sunflower with *Helianthus mollis* and also the crosses with wild *Helianthus annuus* species and cultural type sunflower inbred lines under controlled environmental conditions in Trakya Agricultural Research Institute, Edirne Turkey. Flowering period (day), Physiological Maturity Period (day), Seed Filling Period (day), Growing Degree Days at Flowering and Physiological Maturity Periods, Plant Height (cm), Head Diameter (cm), Average yield per head (g), Oil Content (%), 1000 Seed Weight (g), Leaf number, Leaf Area (cm^2) and Total *Chlorophyll* contents were measured at R3, R5-1 and R6 growing periods. Based on study results, head diameter, leaf area, oil content, seed yield per head and 1000 seed weights of wild crosses were lower than control (Tunca commercial sunflower hybrid) as expected. However, in other observed yield traits, the large variations were observed among crosses existed in the study. *Chlorophyll* contents of crosses were compared relatively at R3, R5-1 and R6 growing periods too. Based on total *chlorophyll* contents of crosses, some promising crosses for drought tolerance levels comparing check Tunca hybrid. As results, these results indicated that wild crosses could be used commonly in the drought tolerance studies and selected ones will be included to sunflower breeding program. Then, they will use directly or indirectly associating initial program to obtain and develop drought tolerant genotypes.

***Keywords*:** Drought tolerance, Sunflower, Wild crosses, Yield traits, Total *Chlorophyll* contents

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oil crops in the world. Sunflower grows mainly in drylands and rainfed areas and recently it pushed into less fertile areas due to considering less profitable than other crops in the rotation. Therefore, each common environmental factor such as drought, acidity, salinity, etc. in that areas decrease and limit the growth of sunflower plants severely then it leads to reduce of seed production, oil yield and quality (Kaya, 2014 b; Kaya 2015 and 2016).

Adequate water and nutrient supply are important factors affecting optimal plant growth and successful crop production. Water stress is one of the severe limitations of crop growth

especially in arid and semi-arid regions of the world as it has a vital role in plant growth and development at all growth stages. However, depending upon plant species, certain stages such as germination and seedling or flowering could be the most critical stages for water stress Griveau et al., 1998; Fernández-Moroni et al., 2012).

Abiotic stress such as drought, higher temperatures especially in lower fertility soils during grain filling period because as a spring crop sunflower grows mostly in rainfeed areas (Pekcan et al., 2015a, b; Pekcan et al., 2016a, b; Hussain et al., 2016). Wild sunflower species are the main sources for drought resistance for sunflower breeding and sunflower breeders screened and transferred many tolerant genes into cultural types (Kaya and Evci, 2007; Kaya, 2014a; Owart et al., 2016; Vassilevska-Ivanova et al., 2016)

Total chlorophyll measurement which is a practical technique and started used widely to evaluate of plant responses for measuring drought stress tolerances recently (Arslan et al., 2020). Therefore, these increasing global warming activities recently lead that sunflower breeders have to deveop and improve drought tolerant hybrids to solve drought and higher temperatures for producing more for enough food for humanity in the future (Rauf, 2008; Kaya, 2014 b; Kaya 2015 and 2016).

MATERIALS AND METHODS

National Sunflower Project conducted by Trakya Agricultural Research Institute (TARI) in Edirne, Turkey have produced and registered many inbred female and restorer (male) lines and F_1 hybrids until today. The National Sunflower Project of Turkey started in 1970s and TARI is a research coordinator of oil crops and have worked efficiently on oilseed research for 50 years. Due to need of the inbred female and restorer (male) lines and F_1 hybrids developed in National Sunflower Project of Turkey tolerance to drought, the TUBITAK (The Scientific and Technological Research Council of Turkey) project was conducted in 2014-17. In this project, their levels of drought tolerance these inbred lines in controlled environments in Edirne, Turkey as well as their hybrids for growing widely in different environmental conditions were evaluated. The study was part of this large and detailed scientific research project.

The study was conducted in TARI research fields with wild sunflower sunflower crosses with early breeding lines resistant to drought in the same project in previous years originated different genetic sources in 2015. Trials were conducted in controlled conditions with randomized complete block design with one row and three replications. Tunca commercial hybrid belonging Limagrain Co were used as control. In each row, there were 16 plants and the distance between rows was 70 cm and in rows was 30 cm.

Trials were conducted in controlled conditions under isolated cages covered when plants were at 50 cm plant heights. Plants were sowed at May 27 and were harvested and threshed by hand in 24 September 2015 in the trials.

Flowering period (day), Physiological Maturity Period (day), Seed Filling Period (day), Growing Degree Days at Flowering and Physiological Maturity Periods, Plant Height (cm), Head Diameter (cm), Average yield per head (g), Oil Content (%), 1000 Seed Weight (g), Leaf number, Leaf Area (cm^2) of sunflower inbred lines were observed and measured in the study. Total *Chlorophyll* contents were measured at R3, R5-1 and R6 growing periods. All measurements were performed with 5 plants in each plot. Soil analysis of the field were given at Table 1 and climatic data of the conducted year and longer years was given in Table 2.

Table 1. Chemical properties of soil in the experiment field

Soil Depth (cm)	Saturation (%)	Total Salinity (%)	pH	CaCO3 (%)	P2O5 (kg/da)	F3O (kg/da)	Organic Matter (%)
0-20	42	0,06	6,88	1,2	17,1	67,3	1,21
20-40	46	0,06	6,97	1,3	18,6	73,2	1,08

Table 2. Some climatic data of longer years and in 2015 during sunflower growth period

Months	Max. Average Temp. (ºC)	Average Temp. (ºC)	Min Average Temp. (ºC)	Average Humidity (%)	Rainfall (mm)
Longer years Averages (1954-2013)					
May	24.7	18.2	11.6	64.4	52.0
June	29.1	22.5	15. 4	60.1	44.7
July	31.7	24.7	17.3	55.9	32.0
August	31.6	24.3	17.1	56.2	23.6
September	27.1	19.8	13.3	62.2	36.8
2015 year					
May	33,3	20,4	10,3	68,4	45,2
June	35,3	22,5	12,1	68,2	31,0
July	40,2	27,1	13,6	60,3	2,0
August	38,3	27,7	11,1	59,9	24,9
September	39,9	24,0	12,2	68,7	29,5

RESULTS AND DISCUSSION

Wild sunflowers are the main source for drought tolerance then the crosses were made at the study. The crosses exhibited enough variability in different yield traits which exist in the research (Table 3 and 4).

The effect of drought stress on yield traits related to phenological obseravations in sunflower the crosses with *Helianthus mollis* and wild *Helianthus* wild were given Table 5 and 6. According the results measured in the study; the number of flowering days of the genotypes varied between 52 days and 62 days, the earliest flowering genotype was interspecific hybrid was No: 18 (F3 R NO: 30 (2015) (010018X wild) x (6973R X Jerusalem artichoke)) and the latest flowering genotype was H. mollis interspecific hybrid No: 5. Among interspecific hybrids in the study, it has been determined that hybrids made with ornamental sunflower and *Helianthus tuberus* ones were earlier than other wild ones.

In the study, the number of physiological death days varied between 76 days and 94 days, and the earliest and latest maturing genotypes were the same as the genotypes in the number of flowering days. Again, total growing degree days obtained according to flowering time and total growing degree days according to physiological maturity time were obtained as the highest and lowest degrees in the same genotypes. Total growing degree days varied between 935.4 and 1396 according to flowering time, and total growing degree days at the time of physiological maturity varied between 1198 and 1720.

Table 3: The effect of drought stress on yield traits in sunflower the crosses of wild sunflower with *Helianthus mollis*

Yield Traits	#	Max	75% Quartile	Median	25% Quartile	Min	Mean	Std Dev.
Flowering period (day)	30	62	59	57	54	53	57	2,6
Physiological Maturity Period (day)	30	91	86	84	82	80	84	2,8
Seed Filling Period (day)	30	30	29	27	27	25	28	1,5
Flowering Growing Degree Days	30	1137	1077	1036	976	956	1026	53
Phy Mat Growing Degree days	30	1066	1576	1540	1504	1468	1546	51
Plant Height (cm)	30	144	112	98	74	63	96	24
Head Diameter (cm)	30	14,8	11,8	10,4	7,8	6,3	10	2,6
Average yield per head (g)	30	53	24,4	20,1	10,1	6	20,2	11,4
Oil Content (%)	30	48,3	43,3	40,3	38,0	24,7	39,6	5,3
1000 Seed Weight (g)	30	54	30	21,6	16,6	12,8	24,6	11
Leaf number	30	41,8	29,5	27,6	24,2	16,5	27,7	5,3
Leaf Area	30	3053	2171	1855	1635	1155	1920	405
Total Chlorophyll at R3 period	30	16,1	11,6	9,1	8,3	6,1	9,8	2,4
Total Chlorophyll at R5-1 period	30	17,3	12,6	9,8	8,9	8,0	10,9	2,5
Relative tolerance at R5-1 to R3 (%)	30	131	115	110	106	102	111	6,3
Total Chlorophyll at R6 period	30	14,2	9,9	7,2	5,4	4,2	7,7	2,6
Relative tolerance at R6 to R3 (%)	30	101	85	75	67	61	77	10,7
Relative tolerance at R6 to R5-1 (%)	30	89	77	67	63	53	69	9,3

Table 4: The effect of drought stress on yield traits in the crosses with wild *Helianthus annuus* species and cultural type sunflower inbred lines.

Yield Traits	#	Max	75% Quartile	Median	25% Quartile	Min	Mean	Std Dev.
Flowering period (day)	32	59	57	56	55	52	55,8	1,7
Physiological Maturity Period (day)	32	88	86	84,5	83	76	83.8	3,0
Seed Filling Period (day)	32	31	30	28	27	24	28,1	2,2
Flowering Growing Degree Days	32	1077	1036	1016	996	935	1011	34,0
Phy Mat Growing Degree days	32	1612	1576	1549	1522	1396	1536	54,0
Plant Height (cm)	32	163	133	125	114	106	125	14,0
Head Diameter (cm)	32	16,8	11,8	11,3	10,4	8,5	11,3	1,4
Average yield per head (g)	32	45	29,7	23,8	17,1	7,5	23,9	9,2
Oil Content (%)	32	51	45,2	41,3	38,1	29,9	41,5	5,1
1000 Seed Weight (g)	32	87	32	26,7	19,2	11,6	27,8	13,9
Leaf number	32	33,8	27,4	25,3	21,9	19,5	24,8	3,8
Leaf Area	32	6606	2861	2104	1793	1393	2778	155
Total Chlorophyll at R3 period	32	13,1	11,1	10,1	9,4	7,0	10,2	1,4
Total Chlorophyll at R5-1 period	32	17,2	12,6	11,2	10,1	8,2	11,4	1,9
Relative tolerance at R5-1 to R3 (%)	32	139	117	112	107	86	112	11,3
Total Chlorophyll at R6 period	32	12,5	9,6	8,6	7,4	4,1	8,5	2,1
Relative tolerance at R6 to R3 (%)	32	112	96	83	72	54	83	15,6
Relative tolerance at R6 to R5-1 (%)	32	94	80	77	72	50	74	11,3

Table 5: The effect of drought stress on yield traits related to phenological observations in the crosses with wild *Helianthus mollis x Helianthus annuus* species.

	The crosses	Flower period (day)	Physiolg Maturity Period (day)	Seed Filling Period (day)	Flower Growng Degree Days	Phy Mat Growng Degree days	Plant Height (cm)	Head Diam (cm)
1	*H. mollis x H. annuus*	57	86	29	1036,4	1576	124	10,8
2	*H. mollis x H. annuus*	55	82	27	996	1504	122	12,8
3	*H. mollis x H. annuus*	55	84	29	996	1540	142	14,5
4	*H. mollis x H. annuus*	59	88	29	1076,8	1612	106	6,5
5	*H. mollis x H. annuus*	62	91	29	1137,4	1666	99	6,3
6	*H. mollis x H. annuus*	60	89	29	1097	1630	114	8,0
7	*H. mollis x H. annuus*	59	86	27	1076,8	1576	98	11,5
8	*H. mollis x H. annuus*	58	88	30	1056,6	1612	102	11,8
9	*H. mollis x H. annuus*	59	86	27	1076,8	1576	99	11,3
10	*H. mollis x H. annuus*	59	86	27	1076,8	1576	80	7,8
11	*H. mollis x H. annuus*	60	85	25	1097	1558	78	7,5
12	*H. mollis x H. annuus*	55	82	27	996	1504	72	7,8
13	*H. mollis x H. annuus*	53	82	29	955,6	1504	81	7,8
14	*H. mollis x H. annuus*	54	81	27	975,8	1486	69	9,5
15	*H. mollis x H. annuus*	54	80	26	975,8	1468	64	11,0
16	*H. mollis x H. annuus*	54	84	30	975,8	1540	72	10,0
17	*H. mollis x H. annuus*	53	81	28	955,6	1486	63	9,5
18	*H. mollis x H. annuus*	53	83	30	955,6	1522	74	11,0
19	*H. mollis x H. annuus*	59	85	26	1076,8	1558	81	7,0
20	*H. mollis x H. annuus*	58	85	27	1056,6	1558	82	6,5
21	*H. mollis x H. annuus*	57	84	27	1036,4	1540	97	7,8
22	*H. mollis x H. annuus*	57	84	27	1036,4	1540	94	6,3
23	*H. mollis x H. annuus*	54	81	27	975,8	1486	138	13,0
24	*H. mollis x H. annuus*	53	80	27	955,6	1468	135	13,8
25	*H. mollis x H. annuus*	53	82	29	955,6	1504	144	14,8
26	*H. mollis x H. annuus*	55	81	26	996	1486	68	13,0
27	*H. mollis x H. annuus*	55	84	29	996	1540	70	11,8
28	*H. mollis x H. annuus*	60	86	26	1097	1576	101	10,0
29	*H. mollis x H. annuus*	57	87	30	1036,4	1594	105	10,8
30	*H. mollis x H. annuus*	57	87	30	1036,4	1594	111	10,8
C	*Control (Tunca)*	60	89	29	1097	1630	162	18,1

When the plant height values are examined, the shortest plant height was 63 cm from *H. mollis* hybrids No: 17 and the highest plant height was 163 cm from the genotype of crosses with wilds No: 17 (F3 R NO: 27 (2015) 010018 x sunflower ornamental plant)). Table diameter values varied between 6.3 cm and 16.8 cm, the smallest head diameter was obtained from *H. mollis* hybrids No: 5 and 22, the largest head diameter was measured at wild crosses No: 16 K2RSN: 21 (2015) (9702Rx0536R) x wild *H. annuus* in the study (Table 5 and 6).

Table 6: The effect of drought stress on yield traits related to phenological observations in the crosses with common sunflower genotypes x *Helianthus species*.

#	The crosses	Flow Prd (day)	Ph M P (day)	Seed Fll P (day)	Flow GDDs	Phy Mat GDD	Plant Hght (cm)	Head Diam (cm)
1	F2 R NO:2(2015) 0536 R x Wild *H. annuus*	57	88	31	1036,4	1612	122	11,3
2	F2 R NO:3(2015) 0536 R x Wild *H. annuus*	57	85	28	1036,4	1558	106	10,3
3	F2 R NO:4(2015) 6973 R x Wild *H. tuberosus*	55	80	25	996	1468	113	12,0
4	F2 R NO:5(2015)01001R x Wild *H. annuus*	56	86	30	1016,2	1576	118	11,0
5	F2 R NO:6(2015) 6973 R x Wild *H. annuus*	56	80	24	1016,2	1468	108	10,3
6	F2 R NO:7(2015) 6973 R x Wild *H. tuberosus*	55	83	28	996	1522	107	11,8
7	F2 R NO:8(2015)62303R x Wild *H. annuus*	57	87	30	1036,4	1594	130	11,5
8	F2 R NO:9(2015)62303R x Wild *H. annuus*	55	85	30	996	1558	137	12,5
9	F2RNO:11(2015)TTAE gene poolx Wild *H. annuus*	55	84	29	996	1540	158	11,8
10	F3RNO:4(2015) (6973RX0536R) x Wild *H. annuus*	54	78	24	975,8	1432	109	10,5
11	F3RNO:5 (2015)(6973RX0536R) x Wild *H. annuus*	55	83	28	996	1522	126	11,0
12	F3 R NO:7 (2015)6973R X Wild *H. tuberosus*	54	78	24	975,8	1432	112	10,8
13	F3 R NO:8 (2015)6973R X Wild *H. tuberosus*	56	86	30	1016,2	1576	122	9,8
14	F3 R NO:10 (2015)01001Rx Wild *H. annuus*	59	87	28	1076,8	1594	130	10,8
15	F3 R NO:14 (2015)0536Rx Wild *H. annuus*	58	83	25	1056,6	1522	129	10,0
16	F3 RNO:21(2015)(9702Rx0536R)xWild *H. annuus*	54	84	30	975,8	1540	138	16,8
17	F3 R NO:27 (2015)010018xWild *H. annuus*	54	85	31	975,8	1558	163	11,5
18	F3 R NO:30(2015)(010018 xWild *H. tuberosus*	52	76	24	935,4	1396	109	8,5
19	F3 R NO:32 (2015)(010018x Wild *H. annuus*	53	83	30	955,6	1522	118	10,5
20	F4RNO:4(2015) Wild *H. annuus* x (0536Rx6973R)	57	85	28	1036,4	1558	124	11,3
21	F4 R NO:10(2015)9702R x Wild *H. annuus*	53	79	26	955,6	1450	126	13,5
22	F4 R NO:14(2015)6973R x Wild *H. annuus*	57	87	30	1036,4	1594	137	11,3
23	F4 R NO:15(2015)0536R x Wild *H. annuus*	57	85	28	1036,4	1558	138	11,3
24	F4RNO:16(2015) 0536 R x Wild *H. annuus*	58	88	30	1056,6	1612	125	10,0
25	F4RNO:18(2015)(010018 x Wild *H. annuus*	58	85	27	1056,6	1558	107	10,0
26	F2ADNO:2 (2015)9725B x Wild *H. annuus*	56	86	30	1016,2	1576	120	11,0
27	F2ADNO:3(2015) 9725B x Wild *H. annuus*	55	84	29	996	1540	124	11,8
28	F2ADNO:4(2015)9725B x Wild *H. annuus*	55	85	30	996	1558	126	11,8
29	F3ADNO:5(2015)6535B x Wild *H. annuus*	56	84	28	1016,2	1540	132	12,3
30	F3ADNO:6(2015)6522B x Wild *H. annuus*	55	83	28	996	1522	119	11,5
31	F3ADNO:14(2015)(6522Bx2478B)xWild *H. annuus*	58	86	28	1056,6	1576	141	12,3
32	G2F6ADNO:2(2015)6522B x Wild *H. annuus*	57	84	27	1036,4	1540	133	9,8

The effect of drought stress on yield traits related to seed quality and leaf observations in sunflower the crosses with *Helianthus mollis* and wild *Helianthus* wild were given Table 7 and 8. Single head yields varied between 6 g and 53.3 g and the minimum single table yield was obtained from *H. mollis* hybrids No: 11 genotype, and the highest single head yield was obtained from the control Tunca genotype. When the 1000-grain weight of the genotypes was examined, the lowest 1000-grain weight hybrids was observed in No: 25 K3 R SN: 18 (2015) (010018) x ornamental plant genotype from sunflower wilds as 11.6 g. The maximum 1000-grain weight was measured as 87.3 g in No: 16 K2 R SN: 21 (2015) (9702 RX0536R) x Wild *Helianthus* annuus genotype.

When the 1000-grain weight is examined of the genotypes, the lowest 1000-grain weight were measured in the hybrid No: 25 K3 R SN: 18 (2015) (010018 x Ornamental plant genotype as 11.6 g. The highest 1000-grain weight were observed as 87.3 g in the hybrid No: 16 K2 R SN: 21 (2015) (9702 RX0536R) x Wild *Helianthus* annuus genotype.

Table 7: The effect of drought stress on yield traits related to seed quality and leaf observations in the crosses with wild *Helianthus mollis x Helianthus annuus* species.

The Crosses		Average yield per head (g)	Oil Content (%)	1000 Seed Weight (g)	Leaf number	Leaf Area (cm²)
1	*H. mollis x H. annuus*	17,5	40,1	17,4	26,8	2072,5
2	*H. mollis x H. annuus*	19,5	48,3	33,5	26,5	1655,0
3	*H. mollis x H. annuus*	53,0	41,4	41,7	29,3	1867,5
4	*H. mollis x H. annuus*	10,3	38,5	13,4	30,3	2257,5
5	*H. mollis x H. annuus*	20,5	38,8	15,9	28,8	2112,5
6	*H. mollis x H. annuus*	9,5	37,2	12,8	41,8	3052,5
7	*H. mollis x H. annuus*	18,5	39,9	21,5	26,5	1725,0
8	*H. mollis x H. annuus*	20,8	40,5	19,2	30,8	2292,5
9	*H. mollis x H. annuus*	27,5	45,0	21,2	28,0	1760,0
10	*H. mollis x H. annuus*	9,5	43,1	23,1	28,5	1765,0
11	*H. mollis x H. annuus*	6,0	33,1	18,6	33,8	2242,5
12	*H. mollis x H. annuus*	10,5	24,7	14,9	16,5	1155,0
13	*H. mollis x H. annuus*	7,5	25,6	15,1	18,5	1445,0
14	*H. mollis x H. annuus*	14,5	40,1	21,7	28,8	1812,5
15	*H. mollis x H. annuus*	24,3	45,6	22,4	29,8	1842,5
16	*H. mollis x H. annuus*	21,5	43,8	21,2	27,8	2172,5
17	*H. mollis x H. annuus*	24,3	42,6	23,8	23,8	1522,5
18	*H. mollis x H. annuus*	25,0	41,0	28,9	29,0	2170,0
19	*H. mollis x H. annuus*	6,0	33,7	16,8	25,5	1635,0
20	*H. mollis x H. annuus*	6,3	34,2	19,6	40,5	2835,0
21	*H. mollis x H. annuus*	13,3	38,2	15,0	34,3	2397,5
22	*H. mollis x H. annuus*	9,3	35,8	14,4	28,3	1977,5
23	*H. mollis x H. annuus*	34,3	39,5	51,2	22,8	1452,5
24	*H. mollis x H. annuus*	47,3	40,9	53,8	24,0	1540,0
25	*H. mollis x H. annuus*	35,8	38,2	44,1	23,3	1807,5
26	*H. mollis x H. annuus*	24,8	44,4	34,4	23,8	1522,5
27	*H. mollis x H. annuus*	23,3	44,4	33,9	24,3	1867,5
28	*H. mollis x H. annuus*	19,8	41,2	23,1	25,5	1635,0
29	*H. mollis x H. annuus*	20,8	42,8	23,1	27,3	2057,5
30	*H. mollis x H. annuus*	23,5	44,2	21,9	25,8	1962,5
C	Control (Tunca)	53,3	42,1	42,4	29,7	5988,3

The values of oil content ratios of hybrids obtained in the study varied between 24.7% and 51%. While the highest oil ratio was obtained from the hybrids No: 4 (F2 R NO: 5 (2015) 01001 R x *Wild Helianthus annuus*) genotype, the lowest oil content one was obtained from *H. mollis hybrids* No: 12. When the oil ratio values were examined, it was seen that the crossses made with artichoke had higher oil contents (Table 7 and 8).

Leaf number and leaf area were measured in the R5-1 period at the study. When the number of leaves of the genotypes was examined, the minimum and maximum number of leaves was obtained from *H. mollis* hybrids. The minimum number of leaves was determined as 16.5 from No: 12 and the maximum number of leaves as 41.8 from No: 6. Leaf area values of the genotypes varied between 1155 cm² and 6606.7 cm². When the leaf areas of the genotypes were examined, the lowest leaf area was determined in *H. mollis* hybrid No: 12, which was the

least number of leaves, while the highest leaf area value was determined in the hybrid with No: 29 (F3 AD NO: 5 (2015) 6535 B x wild *Helianthus annuus*) (Table 7 and 8).

Table 8: The effect of drought stress on yield traits related to seed quality and leaf observations in the crosses with common sunflower genotypes x *Helianthus species*.

#	The crosses	Average yield per head (g)	Oil Content (%)	1000 Seed Weight (g)	Leaf number	Leaf Area (cm²)
1	F2 R NO:2(2015) 0536 R x Wild *H. annuus*	18,0	41,0	23,6	22,0	2126,2
2	F2 R NO:3(2015) 0536 R x Wild *H. annuus*	10,8	44,3	12,6	22,3	1902,2
3	F2 R NO:4(2015) 6973 R x Wild *H. tuberosus*	28,8	44,6	20,6	21,3	1579,6
4	F2 R NO:5(2015)01001R x Wild *H. annuus*	17,0	51,0	30,7	26,0	2396,6
5	F2 R NO:6(2015) 6973 R x Wild *H. annuus*	26,0	41,2	38,2	19,8	1481,5
6	F2 R NO:7(2015) 6973 R x Wild *H. tuberosus*	23,8	40,1	34,5	23,5	1882,4
7	F2 R NO:8(2015)62303R x Wild *H. annuus*	22,0	47,9	32,1	30,5	2482,1
8	F2 R NO:9(2015)62303R x Wild *H. annuus*	30,0	40,5	39,5	29,3	2709,9
9	F2RNO:11(2015)TTAE gene poolx Wild *H. annuus*	45,0	39,6	42,1	27,5	2367,8
10	F3RNO:4(2015) (6973RX0536R) x Wild *H. annuus*	17,5	42,6	19,2	22,0	1632,2
11	F3RNO:5 (2015)(6973RX0536R) x Wild *H. annuus*	16,3	43,0	13,0	25,8	2231,1
12	F3 R NO:7 (2015)6973R X Wild *H. tuberosus*	23,8	36,9	39,7	22,5	1742,3
13	F3 R NO:8 (2015)6973R X Wild *H. tuberosus*	17,5	37,7	28,7	21,8	1935,7
14	F3 R NO:10 (2015)01001Rx Wild *H. annuus*	18,8	49,6	28,2	27,5	2302,8
15	F3 R NO:14 (2015)0536Rx Wild *H. annuus*	12,5	44,1	12,8	25,0	1802,5
16	F3 RNO:21(2015)(9702Rx0536R)xWild *H. annuus*	38,8	34,2	87,3	19,8	1811,0
17	F3 R NO:27 (2015)010018xWild *H. annuus*	30,5	45,0	30,8	33,8	2910,9
18	F3 R NO:30(2015)(010018 xWild *H. tuberosus*	13,3	43,2	19,0	20,3	1482,0
19	F3 R NO:32 (2015)(010018x Wild *H. annuus*	26,0	47,1	19,2	21,0	1790,1
20	F4RNO:4(2015) Wild *H. annuus* x (0536Rx6973R)	15,5	39,2	13,8	23,5	2082,4
21	F4 R NO:10(2015)9702R x Wild *H. annuus*	32,0	37,0	37,4	19,5	1393,0
22	F4 R NO:14(2015)6973R x Wild *H. annuus*	32,5	46,6	27,3	28,0	2674,8
23	F4 R NO:15(2015)0536R x Wild *H. annuus*	23,5	47,3	19,1	25,8	2014,1
24	F4RNO:16(2015) 0536 R x Wild *H. annuus*	15,8	29,9	19,7	22,3	1942,2
25	F4RNO:18(2015)(010018 x Wild *H. annuus*	7,5	32,3	11,6	21,0	1540,1
26	F2ADNO:2 (2015)9725B x Wild *H. annuus*	26,0	39,7	24,5	27,0	5490,7
27	F2ADNO:3(2015) 9725B x Wild *H. annuus*	44,0	40,0	31,4	31,8	6206,7
28	F2ADNO:4(2015)9725B x Wild *H. annuus*	18,5	35,6	31,4	25,5	5242,6
29	F3ADNO:5(2015)6535B x Wild *H. annuus*	26,3	33,3	21,0	31,8	6606,7
30	F3ADNO:6(2015)6522B x Wild *H. annuus*	25,0	45,2	21,9	25,5	4842,6
31	F3ADNO:14(2015)(6522Bx2478B)xWild *H. annuus*	36,5	46,0	31,8	25,8	5311,1
32	G2F6ADNO:2(2015)6522B x Wild *H. annuus*	26,0	41,3	26,1	25,8	4985,6

When the total chlorophyll amount values of the genotypes were examined in the R3 period, the highest chlorophyll amount was determined as 16.1 in *H. mollis* hybrid No: 6 and the lowest chlorophyll amount in *H. mollis* hybrid No: 14 was determined as 6.1. In the R5-1 period, the highest chlorophyll amount was measured as 17.3 in *H. mollis* hybrid No: 6 and the lowest chlorophyll amount was measured as 8.0 in *H. mollis* hybrid No: 6, 17 and 18. In the R6 period,

the highest chlorophyll amount was measured as 14.2 in *H. mollis* hybrid No: 6, and the lowest chlorophyll amount was measured as 4.1 in the genotype of wild *Helianthus annuus* No: 27 F2 AD SN: 3 (2015) 9725 B x wild *Helianthus annuus* (Table 9 and 10).

Table 9: The effect of drought stress on yield traits related to Chlorophyll content observations in the crosses with wild *Helianthus mollis x Helianthus annuus* species.

The crosses		Total Chlorophyll at R3 period	Total Chlorophyll at R5-1 period	Relative tolerance at R5-1 to R3 (%)	Total Chlorophyll at R6 period	Relative tolerance at R6 to R3 (%)	Relative tolerance at R6 to R5-1 (%)
1	*H. mollis x H. annuus*	9,7	11,0	113,4	9,8	101,0	89,1
2	*H. mollis x H. annuus*	11,7	12,1	103,4	7,9	67,5	65,3
3	*H. mollis x H. annuus*	11,7	12,2	104,3	9,9	84,6	81,1
4	*H. mollis x H. annuus*	11,4	12,7	111,4	9,5	83,3	74,8
5	*H. mollis x H. annuus*	13,4	14,7	109,7	11,2	83,6	76,2
6	*H. mollis x H. annuus*	16,1	17,3	107,5	14,2	88,2	82,1
7	*H. mollis x H. annuus*	8,4	8,8	104,8	5,4	64,3	61,4
8	*H. mollis x H. annuus*	8,9	9,8	110,1	7,1	79,8	72,4
9	*H. mollis x H. annuus*	9,5	9,7	102,1	6,3	66,3	64,9
10	*H. mollis x H. annuus*	8,2	8,9	108,5	5,7	69,5	64,0
11	*H. mollis x H. annuus*	7,9	9,2	116,5	5,2	65,8	56,5
12	*H. mollis x H. annuus*	14,2	15,6	109,9	12,1	85,2	77,6
13	*H. mollis x H. annuus*	11,5	13,6	118,3	10,7	93,0	78,7
14	*H. mollis x H. annuus*	6,1	8,0	131,1	4,2	68,9	52,5
15	*H. mollis x H. annuus*	7,1	8,1	114,1	4,5	63,4	55,6
16	*H. mollis x H. annuus*	8,4	8,8	104,8	6,1	72,6	69,3
17	*H. mollis x H. annuus*	7,5	8,0	106,7	5,4	72,0	67,5
18	*H. mollis x H. annuus*	6,9	8,0	115,9	5,3	76,8	66,3
19	*H. mollis x H. annuus*	8,6	9,2	107,0	5,2	60,5	56,5
20	*H. mollis x H. annuus*	9,0	9,8	108,9	6,4	71,1	65,3
21	*H. mollis x H. annuus*	9,9	11,1	112,1	7,2	72,7	64,9
22	*H. mollis x H. annuus*	9,2	11,1	120,7	7,4	80,4	66,7
23	*H. mollis x H. annuus*	12,9	14,2	110,1	10,3	79,8	72,5
24	*H. mollis x H. annuus*	10,5	12,5	119,0	9,4	89,5	75,2
25	*H. mollis x H. annuus*	12,9	14,0	108,5	10,2	79,1	72,9
26	*H. mollis x H. annuus*	7,9	9,1	115,2	5,4	68,4	59,3
27	*H. mollis x H. annuus*	8,4	8,8	104,8	5,6	66,7	63,6
28	*H. mollis x H. annuus*	8,9	9,2	103,4	5,7	64,0	62,0
29	*H. mollis x H. annuus*	9,7	11,1	114,4	8,8	90,7	79,3
30	*H. mollis x H. annuus*	8,3	9,1	109,6	7,7	92,8	84,6
C	Control (Tunca)	9,3	10,2	109,7	8,0	86,0	78,4

It was found that the average amount of chlorophyll in R5-1 period increased by 11.2% compared to R3 period and 19.3% in R6 period compared to R3 period and 27.4% compared to R5-1 period. When the changes in chlorophyll amounts were examined, the genotypes that showed less change compared to the chlorophyll amounts in R3 in the R5-1 period, H. mollis hybrids No: 9, 28, 2, 3, 7, 16 and 27, hybrids with wilds No: 18, 29, 25 and 2 genotypes were identified as the least changed genotypes. Genotypes with less variation than chlorophyll amounts in R3 in the R6 period were identified as *H. mollis* crosses No: 1, 13 and 30, No: 4,

31, 28, 3 and 23 genotypes from wild crosses. Genotypes that showed less change than chlorophyll amounts in R5-1 in the R6 period were determined as *H. mollis* crosses No: 1, 30 and 6, and from wild crosses Noo: 17, 13, 1 and 31 genotypes (Table 9 and 10).

Table 10: The effect of drought stress on yield traits related to Chlorophyll content observations in the crosses with common sunflower genotypes x *Helianthus species*.

#	The crosses	Total Chlorophyll at R3 period	Total Chlorophyll at R5-1 period	Relative tolerance at R5-1 to R3 (%)	Total Chlorophyll at R6 period	Relative tolerance at R6 to R3 (%)	Relative tolerance at R6 to R5-1 (%)
1	F2 R NO:2(2015) 0536 R x *H. annuus*	10,2	8,7	85,5	7,4	72,9	85,3
2	F2 R NO:3(2015) 0536 R x *H. annuus*	13,1	13,8	105,3	8,1	61,8	58,7
3	F2 R NO:4(2015) 6973 R x *H. tuberosus*	11,6	14,0	120,7	11,2	96,6	80,0
4	F2 R NO:5(2015)01001R x *H. annuus*	12,5	17,2	137,6	12,5	100,0	72,7
5	F2 R NO:6(2015) 6973 R x *H. annuus*	10,4	11,5	110,2	8,3	79,5	72,2
6	F2 R NO:7(2015) 6973 R x *H. tuberosus*	9,5	11,1	116,8	6,8	71,6	61,3
7	F2 R NO:8(2015)62303R x *H. annuus*	11,1	12,6	113,5	9,1	82,0	72,2
8	F2 R NO:9(2015)62303R x *H. annuus*	12,1	11,3	93,4	8,8	72,7	77,9
9	F2RNO:11(2015)TTAE gene pool x *H. annuus*	11,1	12,8	115,3	10,6	95,5	82,8
10	F3RNO:4(2015) (6973RX0536R) x *H. annuus*	10,0	11,1	111,0	8,8	88,0	79,3
11	F3RNO:5 (2015)(6973RX0536R) x *H. annuus*	10,6	13,1	123,6	10,1	95,3	77,1
12	F3 R NO:7 (2015)6973R X *H. tuberosus*	10,3	11,7	113,3	8,8	85,2	75,2
13	F3 R NO:8 (2015)6973R X *H. tuberosus*	10,1	11,2	110,9	9,7	96,0	86,6
14	F3 R NO:10 (2015)01001Rx *H. annuus*	12,9	15,3	118,6	12,1	93,8	79,1
15	F3 R NO:14 (2015)0536Rx *H. annuus*	9,4	10,8	114,9	7,7	81,9	71,3
16	F3 RNO:21(2015)(9702Rx0536R)x *H. annuus*	9,5	13,2	138,9	10,6	111,6	80,3
17	F3 R NO:27 (2015)010018x *H. annuus*	11,1	12,5	112,6	11,8	106,3	94,4
18	F3 R NO:30(2015)(010018 x *H. tuberosus*	9,7	9,9	102,1	7,4	76,3	74,7
19	F3 R NO:32 (2015)(010018x *H. annuus*	10,1	11,2	110,9	9,4	93,1	83,9
20	F4RNO:4(2015) *H. annuus* x (0536Rx6973R)	11,0	9,8	89,1	7,6	69,1	77,6
21	F4 R NO:10(2015)9702R x *H. annuus*	9,5	10,5	110,5	7,9	83,2	75,2
22	F4 R NO:14(2015)6973R x *H. annuus*	9,4	11,2	119,1	8,6	91,5	76,8
23	F4 R NO:15(2015)0536R x *H. annuus*	8,6	9,3	108,1	4,7	54,7	50,5
24	F4RNO:16(2015) 0536 R x *H. annuus*	8,2	9,5	115,9	7,9	96,3	83,2
25	F4RNO:18(2015)(010018 x *H. annuus*	11,0	11,3	102,7	8,6	78,2	76,1
26	F2ADNO:2 (2015)9725B x *H. annuus*	8,6	9,3	108,1	7,1	82,6	76,3
27	F2ADNO:3(2015) 9725B x *H. annuus*	7,0	8,2	117,1	4,1	58,6	50,0
28	F2ADNO:4(2015)9725B x *H. annuus*	9,0	11,3	125,6	8,7	96,7	77,0
29	F3ADNO:5(2015)6535B x *H. annuus*	9,3	9,5	102,2	5,4	58,1	56,8
30	F3ADNO:6(2015)6522B x Wild *H. annuus*	10,1	10,8	106,9	6,2	61,4	57,4
31	F3ADNO:14(2015)(6522Bx2478B)xWild *H. annuus*	9,4	10,7	113,8	9,1	96,8	85,0
32	G2F6ADNO:2(2015)6522B x Wild *H. annuus*	9,6	10,5	109,4	5,2	54,2	49,5

Total chlorophyll content of genotypes responded differently at different periods. The amount was increased 11.2% at R5-1 period comparing to R3 period in this study. However,

Total chlorophyll content were decreased 19.3% at R6 comparing with at R3 period, and 27.4% comparing with R5-1. There were big changes were observed both in some yield traits and also other phenological observations. Based on study results, head diameter, leaf area, oil content, seed yield per head and 1000 seed weights of wild crosses were lower than control (Tunca commercial sunflower hybrid) as expected. However, there are similar data were observed in phenological traits comparing control due to different genetic characters.

CONCLUSION

In photosynthetic activity results, the genotypes of *H. mollis* hybrids as numbered 1, 3, 4 and 5 were determined sensitively. Generally, *H. mollis* hybrids were not as durable as expected. While looking at the single selected plants, it is seen that no plants were selected from these genotypes as drought tolerants. However, in some other *H. mollis* genotypes single plants were selected. As a result of the measurements and analysis, it was observed that the hybrids made with *H. mollis* and Jerusalem artichoke were earlier and the parent lines resistant to IMI group herbicides were later.

It has been determined that the plant height of *H. mollis* hybrids is shorter and their head diameter is smaller. When examined in terms of seed yield, it was determined that higher results were obtained from safflower and sunflower ornamental hybrids. It was found that higher oil contents were obtained with artichoke and *Cirsium* hybrids. *H. mollis* hybrids had more leaves, higher values were not obtained in terms of leaf area as expected. It was determined that the hybrids made with ornamental sunflower plants had less leaves than other wild hybrids. Higher chlorophyll values were measured in crosses made with artichoke compared to other wild ones.

As conclusion, drought mostly influenced almost all yield traits. Study results indicated that wild crosses could be used commonly in the drought tolerance studies and selected ones will be included to sunflower breeding program. Then, they will use directly or indirectly associating initial program to obtain and develop drought tolerant genotypes for the future breeding research furtherly to develop tolerant sunflower hybrids.

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THE YIELD PERFORMANCES OF SOME CONFECTIONERY SUNFLOWER HYBRIDS IN TRAKYA REGION

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ABSTRACT

Confectionary sunflower is the most consumed confectionery product in Turkey. Although there is about 100.000 ha confectionery sunflower planted area in Turkey, Turkish seed confectionery sunflower production is not enough for domestic consumption and there is over 100 million USD imports every year. The most important reasons are lower yields due to using standard seeds then resulted lower quality, some diseases and broomrape parasite problem, higher costs due to lower mechanization uses, etc. The confectionery sunflower seed import mostly from China because of the longer grains and these type seeds invade recently almost all Turkish confectionery market. Beside, Turkey has very big potential for confectionery export due to geographical advantages. However, hybrids having higher yields, quality and uniform seed are preferred both by industry and also by farmers are few in Turkey. Furthermore, certified seed use by producers also lower too. The study was conducted to determine yield performance of candidate sunflower confectionery hybrids developed by Tragen R&D Co in Edirne and Tekirdag locations in Trakya region in 2019. Based on study results, some candidate hybrids exhibited higher performances than control hybrid. TG-400 candidate hybrid showed promising performance in both locations and then it sent to registration trials and obtained production permission in 2020.

Key words: Confectionery sunflower, Yield performance, Hybrid, Trakya region

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated as oil crops in the world. However, sunflower grows also for confectionery, birdfeeding and ornamental purposes (Lofgren, 1997). For instance, confectionery sunflower is one of the most preferred snack in Turkey, as well as in other countries such as Eastern Europe, US, Canada China, Pakistan, Iran, Middle East countries, etc.

Except Russia and some Slavic countries, confectionery sunflower has generally white color with grey stripes, and larger seeds (Gholinezhad et al, 2013, 2014; Gontcharov, 2011; Hladni, 2016; Hladni et al., 2009, 2011, 2012, 2015a, b, c, 2018; Hladni and Miladinović, 2019; Kaya, 2002, 2004; Kaya et al., 2008, 2009 and b, 2013; Kaya and Beşer 2018; Kholghi, 2011; Levytska and Kotsiubanska, 2020; Velasco et al., 2014).

Seed quality is so important in confectionery sunflower because when consumers consider mostly bigger sizes, good tastes, lower oil content, longer shelf life, etc. Therefore, 1000 seed weight and larger seed sizes are important traits in the confectionery breeding in addition to seed yield. Furthermore, the confectionary sunflower sectors request higher quality seeds such as homogenity of the seeds as well larger and longer seeds both for snack and hulled

sunflowers (De Figueiredo, 2015; Ergen and Saglam; 2005; Jovanović, 2001; Jovanović et al., 1998a and b; Hofland and Kadrmas, 1989; Shevchenko and Aliiev, 2018). These quality of seeds could be possible only from hybrids. Therefore, hybrid sunflower cultivars satisfy to industry for giving higher quality seeds as well as farmers for presenting high yielding that are still food-grade quality (De Figueiredo, 2015; Demurin, 2018; Dijanović et al., 2003; Gontcharov and Beresneva, 2011; Evci et al., 2011; Hladni et al., 2016, 2019; Jocić et al., 2015; Li et al., 2010; Mamonov, 2004; Nabloussi, 2011; Pekcan et al., 2015; Pérez-Vich et al., 2018; Sincik and Goksoy, 2014; Stanojević, 1992; Velasco et al., 2014).

Turkish confectionery sunflower production is about 100,000 MT in recent years and planting areas are mostly located in Middle Anatolia, as well as in South Marmara and Denizli and Kahramanmaraş provinces (Kaya et al., 2009; 2013; Kaya and Beşer 2018; Tan et al., 2016). However, domestic production of confectionery sunflower is not enough and Turkey imports confectionery sunflower seeds from abroad mostly from China with paying about 100 million USD in each year (Kaya and Beşer 2018).

Open pollinated types are generally planting in Turkey and but hybrids are less in the market. Therefore, hybrids should exist in the market both for increasing production and also supplying high quality seeds both for consumers and industry. The aim of the study was to determine of yield and seed quality performances of some hybrids in confectionery sunflower to supply promising cultivars for sector.

MATERIALS AND METHODS

The yield trials were conducted in Edirne and Tekirdag location in 2019 to determine yield performances of candidate confectionery sunflower hybrids. There were 14 hybrids including five controls from commercial sunflower hybrids (AHMETBEY, METİNBEY, MURATBEY and TRÇ107) in the market. The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,60-m long with the 70 x 40 cm plant spacing.

Total plot area at planting was 7,6*2,8 as 21,28 m^2. The middle two rows were harvested and the border rows were discarded, and plot size was 9.52 m^2 at harvest. The compose fertilizers (20-20-0, Zn) were applied 200 kg/ha dose at planting. Statistical analysis was performed with JMP statistical program.

Tekirdag location was conducted in Beyazkoy village fields, Saray County and the trials were planted by hand in 15 April 2019. Emergence date of sunflower plants was in 25 April 2019 and left only one plant each as mentioned plant density above. The trials were harvested by hand in 2 September 2019 as middle two rows except one plant at the beginning of the middle rows. Edirne location was conducted in Sarayakpinar village fields and the trials were planted by hand in 22 April 2019. Emergence date of sunflower plants was in 2 May 2019 and the trials were harvested by hand in 16 September 2019.

Seed yield (SY), 1000 seed weight (TSW), flowering and physiological maturity period, plant height (PH), head diameter (HD), husk (HC) and oil content (OC) were measured in the study. The flowering period (FP) of hybrids were measured at stage which 50% of plants in the plot with first open ligule petals. The physiological maturity period (PM) of hybrids were counted at the stage which the back of heads was yellow and 10 % were brown color.

The plant height and head diameter of hybrids were measured from 3 plants at mid rows of the plots in each replication at PM stage. Oil content of the hybrids were determined utilizing Nuclear Magnetic Resonance (NMR) analysis. Disease observations were performed in natural

conditions. In the center seed filling (1-5) and general uniformity (1-5) observations they were evaluated as the five is the best and the one is the lowest.

RESULTS AND DISCUSSION

The study was conducted to determine yield performance of candidate sunflower confectionery hybrids developed by Tragen R&D Co located in Trakya University Technopark in Edirne and Tekirdag locations in Trakya region in 2019. Based on study results, flowering days are changed between 59 and 77 days in Edirne location and between 59 and 79 days in Edirne location (Table 1 and 2). In the physiological maturity days, the hybrids exhibited as between 93 and 111 days.

Seed yield of the hybrids exhibited larger variation and some candidate hybrids exhibited higher performances than control hybrids in the trials (Table 3, 4 and 5). Edirne location had higher seed yields than Tekirdağ location because soil quality and humidity was higher in Edirne location. Seed yield was changed between 313,5 and 474,8 kg/da in Edirne location and 187,3 and 279,4 kg/da in Tekirdağ location. The highest yield was obtained from TG-400 candidate hybrid and the lowest seed yield was obtained from TRÇ107 control hybrid in both locations.

In the thousand seed weight as the most important seed yield quality for confectionery sunflower, the highest values were obtained from TG ÇRZ 01A/TGCRZ01-R candidate hybrids in both locations as 236,1 g in Edirne location and 208,4 g in Tekirdağ location respectively.

Based on the average values, the highest seed yield was obtained from TG-400 candidate hybrid as 377,1 kg/da. The highest seed yield was obtained from MURATBEY as 320,1 kg/da in the control hybrids and the average values of the control hybrids was 295,2 kg/da. Except two candidate hybrids, eight candidate hybrids exhibited higher performance than the control average in the study (Table 5 and 6). These candidate hybrids will be tested in the next year again. If they still show the higher performances in the trials, they will be evaluated again and the best ones will be registration trials in the next year.

Based on the current year study in 2019, TG 400 candidate hybrid showed promising performance in both locations and then it selected to send registration trials after preparing all necessary documents in 2020.

Table 1. The yield traits of sunflower hybrids in Edirne location

Hybrids	%50 Flower (day)	Phy Mat (day)	Plant Height (cm)	Head Diam (cm)	Self Pollin (1-5)	Cennter Seed filling (1-5) **	General Uniformity (1-5) ***	Harvest humidity (%)	Broomrape Plant Number	Broomrape plants per plant	*Plasmopara halstedii* (Mildew)	*Puccinia helianthi* (Rust)
235A/ TG CRZ 05-R	65	98	199	29,2	4	5	1	14	1	2	MS	MR
235A/ TG CRZ 04-R	61	94	216	29.8	5	5	1	14	0	0	MS	MS
235A/ TG CRZ 03-R	65	97	212	31,0	4	5	1	13	0	0	MS	MR
G-1	70	105	228	24,1	5	5	1	15	3	5	MR	MR
G-10	71	107	229	23,9	5	5	1	12	0	0	MS	MR
235A/ TG 06-R	59	94	208	30,0	4	4	1	11	1	2	MS	MS
GL-336	77	113	241	24,0	5	5	1	15	5	6	MS	MS
235A/ TG 07-R	65	94	222	31,8	4	4	1	13	1	4	M	MR
TGÇRZ01A/TGCRZ 01-R	63	101	216	24,2	5	5	1	16	4	5	S	MR
S 400 TG	63	103	232	24,8	5	5	1	12	5	3	S	MR
AHMETBEY (C)	62	98	200	25,4	4	4	1	14	4	3	MS	MR
METİNBEY (C)	63	98	190	17,2	4	4	1	12	5	4	MS	MR
MURATBEY (C)	58	96	229	26,2	4	4	1	15	2	5	MS	MR
TRÇ107 (C)	59	96	189	21,2	4	4	1	13	3	3	MS	MR

Table 2. The yield traits of sunflower hybrids in Tekirdağ location

Hybrids	%50 Flower (day)	Phy Mat (day)	Plant Height (cm)	Head Diam (cm)	Self Pollin (1-5)	Cennter Seed filling (1-5) **	General Uniformity (1-5) ***	Harvest humidity (%)	Broomrape Plant Number	Broomrape plants per plant	*Plasmopara halstedii* (Mildew)
235A/ TG CRZ 05-R	62	97	185	24,6	4	4	1	12	1	2	MS
235A/ TG CRZ 04-R	60	92	204	22,3	4	4	1	16	0	0	MS
235A/ TG CRZ 03-R	62	94	208	22,7	4	3	1	11	0	0	MS
G-1	71	103	219	20,1	4	5	1	14	2	4	MR
G-10	69	108	217	19,6	5	5	1	16	0	0	MS
235A/ TG 06-R	59	92	197	24,1	4	4	1	12	1	2	MS
GL-336	78	111	222	22,1	5	4	1	15	3	4	MS
235A/ TG 07-R	66	93	206	25,2	4	4	1	13	1	3	MS
TG ÇRZ 01 A/TG CRZ 01-R	64	100	205	19,9	4	5	1	14	3	3	MS
S 400 TG	62	101	221	20,8	4	4	1	14	2	2	MS
AHMETBEY (C)	60	97	198	19,0	4	4	1	15	4	3	MS
METİNBEY (C)	62	99	178	15,7	4	4	1	12	3	2	MS
MURATBEY (C)	59	99	201	22,1	4	4	1	14	1	2	MS
TRÇ107 (C)	59	95	177	18,8	4	4	1	14	2	2	MS

Table 3. The yield traits related to seed of sunflower hybrids in Edirne location

Çeşit No	Husk Content (%)	Hectoliter Weight (g)	1000 Seed Weight (g)	Oil Content (%)	Oil yield (kg/da)	Seed yield (kg/da)
235A/ TG CRZ 05-R	43	31,2	165,8	32,13	152,6	379,5 DE
235A/ TG CRZ 04-R	47,1	33,6	188,8	25,47	90,9	322,5 F
235A/ TG CRZ 03-R	40,0	34,2	165,2	31,43	136,2	410,5 CD
G-1	47,1	29,6	201,0	23,23	111,1	420,2 BCD
G-10	52,3	32,4	231,0	24,67	115,4	464,7 AB
235A/ TG 06-R	41,7	34,8	164,4	29,53	89,6	325,4 F
GL-336	44,3	34,0	209,0	27,17	125,4	424,4 BC
235A/ TG 07-R	45,0	37,2	148,6	28,83	127,5	428,9 BC
TG ÇRZ 01A/TGCRZ01-R	53,5	32,3	236,1	23,60	112,9	420,5 BCD
S 400 TG	49,0	32,4	187,7	28,50	136,9	474,8 A
AHMETBEY (C)	48,7	31,7	158,2	25,57	105,7	392,2 CD
METİNBEY (C)	53,4	30,8	146,4	27,43	99,5	338,1 EF
MURATBEY (C)	47,6	32,0	143,2	29,43	115,9	405,6 CD
TRÇ107 (C)	46,4	35,5	128,5	28,90	89,4	313,5 F

Table 4. The yield traits related to seed of sunflower hybrids in Tekirdag location

Hybrid	Husk Content (%)	Hectoliter Weight (g)	1000 Seed Weight (g)	Oil Content (%)	Oil yield (kg/da)	Seed yield (kg/da)
235A/ TG CRZ 05-R	45,1	30.7	151,2	32,07	60,7	211,1 EF
235A/ TG CRZ 04-R	48,1	32,6	172,6	25,33	52,0	210,3 EF
235A/ TG CRZ 03-R	42,1	31,2	155	31,10	75,0	258,0 ABC
G-1	48,1	27,6	188,2	23,57	58,4	247,6 ABCD
G-10	53,4	32,4	206	25,00	59,6	273,6 AB
235A/ TG 06-R	39,5	32,6	148,9	29,80	57,7	203,8 EF
GL-336	47,1	31,8	192	26,90	68,3	249,8 ABCD
235A/ TG 07-R	48,8	35,2	141,2	28,70	53,5	220,5 DE
TGÇRZ01A/TGCRZ01R	54,1	31,1	208,4	23,47	55,7	249,3 ABCD
S 400 TG	50,8	12,3	173,6	28,30	64,3	279,4 A
AHMETBEY (C)	49,6	30,1	139	25,93	59,4	246,0 BCD
METİNBEY (C)	54,9	30,1	136,1	26,83	66,0	244,1 BCD
MURATBEY (C)	49,3	31,8	128,9	27,60	-	234,6 CDE
TRÇ107 (C)	47,2	33,6	115,1	28,90	52,5	187,3 F

Table 5. Edirne, Tekirdag and the average seed yield data

#	Hybrid name	Edirne Seed Yield (Kg/da)	Tekirdağ Seed Yield (Kg/da)	Average Seed Yield (Kg/da)
1	235A/ TG CRZ 05-R	379,5 DE	211,1 EF	295,3 DEF
2	235A/ TG CRZ 04-R	322,5 F	210,3 EF	266,4 FG
3	235A/ TG CRZ 03-R	410,5 CD	258,0 ABC	334,3 C
4	G-1	420,2 BCD	247,6 ABCD	333,9 C
5	G-10	464,7 AB	273,6 AB	369,1 AB
6	235A/ TG 06-R	325,4 F	203,8 EF	264,6 FG
7	GL-336	424,4 BC	249,8 ABCD	337,1 BC
8	235A/ TG 07-R	428,9 BC	220,5 DE	324,7 CD
9	TG ÇRZ 01 A/TG CRZ 01-R	420,5 BCD	249,3 ABCD	334,9 C
10	S 400 TG	474,8 A	279,4 A	377,1 A
11	AHMETBEY (C)	392,2 CD	246,0 BCD	319,1 CDE
12	METİNBEY (C)	338,1 EF	244,1 BCD	291,1 EF
13	MURATBEY (C)	405,6 CD	234,6 CDE	320,1 CDE
14	TRÇ107 (C)	313,5 F	187,3 F	250,4 G
Average		394,3	236,8	315,6
LSD: (0.05) (kg/da)		44,8	35,8	33,2
CV (%)		8,0	9,7	10,5
F		10,97 **	5,42 **	10,27 **

Table 6. Comparing seed yields of the candidate S 400 TG hybrid with standards

Hybrids	Edirne Location Kg/da	Tekirdag location Kg/da	Average Kg/da
AHMETBEY (C)	392,2 CD	246,0 BCD	319,1 CDE
METİNBEY (C)	338,1 EF	244,1 BCD	291,1 EF
MURATBEY (C)	405,6 CD	234,6 CDE	320,1 CDE
TRÇ107 (C)	313,5 F	187,3 F	250,4 G
The best standard	405,6	246,0	320
S 400 TG	**474,8 A**	**279,4 A**	**377,1 A**
The difference with best standard	% 17	% 13	% 17,8

CONCLUSION

Based on study results, some candidate hybrids exhibited higher performances than control hybrids. As conclusion, TG-400 candidate hybrid showed promising performance in both locations and then it sent to registration trials and obtained production permission in 2020.

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DETERMINING YIELD STABILITY IN CONFECTIONERY SUNFLOWER

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ABSTRACT

As the most consumed snack in Turkey, domestic confectionary sunflower production is not enough and Turkey pay every year about 100 million USD imports. Furthermore, Turkey has excellent location for confectionery sunflower seeds because almost all neighbour countries consume a lot sunflower snacks so has huge advantages for export too. However, mostly open pollinated cultivars are in the market with having less uniform seeds in the confectionery planted areas (about 100.000 ha in Turkey). Therefore, hybrid confectionery sunflower cultivar should exist urgently and they have to have higher adaptation capabilities as well as stability to increase confectionery sunflower production in Turkey. The study was conducted to determine seed yield stability of some sunflower confectionery cultivars in different climatic conditions in 2014-2018 period. Based on study results of the eight stability parametes, Palanci cultivar had higher performance for seed yield as well as could be determined as the most stabile hybrid among cultivars existed in the research.

Key words: Confectionery sunflower, Seed yield performance, Stability, G x E interactions

INTRODUCTION

Although sunflower (*Helianthus annuus* L.) mostly cultivated for vegetable oil, it grows also for confectionery as human food both as snack and also hulled seeds for patisserie products. As confectioney purposes, it is one of the most consumed product in Turkey, Eastern Europe, Balkans, Middle East countries, US, Canada, China, Pakistan, Iran, etc. (Kaya et al., 2013). The size, the taste, longer shelf life, etc., are the most important seed quality traits in confectionery sunflower determined by mostly 1000 seed weight, seed size, fatty acid and tocopherol (E vitamine) contents (Ergen and Saglam; 2005). In addition to these seed quality traits, seed yield is the most important trait in confectionery sunflower production in the world and higher seed yield could be obtaine only from hybrid cultivars for satisfying both industry as giving higher food-grade quality seeds and also as farmers for presenting high yielding (Hladni et al., 2015; Li et al., 2010; Pekcan et al., 2015).

Turkish confectionery sunflower planted areas is about 50-60.000 ha and production 100,000 MT recently. Kayseri, Konya, Kahramanmaraş, Denizli and Bursa provinces have confectionery sunflower planting areas in Turkey (Kaya et al., 2013; Kaya and Beşer 2018). White color with grey stripes confectionery sunflower types are growing and preferring mostly in Turkey as well as larger seeds while Russia, Ukraine and some Balkan countries prefer black colors generally (Gontcharov, 2011; Hladni et al., 2015; Kaya et al., 2013; Kaya and Beşer 2018).

Some confectionery sunflower varieties are more preferred by producers in different regions. The basis of this is that those varieties adapt more easily to the climatic conditions in that region and show higher grain and oil yield performance. For this reason, it is extremely important that hybrids have agronomic and physiological characteristics suitable for the region according to the needs of the local farmers (Kaya and Atakisi, 2002; Kaya et al., 2005).

Genotype x environment (GxE) interaction is defined as the variation performances of the cultivars developed for long years of intensive studies according to changing environmental conditions. For plant breeders, the fact that GxE interaction causes differences in yield sequences of genotypes in different locations poses a problem in terms of efficiency of selection. However, if this interaction does not change the yield order of genotypes in different environments, then there is no problem in terms of variety recommendation (Kaya and Atakisi, 2002; Rao et al., 2004; Kaya et al., 2005; Kaya et al., 2012; Sheoran et al., 2012; Cvejic et al., 2019; Ahmed et al., 2020).

GxE interactions occur in efficiency performance trials. These interactions are important both in terms of plant breeding. Some researchers ignore the GxE interaction by taking the general average of yields at different locations, especially in short-term yield trials, with the idea of choosing the best variety and combining more criteria in terms of potential yield. However, the main purpose of setting up yield trials is to predict the performance of the best variety to be planted in future farmer fields, using available data. Therefore, this neglect in trials leads to a great financial loss for the producers in the future (Kaya and Atakisi, 2002; Kaya et al., 2012; Bhoite et al., 2010; Cvejic et al., 2019; Ahmed et al., 2020).

It could be theoretically desirable that each developed variety will show high performance in all the environments where it is cultivated and in years with different climates although it is not possible in practice. For this reason, as a result of the stability analysis of the varieties tested in as many different regions as possible, some of the cultivars show superior performance only in good conditions, while some of them could maintain their performance even in bad conditions (Kaya and Atakisi, 2002; Kaya et al., 2005; Cvejic et al., 2019).

Various methods are used to evaluate and compare the performance of plants in different environmental conditions and years. Regression analysis which investigates the relationship between the genotypic averages of each genotype in the trials and the averages in different environments, is one of the most used stability measurement methods. Linear regression coefficient (b) determines the performance of each genotype in different environments, while deviations from regression are squared (Kaya and Atakisi, 2002; Kaya et al., 2005; Sheoran et al., 2012; Cvejic et al., 2019; Ahmed et al., 2020).

Finlay and Wilkinson (1963) accepted the difference between the average performance of the cultivars in each different location from the general average as the environmental index and evaluated the linear regression (bi) of the average yields of the cultivars on this index as a measure of adaptation. To this regression model, Eberhart and Russell (1966) suggested that regression mean square deviation (Sdi^2) is another determinant of the stability of adaptation to the environment and this model has been accepted by many breeders until today.

Perkins and Jinks (1968) and Baker (1969) approached this regression model differently and accepted the value of, known as the corrected regression coefficient and equal to "0" instead of "1", as the stability measure. Wricke's (1962), Wi^2, which is called the equvalence value, and Vi, known as the stability variance parameter of Shukla (1972), determines the order of the stability of the varieties according to the contribution ratio in the GxE interaction variance of each studied genotype.

According to Baker (1969), the calculated coefficient on the environmental index of the GxE interaction of each genotype is equal to the corrected regression coefficient of Perkins and Jinks (1968) and the expected value is zero. If this coefficient is zero, it indicates that the genotype will adapt well to all environments.

Aguero et al. (2000) compared the high oleic acid-containing sunflower hybrids according to their LSDs, the best hybrid in the same environment and their relative yields calculated as the deviation of the mean of the environment, and the variety with the lowest standard deviation in different environments and the closest to the best hybrid, they emphasized that it would be the most stable ones.

The aim of the study was to determine the effect of GxE interaction and determine their stability by examining the performance some confectionery sunflower cultivars in different environmental conditions.

MATERIALS AND METHODS

The yield trials were conducted in Edirne, Luleburgaz, Cayırova-Kocaeli and Manisa locations in 2015-2018 period to determine yield performances of candidate confectionery sunflower cultivars. There were six sunflower cultivars (AHMETBEY, METİNBEY, PALANCI, ÇIGDEM, CONFETA and INEGOL) existing as controls in testingcommercial sunflower hybrid trials in this four years' period. From these cultivars, Inegol and Cigdem are the open pollinated other three cultivars are confectionery hybrid.

The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,60-m long with the 70 x 40 cm plant spacing. Total plot area at planting was 7,6*2,8 as 21,28 m^2. The middle two rows were harvested and the border rows were discarded, and plot size was 9.52 m^2 at harvest. The compose fertilizers (20-20-0, Zn) were applied 200 kg/ha dose at planting. Statistical analysis was performed with JMP statistical program and stability analysis were performed in the TARPOPGEN program in the study. The trials were planted by hand in April generally and left only one plant each as mentioned plant density above. The trials were harvested by hand generally in September in each year as middle two rows except one plant at the beginning of the middle rows.

In the trials, seed yield (SY), 1000 seed weight (TSW), flowering and physiological maturity period, plant height (PH), head diameter (HD), husk (HC) and oil content (OC) were measured in the study. The plant height and head diameter of hybrids were measured from 3 plants at mid rows of the plots in each replication at PM stage. Oil content of the hybrids were determined utilizing Nuclear Magnetic Resonance (NMR) analysis. Disease observations were performed in natural conditions. In the center seed filling (1-5) and general uniformity (1-5) observations they were evaluated as the five is the best and the one is the lowest.

In order to determine and compare the performance of genotypes in different environments, researchers have developed many methods and measured the stability and adaptability of varieties using them. In the study, the stability status of hybrids was examined according to 8 of the most widely used stability analysis method in the world. The stability parameters in these methods used in the research are as follows:

1. Finlay and Wilkinson (1963); Coefficient of linear regression (bi) on the environmental index, which is the difference between the mean of the cultivars in different environments and the general mean
2. Eberhart and Russell (1966); regression of phenotypic values of each genotype in different environments on environmental indices (bi) and deviation from regression mean square (Sdi^2)
3. Perkins and Jinks (1968); corrected regression coefficient (= bi - 1),
4. Baker (1969); corrected regression coefficient (= bi - 1),
5. Wricke (1962); Equivalence value Wi^2, sum of squares of GxE interaction effects calculated over all circles for each genotype,
6. Shukla (1972); stability variance parameter Vi,

7. Francis and Kannenberg (1978); variance of phenotypic values of each genotype in different environments (Si ²) and% coefficient of variation (CV),

8. Ketata (1990); Average and Standard Deviation coefficient,

RESULTS AND DISCUSSION

It is extremely important for the breeder to know the performance of the varieties developed as a result of many years of efforts under different environmental conditions. The determination of whether these varieties adapt to different environments and whether they are stable by maintaining their superior performance in different conditions is determined by stability analysis. Therefore, the in terms of yield and yield elements, the cultivars planted in three locations each year, and preliminary analysis of variance were performed over the values combined as four locations for the years 2015 to 2018, and the statistical significance of the GxE interaction was tested. In order to perform stability analysis, GxE interaction should be important so both genotypes and the G x E interactions are found as significant in ANOVA table. However, when the analyzed of the genotypes in the 2nd ANOVA, only 1 and 5th genotypes were found significant (Table 1 and 2).

The seed yield performances of confectionery sunflower cultivars were analyzed by eight stability parameters in the study. While Finlay and Wilkinson (1963) (bi) and Eberhart and Russel (1966) (bi) coefficients as well as Perkins and Jinks (1968) (β=bi– 1) and Baker (1969) (β =bi– 1) coefficients were the same, then bi and β values could be considered as the same stability values while evaluating. When it was examined the tables, Ahmetbey, Metinbey, Palanci and Çigdem cultivars, existed over the level in these values and Confeta and Inegol cultivars had lower values (Table 3, 4 and 5). In terms of the values, the closest values to bi = 1 and β = 0 from the chart could be accepted and considered the more stable cultivars in the study. Therefore, it seems that Palanci was the most stabile cultivar in the study. Among the cultivars, Confeta as the lowest bi value (Table 3).

In terms of Sdi² values, Cigdem had the lowest values so can be considered stable in terms of this parameter, having lower Sdi² values. Palanci and Metinbey followed this cultivar respectively. In terms of Wricke's Wi², Palanci cultivar had the lowest value then it could be determined the most stable cultivar based on this parameter. When it was evaluated the stability parameter of Shukla in Vi values, like Wricke's Wi², Palanci had the lowest value and followed by Cigdem were accepted as stable in terms of seed yield (Table 4).

In the Francis and Kannenberg's Si ² values, Confeta and Inegol were measured as the most stable cultivars. Again, according to the stability parameter CVs of the same cultivars had the lowest values as were observed more stably than others. In terms of average and Standard Deviation coefficient of Ketata, Inegol, Cigdem and Metinbey were determined as the most stable cultivars by taking the lowest values (Table 5).

Table 1. The ANOVA table of seed yield

Source of variation	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Environment X Reps	12	25.806,09	2.150,50	1,03ns	0,4301
Genotype (G)	5	185.849,30	37.169,86	17,89**	0,0000
Environment (E)	3	2.798.266,86	932.755,62	449,03**	0,0000
G X E	15	266.782,57	17.785,50	8,56**	0,0000
Error	60	124.634,65	2.077,24		
General	95	3.401.339,48			

** = %1 level significant, * = %5 level significant, ns = non significant

Tablo 2: Stability ANOVA table.

Source of variation	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**P(>F)**
Genotype	5	46.462,32	9.292,46	4,139*	0,0204
E(G x E)	18	766.262,35			
Environment	1	699.566,71			
G X E	5	39.752,81	7.950,56	3,541*	0,0336
Deviations	12	26.942,82	2.245,23		
1. Genotype	2	6.901,91	3.450,95	6,645**	0,0028
2. Genotype	2	1.445,62	722,81	1,392ns	0,2555
3. Genotype	2	982,76	491,38	0,946ns	0,3961
4. Genotype	2	487,92	243,96	0,470ns	0,6331
5. Genotype	2	14.882,09	7.441,04	14,329**	0,0001
6. Genotype	2	2.242,50	1.121,25	2,159ns	0,1224
Error	60	31.158,66	519,31		
General	23	812.724,68			

** = %1 level significant, * = %5 level significant, ns = non significant

Tablo 3: Stability Parameters

		Finlay and			Eberhart and	
	Average	Wilkinson	Baker		Russel	
Genotype	Yield (Kg/da)	bi	bi	Sd^2	bi	Sd^2
Ahmetbey 1	442,06	1,122	0,122	3450,95	1,122	3450,95
Metinbey 2	433,81	1,305	0,305	722,81	1,305	722,81
Palanci 3	433,25	1,058	0,058	491,38	1,058	491,38
Çigdem 4	408,56	1,118	0,118	243,96	1,118	243,96
Confeta 5	312,69	0,568	-0,432	7441,04	0,568	7441,05
İnegol 6	416,19	0,829	-0,171	1121,25	0,829	1121,25

Tablo 4: Stability Parameters

				Francis and	
	Average	Wricke	Shukla	Kennenbert	
Genotype	Yield	Wi	Vi	S^2	CV
1	442,06	8624,859	2338,350	51188,724	51,180
2	433,81	12324,276	3818,116	66715,891	59,541
3	433,25	1375,703	-561,313	43835,792	48,325
4	408,56	2106,192	-269,117	48724,307	54,027
5	312,68	36601,786	13529,120	17516,807	42,327
6	416,18	5662,828	1153,537	27439,266	39,801

Based on Finlay and Wilkinson (1963) bi values, while Ahmetbey, Metinbey, Palanci and Çigdem cultivars had better yields especially in the better conditions as existed over the average level in these values. Inegol as open pollinated cultivar which also exhibited also over general average in th seed yielding could peform better performance even in the bad environmental conditions too. Confeta confectionery sunflower hybrid keep better performance in the bad conditions generally (Figure 1).

Tablo 5: Stability Parameters

		Perkins and Jinks		Ketata	
Genotype	Average Yield	Bi	Sd²	Average	Standard Deviation
1	442,06	0,122	3450,95	4,250	5,583
2	433,81	0,305	722,81	3,500	3,000
3	433,25	0,058	491,38	4,000	0,667
4	408,56	0,118	243,96	2,500	3,000
5	312,68	-0,432	7441,05	2,500	3,667
6	416,18	-0,171	1121,25	4,250	2,917

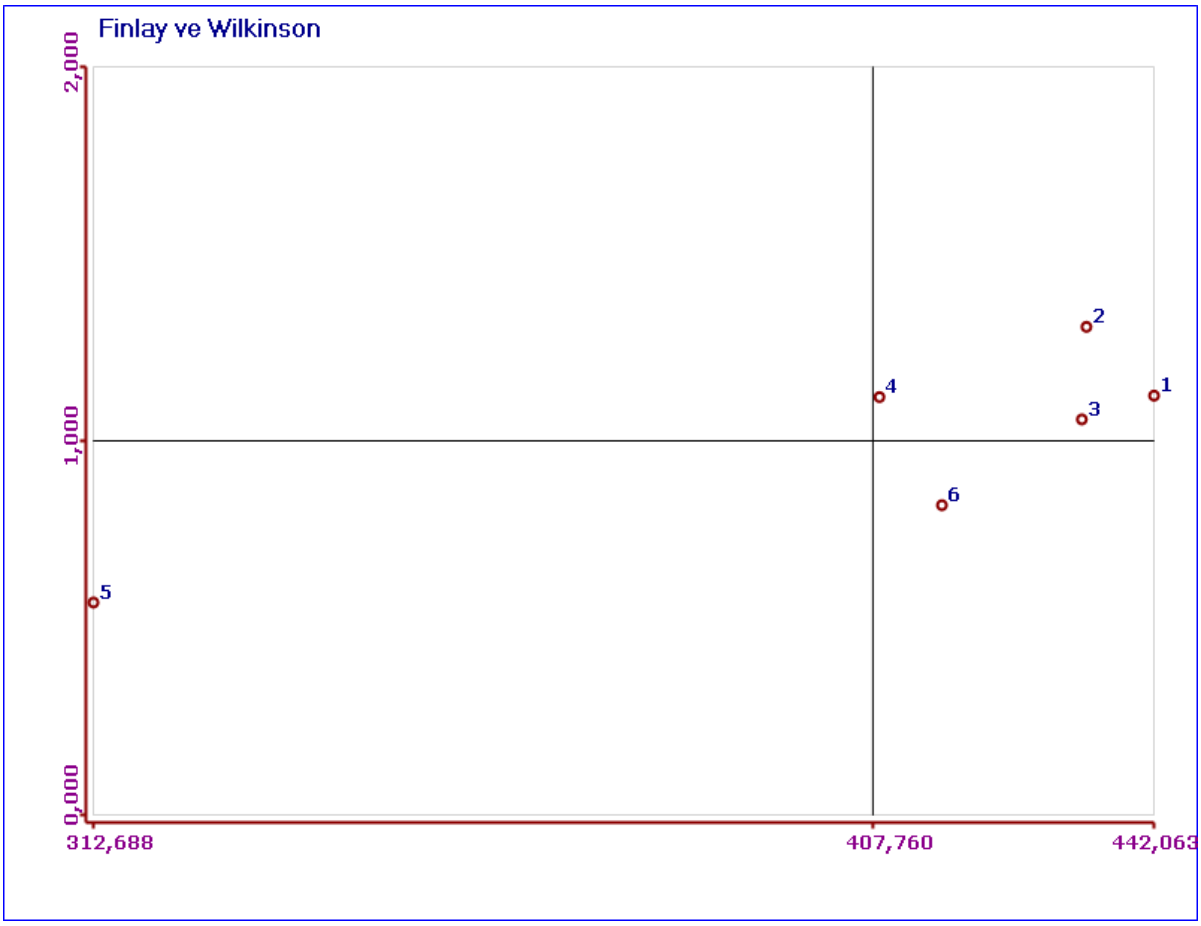

Figure 1. The cultivars based on different stability parameters

Based on Ketata average values, while Ahmetbey cultivars had better yields especially in the better conditions as better yielding higher standard deviation. Palanci had higher performance for seed yield as well as had the lowest standard deviation so it is the best stabile cultivar among cultivars existed in the study. Similarly, Metinbey hybrid and Inegol open pollinated cultivar had higher seed yielding but lower standard deviation too. Çiğdem as open pollinated cultivar lower yielding and lower standard deviation but Confeta confectionery sunflower hybrid lower seed yielding performance with higher standard deviation (Figure 2).

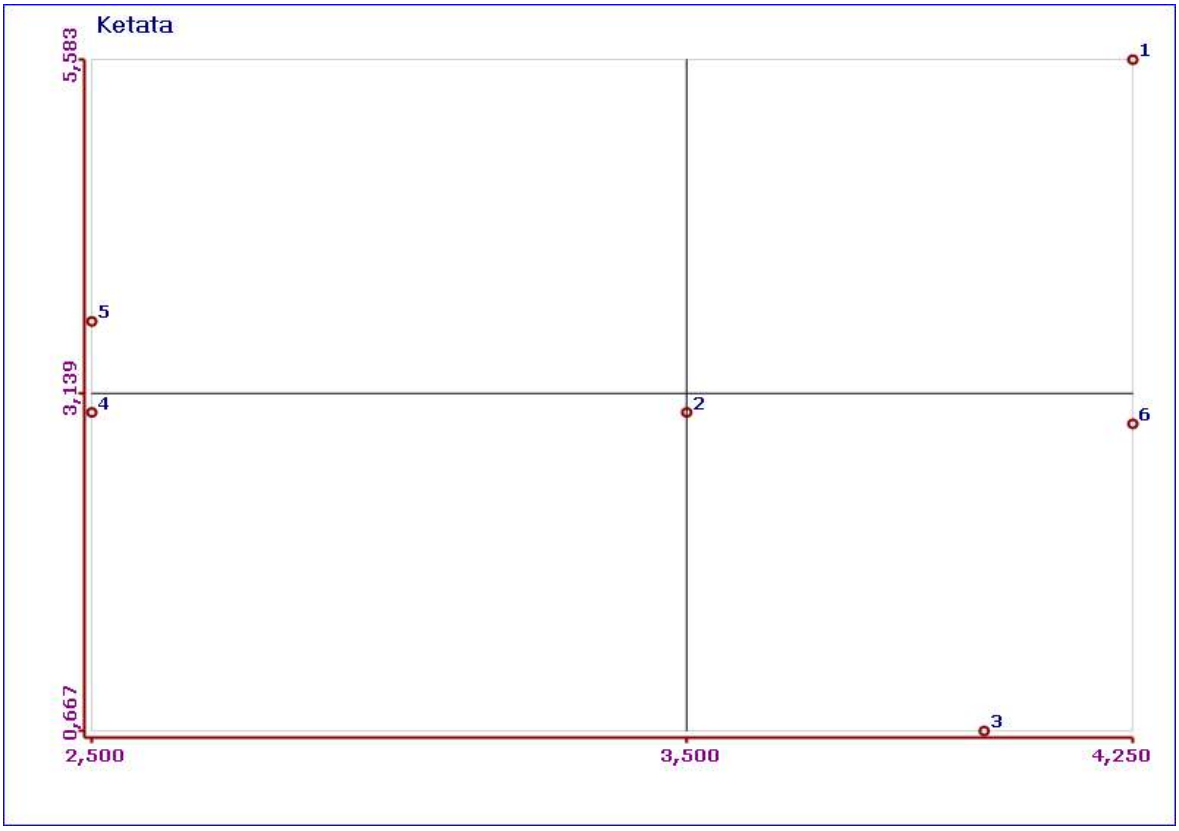

Figure 2. The cultivars based on different stability parameters

CONCLUSION

In the evaluation of adaptation and stability analysis, what is important is whether the varieties with high yield potential can maintain this performance under different environmental conditions, ie whether this potential is stable. On the contrary, very stable varieties with low yields are of no importance in sunflower production. Based on study results of the eight stability parametes, Palanci cultivar had higher performance for seed yield as well as could be determined as the most stabile hybrid among cultivars existed in the research.

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MOLECULAR APPROACHES IN DETERMINING MEAT QUALITY

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ABSTRACT

Consumer requests are come at the beginning of the most important features in the quality of a meat. Meat color, pH, marbling score (MS) and tenderness of meat (texture) are the most important features that determine meat quality and are among the consumer requests. Characters such as tenderness and color of the meat come hereditary. Therefore, the tenderness of the meat stands out as quite an important feature. As well as meat tenderness, food analysis such as cooking loss, water holding capacity, MS, color, pH-temperature measurements are also taken as the basis for determining the quality of the meat offered for consumption. It is an important problem that beef is not as high quality as the consumers want. This is related to the animal's genotype and environmental conditions (age, species, race, care and feeding conditions, etc.). The fact that fattening is economical and qualified is a must for quality and economical meat production. This means too, that cattle of genotype known to produce better quality meat, By determining the genetic variations in the quantitative character locus (QTL) with technological and molecular genetic methods, today it is used in animal breeding studies for the production of quality beef. Gene markers are used to determine the genetic value of cattle and to increase genetic progression in populations by indirect selection. Marker Supported Selection (MAS) is performed by using QTL, which enable for the selection of cattle at a young age, regardless of gender and is not affected by environmental conditions. QTL analyzes are based on the link between some gene markers and QTL. The main purpose of the formed QTL maps is to identify the genes that cause variation in quantitative properties and mutations in these genes. Genetic studies have been performed on this topic in beef cattle, linear correlation relationship between single nucleotide polymorphisms (SNP) on meat quality has been revealed. Nowadays, Sequencing and PCR-RFLP method is most commonly used to detect mutations in marker genes.

Keywords: Meat quality, marker genes, QTL, MAS, SNP

INTRODUCTION

The importance of meat

Red meat protein is one of the main elements of a balanced diet in humans and an indispensable staple food that constitutes our best nutrient source in terms of biological value (Lawrie and Ledward, 2006). Animal-derived food products are important for nutrition and human health (Tosun and Hatırlı, 2006). High nutritional value is considerably important for consumers and red meat is known to be rich in proteins of high-quality essential amino group acids as well as fat, vitamins, minerals, iron, and zinc (Biesalski, 2005). Important essential amino acids such as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are not found in plant proteins, but only in animal proteins (Kutlu et al., 2005). Consumer demands constitute the most important characteristics that define the quality of meat, and the fact that beef may often fail to meet the quality consumers demand creates a

major problem. This issue is related to both the genotype of the animal and the environmental conditions. Features such as meat colour, friability, marbling score (MS) and pH are among the consumer demands that determine meat quality.

Meat colour is the first important quality factor that determines whether the consumer is likely to purchase the product, and also an important feature that affects consumer preferences (Font-i-Furnols and Guerrero, 2014; Suman et al., 2014). The colour of meat is an inherited attribute and represents a key factor that defines meat quality (Girolami et al., 2013). Fresh and ripe meat of high quality is expected to be velvety red. In production and processing of food products, pH-metry is a commonly utilised measuring method. Technological features such as product yield and durability of the product, water-holding capacity, colourisation, and stability of the product as well as ripening and softness of the meat are all closely related to the final value pH reaches in 24 hours. The changes in pH in the post-mortem phase significantly affect the quality and appearance of the meat. The pH value of meat should be ideally between 5.2 with 5.5. Any pH above 6.2 indicates the onset of bacterial spoilage in meat. Cooking loss and water-holding capacity of meat are also taken into account in food analyses when determining the quality of meat offered for consumption (Mullen, 2002; Kök and Atalay, 2018).

Meat Texture and Marbling (Mosaic Fat Content)

The flavour and juiciness of are attributes of meat that can be improved by various methods of processing and cooking. However, tenderness is an inherited attribute that is directly associated with the animal breed (Thu, 2006). For this reason, the tenderness of meat stands out as a substantially important feature. The texture of meat determines juiciness and softness, thereby determining acceptability by customers and increasing the value of the meat (Weber et al., 2020). Texture is defined as the degree of hardness or softness that remains in the mouth as a result of chewing meat. In other words, tenderness of meat is the texture of cooked meat. The Warner-Bratzler Shear Force (WBSF) device is a mechanical texture fragmentation knife that simulations the process of chewing and shredding meat in the mouth. The WBSF texture device can measure that cooked and uncooked beef *Musculus Longissimus dorsi* (MLD) as mechanical and predict value sensory panel tenderness. This also refers to the time it takes to swallow the meat after chewing and break-down in the mouth. Force (kg) × Time (sec) graph showing the WBSF peak and mean values of the three samples obtained from each MLD beef (Figure 1). Tenderness is therefore a factor to be evaluated for cooked meat as well. The hardness and softness (tenderness) of meat is also related to the mosaic fat content, i.e. the marbling score. As showed in figure 2, Marbling is defined as the fat deposition between MLD fibres with a mosaic-like pattern, and the distribution of fat between these muscle fibres affects the meat texture (Barendse, 1999; George-Evins et al., 2004).

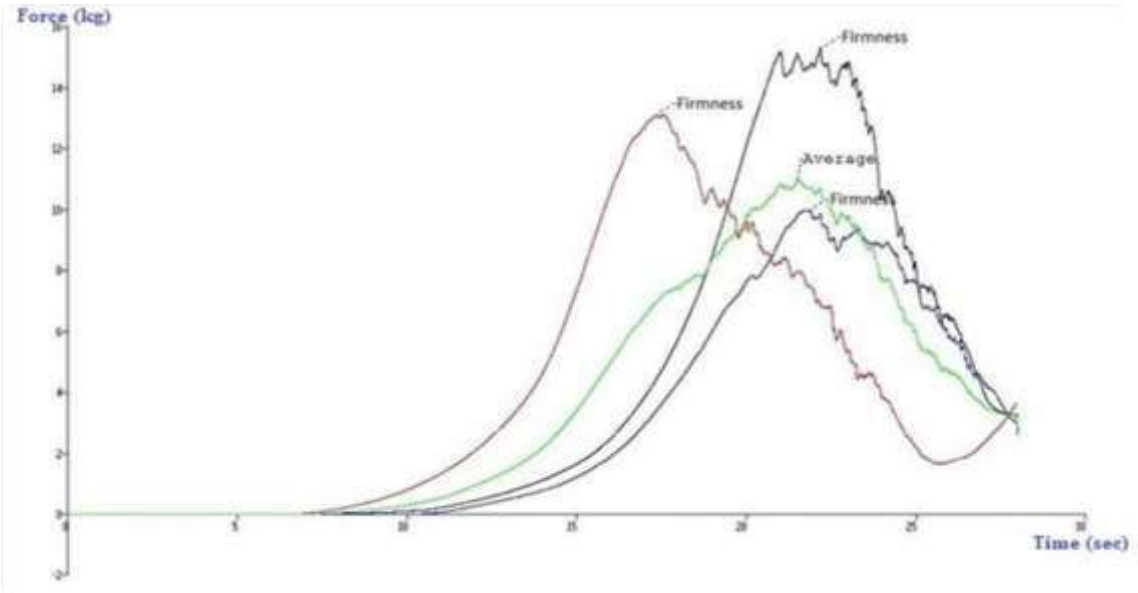

Figure 1. Force (kg) × Time (sec) graph showing the WBSF peak and mean values of the three samples obtained from each MLD beef, kg: Kilogram, sec: second (Vapur, 2019)

Measurement	Area	Unit
Total Area	142505,6819	px
Area	3012,620027	px
Area	5480,239694	px
Area	1955,281207	px
Area	2890,260631	px
Area	3896,021948	px
Area	969,2729767	px
Area	320,1646091	px
Area	488,340192	px
Area	341,2894376	px
Area	677,6406036	px
Area	307,2702332	px
Area	208,5048011	px
Area	436,7626886	px
Area	165,7064472	px
Area	225,5144033	px
Area	513,5734072	px
Total Fat	21888,46331	

Figure 2. Rib steak (MLD) cross-sectional area and rate of fat distribution, (Px): Pixel (Vapur, 2019)

Cattle are the main animal species that produce red meat worldwide. These consist of beef cattle and combined cattle breeds that have been specifically improved for meat production and quality, with ongoing efforts for further improvement. By using molecular genetic methods to detect genetic variations in quantitative trait loci (QTL) of cattle, the quality of meat may be determined at the calf stage, allowing cost-effective and high-quality meat yield in livestock production. In this respect, gene markers are used to determine the genetic value of cattle and to increase genetic progress in populations by indirect selection (Corva et al., 2007). The effect of marker-assisted selection (MAS), i.e. indirect selection using gene markers in cattle is a gender-independent process. Gene markers allow selection of young cattle and are not affected by environmental conditions (Andersson, 2001; Yamada et al., 2003; Andersson and Georges 2004). QTL analysis relies on the correlation between these markers and QTL (Knott and Haley 1992). The main aim of QTL mapping in reference cattle breeds is to identify the genes and

mutations that result in variation in quantitative traits (Gao et al., 2007; Hocquette et al., 2007; Allan and Smith, 2008). The yield characteristics of the same breed are determined by using QTL in different herds, leading to the appropriate use of these animals in terms of economics. Genetic studies have been conducted mostly in beef cattle and a linear correlation has been demonstrated between single nucleotide polymorphisms (SNPs) and the quality of meat (Zwierzchowski et al., 2001). A number of genes associated with meat quality have been identified and several candidate genes have been determined to be QTL with specific SNPs (Li et al., 2013). The functional candidate gene approach has been used to detect the polymorphisms associated with the quality and yield of meat (Georges, 2007; Pannier et al., 2010). DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) have been suggested to be the most reliable and convenient tools in detecting genetic polymorphisms (Ahani Azari et al., 2012; López-Rojas et al., 2017). To date, candidate gene studies have focused on a number of genes in order to reveal the molecular basis of carcass and meat quality, and various genes have been investigated across different studies. Some of these genes are described below.

The 200 candidate genes have used for carcass and meat quality in beef cattle

Some of the marker genes most studied in cattle; Leptin (LEP) (Lagonigro et al., 2003; De Oliveira et al., 2013; Vapur, 2019), Thyroglobulin (TG) (Casas et al., 2005; Fortes et al., 2009; Vapur, 2019), Calpastatin (CAST), Calpains (Calcium Active Neutral Protease, CAPN1, Regulation of Calpain Activation) (Curi et al., 2010; Giusti et al., 2013; Kök et al. 2019), Diacylglycerol O-Acyltransferase 1 (DGAT1) (Giusti et al., 2013), Stearyl CoA deraturase (SCD), Peroxisome Proliferator Active Receptor –γ (PPARγ), Nebulin (NEB), Phosphatidylinositol 4-kinase alfa (Pik-4), Calcium / Calmodulin Dependent Protein Kinase II (CaM-K II), fatty acid binding protein (FABP4), Titin (TTN) (Lee et al., 2008), Ribosomal protein L27a (RPL27A), Akirin 2 (AKIRIN2) (Watanabe et al., 2011), endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1) (Sukegawa et al., 2010), Myostatin (Double Muscle Gene -GDF8), (Kambadur et al., 1997), Growth Hormone Receptor Gene (GHR) (Waters et al., 2011).

The Leptin gene (LEP)

The LEP gene in cattle consists of 3 exons and 2 introns. Containing 16,824 nucleotides, this gene is located on chromosome 4 (4q32) (Perucatti et al., 2006) and encodes the LEP protein, which consists of 167 amino acids (a.a). LEP has been considered a potential candidate gene associated with body size, muscle fattening, carcass characteristics and meat quality based on QTL studies in cattle (Lagonigro et al., 2003; De Oliveira et al., 2013; Vapur, 2019). The first study on the LEP gene was conducted by Zhang et al. in 1994 and Stone et al. mapped the LEP gene in 1996. Pomp et al. (1997) detected the polymorphisms in the intronic region of this gene by PCR-RFLP using the *Sau3AI* restriction enzyme. Konfortov et al. (1999) sequenced both the intronic and exonic regions of the LEP gene. The discovery of the association between the LEP gene and meat quality has led to an increase in relevant research studies (Shin and Chung, 2007a; Corva et al., 2009).

Table 1. The relationship of the LEP gene with meat quality (intramuscular fat, back fat thickness, meat texture, marbling, meat color, pH, water holding capacity, etc.)

Location / Position	**SNP Name**	**SNP**	**Phenotypic trait**	**Literature**
2. exon 305 (877-1342)	E2FB (C1180 T)	C/T	Carcass fat, Feed intake, Drip Loss (DL), Water holding Capacity (WHC), Meat color (L*,a*, b*), pH, Marbling score, Back fat thickness, WBSF, Meat quality	Buchanan et al., 2002; Nkrumah et al., 2004; Pinto et al., 2011; Papaleo Mazzucco et al., 2016; Schenkel et al., 2005; Da Silva et al., 2012; De Oliveira et al., 2013; Souza et al., 2009; Konfortov et al., 1999 ;Vapur G., 2019; Shin and Chung, 2007a; Gill et al., 2009
2. exon 252 (1008-1201)	E2JW (A1127 T)	A/T	Marbling score, Back fat thickness, meat color (L*,a*, b*), pH, WBSF, Intramuscular fat, Carcass weight	Yazdani et al., 2010; Kulig and Kmie´c, 2009; Geary et al., 2003; Silva et al., 2014; Ardıçlı et al., 2017; Shin and Chung, 2007a
2.exon (118026)	SNP2	C/T	Carcass yield, Backfat thickness	Corva et al., 2009
3. exon (2961-3456)	C3100T	C/T	-	Shin and Chung, 2007
3. exon 140	-	C/T	Carcass fat Intramuscular fat	Lagonigro et al., 2003
3. exon	A59V	C/T	Meat color (L*,a*, b*), Intramuscular fat, Carcass weight, meat quality	Friedman and Halaas, 1998; Yazdani et al., 2010; Da Silva et al., 2012; Kulig and Kmie´c, 2009; Geary et al., 2003; Silva et al., 2014; Ardıçlı et al., 2017
3. exon	A80V	C/T		
Promoter Region 207	UASMS 1	C/T	Marbling, Carcass fat, meat quality, Backfat, Body Weight, Serum lep concentration	Gill et al., 2009; Nkrumah et al., 2005; Da Silva et al., 2012
Promoter Region 528	UASMS 2	C/T		
Promoter Region	C963T	C/T	Marbling, Back fat thickness	Da Silva et al., 2012
Microsatellite	BM150 0	-	Back fat thickness	Silva et al., 2014

As shown in Table 1, SNPs in exons 2 and 3, and the promoter region in the LEP gene have been associated with marbling and muscle fattening in different cattle breeds (Lagonigro et al., 2003; Gill et al., 2009; Da Silva et al., 2012; De Oliveira et al., 2013; Aslaminejad et al., 2010; Silva et al., 2014). Several investigators have investigated SNPs in the LEP gene in relation to their effect on meat quality, associated the SNPs in exon 2 (E2JW, E2FB) with marbling, and identified these SNPs as markers (Lagonigro et al., 2003; Schenkel et al., 2005; Da Silva et al., 2012; De Oliveira et al., 2013; Vapur, 2019). Furthermore, these SNPs have been linked to attributes such as back fat thickness, muscle fattening, milk yield, live weight (LW) and feed consumption in addition to marbling. (Buchanan et al., 2002; Nkrumah et al.,

2004; Shin and Chung, 2007a; Souza et al., 2009; De Oliveira et al., 2013). The A80V polymorphism in exon 3 of the LEP gene has been reported as a candidate marker for milk yield and composition, while its association with attributes of meat quality currently remain unclear (Liefers et al., 2003; Kulig, 2005; Kulig et al., 2010). The A80V polymorphism of the LEP gene has also been reported to be an effective marker for weight and average daily body weight gain, marbling, and carcass characteristics (Kulig and Kmie´c, 2009, Geary et al., 2003; Silva et al., 2014). Friedman and Halaas (1998) and Yazdani et al. (2010) have reported that this polymorphism, also known as A59V, is a candidate marker for carcass characteristics and meat attributes. Briefly, QTL studies have revealed that the LEP gene is a potential candidate gene for performance, meat quality, marbling, carcass characteristics and muscle fattening in cattle. It has therefore been recommended to be included in MAS programmes (Buchanan et al., 2002; Gill et al., 2009).

The Thyroglobulin gene (TG)

The TG gene is located on chromosome 14q with a length of 235,941 nucleotides and consists of 48 exons and 47 introns in cattle (Smith et al., 1997; Vapur, 2019). The TG gene encodes 2769 amino acids, which constitute the thyroglobulin protein. Thyroid hormones have long been known to contribute to fat deposition between myocytes. Upon evaluation as a potential candidate gene in QTL studies, TG gene polymorphisms have been considered a candidate gene marker owing to their effects on yield grade, weaning, meat colour, meat pH, marbling, average daily live weight (LW) gain, birth weight and weaning characteristics, carcass fat thickness and fat deposits. TG is one of the important genes that affect the lipid metabolism. (Thaller et al., 2003; Barendse et al., 2001; Kneeland et al., 2004; Maltecca et al., 2009; McClure et al., 2010). Table 2 shows the association between marbling and the SNPs in the TG gene of various cattle breeds and buffalo (Pannier, et al., 2010; Yardibi et al., 2013; Dubey et al., 2014; Dubey et al., 2015). The marker SNPs in TG have been confirmed to affect intramuscular fattening in different cattle breeds (Barendse, 2004; Bonilla et al., 2010; Savaşçı and Atasoy, 2016). Genetic variations in the 5′ promoter region of the TG gene are widely used in MAS programmes to increase the marbling score of beef. Hou et al. (2011a) identified 6 new SNPs in the 3′ flanking region of the TG gene. They associated MLD marbling with the SNPs T354C, G392A, A430G and T433G. Thaller et al. (2003) demonstrated the association between the TG5 (C422T) marker and MLD muscle fat content in German Holstein cattle. Gan et al. (2008) showed the effect on marbling caused by a SNP in the 3′ flanking region of the TG gene. However, Fortes et al. (2009) reported a lack of correlation between TG gene polymorphism and fat accumulation or tenderness of meat. Three positions of the TG gene, namely position 257 (C257T), position 335 (A335G) and position 422 (C422T) have been investigated in Korean cattle and a positive correlation with marbling has been reported for these polymorphisms (Shin and Chung, 2007b). Unlike Shin and Chung, some studies report that cattle with the TT genotype at position 422 of the 5′ promoter region have higher MS than cattle with (C422T) CT and CC genotype (Barendse, 1999; Barendse et al., 2001; Thaller et al., 2003; Burrell et al., 2004). Casas et al. (2007) reported that the TT genotype has the highest MS across three different hybrid populations. Barendse et al. (2004), Wood et al. (2006) and Bonilla et al. (2010) revealed an important correlation between the T allele and marbling. On the other hand, Johnston and Graser (2010), McClure et al. (2010), Pannier et al. (2010) found no evidence of a linear correlation between marbling and the T allele.

Table 2. The relationship of TG gene with meat quality (Body Weight, intramuscular fattening, back fat thickness, meat texture, marbling, meat color, pH, water holding capacity)

Location	**SNP Name**	**SNPs**	**Phenotypic traits**	**Literature**
5′ Promoter Region	C422T (C422T)	C/T	Feed intake, marbling, intramuscular fat, fat thickness,*LMA, meat yield, meat color (l*, a*), meat quality	Barendse, 1999-2001; Thaller et al., 2003; Barendse et al., 2004; Burrell et al., 2004; Shin and Chung, 2007b; Casas et al., 2005-2007; Ağaoğlu et al., 2015; Savaşçı and Atasoy, 2016; Ardıçlı et al., 2018
	C257T	C/T	Marbling	Shin and Chung, 2007b
	A335G	C/T	Meat color (a*, b*), LMA, Marbling	Ribeca et al., 2014; Shin and Chung, 2007b
3′ Flanking Region	G133C G156A C220T A506C	G/C G/A C/T A/C	Marbling	Gan et al., 2008
	T354C G392A, A430G T433G	C/T G/C A/G T/G		Hou et al., 2011a;
5' Flanking Region	T1355C G1356A	T/C G/A	Meat percentage, body weight, LMA*	Zhang et al., 2015a
	G275A G277C G280A C281G	G/A G/C G/A C/G	Average daily earnings, carcass composition, meat quality traits, growth, intramuscular fat	Zhang et al., 2015b

*LMA; longissimus muscle area

The attempts to associate TG5 (C422T) markers with marbling or other traits of fat accumulation have led to the inclusion of TG5 in commercial SNP panels (Barendse, 1999; Thaller et al., 2003; Barendse et al., 2004). GeneSTAR is a DNA test to assess the quality and production characteristics in all cattle breeds. In order to improve the estimability of the quality and marbling level of beef, the SNP at position 422 of the 5′ promoter region (C422T) has been approved and launched by the company, GeneSTAR, as a TG5 marker in 2000. GeneSTAR marbling is a DNA-based diagnostic test for the TG gene and an available commercial marker for carcass quality attributes that is recommended for use in MAS programmes (Van Eenennaam et al., 2007; Pfizer, 2013). These DNA tests are used in USA, Japan, Canada, Argentina, and Australia (Shin and Chung, 2007b).

The Calpain gene (CAPN)

The decrease in the pH of meat upon slaughter activates the Calpains family. Calpains, which exert a calcium-dependent activity as natural proteinases, denature myofibrillar proteins, thereby increasing the texture of the meat. The CAPN gene is located on chromosome 29 in cattle (Smith et al., 2000). Among the Calpains, Calpain 1 and 2 have been shown to affect the tenderness of meat (Barendse, 2002; Kök et al., 2019; Page et al., 2002; White et al., 2005) while the exact effects of other Calpains (CAPN 5,7,10,12,14,15) currently remain unelucidated (Morris et al., 2006). The effect of relevant Calpains on palatability, juiciness, texture and meat

quality parameters of beef have been confirmed as direct effects on the phenotype by WBSF tests applied to MLD and with the genotype results obtained in polymorphism and SNP studies in CAPN1 loci (Curi et al., 2009; Van Eenennaam et al., 2007; Kök and Atalay, 2018). Studies have so far focused on three of the CAPN1 markers shown in Table 3 (CAPN 316, CAPN 530 and CAPN1 4751), which are known to be associated with the texture of meat (Barendse, 2002; Kök et al., 2017; Page et al., 2002; White et al., 2005). Page et al. (2002) have associated the μ-calpain (CAPN1) gene polymorphisms, CAPN1 316 and CAPN1 530, with the texture of meat. CAPN1 (316) is located in exon 9. The CAPN 316 C allele frequency has been shown to have a positive correlation with meat texture in *B. taurus* and *B. indicus* cattle (Curi et al., 2010; Gill et al., 2009; Kök et al., 2019; Page et al., 2002; Van Eenennaam et al., 2007; White et al., 2005). Page et al. (2004), Allais et al. (2011), Gill et al. (2009), Kök and Atalay (2018), and Costello et al.(2007) have shown increased tenderness in beef from cattle bearing the CC and CG genotype with the C allele compared to those of the GG genotype. CAPN1 (4751) is a polymorphism located on intron 17. Curi et al., (2009) have reported that the C allele of the CAPN1 4751 polymorphism is a favourable factor for the texture of meat (Kök and Atalay, 2018; Kök et al., 2017, White et al., 2005; Smith et al., 2009; Van Eenennaam et al., 2007; Curi et al., 2009). Overall, study results have shown that the cattle with the CAPN1 4751-CC genotype produce meat with increased tenderness, i.e. improved texture compared to those of the other genotypes (CC and CT) (Curi et al., 2009; Kök and Atalay, 2018; Smith et al., 2009; White et al., 2005). CAPN 530 is an amino acid polymorphism that occurs in exon 14 (Page et al., 2002,2004; White et al., 2005). For the CAPN 530 marker, the G allele is the allele that shows a positive correlation with tenderness (Allais et al., 2011; Corva et al., 2007; Costello et al., 2007; Page et al., 2004). Results of the studies in this field (Corva et al., 2007; Page et al., 2002, 2004), support the notion that beef with the CAPN 530 GG genotype is more friable than beef of other genotypes (AG and AA) based on the WBSF value. Investigators have also investigated the SNP, CAPN II/*HhaI* in Irish cattle breed (Costello et al., 2007) and the SNPs, CAPN1 5331 and 4753 in the Brahman breed (Casas et al., 2006; White et al., 2005); however, without concluding any positive correlation between the texture of meat and these SNPs. During the analysis of the CAPN1 polymorphism in Chinese Yanbian Yellow cattle, Xin et al. (2011) identified an important correlation between variants of this gene (E7-1, E7-2, E14-4, E14-5, E14-6) and pH, colour scores and other quality attributes such as fatty acids and amino acid content of meat. Hou et al. (2011b) evaluated the 3553A>G polymorphism and demonstrated higher marbling scores in those with the AA genotype compared to the other genotypes (AG and GG). Li et al. (2013) reported greater MS in animals of the CC genotype with the c.947G>C polymorphism than the scores in those bearing the CG or GG genotype among Angus, Charolais, Hereford and Limousin breeds. Cheong et al. (2008) determined that the T allele with the c.2151*479C>T polymorphism in the 3′ UTR region of cattle is responsible for low marbling scores in Korean Hanwoo cattle. Intramuscular fat content has been shown to be the lowest in cattle of the TT genotype and the highest in those of the CC genotype.

The Calpastatin gene (CAST)

The calpastatin gene is located on chromosome 7 in cattle (Bishop et al., 1993). Studies involving the CAST gene has revealed QTL, requiring this gene to be evaluated as a candidate gene in MAS since the SNPs, CAST-T1, and U*oG*-CAST are associated with the quality of meat (Van Eenennaam et al.,2007; Curi et al.,2010; Kök and Atalay 2018). CAST shows its effect on meat texture in an indirect manner, i.e. through CAPN (Barendse et al., 2007). The CAST gene also affects the inter-muscular fattening of the carcass (Juszczuk-Kubiak et al., 2008; Xiaomei et al., 2018). Juszczuk- Kubiak et al. (2008) identified 4 SNPs (using *AluI*, *BseYI* and *NdeI* enzymes) within intron 12 of the CAST locus in cattle. Investigating the CAST gene in *Bos taurus* breed, Allais et al., (2011) identified a novel SNP in intron 8, and two novel SNPs

in exon 30 and in the 3′ UTR region. In *Bos indicus* cattle, an A/T SNP in the 3′ UTR region, referred to as CAST-Brahman, has been identified and associated with the texture of meat (Frylinck et al., 2009) as well as a C/T SNP identified in hybrids (*Bos taurus* × *Bos indicus)* (Casas et al., 2006; Frylinck et al., 2009). SNPs have been determined by means of PCR-RFLP (*EcoRI* and *BamHI*) in the 3′ UTR region of the CAST gene in cattle and these polymorphisms have been recommended for use as gene markers to predict the texture of meat (Lonergan et al., 1995). In the CAST gene of *Bos taurus* and *Bos indicus* breeds, a positive correlation with the texture of meat has been mostly shown for two marker SNPs, namely UoG-CAST (Curi et al., 2010; Gill et al., 2009; Kök and Atalay 2018; Quaas et al., 2006; Reardon et al., 2010; Schenkel et al., 2006) and CAST-T1 (Allais et al., 2011; Corva et al., 2007; Curi et al., 2010; Smith et al., 2009; Van Eenennaam et al., 2007) (Table 3). The G/C base substitution in intron 5 of the CAST gene in cattle has been identified as the UoG-CAST polymorphism (Schenkel et al., 2006). The same polymorphism is also known as CAST/*RsaI* (Kök et al., 2013; Reardon et al., 2010). In the SNP, UoG-CAST, the C allele is responsible for the favourable effect on meat texture (Curi et al., 2010; Kök and Atalay, 2018; Schenkel et al., 2006). The UoG-CAST (CAST/*RsaI)* polymorphism has been reported in cattle of the CC genotype that produce meat with increased friability compared to those with the CG or GG genotype (Curi et al., 2010; Gill et al., 2009; Kök and Atalay, 2018; Schenkel et al., 2006; Van Eenennaam et al., 2007). The CAST-T1 polymorphism of the CAST gene in cattle has been found to have two different genetic variants (Allais et al., 2011; Corva et al., 2007; Curi et al., 2010; Morris et al., 2006; Smith et al., 2009; Van Eenennaam et al., 2007). One of these variants is located in exon 30 (2959A>G) while the other is the polymorphism (C/T) in the 3′ UTR region of the gene. The nucleotide polymorphism in the 3′ UTR region of the CAST gene in *Bos indicus* cattle, referred to as "CAST-Brahman SNP", has been associated with increased friability in beef from animals of the AA genotype compared to beef from animals bearing other genotypes (AT, TT) (Frylinck et al., 2009). In studies on other SNPs in the CAST gene, no positive correlation was observed between the texture of meat and the M/N polymorphisms in exon L region and intron 1 of the CAST gene (L14450-622) in Holstein cattle and buffalo (with frequency of the M allele 73% in Holstein and 22.5% in buffalo) (Yousefi and Azari 2012), the A/B polymorphism in intron 6 of the CAST gene in Nellore cattle breed (frequency of the B allele, 58%) (Alireza et al., 2009), the SNP2870 (AF159246) A/G polymorphism in the CAST gene in *B. taurus* cattle (frequency of the A allele, 33-53%) (Corva et al., 2007) and the Calpain II polymorphisms in Irish beef cattle (Costello et al., 2007) ($p>0.05$).

Table 3. The relationship of CAPN1 and CAST gene with meat quality (intramuscular fattening, meat texture, marbling, meat color, pH, water holding capacity, etc.)

Gene Name	SNP Name	Location	SNP	Phenotypic traits	Literature
Calpain-CAPN1	CAPN1 316	9. exon	G/C	Texture	Kök et al., 2017; Allais et al., 2011; Curi et al., 2010; Gill et al., 2009; Corva et al., 2007 Costello et al., 2007; Page et al., 2004
	CAPN1 4751	17. intron	C/T		Kök et al., 2017; Curi et al., 2009; Gill et al., 2009; Smith et al., 2009; Casas et al., 2006; White et al., 2005
	947G>C	1. intron	G/C	Marbling	Li et al., 2013
	SNP15	4.exon	C/T	Lean meat color score	Liu et al., 2015
	3553A>G	4. intron	A/G	Marbling	Hou et al.,2011b
	E7-1 E7-2 E14-4 E14-5 E14-6	7. exon 7. İntron 8. İntron 16. intron 17. Exon 18.exon	27 pcs SNPs	pH, meat color	Xin et al., 2011
	c.2151*479	3′ UTR	C/T	Marbling, carcass quality	Cheong et al., 2008
Calpastatin-CAST	*Uo*G-CAST	5. intron	G/C	Texture Tenderness	Kök et al., 2013; Costello et al., 2007; Gill et al., 2009; Reardon et al., 2010; Schenkel et al., 2006; Van Eenennaam et al., 2007
	CAST-T1	30. exon 3′ UTR	A/G C/T		Corva et al., 2007 Van Eenennaam et al., 2007
	c.155C>T	1. exon	C/T		Barendse et al.,2007; Kaplanová et al. 2013; Ekerljung, 2012
	61G>C		G/C	Tenderness, cooking lose	Juszczuk-Kubiak et al. 2004

CONCLUSION

In conclusion, genetic marker technology appears to be promising for the breeding of livestock. The SNPs in LEP, TG, CAST and CAPN1 gene loci are known to be associated with the quality of meat (texture, pH, MS, meat colour) and are therefore considered potential candidate genes. The gene markers, LEP (E2JW, E2FB), TG5 (C422T), CAST (UoG-CAST, CAST-T1) and CAPN1 (CAPN1 316, CAPN1 530 and CAPN1 4751), have been established to be among the markers with identified genetic potential for the production of meat with

improved quality. In the event that the LEP, TG, CAST and CAPN1 genes of a given cattle bear the favourable markers associated with improved meat quality, the genotype of these animals should be determined based on QTL by means of the MAS method without the impact of environmental factors at young age so that they may be separated from the cattle of other genotypes in livestock and allocated as breeding cattle or beef cattle for the production of high-quality meat.

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THE DETERMINATION OF YIELD PERFORMANCES OF SOME FORAGE PEA VARIETIES IN TRAKYA REGION

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ABSTRACT

Forage pea (*Pisum sativum ssp. arvense* L.) grows drylands and winter conditions in Turkey and other parts of the world. Therefore, both dry and fresh hay yield is higher and preferred by producers largely for animal feeding. Furthermore, its nutritional value such as fiber value, protein content. etc. is higher and present delicious feed both fresh hay and dry as well as grain for animals. Forage pea production has increased recently in Turkey in different regions especially after government subsidies started on forage pea. The study was conducted to determine yield performance of some candidate forage pea varieties developed by Tragen R&D Co in Edirne and Tekirdag locations in Trakya region in 2017-2018 growing season. Based on study results, some candidate varieties exhibited higher performances both fresh and hay yield than controls. Among candidate forage pea varieties, TG-601 candidate forage pea variety showed promising performance in both locations. Therefore, it sent to registration trials and obtained production permission in 2019 Fall.

Key words: Forage pea, Yield traits, Fresh and dry hay yield, Trakya region, Animal feed

INTRODUCTION

Forage pea (*Pisum sativum ssp. arvense* L.) is a multi-purpose plant used as herbage, dry hay and silage as well as manure crop and is quite available for production mostly in temperate zones. Since it has quite short growth period, it is commonly preferred in crop rotations and could be beneficial in both animal feeding and development of crop rotation systems. Forage pea grows generally by farmers mixed with wheat, barley, oat, triticale to reduce lodging in Turkey (Tan et al., 2013; Özdemir and Tamkoç, 2019; Sürmen et al., 2019).

In coastal regions, forage pea could be intercropped with cereals in autumn, winter and early spring months and could be used as a short-term pasture with heavy grazing activities in the world. Forage pea grows drylands and winter conditions in Turkey, because it has tolerant to cold and has winter hardiness then could grow winter season in Turkey and other countries too (Özköse, 2017; Kadıoglu and Tan, 2018; Cacan et al., 2019).

Forage pea herbage is quite edible and nutritious for almost all animals. Its grass is nutritious and delicious for almost all kinds of livestock. Furthermore, its seeds are a valuable feed as a source of energy and protein for both ruminants and poultry as well as a good pre-plant for grains widely for collecting the atmosphere nitrogen to the soil (Tan et al., 2012; Açıkgöz, 2013; Kosev and Vassileva, 2019). Therefore, both dry and fresh hay yield is higher and preferred by producers largely for animal feeding. Furthermore, its nutritional value such as fiber value, protein content. etc. is higher and present delicious feed both fresh hay and dry as well as grain for animals. If the hay harvested at proper period, it could have around 20%

crude protein content. Herbage yield per decare under dry conditions is around 1 ton and hay yield is around 250-300 kg/da. Herbage yield could reach to 2-4 ton da-1 under irrigated conditions or in coastal zones (Acikgoz et al, 2007; Bilgili et al., 2010; Açıkgöz, 2013; Tan et al., 2013).

Forage pea studies conducted in Turkey indicated that it is adapted in almost all regions of Turkey (Tekeli and Ateş, 2003; Tamkoç, 2007; Sayar, 2007; Çil, 2007; Uzun et al., 2005 and 2012; Geren and Alan, 2012; Kavut et al., 2016; Tan et al., 2012 and 2013; Karaköy et al., 2016; Kadıoğlu and Tan 2018). Forage pea is one of the most winter planted forage legumes in Turkey. Turkey is huge market for forage crops because animal husbandry is well developed and there is big demand for dairy and meat production. Therefore, government gives big subsidies to increase forage productions. Forage pea production has increased recently in Turkey in different regions especially after government subsidies started on forage pea. Based on the statistical data, planting areas have increased five times more in the last five years (2014 to 2019) reached about 140.000 da (Ozkan, 2020)

Forage pea planting area exist mostly in Marmara region (%60) and Middle Anatolia (%17) and Blacksea region (%13) follow it in Turkey (Okcu, 2020). There are some registered cultivars belong to government institutes and also foreign varieties recently in the market. National private companies do not have breeding program only government institutes are working as regional basis. TRAGEN R&D Co is located in Trakya University Technopark Edirne and has breeding materials on forage pea at different breeding levels and sent registration one variety in 2019-20 growing season. TRAGEN has some finished lines and also send registration in next growing seasons. The study was conducted to determine yield performance of some candidate forage pea varieties developed by Tragen and Tekirdag locations in Trakya region in 2017-2018 growing season.

MATERIALS AND METHODS

The yield trials were conducted in Corlu and Hayrabolu in Tekirdag province and Edirne and location in 2017-2018 growing season to determine yield performances of some candidate forage pea lines. There were 7 cultivars in the trials including three controls from commercial forage pea cultivars (Taşkent, Töre and Gap Pembesi) in the market. The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,50-m long and total plot area at planting was 9,00 m^2 and 4,5 m^2 at harvest. The middle two rows were harvested and the border rows were discarded, and plot size was 9.52 m^2 at harvest. The compose fertilizers (20-20-0, Zn) were applied 200 kg/ha dose at planting. Statistical analysis was performed with JMP statistical program.

In Corlu location the trials were planted by hand in 24 November 2017. Emergence date of sunflower plants was after one week from planting. The trials were harvested by hand in 16 May 2018 for fresh hay and after maturity for seeds. Edirne location was conducted in Edirne suburban area fields and the trials were planted by hand in 20 November 2017 and the trials were harvested by hand in 14 May 2018. Hayrabolu location was planted by hand in 16 November 2017 and the trials were harvested by hand in 16 May 2018.

Seed yield, fresh and dry hay yield, 1000 seed weight, flowering and physiological maturity period, plant height, branching, lodging, winter hardiness, pod number per plant and grain number per pod, humidity, protein and oil content were measured in the study. Winter hardiness and lodging observations were performed in the trials and lines were evaluated for lodging as the five is the best and the one is the lowest.

RESULTS AND DISCUSSION

The study was conducted to determine yield performance of some candidate forage pea lines developed by Tragen R&D Co located in Trakya University Technopark in Edirne and Tekirdag locations in Trakya region in 2017-2018 growing season. Based on study results, seed yield of the forage pea lines exhibited larger variation and some candidate forage pea lines exhibited higher performances than control forage pea cultivars in the trials (Table 1). The highest seed yield from TG 601 line among the forage lines. Edirne location had higher seed yields than both Corlu and Hayrabolu locations because soil quality and humidity was higher in Edirne location. Seed yield was changed between 63 and 178 kg/da in Corlu location, 70 and 218 kg/da in Hayrabolu location and 264-401 kg/da Edirne location and 851 and 1516 kg/da in average values in all locations. The highest yield was obtained from TG 601 candidate forage pea and followed by TGFP MR line the lowest seed yield was obtained from Taşkent control cultivar in all locations Based on the location and average values, almost all candidate forage pea lines exhibited higher performance in the seed yields than the control average in the study (Table 1).

Fresh hay yield was changed between 908 and 1433 kg/da in Corlu location, 795 and 1636 kg/da in Hayrabolu location and 851-1479 kg/da Edirne location and 143 and 201 kg/da in average values in all locations. The highest fresh hay yield was obtained from TG 601 candidate forage pea and followed by TGFP 6 line and the lowest fresh hay yield was obtained from TGFP MR line in all locations (Table 2).

In the dry hay yields of the cultivars, the values were changed between 472 and 711 kg/da in Corlu location, 473 and 766 kg/da in Hayrabolu location and 474-711 kg/da Edirne location and 473 and 729 kg/da in average values in all locations. The highest dry hay yield was obtained from Gap Pembesi control forage pea cultivar and followed by TG 601 line and the lowest dry hay yield was obtained from TGFP MR line in all locations (Table 3).

Table 1. The locations and the average seed yield data of forage pea lines

#	Cultivars	Corlu Seed Yield (Kg/da)	Hayrabolu Seed Yield (Kg/da)	Edirne Seed Yield (Kg/da)	Average Seed Yield (Kg/da)
1	Gap Pembesi (C)	199 A	194 B	300 B	231A
2	Taşkent (C)	63 D	70 E	296 B	143 C
3	Töre (C)	94 C	101 D	285 B	160 BC
4	TGFP 6	64 D	94 D	401A	186 B
5	TG 601	178 B	218 A	359 A	251 A
6	TGFP 13	84 C	92 D	264 B	147 C
7	TGFP MR	169 B	169 C	385 A	241 A
Average		122,0	134,4	327,4	194,6
LSD: (0.05) (kg/da)		6,47	7,42	23,76	15,42
CV (%)		7,47	7,56	10,26	19
F		**	**	***	**

In the dry hay yields of the cultivars, the values were changed between 472 and 711 kg/da in Corlu location, 473 and 766 kg/da in Hayrabolu location and 474-711 kg/da Edirne location and 473 and 729 kg/da in average values in all locations. The highest dry hay yield was obtained from Gap Pembesi control forage pea cultivar and followed by TG 601 line and the lowest dry

hay yield was obtained from TGFP MR line in all locations (Table 3). There were not much differences in the quality results of the forage pea lines (Table 4). The highest quality values was obtained from TG 601 line in the study.

Table 2. The locations and the average fresh hay yield data of forage pea lines

#	Cultivars	Corlu Fresh hay yield (Kg/da)	Hayrabolu Fresh hay yield (Kg/da)	Edirne Fresh hay yield (Kg/da)	Average Fresh hay yield (Kg/da)
1	Gap Pembesi (C)	1241 BC	1304 B	1328 B	1291 BC
2	Taşkent (C)	1108 CD	1296 B	1202 BC	1202 CD
3	Töre (C)	1094 CD	1309 B	1146 CD	1183 DE
4	TGFP 6	1333 AB	1269 B	1329 B	1310 B
5	TG 601	1433 A	1636 A	1479 A	1516 A
6	TGFP 13	1061 D	1170 B	1060 D	1097 E
7	TGFP MR	908 E	795 C	851 E	851 F
Average		1168,3	1254,1	1199,3	1207,1
LSD: (0.05) (kg/da)		71.1	103,81	65,16	48,21
D.K (C.V) (%)		8,6	11,6	7,6	16
F		**	**	**	**

Table 3. The locations and the average dry hay yield data of forage pea lines

#	Cultivars	Corlu Fresh hay yield (Kg/da)	Hayrabolu Fresh hay yield (Kg/da)	Edirne Fresh hay yield (Kg/da)	Average Fresh hay yield (Kg/da)
1	Gap Pembesi (C)	711 A	766 A	711 A	729 A
2	Taşkent (C)	583 BC	644 AB	613 ABC	613 B
3	Töre (C)	427 D	600 AB	513 BC	513 C
4	TGFP 6	591 B	712 BC	651 AB	651 AB
5	TG 601	569 BC	741 C	655 AB	655 AB
6	TGFP 13	500 CD	573 CD	481 C	518 C
7	TGFP MR	472 D	473 D	474 C	473 C
Average		644,0	585,1	593,0	644,0
LSD: (0.05) (kg/da)		40,96	96,94	70,86	39,78
D.K (C.V) (%)		10,5	21	17	16
F		**	**	**	**

Table 4. The quality results of forage pea lines

Cultivar	Humidity (%)	Hectoliter weight (g)	Protein Content (%)	Oil Content (%)
Gap Pembesi	14,1	78,9	23,9	0,7
Taşkent	14,4	81,3	22,4	0,2
Töre	14,9	78,4	24,5	0,1
TGFP 6	14,6	81,0	24,0	0,1
TG 601	14,4	81,5	24,6	0,6
TGFP 13	14,6	81,0	24,0	0,1
TGFP MR	14,2	79,9	24,5	0,7

The flower color was mostly purple except TGFP MR being white. Winter hardiness was mostly higher in almost all cultivars in the study (Table 5, 6 and 7). Flowering days are changed between 151 and 170 days comparing all locations (Table 8, 9 and 10). In the physiological maturity days, the forage pea lines exhibited the values as between 171 and 190 days. In the thousand seed weight, the forage pea lines observed large ranges and the values were changed between 86,7 g and 253 g. the highest value were obtained from Gap Pembesi control lines and the lowest value observed in the Taşkent control forage pea cultivar in the study (Table 8, 9 and 10).

Based on the study results, TG 400 candidate hybrid showed promising performance in almost all locations and then it selected to send registration trials after preparing all necessary documents in 2020. TG 601 forage pea candidate line has purple flower, higher leaf rate and number, 110-120 cm plant height. Its herbage and hay yield is higher and exhibited bigger seed size tolerant to lodging and some diseases as well as winter hardiness in the study. Furthermore, TG 601 line had higher quality traits such as hectoliter, protein rate, etc., and it could be suitable for both herbage and hay yield. TG 601 had higher herbage yield in yield trials in Trakya region then it could be suggested for planting large areas in the region which has the most planted areas in Turkey (Picture 1).

Picture 1: TG 601 forage pea line

Table 5. The yield traits of forage pea cultivars in Corlu location

Cultivars	Flower color	Branch number	Lodging (1-5)	Winter hardiness (%)	Fresh hay yield (kg/da)	Dry hay yield (kg/da)	Seed Yield (kg/da)
TAŞKENT (C)	Purple	11,75	3	90	1108,34	583,34	63,72
GAP PEMBESI (C)	Purple	9,50	3	90	1241,67	711,11	199,72
TÖRE (C)	Purple	12,75	3	90	1094,44	427,78	94,33
TGFP-MR	White	9,75	2	90	908,34	472,22	169,17
TGFP-13	Purple	14,75	3	90	1061,11	500,00	84,84
TGFP -6	Purple	14,50	2	90	1333,33	591,67	62,89
TG 601	Purple	12,00	3	90	1433,34	569,45	178,00

Table 6. The yield traits of forage pea cultivars in Hayrabolu location

Cultivars	Flower color	Branch number	Lodging (1-5)	Winter hardiness (%)	Fresh hay yield (kg/da)	Dry hay yield (kg/da)	Seed Yield (kg/da)
TAŞKENT (C)	Purple	11,75	3	90	1296,11	644,45	70,06
GAP PEMBESI (C)	Purple	9,50	3	90	1304,45	766,67	194,33
TÖRE (C)	Purple	12,75	3	90	1309,44	600,00	101,78
TGFP-MR	White	9,75	2	90	795,00	472,22	169,45
TGFP-13	Purple	14,75	3	90	1169,95	573,33	92,22
TGFP -6	Purple	14,50	2	90	1269,44	712,22	94,00
TG 601	Purple	12,00	3	90	1636,11	741,11	218,89

Table 7. The yield traits of sunflower forage pea lines in Edirne location

Cultivars	Flower color	Branch number	Lodging (1-5)	Winter hardiness (%)	Fresh hay yield (kg/da)	Dry hay yield (kg/da)	Seed Yield (kg/da)
TAŞKENT (C)	Purple	11,75	3	90	1202,22	613,89	296,00
GAP PEMBESI (C)	Purple	9,50	3	90	1328,61	711,11	300,00
TÖRE (C)	Purple	12,75	3	90	1146,39	513,89	285,33
TGFP-MR	White	9,75	2	90	851,67	472,22	385,67
TGFP-13	Purple	14,75	3	90	1060,00	481,11	264,00
TGFP -6	Purple	14,50	2	90	1329,17	651,95	393,33
TG 601	Purple	12,00	3	90	1479,17	655,28	359,00

Table 8. The yield traits of forage pea cultivars in Corlu location

Cultivars	Flowering (day)	Physiological Maturity (day)	Plant Height (cm)	Thousand Seed Weight (g)	Pod number per plant	Grain number per pod	Lodging (1-5)	
							At flowering	At maturity
TAŞKENT (C)	159	179	99	86,70	8	6	3	4
GAP PEMBESI (C)	151	171	104	253,03	7	5	3	4
TÖRE (C)	164	184	102	125,60	12	6	3	4
TGFP-MR	152	172	48	192,28	11	6	2	3
TGFP-13	163	183	120	164,60	8	6	3	4
TGFP -6	160	180	106	169,70	12	8	2	3
TG 601	159	179	103	196,90	11	6	3	4

Table 9. The yield traits of forage pea cultivars in Hayrabolu location

Cultivars	Flowering (day)	Physiological Maturity (day)	Plant Height (cm)	Thousand Seed Weight (g)	Pod number per plant	Grain number per pod	Lodging (1-5)	
							At flowering	At maturity
TAŞKENT (C)	162	182	99	86,70	7	6	3	4
GAP PEMBESI (C)	154	174	104	253,03	7	5	3	4
TÖRE (C)	166	186	107	125,60	12	6	3	4
TGFP-MR	153	173	44	192,28	11	6	2	3
TGFP-13	170	190	95	164,60	8	6	3	4
TGFP -6	167	187	66	169,08	13	6	2	3
TG 601	164	184	103	196,90	10	6	3	4

Table 10. The yield traits of forage pea cultivars in Edirne location

Cultivars	Flowering (day)	Physiological Maturity (day)	Plant Height (cm)	Thousand Seed Weight (g)	Pod number per plant	Grain number per pod	Lodging (1-5)	
							At flowering	At maturity
TAŞKENT (C)	155	178	130	126,25	13	5	3	4
GAP PEMBESI (C)	151	171	104	253,25	7	5	3	4
TÖRE (C)	161	183	121	102,50	9	5	2	3
TGFP-MR	151	171	67	188,00	10	5	3	4
TGFP-13	160	172	134	119,25	11	6	2	3
TGFP -6	160	178	85	160,00	7	6	3	4
TG 601	159	179	128	147,50	10	6	2	3

CONCLUSION

Based on study results, there are some promising forage pea lines and exhibited higher performances than control ones not only fresh and dry hay yield but also for seed yield. TG 601 candidate forage pea line had the highest performances in the trials then sent to registration and had production permission during the registration process in 2020. As results, TRAGEN Ltd Co has some forage pea lines ready for registration both for high fresh and dry hay yielding & also quality and seed yield as well as taste of fresh plants. Other candidate forage pea lines will be tested in the next year again. If they still show the higher performances in the trials, they will be evaluated again and the best ones will be registration trials in the next year.

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SEED QUALITY RELATIONSHIPS IN CONFECTIONERY PUMKIN

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ABSTRACT

Confectionary pumpkin (*Cucurbita pepo* l.) is one of the most consumed confectionery product in Turkey. There are few hybrids in confectionery pumpkin production in Turkey and seed quality is the most important problem in Turkish confectionery market as well as in production. Hybrids are preferred both by industry for homogenous seeds and also by producers for uniform plants and having higher yields and quality. The study was conducted to determine of seed quality characteristics of inbred lines and their F_1 hybrids with other yield traits. The relationships of thousand seed weight, seed length and width as seed quality traits with leaf length and width, vine length, fruit diameter, length and width, flesh thickness, number of seeds and seed weight per fruit were investigated based on correlation and regression analysis among these traits in the study. The correlation analysis indicated that fruit width is the most important character for seed quality having higher positive relationships with these three seed quality traits as well as with seed yield in both inbred lines and hybrid. Fruit diameter and seed number per fruit seems other essential characters for seed quality in confectionery pumpkin other than this trait following it respectively. In conducted regression studies, linear relationships in positive way were observed especially between fruit width and these three seed quality traits.

Key words: Sunflower, Oil content, Drought, Drought tolerance, Inbred lines, Yield traits

INTRODUCTION

Confectionery pumpkin is one of the most preferred and consumed snacks in Turkey. Turkey exists in top ten pumpkin producers' countries (8^{th}) in the world with 2.23% rate of world production (total production is 27.6 million tons and planting area is about 3 million ha) (Seymen, 2020). China (8 million tons), India (5.5 million tons), Ukraine (1.3 million tons) and Russia (1.1 million tons) are major producer countries in the world (FAO, 2018). Turkey confectionary pumpkin production is 50.265 tons at 706.894 da area and these areas are mostly located in the Middle Anatolia (Kayseri (16 706 tons), Nevşehir (16 673 tons), Aksaray (4 849 tons), Konya (4 468 tons) and Eskişehir (2 598 tons)) based on TUİK, 2019 data (Seymen, 2020).

Pumpkin seeds has 28-40% protein as 35-50% oil content as well as having important minerals in human nutrition such as potassium, phosphorus, calcium, magnesium and iron and A, C and E vitamins (Muralidhara et al., 2014; Türkmen et al., 2014 and 2016; Seymen et al., 2012 and 2019). Therefore, confectionery pumpkin has some medical uses in terms of human health in addition to consuming as snack. Some researchers reported that it improves the immune system, reduces the risk of stomach, breast, lung and colon cancer. Furthermore, in some studies, it was mentioned that plays an important role in lowering cholesterol levels and treating advanced prostate utilizing phytosterols (Sultana et al., 2015; Ahmed et al., 2016; Pessarakli, 2016; Erdinç et al., 2018).

The main confectionery pumpkin producer country is China, and the Chinese market is about 20 times bigger of Turkey. While mostly hulless type was consuming in the world, normal confectionery shelled types are common in our country calling as Urgup sivrisi, kemerli, Nevsehir hanim tirnagi (lady nail), etc. However, all these types are open pollinated ones and there is no hybrid commonly in the production. Furthermore, there is no certified seed use in the market. The seed certificate requirement of Turkey is approximately 300 tons under normal conditions. On the other hand, while hybrid varieties are used extensively in the USA, hybrid seeds have just started to enter the Chinese market which is the largest in the world. Therefore, hybrid pumpkin should enter Turkish market soon both to increase production and also to supply high quality seeds for industry and customers. Since, the confectionery industry is highly developed and has very modern facilities in Turkey compared to other producer countries. Furthermore, confectionery pumpkin is a product that has export potential in addition to its intense domestic consumption in our country, but since our country production is not sufficient, some companies also rent lands in neighboring countries such as Ukraine, Russia, Bulgaria, etc. (Türkmen et al., 2014 and 2016; Seymen et al., 2012 and 2019; Kaya and Beşer, 2018).

The production confectionary pumpkin in Turkey is increasing year by year with good prices because the use of mechanization increase in large areas, the yield also increases in both less irrigated semi-arid regions and also irrigated areas. On the other hand, there is no storage problem and it is more profitable than some other crops in the rotation in some regions (Kurtar et al., 2018; Kaya and Beşer, 2018; Seymen, 2020). However, the most common problem is high yielding and high quality varieties. In some breeding studies, enough heterosis exhibited in confectionery pumpkins then some hybrid varieties developed and selling commercially in different countries (Hazra et al., 2007). However, the lack of CMS system or not having any practical applications for male sterility, hybrid production is not easy and has higher cost due to need of hand pollination.

Due to its ecological conditions, Trakya Region, is one of the most suitable regions for pumpkin cultivation in Turkey. The region which has previously more planting areas (hanim tirnagi (lady nail) mostly was planting in there) has still more confectionery pumpkin production areas currently after the Central Anatolia region (1000 tons of production and 1000 ha area) TUIK, 2015). However, the cultivation areas have decreased in both Edirne and the whole region in recent years. Most of the confectionery production areas in the Trakya Region are located in Edirne (Evci et al., 2013 and 2014; Kaya and Beşer, 2018).

National private companies do not have breeding program in confectionery pumpkin only one government institute is working as regional basis. TRAGEN R&D company is located in Trakya University Technopark in Edirne, Turkey and has breeding materials on confection pumpkin at different breeding levels and developed and sent registration two hybrids in 2019. TRAGEN has some finished lines and also send registration in next growing seasons. The study was conducted in Edirne to determine the relationships in seed quality traits in hybrid confectionery pumpkin.

MATERIALS AND METHODS

The study was conducted TRAGEN R&D company research fields in Edirne with 10 inbred lines originated different genetic sources in 2017 and 2018 growing seasons. The inbred lines were used as male and female in the study to generate 20 confectionery pumpkin hybrids in the study. Trials were conducted in controlled conditions with randomized complete block design with two rows. In each plot, there were 14 plants and the distance between rows was 70 cm and in rows was 70 cm.

As the major seed quality traits, leaf length (cm), leaf width (cm), vine lenght (cm), fruit length (cm), fruit width (cm), flesh thickness (cm), fruit diameter (cm), seed number per fruit,

seed yield per fruit (g), thousand seed weight (g), seed lenght (cm) and seed width (cm) were observed and measured both in male and female inbred lines and also hybrids. Correlation and regression analysis were performed to determine the relationships in seed quality traits in confectionery pumpkin utilizing JUMP statistical program.

RESULTS AND DISCUSSION

The observations of seed quality traits of the confectionery pumpkin hybrids were given Table 1. There were big variabilities were observed among seed quality traits in the study. Based the correlation analysis, mostly the seed quality traits as seed width and length and thousand seed weight had more significant results both inbred lines and hybrids, therefore these three yield traits were selected for detailed regression analysis in the study (Table 2, 3 and 4).

In the correlation analysis which was performed to determine the relationships among seed quality traits, fruit diameter had the highest significant characteristics in female lines, fruit width in male lines and hybrids among yield traits examined in the study. The highest negative value was observed between seed length and fruit length in female lines then followed by fruit width and length and it had higher negative values in male and hybrids too. Fruit length has generally negative correlations with other yield traits too. It means that fruit length reduces seed quality as well as seed yield in the confectionery pumpkins (Table 4). On the other hand, fruit width and fruit diameter in female line but fruit width had generally higher positive correlations almost yield traits both inbred lines and hybrids. It means that fruit width has positive effects both for yielding and also for seed quality. Furthermore, the higher positive correlations were observed mostly in female lines then we could conclude that female lines are more contributing behavior to determine seed quality and yielding in confectionery pumpkin.

Based on regression analysis results presented in the given figures in below (Figure 1 to 39), the paralel results were obtained in both female and male inbred lines as well as in hybrids. The significant linear relationships were observed generally both in negative and positive way among seed quality traits as well as higher the regression coefficient values.

In the male inbred lines, the regression analysis between seed width and fruit width, seed width and fruit diameter, seed length and vine length, seed length and fruit width, seed width and seed length, seed length and seed yield per fruit, seed length and 1000 seed weight, 1000 seed weight and vine length, 1000 seed weight and fruit, 1000 seed weight and seed number per fruit, 1000 seed weight and seed yield per fruit width exhibited significant linear relationship in positive way. The significant negative linear relationships were observed only in between seed length and fruit length, 1000 seed weight and fruit length in male lines in the study (Figure 1 to 12).

In the female inbred lines, significant positive linear relationships were observed in conducted the regression analysis between seed width and fruit width, seed width and fruit diameter, seed width and seed yield per fruit, seed width and 1000 seed weight, seed length and fruit width, seed length and fruit diameter, seed length and 1000 seed weight, seed length and seed width, 1000 seed weight and fruit width, 1000 seed weight and fruit diameter in the study. However, the significant negative linear relationships were observed in seed length and leaf length, seed length and leaf width, seed length and fruit length in female lines in the study (Figure 3 to 26).

Table 1: The yield trait observations of confectionery pumkin hybrids

HYBRIDS	Leaf Length (cm)	Leaf Width (cm)	Vine Lenght (cm)	Fruit Length (cm)	Fruit Width (cm)	Flesh Thickness (cm)	Fruit Diameter (cm)	Seed Number per Fruit	Seed Yield per Fruit (g)	Thousand Seed Weight (g)	Seed Lenght (cm)	Seed Width (cm)
TGK-1xTGK-12	28,50	28,33	232,33	30,10	12,10	3,50	14,70	380,00	74,40	254,00	17,93	10,14
TGK-1xTGK-13	31,00	31,33	126,33	23,80	17,00	3,70	16,70	498,00	71,50	267,00	20,92	10,95
TGK-1xTGK-14	28,83	29,16	149,33	26,00	14,80	3,80	15,00	282,00	50,60	215,00	18,56	10,61
TGK-1xTGK-3	35,33	31,16	155,00	19,20	17,60	3,60	19,30	196,00	28,50	225,00	20,80	9,97
TGK-3xTGK-12	28,16	28,73	58,33	19,00	13,00	3,40	13,90	172,00	16,80	153,00	15,65	7,99
TGK-3xTGK-15	30,83	31,00	118,33	20,00	13,80	3,80	14,20	182,00	44,00	207,00	17,12	9,41
TGK-3xTGK-13	30,83	30,16	339,66	25,00	16,00	3,50	15,50	324,00	58,70	225,00	19,45	10,95
TGK-3xTGK-14	31,16	30,70	231,66	28,10	12,10	3,60	14,00	349,00	51,30	191,00	17,52	8,92
TGK-10xTGK-14	30,75	32,16	350,00	27,00	13,80	3,00	14,20	413,00	78,40	222,00	18,64	9,75
TGK-11xTGK-12	25,33	29,16	173,33	32,40	13,00	3,10	13,30	359,00	43,10	235,00	21,18	9,81
TGK-11xTGK-17	23,66	21,66	286,66	26,00	16,10	4,00	16,20	404,00	50,00	258,50	21,41	11,20
TGK-11xTGK-6	24,16	24,66	184,66	26,00	16,30	3,10	16,40	431,00	29,30	262,00	22,03	11,14
TGK-11xTGK-3	34,00	35,00	156,66	22,30	15,30	3,30	15,00	391,00	45,20	192,00	18,31	10,33
TGK-12xTGK-17	31,00	30,83	196,33	24,00	16,00	3,50	18,00	440,00	36,40	226,50	20,34	10,02
TGK-13xTGK-14	26,66	30,50	184,33	33,70	13,80	4,00	14,10	358,00	25,70	225,50	17,77	10,50
TGK-13xTGK-4	27,50	28,33	201,66	29,50	18,50	3,50	17,10	423,00	101,50	256,00	19,58	11,58
TGK-14xTGK-4	35,25	35,66	205,00	28,40	16,10	2,60	14,40	336,00	105,50	280,00	20,35	10,00
TGK-14xTGK-17	29,00	32,83	96,66	27,60	14,90	3,90	17,50	494,00	81,60	223,00	20,20	10,10
TGK-15xTGK-17	24,16	27,00	305,00	23,10	19,30	3,70	19,70	433,00	52,80	302,50	22,68	11,03
TGK-15xTGK-16	23,83	25,83	111,33	36,20	10,80	3,90	14,00	393,00	35,60	193,00	17,05	9,22
AVERAGE	29,00	29,71	193,13	26,37	15,02	3,53	15,66	362,90	54,05	230,65	19,37	10,18
STD DEV	3,54	3,21	77,48	4,51	2,17	0,35	1,83	91,32	23,91	34,24	1,83	0,85

Table 2: Correlation values of confectionery pumkin female lines among yield traits.

	Leaf Length	Leaf Width	Vine Lenght	Fruit Length	Fruit Width	Flesh Thickness	Fruit Diameter	Seed Number per Fruit	Seed Yield per Fruit	1000 Seed Weight	Seed Lenght	Seed Width
Leaf Length	1,0000											
Leaf Width	0,9413**	1,0000										
Vine Lenght	-0,7773*	-0,7392*	1,0000									
Fruit Length	0,8666**	0,7519*	-0,6438ns	1,0000								
Fruit Width	-0,7688*	-0,7338*	0,6171ns	-0,9088ns	1,0000							
Flesh Thickness	-0,1101ns	-0,1155ns	0,4660ns	-0,3439ns	0,5239ns	1,0000						
Fruit Diameter	-0,7983*	-0,7547*	0,6271ns	-0,9015**	0,9830**	0,4888ns	1,0000					
Seed Numbr/Fruit	0,0831ns	0,0667ns	-0,2599ns	0,1679*	-0,4394ns	-0,5741ns	-0,3534ns	1,0000				
Seed Yield/Fruit	-0,2712ns	-0,3431ns	0,3795ns	-0,5037ns	0,5021ns	0,6552ns	0,4969ns	0,1332ns	1,0000			
1000 Seed Weight	-0,4878ns	-0,6011ns	0,3555ns	-0,6476ns	0,7918*	0,5649ns	0,8014**	-0,1277ns	0,8001**	1,0000		
Seed Lenght	-0,8871**	-0,8881**	0,6362ns	-0,9117**	0,8869**	0,2272ns	0,9041**	-0,0197ns	0,5454ns	0,7755*	1,0000	
Seed Width	-0,4066ns	-0,4357ns	0,2922ns	-0,6713*	0,7900*	0,5493ns	0,8111**	-0,0846ns	0,7700*	0,9284**	0,7453*	1,0000

Table 3: Correlation values of confectionery pumpkin male lines among yield traits.

Yield Traits	Leaf Length	Leaf Width	Vine Lenght	Fruit Length	Fruit Width	Flesh Thickness	Fruit Diameter	Seed Number per Fruit	Seed Yield per Fruit	1000 Seed Weight	Seed Lenght	Seed Width
Leaf Length	1,0000											
Leaf Width	0,7956*	1,0000										
Vine Lenght	-0,0072ns	-0,1138ns	1,0000									
Fruit Length	0,5761ns	0,3108ns	-0,5159ns	1,0000								
Fruit Width	-0,5121ns	-0,5347ns	0,6864*	-0,7800*	1,0000							
Flesh Thickness	0,4300ns	0,2676ns	-0,0792ns	0,2850ns	-0,0489ns	1,0000						
Fruit Diameter	-0,6243ns	-0,3751ns	0,4865ns	-0,7685ns	0,8292**	0,0909ns	1,0000					
Seed Numbr/Fruit	-0,4334ns	-0,1425ns	0,4946ns	-0,8186**	0,7048*	0,0980ns	0,8428**	1,0000				
Seed Yield/Fruit	-0,1191ns	0,0573ns	0,6562ns	-0,7652*	0,6946*	0,2265ns	0,7144*	0,8919**	1,0000			
1000 Seed Weight	-0,2148ns	-0,1529ns	0,8836ns	-0,7943*	0,7988**	-0,1392ns	0,6451ns	0,7591*	0,8754**	1,0000		
Seed Lenght	-0,4172ns	-0,4607ns	0,7995**	-0,8476**	0,8564**	-0,2880ns	0,6111ns	0,6395ns	0,6880*	0,8978**	1,0000	
Seed Width	-0,4452ns	-0,5065ns	0,2592ns	-0,4887ns	0,7268*	0,3539ns	0,6774*	0,5542ns	0,5967ns	0,4758ns	0,4661ns	1,0000

Table 4: Correlation values of confectionery pumkin hybrids among yield traits.

Yield Traits	Leaf Length	Leaf Width	Vine Lenght	Fruit Length	Fruit Width	Flesh Thickness	Fruit Diameter	Seed Numb per Fruit	Seed Yield per Fruit	1000 Seed Weight	Seed Lenght	Seed Width
Leaf Length	1,0000											
Leaf Width	0,8449**	1,0000										
Vine Lenght	-0,0698ns	-0,1592ns	1,0000									
Fruit Length	-0,4475*	-0,1806ns	0,1043ns	1,0000								
Fruit Width	0,1228ns	-0,0024ns	0,2611ns	-0,4428*	1,0000							
Flesh Thickness	-0,3637ns	-0,3940ns	-0,2046ns	0,0411ns	-0,0608ns	1,0000						
Fruit Diameter	0,0333ns	-0,1187ns	0,1261ns	-0,4108ns	0,8090**	0,2284ns	1,0000					
Seed Number/Fruit	-0,3010ns	-0,0919ns	0,2684ns	0,4279ns	0,2314ns	0,0215ns	0,2915ns	1,0000				
Seed Yield/Fruit	0,2609ns	0,3407ns	0,3027ns	0,2235ns	0,2608ns	-0,3236ns	0,0576ns	0,4216ns	1,0000			
1000 Seed Weight	-0,1855ns	-0,1828ns	0,4574*	0,1279ms	0,6454**	-0,1660ns	0,4962ns	0,5108*	0,4929*	1,0000		
Seed Lenght	-0,1645ns	-0,2062ns	0,3515ns	-0,0715ns	0,7274**	-0,1605ns	0,6687**	0,5027*	0,2036ns	0,8081**	1,0000	
Seed Width	-0,2142ns	-0,2534ns	0,4270ns	0,0991ns	0,7105**	0,1091ns	0,5037*	0,5377*	0,3505ns	0,7446**	0,6868**	1,0000

Figure 1. The regression analysis between seed width and fruit width in male lines

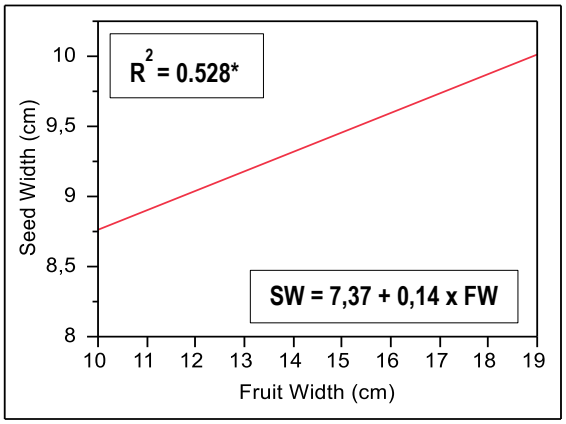


Figure 2: The regression analysis between seed width and fruit diameter in male lines

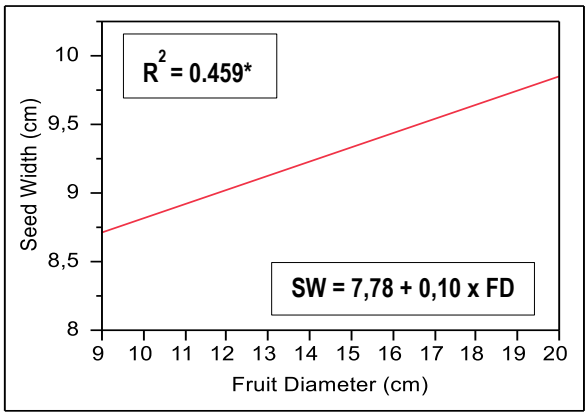


Figure 3. The regression analysis between seed length and vine length in male lines

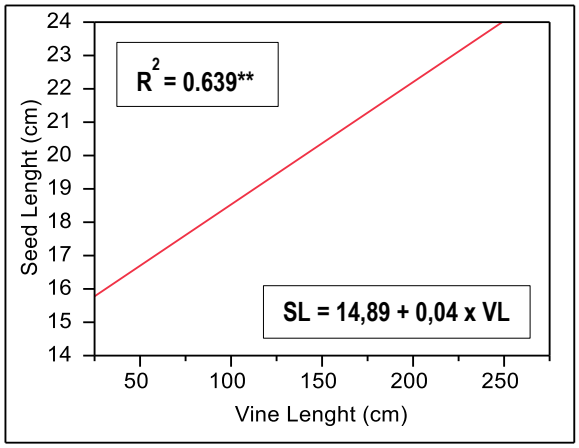


Figure 4: The regression analysis between seed length and fruit length in male lines

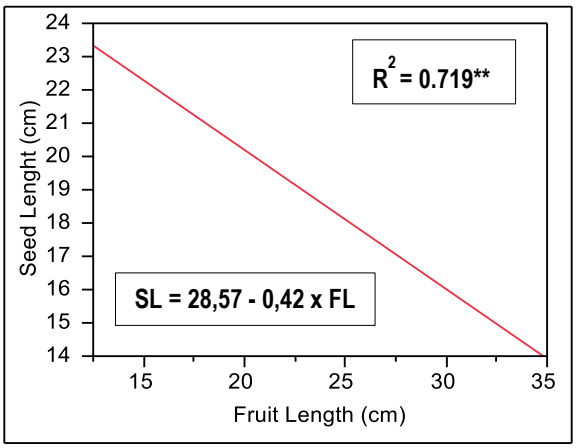


Figure 5. The regression analysis between seed length and fruit width in male lines

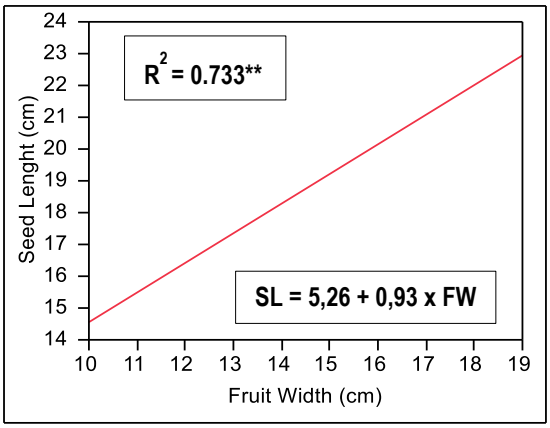


Figure 6: The regression analysis between seed length and seed yield per fruit in male lines

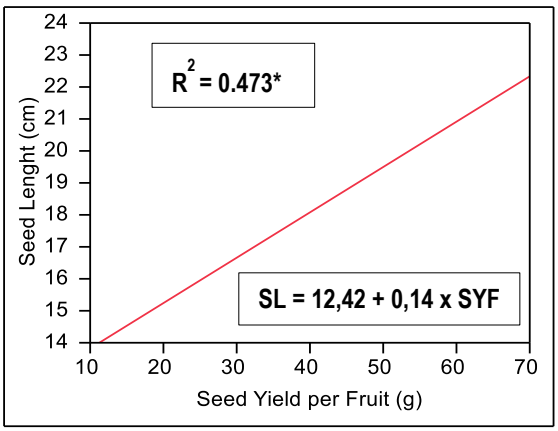


Figure 7. The regression analysis between 1000 seed length &1000 seed weight in male lines

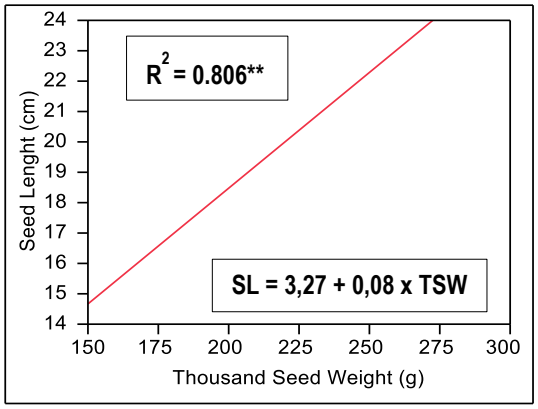


Figure 8: The regression analysis between seed weight and vine length in male lines

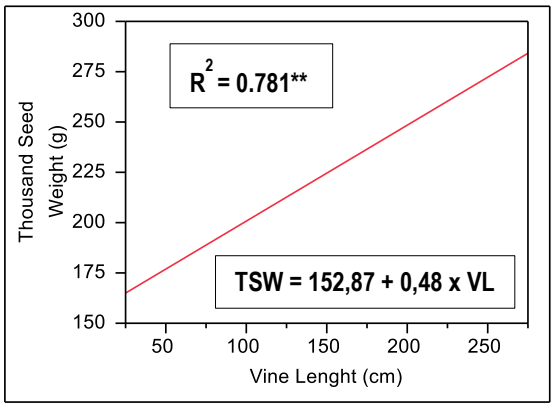


Figure 9. The regression analysis between 1000 1000 seed weight & fruit length in male lines

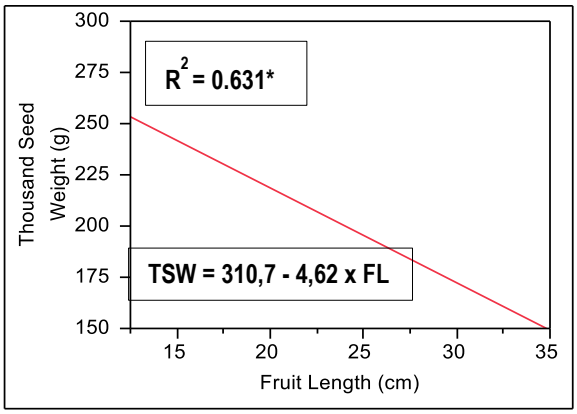


Figure 10: The regression analysis between seed weight & fruit width in male lines

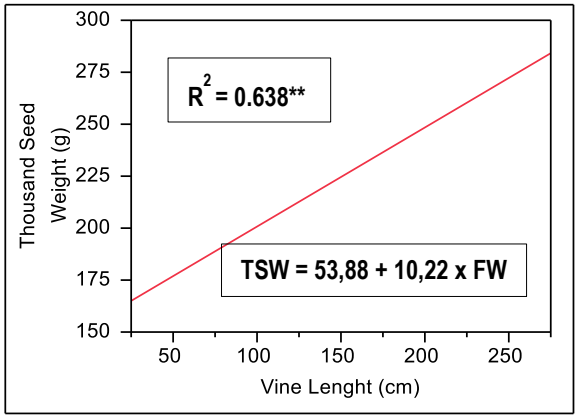


Figure 11. The regression analysis between 1000 1000 seed weight & seed # /fruit in male lines

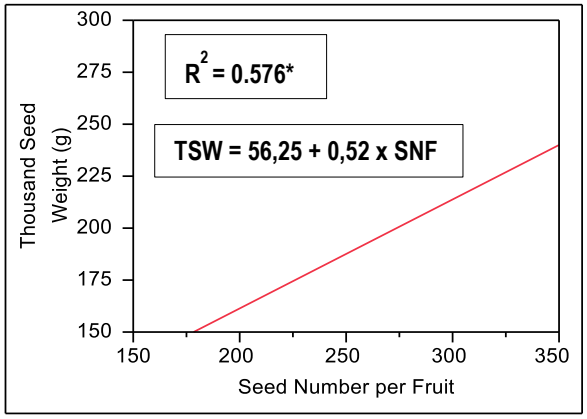


Figure 12: The regression analysis between 1000 seed weight & seed yield /fruit in male lines

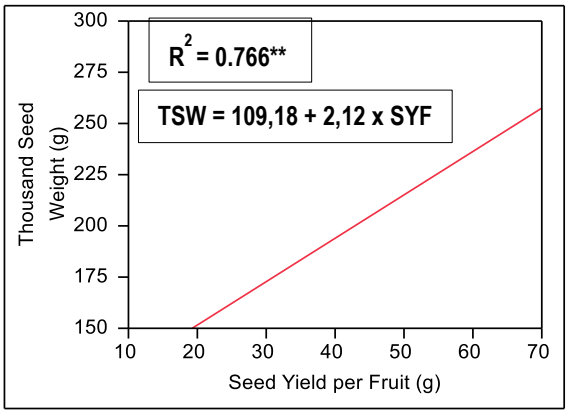


Figure 13. The regression analysis between seed width & fruit width in female lines

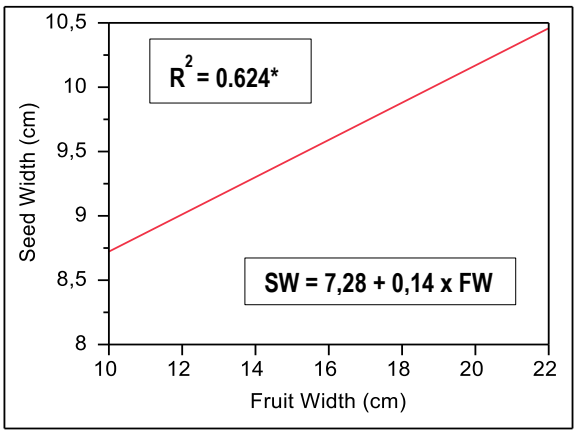


Figure 14: The regression analysis between seed width & fruit diameter in female lines

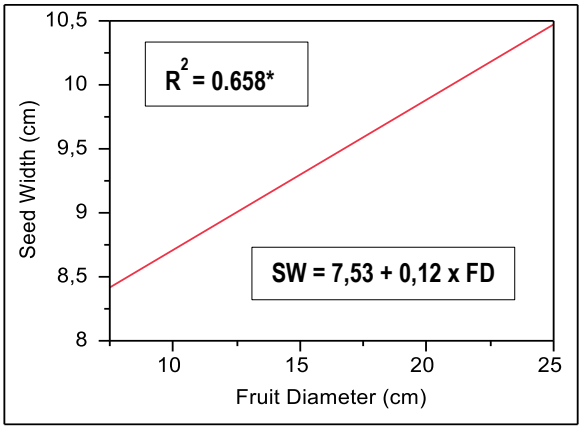


Figure 15. The regression analysis between seed width & seed yield/fruit in female lines

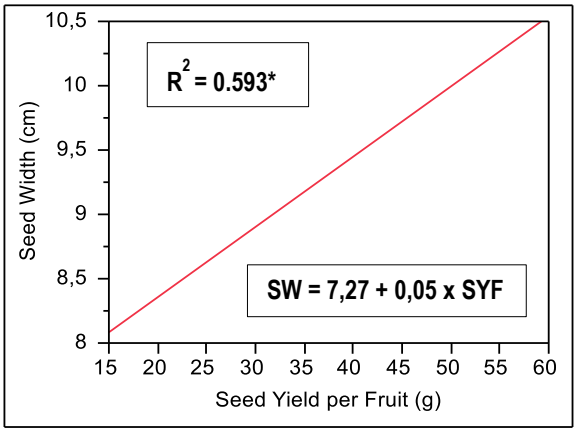


Figure 16: The regression analysis between seed width & 1000 seed weight in female lines

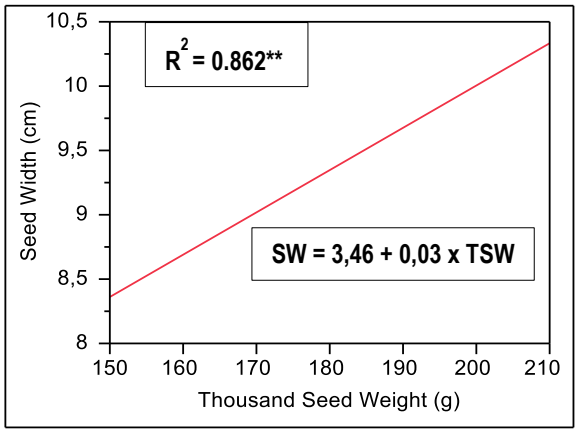


Figure 17. The regression analysis between seed width & seed length in female lines

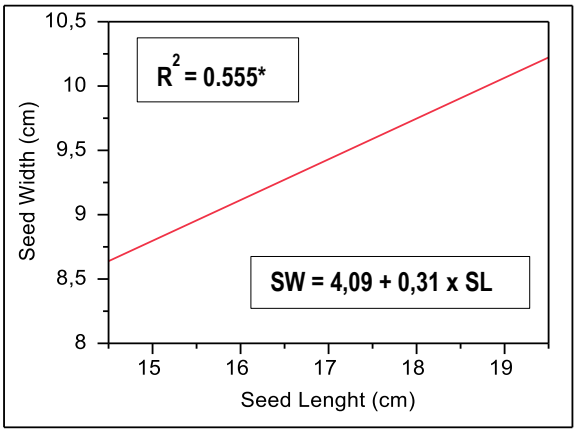


Figure 18: The regression analysis between seed length & leaf length in female lines

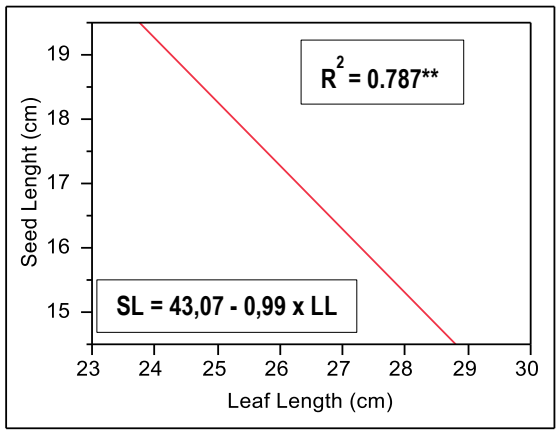


Figure 19. The regression analysis between seed length & leaf width in female lines

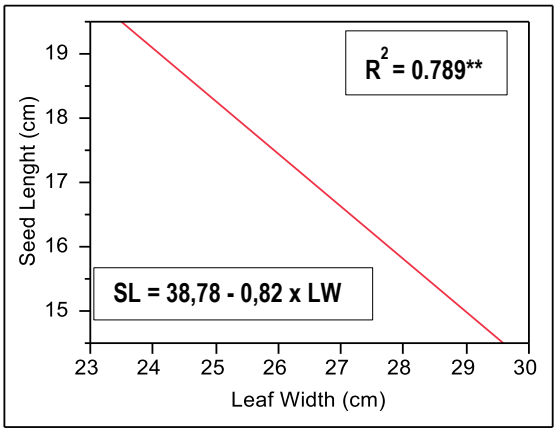


Figure 20: The regression analysis between seed length & fruit length in female lines

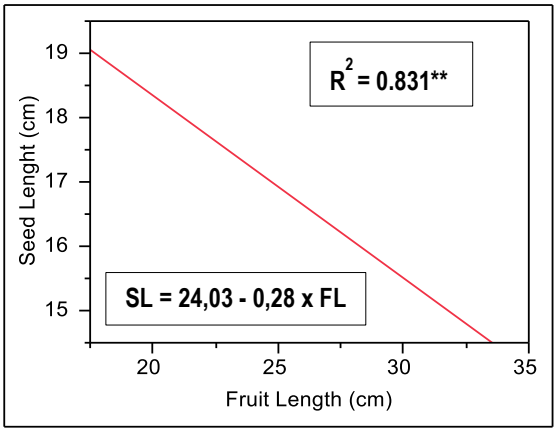


Figure 21. The regression analysis between seed length & fruit width in female lines

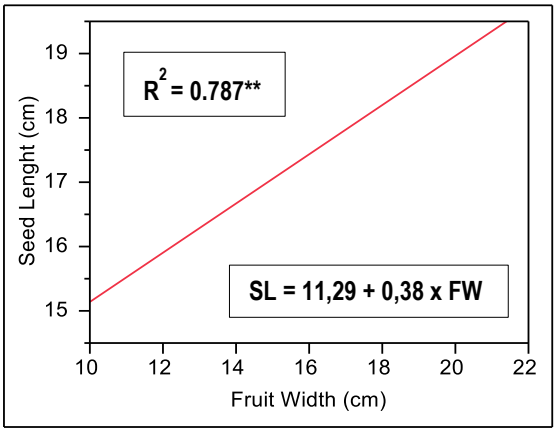


Figure 22: The regression analysis between seed length & fruit diameter in female lines

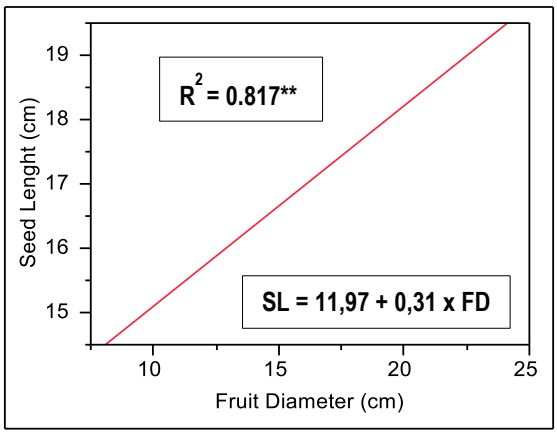


Figure 23. The regression analysis between seed length & 1000 seed weight in female lines

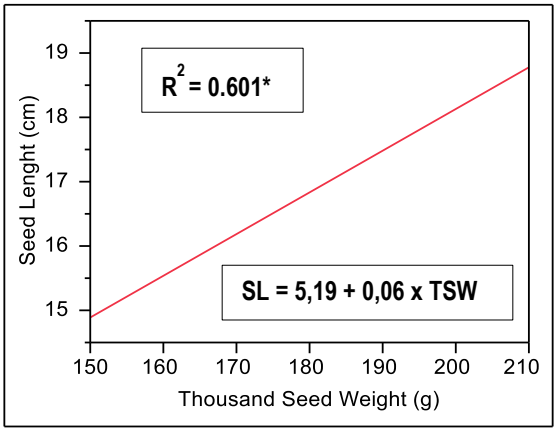


Figure 24: The regression analysis between seed length & seed width in female lines

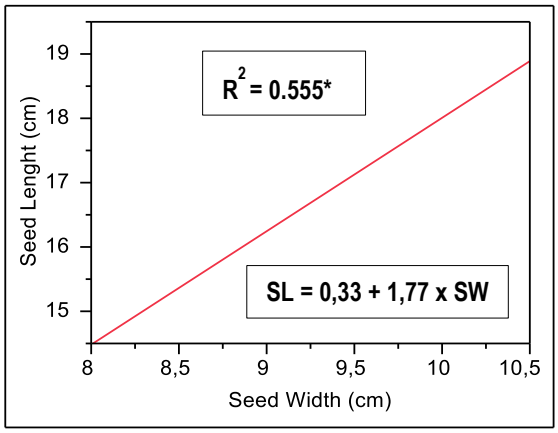


Figure 25. The regression analysis between 1000 seed weight & fruit width in female lines

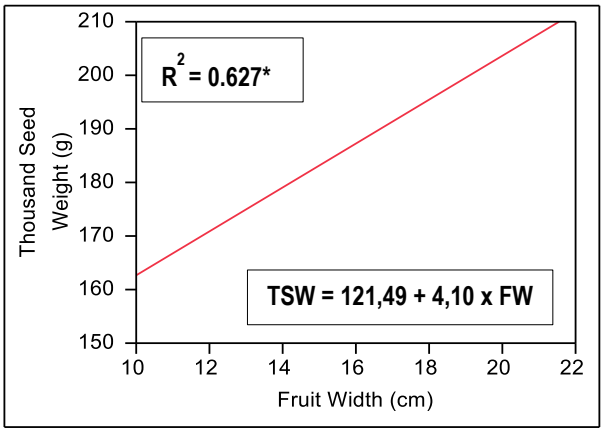
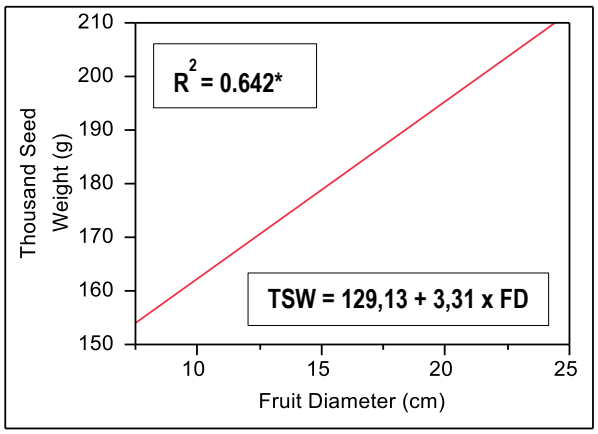


Figure 26: The regression analysis between 1000 seed w. & fruit diameter in female lines



In the hybrids, significant positive linear relationships were observed in conducted the regression analysis between seed width and fruit width, seed width and fruit diameter, seed width and seed yield per fruit, seed width and 1000 seed weight, seed width and seed length, seed length and fruit width, seed length and fruit diameter, seed length and 1000 seed weight, 1000 seed weight and vine length, 1000 seed weight and fruit width, 1000 seed weight and fruit diameter, 1000 seed weight and seed number per fruit 1000 seed weight and seed yield per fruit. However, there is no significant negative linear relationships were observed in in the hybrids (Figure 27 to 39).

Figure 27. The regression analysis between seed width & fruit width in hybrids

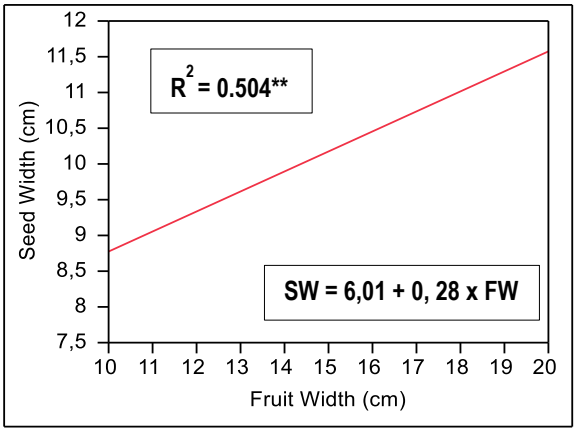


Figure 28: The regression analysis between seed width & fruit diameter in hybrids

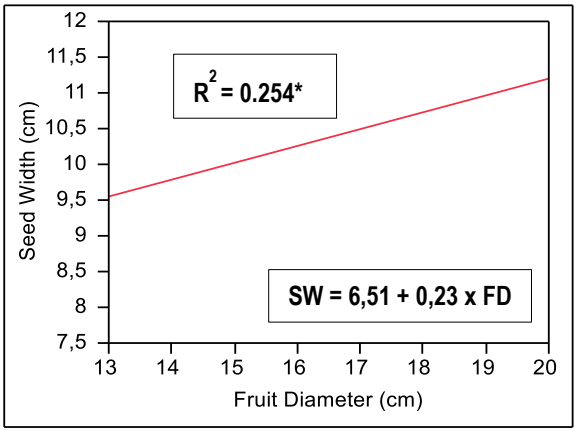


Figure 29. The regression analysis between seed width & seed # / fruit width in hybrids

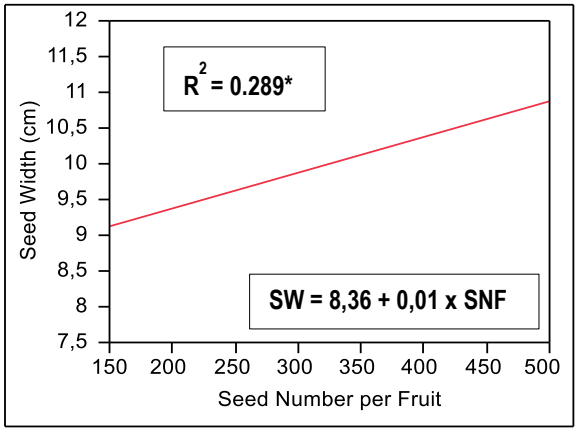


Figure 30: The regression analysis between seed width & 1000 seed weight in hybrids

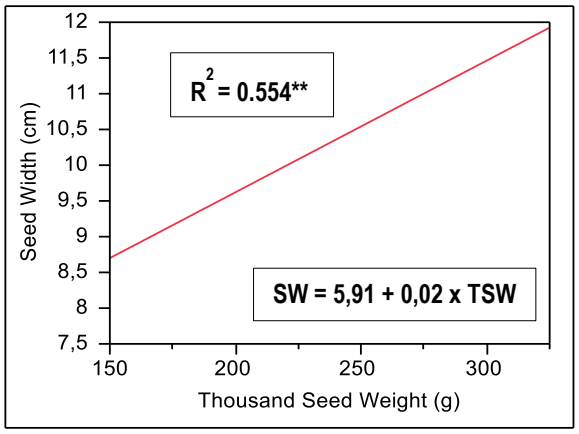


Figure 31. The regression analysis between seed length & fruit width in hybrids

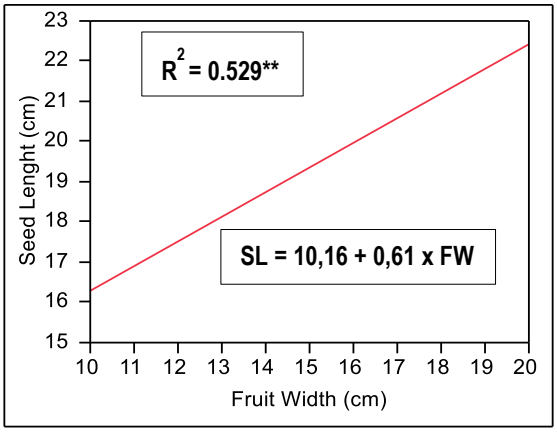


Figure 32: The regression analysis between seed length & fruit diameter in hybrids

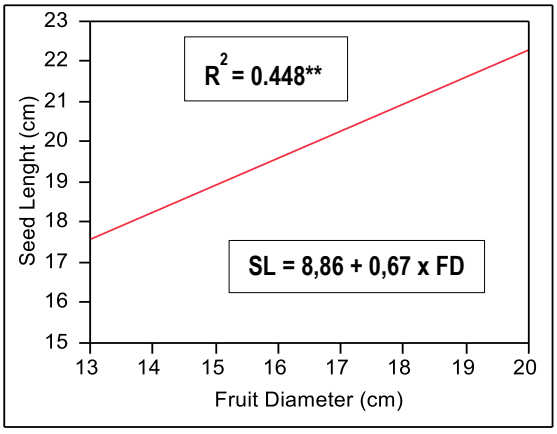


Figure 33. The regression analysis between seed length & 1000 seed weight in hybrids

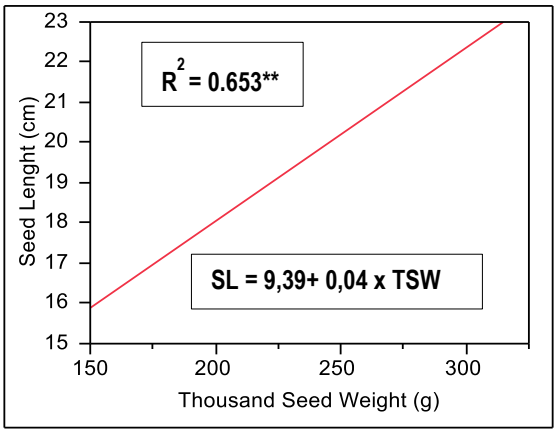


Figure 34: The regression analysis between seed length & seed width in hybrids

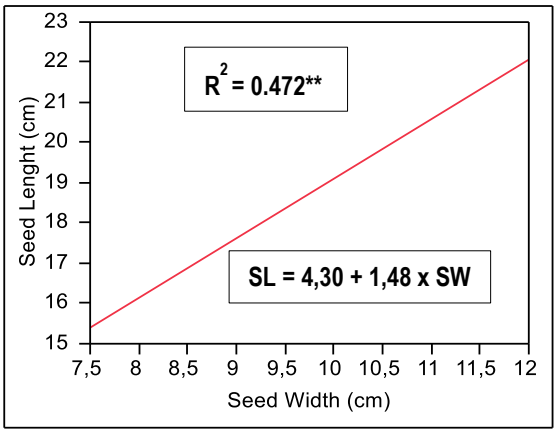


Figure 35. The regression analysis between 1000 seed weight & vine length in hybrids

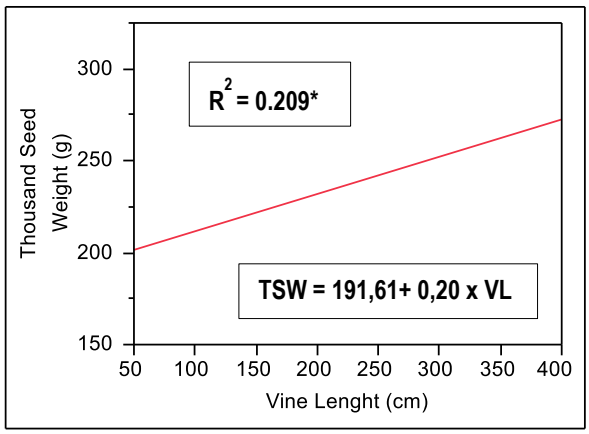


Figure 36: The regression analysis between 1000 seed weight & fruit width in hybrids

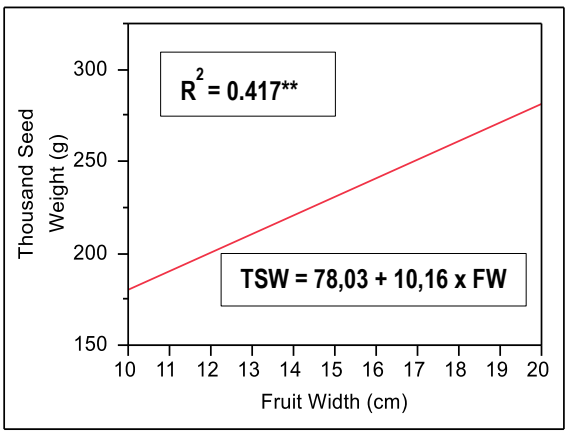


Figure 37. The regression analysis between 1000 seed weight & fruit diameter in hybrids

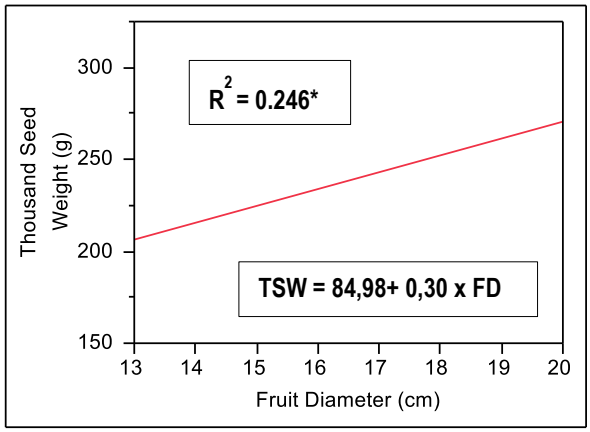


Figure 38: The regression analysis between 1000 seed weight & seed # / fruit in hybrids

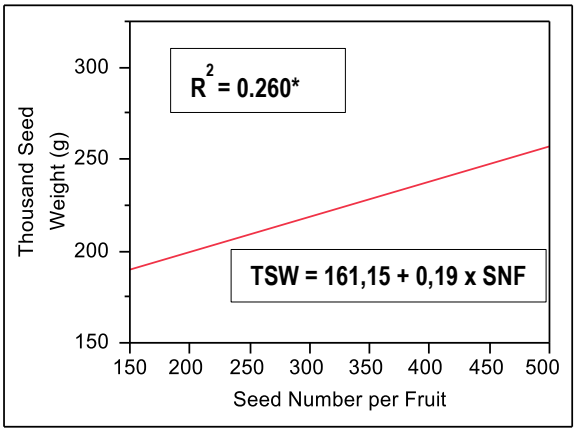
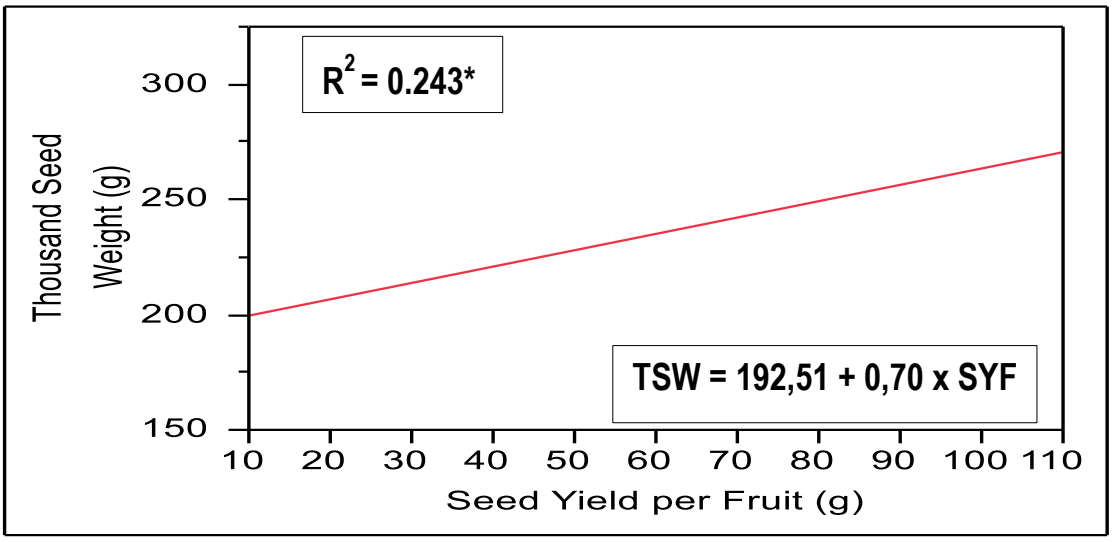


Figure 39. The regression analysis between 1000 seed weight & seed yield per fruit in hybrids



CONCLUSION

As conclusion, significant correlation results indicate that in seed quality traits, confectionery pumpkin breeders should consider more female inbred lines to get better seed quality confectionery pumpkin genotypes in hybrid breeding. Fruit diameter had the highest positive significant characteristics correlated with other yield traits in female lines, fruit width in male lines and hybrids among yield traits examined in the study. The highest negative value was observed between seed length and fruit length correlated with other yield traits in female lines then followed by fruit width and length and they had higher negative values in male and hybrids too. Fruit length has generally negative correlations with other yield traits too so that fruit length reduces seed quality as well as seed yield in the confectionery pumpkins. The highest positive correlation was observed seed width and 1000 seed weight because seed and seed length with fruit diameter so study results indicated that higher fruit diameters increase the possibility to obtain larger seeds. Similar results were obtained in the regression data. On the other hand, it was observed that vine length and leaf length as well as leaf width reduce seed quality in confectionery pumpkin.

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NUMERICAL INVESTIGATION ON THE EFFECTS OF A NATURAL GAS BURNER TIP LENGTH ON THE COMBUSTION PARAMETERS

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ABSTRACT

In this paper, the effects of a natural gas burner tip length on the combustion parameters are studied numerically with the use of the Fluent Code. A back pressure combustion chamber was used as the combustion medium. Pure methane was taken as fuel for the numerical analysis. Four different combustion cases were analysed by changing the burner tip length. The investigated combustion parameters were the flame length, flame temperature and the NO_X emissions in the flue gas. The obtained results show that the present natural gas burner has an optimum tip length in terms of reducing the NO_X emissions and obtaining an acceptable flame length.

INTRODUCTION

Scientific studies in the world have been on the development of less polluting systems and the use of renewable energy systems in recent years. Today, vehicles that are used extensively, heat treatment systems used in industry and central and individual heating systems used in buildings are among the main systems that cause harmful emissions to the environment. In addition, the fact that these types of systems use fossil fuels is one of the conditions that cause energy consumption, while natural gas is the most widely used fossil fuel.

There are various studies in the literature to reduce the pollution caused by the use of fossil fuels. These studies can be in the form of modifying the existing system to produce less emissions and reduce fuel consumption rate and integrating renewable energy sources into existing systems in some way. Khanafer and Aithal (2011) numerically investigated the effects of the vortex velocity and combustion volume wall temperatures provided by a combustor on NOx emissions with the FIDAP program. According to the results, the increased vortex velocity reduced CO emissions and the amount of unburned hydrocarbon components, while NOx emissions did not change in proportion to the vortex speed. Feyz, Esfahani, Pishbin and Razavi (2015) experimentally studied the combustion performance of 55kW premixed natural gas burner. Depending on the ratio of burner length to diameter, combustion performances were investigated. Flame types connected to the burner end and separated from the burner end were observed in the experiments. In the study, it was stated that a better heat transfer rate was achieved with the flame located separately from the burner tip. Zadghaffari, Moghaddas and Rahimiahar (2012) investigated the temperature and emission values of a non-premixed combustion system by changing the values of the burner tip angle. Optimum burner tip angles were determined to ensure uniform flow in the boiler with low NOx values. Hossain and Malalasekera (2001) numerically studied the combustion of syngas gas using different combustion models and compared the results with the experimental results. The combustion models used were eddy - break up, reaction plate and laminar flamelet model. The most proper

result in terms of temperature was obtained from the laminar flamelet model. Hashemi, Hajialigol, Mazaheri, and Fattahi (2014) numerically investigated the effect of the diameter and length of the burner tip containing air and fuel inlets on the flame structure. Büyükakın and Öztuna (2019) conducted an experimental and a numerical study on a non-premixed natural gas burner. According to the results, the increase in the number and angle of the air turbulator blades decreased the flame length while reducing the NOx emissions up to a certain point. In the present study, the effect of tip length of a non-premixed natural gas burner on the flame length and NO_X emissions was investigated by the numerical methods. The Fluent code was used as a numerical tool. In the analyses, the fuel consumption rate is 45 Nm3/h and the air excess coefficient is 1.2.

Mathematical Model

Various mathematical models are available in the Fluent code to model the combustion process of natural gas fuel. In this study, the non-premixed combustion model is used. In the non-premixed combustion model, mass, energy and momentum conservation equations and the average mixture fraction conservation equation are solved. In Eqs.(1-5), the conservation equation for mass, energy, momentum and average mixture fraction and the radiation heat transfer equation are given respectively [ANSYS Fluent 12 Theory Guide, 2009]. The mixture fraction value ranges from 0 to 1 and gives the mass fraction from the fuel at any point.

$$\frac{\partial \rho}{\partial t} + \nabla.(\rho \mathbf{v}) = 0 \quad (1)$$

$$\frac{\partial(\rho \mathbf{v})}{\partial t} + \nabla.\rho\mathbf{v}\mathbf{v} = -\nabla.\mathbf{P} + \rho \sum_{i=1}^{N} Y_i \mathbf{f_i} \quad (2)$$

$$\frac{\partial(\rho h)}{\partial t} + \nabla.(\rho \mathbf{v} h) = \nabla.[\frac{k_t}{c_p} \nabla h] + S_h \quad (3)$$

$$\frac{\partial(\rho \bar{f})}{\partial t} + \nabla.(\rho \mathbf{v} \bar{f}) = \nabla.(\frac{\mu_t}{\sigma_t} \nabla \bar{f}) + S_m + S_{user} \quad (4)$$

$$\frac{d\, I(\vec{r},\vec{s})}{ds} + (\alpha + \sigma_s)\, I(\vec{r}, \vec{s}) = \alpha n^2 \frac{\sigma T^4}{\pi} + \frac{\sigma_s}{4\pi} \int_0^{4\pi} I(\vec{r}, \vec{s}') \, \Phi(\vec{s}.\vec{s}') \, d\Omega' \quad (5)$$

In Eq.(2), **P** is the stress tensor and represents the surface forces. In Eq.(5), $\vec{r}$, $\vec{s}$, $\vec{s}'$, s, α , n, σ_s, σ, I, T, Φ and Ω' are position vector, direction vector, scattering direction vector, path length, absorption coefficient, refractive index, scattering coefficient, Stefan-Boltzmann constant (5.672×10^{-8} W/m^2K^4), total radiation intensity, local temperature (K), phase function and solid angle, respectively. The average value of any scalar value (temperature, density) in the flow field is solved as follows.

$$\bar{\emptyset} = \int \emptyset(f, \bar{H}) P(f)\, df \quad (6)$$

In order to solve the mean $\bar{\emptyset}$ value, the average mixture fraction variance is solved in the Fluent code [ANSYS Fluent 12 Theory Guide, 2009]. Also, in this study, laminar flamelet approach is used for the function $\emptyset$ (f, $\bar{H}$) in Eq. (6).

RESULTS

The Eqs. (1-6) were solved simultaneously for the back-pressure combustion chamber and non-premixed burner system. Pure methane was handled for the fuel type. In addition to Eqs.(1-6), the thermal NO formation equation [ANSYS Fluent 12 Theory Guide, 2009] was solved after all the calculations for Eqs.(1-6) were converged. The original burner and extended burner are given in Fig.1. The obtained results are presented in Fig.2.

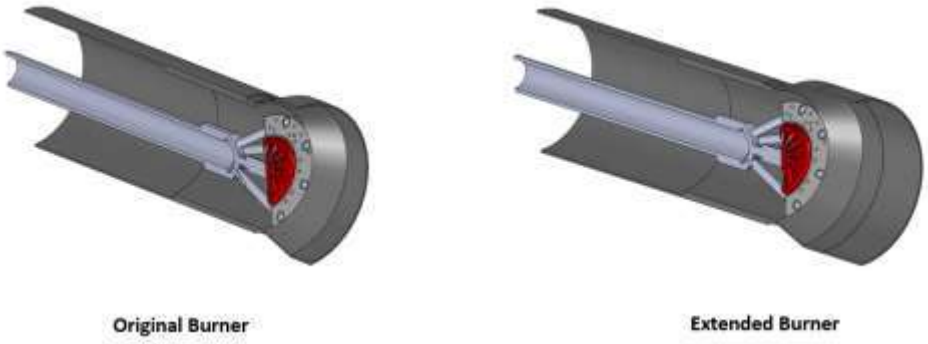

Figure 1 The original and extended burner

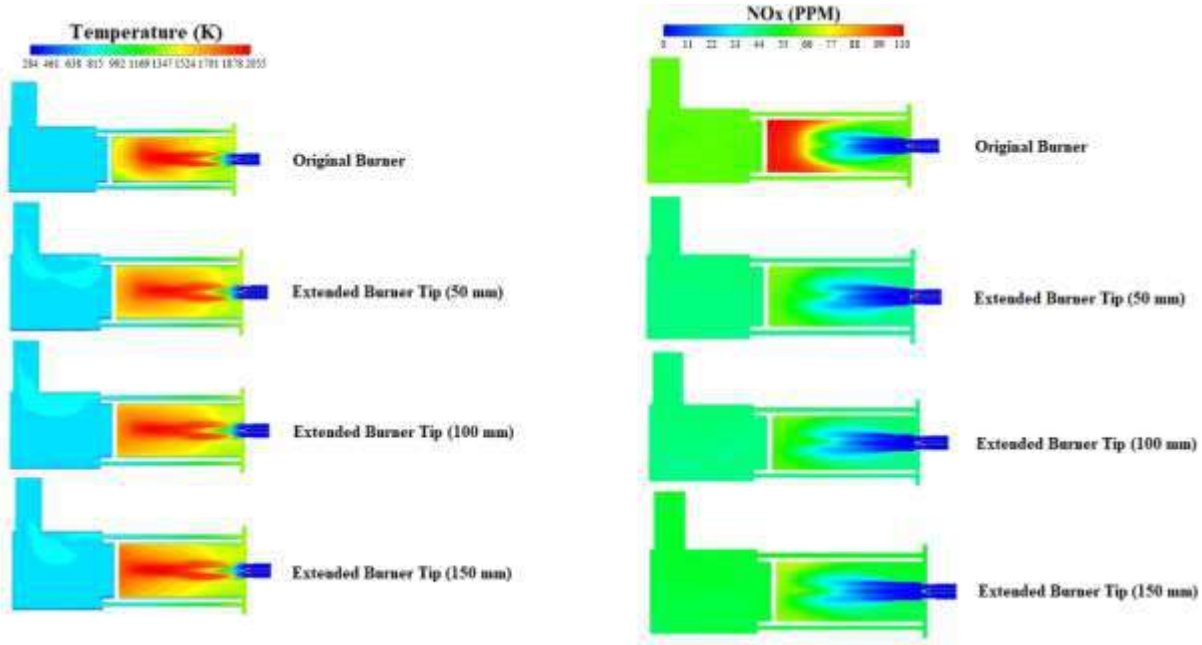

Figure 2 The obtained temperature and NO_X emissions distributions

As seen in Fig.2, if the burner tip is extended beyond 50 mm, the methane flame becomes attached to the opposite wall of the combustion chamber. This shows that it is impractical to extend the burner tip beyond a certain length. The obtained results show that this certain length

for the given burner and boiler system is 50mm. In Fig.3, the calculated NO_X emissions in the flue gas are given.

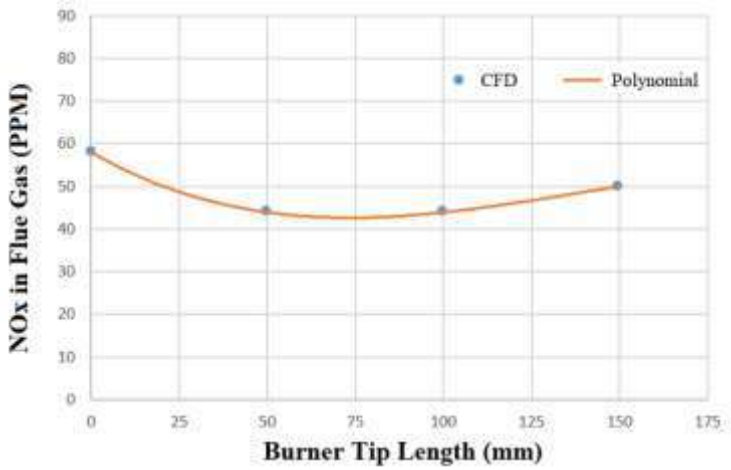

Figure 3 The Calculated NO_X emissions in the flue gas

The results in Fig.3 show that there is an optimum burner tip length, which can reduce the NO_X emission formation. This length also corresponds to the 50 mm burner tip length. It is known that when the burner tip length is extended, the fuel and air becomes mixed better according to the original case. This cools down the flame in the combustion chamber and then the NO_X emissions in the flame and the flue gas are reduced. However, over a certain length of the burner tip, the NO_X emissions in the flue gas tend to increase again. This is attributed to the concentrated flame form, which becomes thinner and longer together with the increase in the burner tip length.

CONCLUSION

The conclusions achieved are given as below;

- The results show that there is a midpoint where we can reduce the NOx emissions to the minimum by extending the burner tip length.
- This point is determined by the length of the combustion flame.
- Increasing the burner tip length excessively is not effective in terms of reducing the NOx emissions. In addition, this will damage the boiler wall since the flame has a direct contact with it.
- Increasing the burner tip length for 50 mm can be accepted as the optimum solution for the given burner-boiler system in terms of reducing the thermal NOx emissions.

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Nomenclature

$\mathbf{c_p}$	Specific heat (kJ/kg K)
$\mathbf{f}$	Mixture fraction
$\bar{\mathbf{f}}$	Average mixture fraction
$\mathbf{h}$	Enthalpy (kJ/kg)
$\mathbf{k_t}$	Heat conductivity (kW/mK)
$\mathbf{S_h}$	Radiation Source Term (kJ/m3s)
$\mathbf{t}$	Time (s)
$\mathbf{v}$	Velocity vector (m/s)
$\mathbf{Y_i}$	Mass fraction for i_{th} species
$\boldsymbol{\rho}$	Density (kg/m^3)
$\boldsymbol{\mu_t}$	Turbulent viscosity (kg/ms)
$\boldsymbol{\sigma_t}$	Constant value

ANTIPROLIFERATIVE EFFECT AND BIOACTIVE PHENOLIC PROFILE OF CULTIVATED TURKISH ARONIA (*ARONIA MELACARPA SP. VIKING*) BASED TEA

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ABSTRACT

Aronia berries, or chokeberries, are small, dark fruits that grow on shrubs of the *Rosaceae* family. They're native to North America but grown in other parts of the world, including across Europe. In this study, aronia berry plant had cultivated in Yalova, Turkey and planted Turkey variety had examined as nutritional values. With analytical determinations, it has been determined phenolic acids, gallic acid, epigallocatechin, catechin, chlorogenic acid, hydroxycinnamic acid, neochlorogenic acid, vanillic acid, siringic acid, caftaric acid, p-coumaric acid, ferulic acid, t-sinapic acid as flavanols (catechins); utin (quercetin-3-glycoside), kaempferol-3-rutinoside, quercitrin (quercetin-O-glycoside), quercetin, astragalin (kaempferol-O-glycoside), kaempferol as flavonols and their glycosides. It was determined that quercetin and chlorogenic acid inhibited the proliferation of SKBR-3 breast cancer cells as a result of their 5-100 μM concentration through 48 hours' incubation. It is seen that the 100 μM quercetin decreases SKBR-3 cells to 56.25% level and inhibits the cell proliferation at the level of 43.75%. It was found that chlorogenic acid diminished SKBR-3 proliferation to 62.5% level and inhibited the SKBR-3 cell proliferation at the level of 37.50%. It is seen that catechin bioactive reduces SKBR-3 cells to 65.66% and inhibits the cell proliferation by 34.34% even at only 10 μM concentration (p <0.05). These are shown that our developed aronia plant tea can be used as healthy antiproliferative supplement.

Keywords: Aronya, *Aronia melanocarpa*, SKBR-3 breast CA cell, phenolic, Q- TOFF-MS

INTRODUCTION

Black chokeberry or aronia (*Aronia melanocarpa*) is a shrub of the Rosaceae family that is a berry plant native to North America and was transferred to Europe about a century ago (Chrubasik et.al.,2010). Edible parts of black chokeberry are mainly small cherry-like berry parts. The genus Aronia (Rosaceae family) includes two species of shrubs, native to eastern North America and Eastern Canada: *Aronia melanocarpa* (Michx.) Ell., known as black chokeberry and *Aronia arbutifolia* (L.) Pers. (red chokeberry).

Aronia melanocarpa, a black chokeberry, has been used for treating cardiovascular diseases, for reducing blood glucose level and for lowering blood pressure through its antioxidant activity. Moreover, it exhibits anti-inflammatory, gastroprotective, antidiabetic, and hepatoprotective properties (Banjari et.al.,2017; Valcheva-Kuzmanova and Belcheva,2014; Bermúdez-Soto et.al.,2007). Aronia includes major level of biologically active constituents including polyphenolic flavonoids (flavanols, flavonols, anthocyanins) and phenolic acids.

The aronia berries contain high levels of flavonoids, mostly proanthocyanidins and anthocyanins, and in vitro and in vivo studies indicate that the berries may have potential health benefits, e.g. hepatoprotective effects, cardioprotective effects, antidiabetes effect and anticancer effects on selected CA cells. The consumption of low levels of antioxidants in the form of fruit and vegetables has been shown to more than double the incidence of certain cancers. (Tokusoglu and Boz, 2019; Tokusoglu 2019; Sidor et.al. 2019; Gavaric et.al.2019; Toli et.al 2015; Savikin et.al.2014).

Unprocessed fresh black chokeberry fruits are generally not consumed routinely owing to their so astringent taste; aronia berries widely consumed as juices, syrups, jams, fruit teas and dietary supplements (Tokusoglu,2019; Tokusoglu and Boz,2019). Chemical composition and biological activity of berries and their tea were analyzed in the study.

MATERIAL AND METHODS

Aronia berry [*Aronia melanocarpa* (Michx.)] (black chokeberry) was harvested at Yalova Research Institute, Yalova, Turkey. In our current research, aronia based new products including aronia berry teas (as decoction and infusion types), was developed by Dokuz Eylul University Technology Development Zone Depark Technopark Spil Innova LLC, Izmir Project. For aronia (chokeberry) powder production, aronia berries were subjected to freeze drying (FD) and spray drying process (B-290, Buchi Labour Technik, AG, Flawil, Switzerland) based on our determined conditions (Tokusoglu,2019).

In manufacturing, decoction method was applied by boiling of aronia berry material in a nonaluminum pot during 8 min until up to two-thirds of the water was evaporated and was strained by home-made tea strain apparatus. Phenolics were extracted according to Tokusoglu (2019). The obtained extract was used for determination of total phenolic content (TPC), for antioxidant capacity assay by DPPH method and chromatographic analyses including HLPC-DAD and Q-TOFF-MS. In Q-TOFF-MS Analyses, 30 °C of column temperature, 2 µL of injection volume, flow rate 0.5 ml/min was performed. Gradient elution was as 0–0.5 min, 5% B; 0,5–2 min, 25% B; 2–4 min, 50% B; 4–6 min, 75% B; 6–10 min, 95% B; for column conditioning için 10-16 min, 5%B. For MS analyses, dryer gas flow rate 140 L/min; nebulizer gas pressure as 35 psi, dryer gas temperature as 290 °C; sheath gas temperature as 400°C; sheath gaz flow as 12 L/min, Agilent Dual Jet Stream elektrosprey ionization (Dual AJS ESI) intermediate surface unit 6550 iFunnel was utilized.

Human prostate cancer androgen-responsive LNCaP and androgen non-responsive DU145 cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The LNCaP cell line was grown in RPMI-1640 medium supplemented with 10% fetal calf serum, 1 mM gluta- mine and 10 µl/ml penicillin-streptomycin. DU145 cells were maintained in Earle's Minimal Essential Medium (EMEM), containing 10% fetal calf serum, 1 mM glutamine, antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin) and 1% non-essential amino acids.

Cell Analyses

The SKBR beat cancer cells were plated at a constant density to obtain identical experimental conditions in the different tests, thereby ensuring high accuracy of the measurements. After 24-h incubation at 37℃ under a humidified 5% CO_2/95% air mixture to allow cell attachment, the cells were treated with different concentrations of individual aronia phenolics (100-500 µM), and incubated for 72 h under the same conditions (n=4).

MTT bioassay. To monitor cell viability, the cells were plated at $8x10^3$ cells per well of a 96-multiwell flat-bottomed 200 µl microplate (24). The optical density of each well sample was measured with a microplate spectrophotometer reader (Titertek Multiskan, Flow Laboratories, Helsinki, Finland) at λ=570 nm.

RESULTS

It has been determined phenolic acids, gallic acid, epigallocatechin, catechin, chlorogenic acid, hydroxycinnamic acid, neochlorogenic acid, vanillic acid, siringic acid, caftaric acid, p-coumaric acid, ferulic acid, t-sinapic acid as flavanols (catechins); utin (quercetin-3-glycoside), kaempferol-3-rutinoside, quercitrin (quercetin-O-glycoside), quercetin, astragalin (kaempferol-O-glycoside), kaempferol as flavonols and their glycosides (Figure 1).

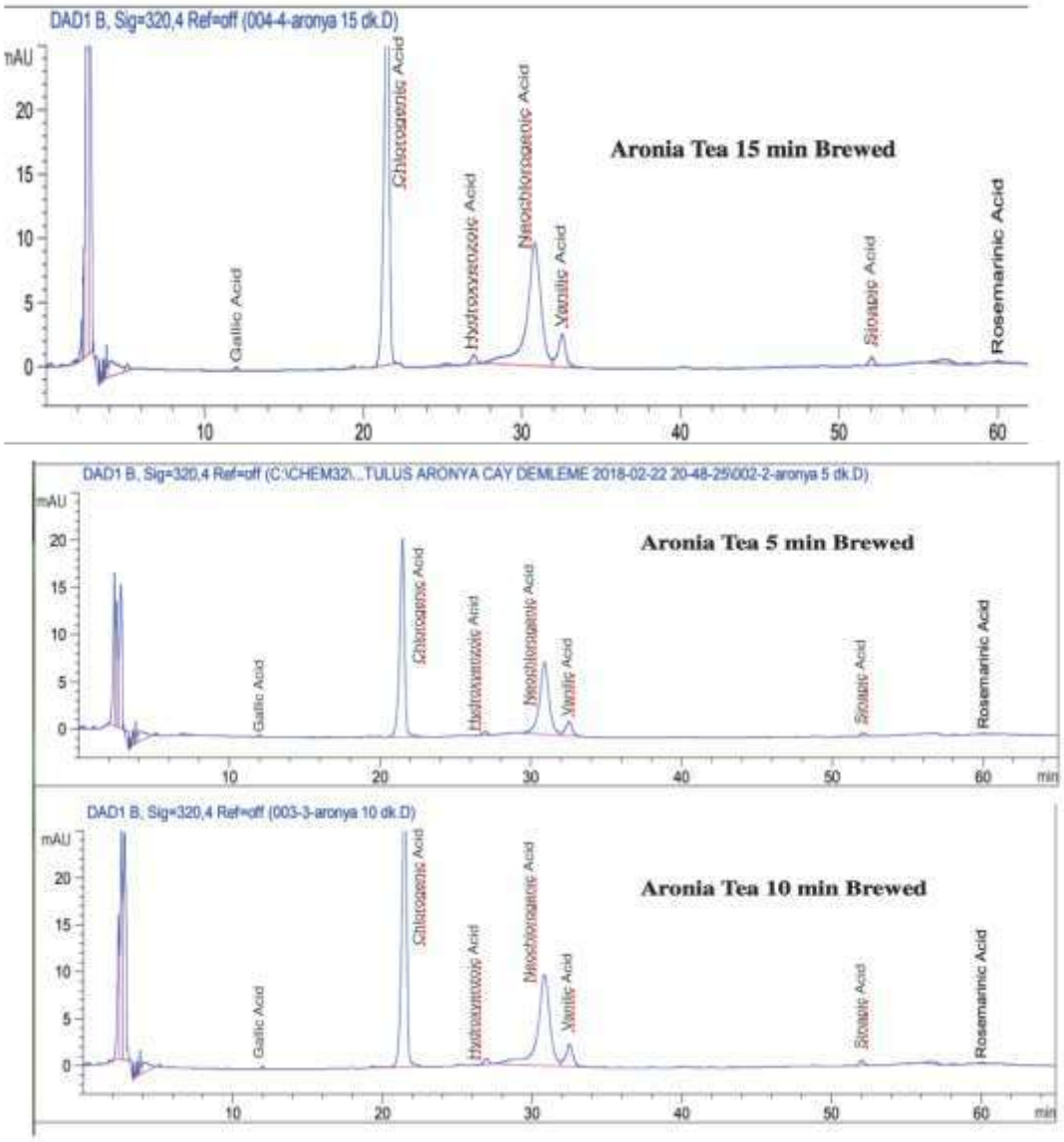

Figure 1. Simultaneously Chromatograms of Phenolic Acids in Aronia Berry Teas Brewed as 5,10,15 min

It was determined that quercetin and chlorogenic acid inhibited the proliferation of SKBR-3 breast cancer cells as a result of their 5-100 μM concentration through 48 hours incubation. It is seen that the 100 μM quercetin decreases SKBR-3 cells to 56.25% level and inhibits the cell proliferation at the level of 43.75%. It was found that chlorogenic acid diminished SKBR-3 proliferation to 62.5% level and inhibited the SKBR-3 cell proliferation at the level of 37.50%. It is seen that catechin bioactive reduces SKBR-3 cells to 65.66% and inhibits the cell proliferation by 34.34% even at only 10 μM concentration (p <0.05). (Figure 2). These are shown that our developed aronia plant tea can be used as healthy antiproliferative supplement.

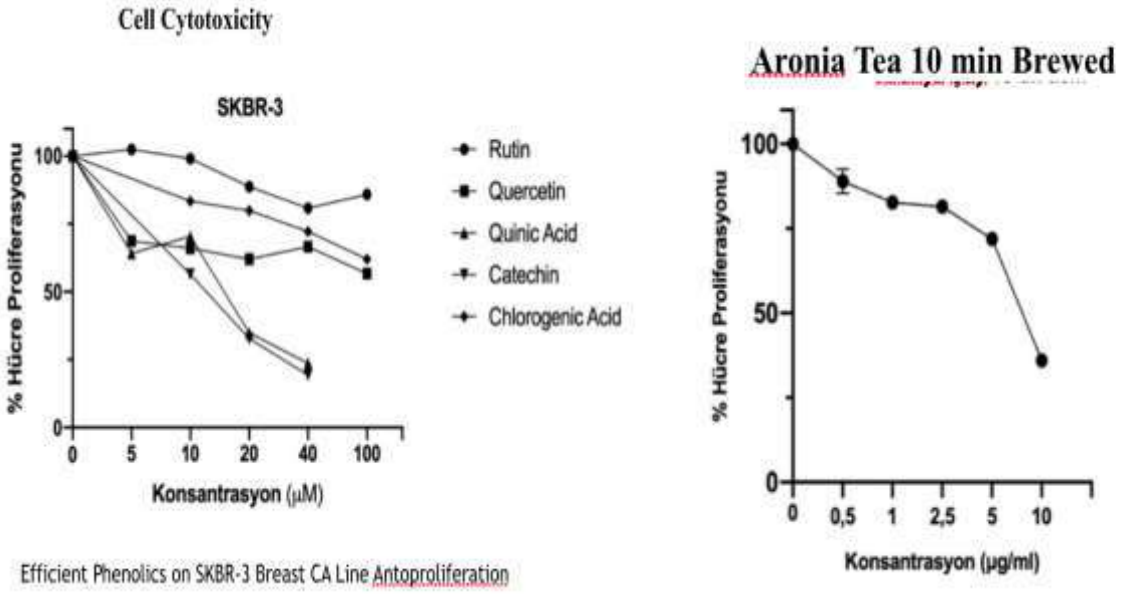

Figure 2. SKBR-3 Cell Cytotoxicity Levels of Aronia Phenolics and Antiproliferation Statue of Aronia Tea Brewed at 10 min

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BREEDING CLIMATE RESILIENT POTATO CULTIVARS USING CONVENTIONAL AND NEXT GENERATION BREEDING TECHNIQUES

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ABSTRACT

Potato is a cool season crop with an optimal growth temperature between 17 and 21 °C, and it is very sensitive to heat and drought stress. All climate scenarios indicate that the global climate is changing and will continue to change in the near future. The main challenges from climate change to agriculture and food production are the more frequent and severe drought and floods as well as adverse effects of high growth temperatures. Climate change will also bring other problems such as salinity, frost, epidemics of new diseases and pests. The total global yield in the regions currently cropped with potato was calculated to decline up to 32% without adaptation to climate change. The breeding of heat and drought tolerant potato cultivars is one of the most feasible and practical approaches to cope with global warming. However, breeders are generally focused on development of heat or drought tolerant potato cultivars instead of dual tolerance to both stresses. Previous studies indicate that tolerance mechanism for heat and drought is different in potato. Screening of many breeding lines against heat and drought stress under field conditions during early generations is not feasible for many breeding programs due to high cost and labor requirements. Therefore, rapid and reliable screening methods are needed to evaluate large populations in early generations. Biotechnological tools and -omics technologies offer some advantages to breeders for screening large populations especially against biological stress factors, but no sound achievements obtained for abiotic stress factors in potato up to now. Therefore, inclusion of these nest generation tools to breeding program is also essential to accelerate breeding process of climate resilient potato cultivars. Currently our research group has several projects to develop novel screening tools to identify heat and drought tolerant genotypes. Main achievements and outputs of our studies will be presented and discussed in this presentation.

Key words : Potato, plant breeding, stress tolerance, climate change, selection

INTRODUCTION

Potato is domesticated in the Andes of South America between Peru and Bolivia around 8-10 thousand years ago, and it became a world crop after the first introduction to Europe in 1567 by Spanish. Nowadays it is the fourth most produced food crop and the first non-cereal crop in the world with an annual production of over 370 million tons on 17,3 million ha area (Anonymous, 2020). The majority of the annual world potato production is contributed by the developing countries, where it is cultivated in marginal areas prone to environmental fluctuations. Potato yields vary among countries between 9.2 to 62.3 t/ha, with developing countries obtaining the lowest potato yields (Anonymous, 2020). Although the average annual potato production has increased in the last two decades, this was mainly due to an increase in the cultivated area, whereas the average yield rates remained nearly stable in developing countries (Walker et al., 2011). Potato is a staple food with its potential in fighting against

malnutrition in both developed and developing countries since potato tubers are known sources of carbohydrates, proteins, vitamins and minerals (Çalışkan et al., 2010). Besides being a highly nutritious food, potato is considered as one of the most promising crops to reduce human hunger and poverty in the world due to its high yield potential as reflected by a very high Harvesting Index value above 75% (Scott et al., 2000; Thiele et al., 2010). For these reasons, sustainable potato production is crucial for food security and social sustainability in the future under ever-changing environmental conditions.

The potato is a cool season crop with an optimal growth temperature between 17 and 21 ºC (Struik and Ewing, 1995; Levy and Veilleux, 2007). The higher temperatures than the optimum significantly affect several physiological processes related with yield and quality such as haulm growth, dry matter production and partitioning, tuber initiation and growth, photosynthetic rate, synthesis of hormones, enzymes and other metabolites (Levy and Veilleux, 2007). Although potato shows a higher harvest index than other major crops, and exhibits a higher energy productivity than other crops (Renault and Wallender, 2000), it is more sensitive to drought stress due to its shallow root system (Yuan et al., 2003). Limited water supplies lead to potato production losses in areas with inconsistent rainfall or poor irrigation (Evers et al., 2010; Thiele et al., 2010). Due to global climate change, the frequency and severity of drought conditions are expected to worsen in the following decades, leading to reductions in potato production (Hijmans, 2003). The loss in total potato yield is expected to reach up to 32% by 2050 (Hijmans, 2003). Drought could also affect the nutritional quality of tubers by reducing dry matter and accumulation of nutritional compounds (Haverkort and Verhagen, 2008). Yield losses combined with low crop quality could drastically affect the economic output and the overall human food supply (Simelton et al., 2012). As potato production is mainly done based on irrigation or rainfall in Turkey and Poland, any water shortages in near future puts the potato production at high risk in both countries. Therefore, it is necessary to develop new climate resilient potato cultivars to sustain potato production in future.

BREEDING CHALLENGES IN POTATO

New potato varieties with high yield and quality traits are mainly achieved by breeding programs. Three main characteristics of potato, which are vegetative propagation, heterozygous genetic structure of cultivars and tetrasomic inheritance, make potato breeding unique and different from many other crops. Although potato has "true" seeds within the berries formed after sexual reproduction, it is commercially propagated by asexually using tubers. The new genotype is fixed in the first generation after sexual hybridization of two parents. All seedlings that arise from the germination of hybrid true potato seeds characterize an entirely distinctive individual having entirely new combination of genes due to heterozygote structure of parents. Due to the segregation occurring in F_1 generation, breeders should start considerably higher number of hybrid true seeds at the beginning of breeding program.

Being a tetraploid plant, generation of new potato varieties having the desired traits can take about 10-15 years in classical breeding approach. At the level of tetraploid, a locus is to be characterized by up to four divergent alleles of a gene, whereas in the case of diploid, a maximum of two different alleles. Hence, the segregation of traits becomes much more complex in tetraploids than in diploids. Therefore, using lab-based screening techniques for

selection of breeding lines with proposed characteristics is important to get reliable and faster results.

BREEDING STRATEGIES FOR CLIMATE RESILIENT CULTIVARS

It is expected that climate change will make potato production more challenging in future due to aforementioned effects of drought and heat stress on potatoes. Hence the necessity of climate resilient potato cultivars are growing in all parts of the world. In addition, climate change will also create other problems such as salinity, frost, epidemics of new diseases and pests. However, breeders generally focus on improve only one of abiotic or biotic threats such while we will need climate resilient cultivars indeed. Especially dual tolerance to heat and drought stresses will be very important since plants will generally face with both stresses simultaneously with changing climate in future (Caliskan, 2016). Where heat and drought stress occur simultaneously, they generally result in more extreme detrimental effects than would each stress separately (Lipiec et al, 2013). Combined effect can also alter plant metabolism in novel ways compared to each applied individually. Since potatoes are grown mainly in Central Anatolia, where the lowest annual rainfall occur in Turkey, irrigation up to 20 times per season were applied for satisfactory yield (Figure 1). This irrigation practice also results in decrease of water reservoirs, and threats the sustainability of whole agricultural production in the Central Anatolia. Therefore climate resilient potato cultivars are also very important for Turkey.

There are two prerequisites for success of a potato breeding program aims to development of heat and drought tolerant cultivar: choosing the most appropriate parents, and using of the reliable screening methods in early generations (Hijmans, 2003; Levy and Veilleux, 2007). User friendly and cost-efficient breeding tools are also essential to apply more precise and accelerated selection scheme. It is possible to find useful germplasm for these traits (Caliskan, 2016). However direct selection for drought and heat tolerance by assessing performance and yield is very complex and time-consuming, as it requires trials on managed field sites either in areas with frequent droughts or under expensive rain-out.

Although plant breeding defined as "*Plant breeding is the art and the science of improving the heredity of plants for the benefit of humankind*" (Poehlman and Sleper, 1995), it has been becoming more science year by year (Figure 2). With these developments in genetics, molecular biology, and their implementation in to plant breeding, a new concept "next generation breeding" is emerged in recent years (Zia et al. 2017). Therefore, a successful potato breeding program should benefit all innovative tools to improve the efficiency of breeding program. Phenomics and genomics are two important and trendy tools in developing stress tolerant cultivars (Caliskan, 2016). A high-throughput phenotyping is a key step to identify individuals tolerant to targeted stress factors (Ghanem et al, 2015). Screening of individuals for morphological (i.e. number and size of leaves, plant height, root size, etc.), physiological (i.e. chlorophyll content, carbon exchange rate, transpiration rate, etc.), and biochemical (i.e. stress enzymes, plant hormones, etc.) traits can give very useful information to define and ideotype under stressful condition (Ghanem et al, 2015). In recent years, imaging and monitoring systems have been used as a high-throughput phenotyping platform especially against stress factors (Araus and Cairns, 2014). These platforms generally use sensors, robotics, aeronautics, and high-performance computing tools, but more practical and low-cost approaches are also needed (Araus and Cairns, 2015; Ghanem et al, 2015).

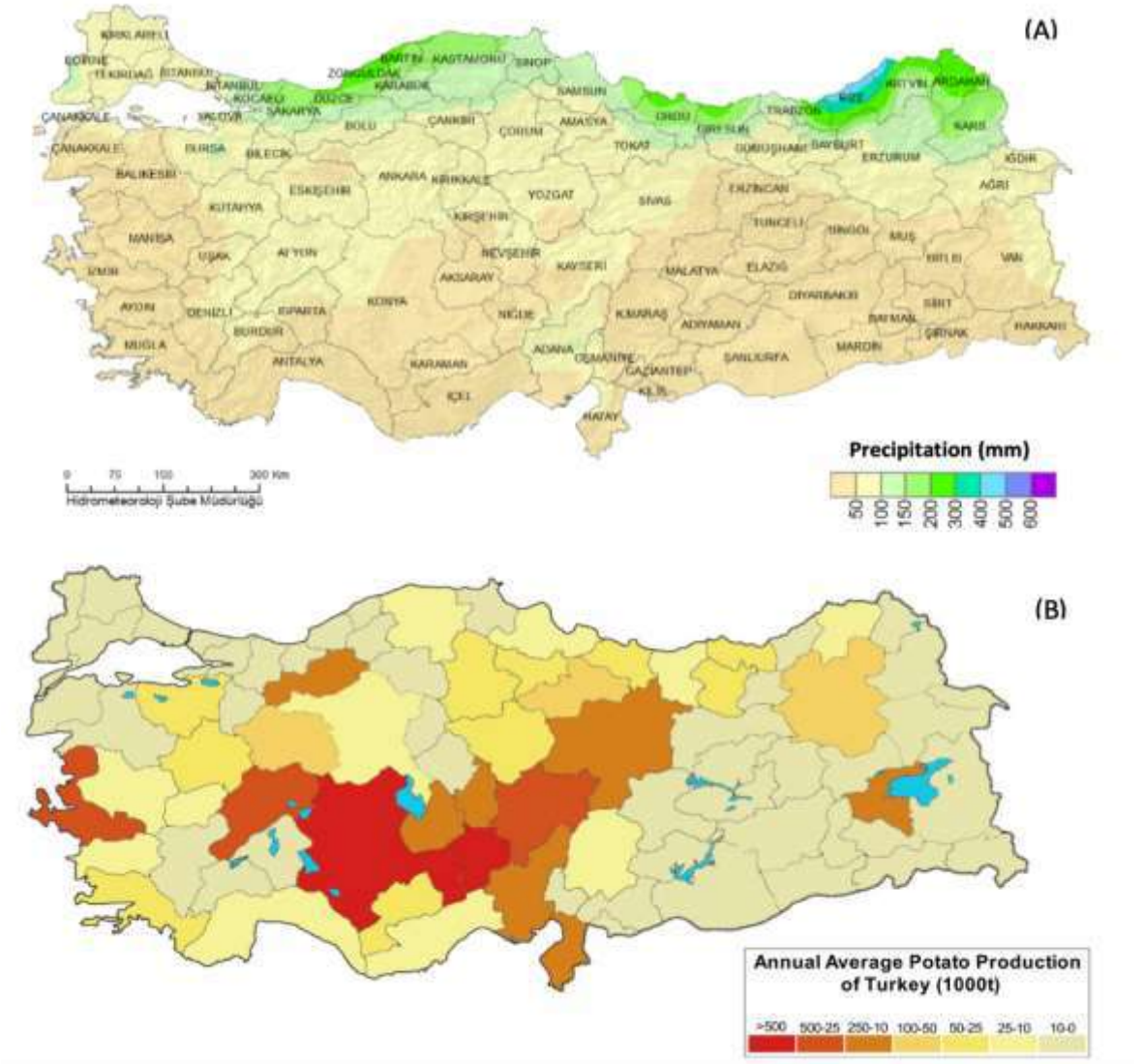

Figure 1. Average annual rainfall (A), and main potato production areas (B) in Turkey.

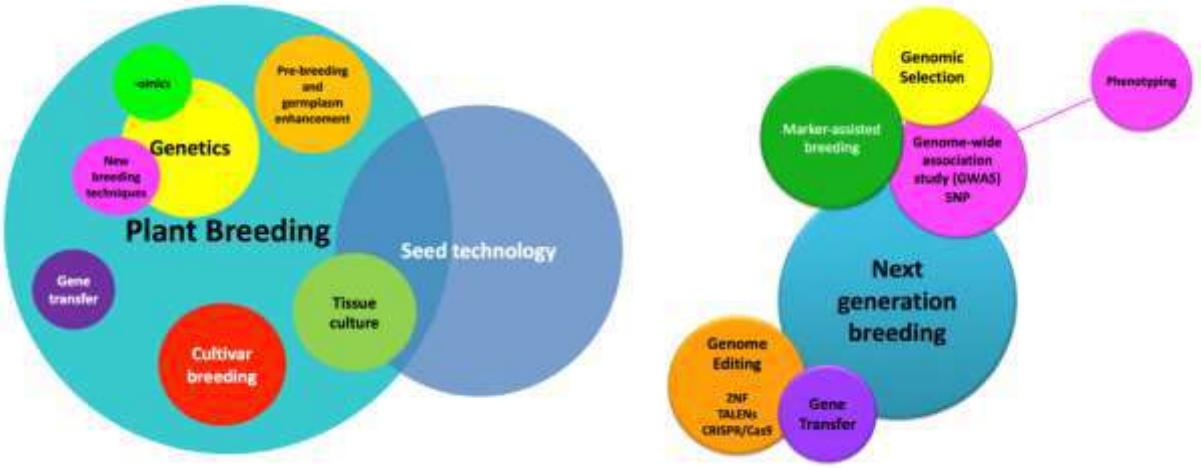

Figure 2. Plant breeding has becoming more science than art year by year.

Our Potato Research Group at Nigde Omer Halisdemir University (NOHU) has been conducting several projects on understanding the tolerance mechanism of heat and/or drought tolerance as well as development of reliable selection tools and heat and/or drought tolerant genotypes in potato. The first project started with assessment effects of heat stress on potato genotypes, determination of morpho-physiological traits for selection of heat-tolerant potato genotypes and selection of heat tolerant breeding lines using determined selection criteria. At the end of the project, we determined that high haulm dry weight, leaf area index, chlorophyll index (SPAD), photosynthetic rate, and low canopy temperature values are highly associated with heat tolerance in potato (Demirel et al. 2017). We also developed 11 potato breeding lines having tolerance to heat stress.

The second project was initiation of a breeding program to develop drought tolerant potato cultivars and development of molecular markers to identify drought tolerant genotypes (Çalışkan et al. 2020). The project was consisted of three consecutive steps: (i) association mapping of drought related traits using phenotyping 240 diverse potato genotypes under normal and deficit (%35) irrigation in two locations, (ii) selection of associated single nucleotide polymorphism (SNP) markers associated with drought-tolerance related traits, and (iii) validation of selected markers in a breeding population created by crossing in the project. The general framework of the project are illustrated in Figure 3. By this project, 419 novel SNP's linked to drought-tolerant traits were determined, and then 16 KASP markers were designed to validate the most promising SNP's. The 16 KASP markers were tested using a different population having 50 genotypes, and 8 KASP markers were selected to identify high-yielding genotypes under drought conditions. Breeding lines developed during project were screened with 8 KASP markers to identify drought-tolerant lines, and 1054 breeding lines having drought-tolerance and high yield potential were selected for further evaluation.

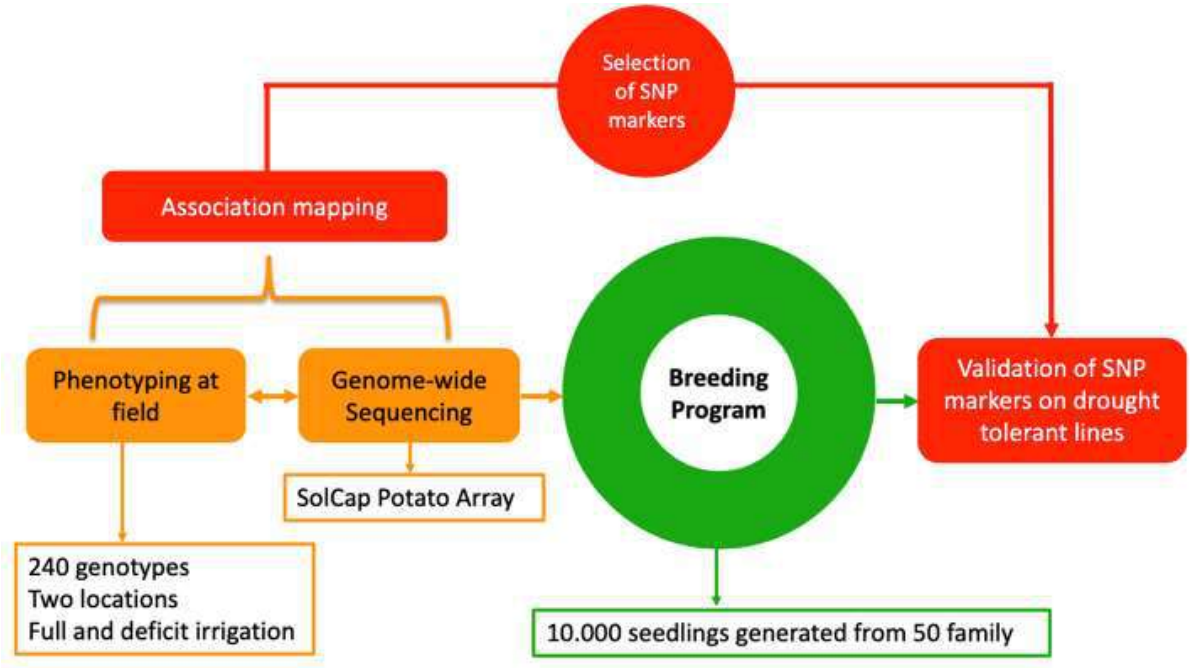

Figure 3. The general framework of the project to develop drought tolerant potato breeding lines and molecular markers related with drought tolerance in potato.

The third project was on the investigation of physiological traits and mechanisms for resilience to multiple abiotic stress in potato with collaboration with The James Hutton Institute of Scotland. We identified unique changes not only in morphology of potato cultivars, but also in transcripts and metabolites that were specific to individual and combined heat and drought stresses (Figure 3). Our experiments indicate that stress tolerant cultivars respond to stress by (i) reducing light harvesting capacity and increasing non-photochemical quenching and (ii) maintaining capacity for growth and development in part by (iii) rerouting metabolism to compensate for reduced photosynthesis (Demirel et al. 2020).

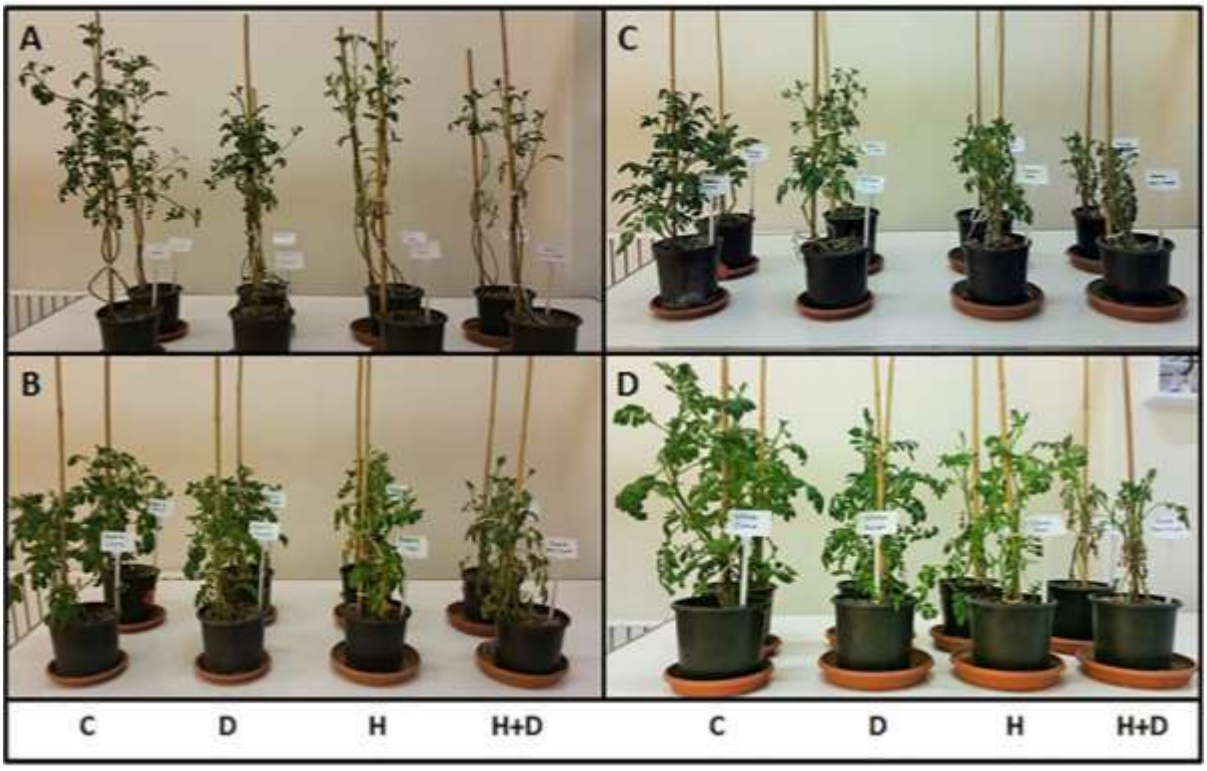

Figure 3. Above ground phenotype of two drought and heat sensitive (A, B) and two drought and heat tolerant (C, D) potato varieties after 12 days of abiotic stress treatment. A, Agria; B, Russet Burbank; C, Desiree; D, Unica. Treatments were control (C), drought (D), heat (H), and heat with drought (H+D) (Demirel et al. 2020).

CONCLUSION

It is clear that climate change threats the sustainability of potato production in most regions throughout the world including Turkey. Therefore, potato breeders should consider to develop climate resilient potato cultivars to adapt upcoming changing environment. However, a robust and cost-efficient cultivar breeding programs are needed to not only to overcome combined effects of different stresses, but also for practical consideration. Inclusion of phenomics and genomics tools to breeding program is very useful to accelerate breeding process of climate resilient potato cultivars.

After completing three projects on heat and/or drought tolerance in potato, we derived several conclusion as follows:

- Responses of potato genotypes to drought and heat stress were considerably different
- Some genotypes had better performances for total tuber yield under drought and heat stressed conditions
- High SPAD and LAI values along with low CT can be good indicator of high productivity under water stress
- Selection of genotypes having dark green leaves with high LAI and high yielding genotypes under well-watered conditions can be useful strategy to develop drought-adapted potato cultivars.
- Molecular markers for correlated traits may be useful
- High yielding genotypes under non stress conditions tended to give high yield under drought and/or heat stress conditions

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THE AGRICULTURAL PRODUCTION AND ANIMAL HUSBANDRY IN TRAKYA REGION BASED ON THE FIRST AGRICULTURAL CENSUS DATA OF THE REPUBLIC OF TURKEY

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ABSTRACT

One of the most important dynamics of Turkey's development is agriculture and animal husbandry. For this reason, in the early years of the Republic, industrialization was in the industrial sector based on agriculture and the first sugar and agricultural tools factories were established in this period. The Republic made its first agricultural census in 1927. Thereafter, no census was made until 1950, after which an agricultural census took place every ten years. These censuses contain important data to determine the socio-economic development of the country. These data include farmer families, cultivated areas, production amount and value, and the number of animals and agricultural tools. Turkey, despite being an agricultural country, while 32% of the arable land in the country in 1927, was only able to farming 4,86's. During the foundation years of the Republic, the state has divided the country's geography into 9 regions in accordance with its agricultural policies. Trakya is located in the 3rd district covering the majority of the Marmara region. In this study, based on the 1927 Census of Agriculture (Edirne, Kırklareli, Tekirdağ), we will examine the agricultural data of Trakya and the income status of the farmers. In this context, data on farmers' population, cereals, pulses and seed production in Edirne, Kırklareli and Tekirdağ provinces will be presented. In addition, the number of agricultural tools and machines used during production will be given and the basic structure of agriculture will be emphasized. In addition, the number of animals used in farming and the number of livestock and poultry will be given. Thus, the socio-economic structure of the Trakya farmers will be revealed by using the data on the Trakya agriculture and animal husbandry during the foundation years of the Republic.

Keywords: Turkey Republic, Agriculture and Livestock, Agriculture Census, Trakya, Farming

INTRODUCTION

Agriculture data in transition from Ottoman Empire to Republic of Turkey

In the last period of the Ottoman Empire, the 75-80% of the country's population was engaged in agriculture and animal husbandry. However, since mechanization could not be achieved in agriculture and classical animal husbandry methods were used, the efficiency in agriculture and animal husbandry was very low (Yazan, 2018). Therefore, the only 32% of the country's tax revenues were derived from agriculture and animal husbandry (Güran, 2003). Although the last period Ottoman governments imported and distributed agricultural machinery

to farmers in addition to infrastructure applications in order to increase agricultural income, despite the partial increase in production, it was not possible to achieve the basic targets in agricultural production (Erdogan, 2019).

The first official statistics on the last period of the Ottoman Empire belong to the year 1897. The Ottoman State had developed separate tax systems according to the classical and modern periods in order to determine its income. The "Tahir Notebooks" in the classical period and "Temettuat Books" in the modern period cover information about population, production and income sources, then, acccording to the records in these books; country "Population, Agriculture and Livestock" data were emerging (Öz, 2010). Based on these data of 1897; the Turkey land used for agricultural production in 21 provinces across the country is 53,801,141 decares. In the same period, the Turkey number of animals was 24,851,791 (Güran, 2003). Between 1878-1913, the state had to import an average of 75,000 tons of flour, 65,000 tons of rice and 10,000 tons of wheat each year. On the one hand, the Ottoman Empire who had difficulty in paying their debts to the General Düyun-ı Umumiye, paid 12,000,000 gold liras every year due to their agricultural needs for importing (Yavuz and Caglayan, 2005).

In this period, when compared with Europe, the number of machinery used in agricultural production in the Ottoman Empire was almost negligible. Agriculture; Except for a small number of agricultural establishments in the Aegean, Eastern Mediterranean and Çukurova, it was mostly carried out by classical methods and using simple agricultural tools throughout the country (Baskıcı, 2003). This situation had also spread from the Ottoman to the Republic. Moreover, Turkish agriculture was negatively affected by the lack of human resources brought by the war years (1914-1918) during the transition period, a significant part of the agricultural land was left empty, there was a decrease in production and livestock breeding as well as agriculture. Industry has also been adversely affected by the developments in the war years. This situation continued during the Armistice and the War of Independence, 1920, 1921 and 1922 were the years when agricultural production was the lowest. Being aware of this situation, the governments of the Republic, on the one hand, took measures to improve Turkish agriculture (Eldem, 1994). On the other hand, they started the industrialization move based on agriculture. Agriculture was among the topics discussed in the 1923 Izmir Economic Congress. Here, the abolition of the Ashar tax was brought to the agenda, emphasis was placed on the provision of vitality in the village and the mechanization of agriculture. After the Economic Congress, legislative changes and practices regarding the villagers came one after another, the Village Law was enacted in 1924, and the Ashar tax was abolished in 1925; Land was distributed to landless peasants, provided that the cost of land was paid in twenty years for the purpose of landing the villagers, Ziraat Bank provided credit facilities to small farmers, cooperatives were encouraged, and cheap agricultural equipment and machinery were distributed to the farmers in order to encourage and encourage modern agriculture, and sample farms were opened (Gülçubuk, 2005). For the same purpose, the first sugar factories were established, the workshops producing agricultural machinery increased and the textile industry was encouraged (Kaştan, 2007). As a result, a relative increase has been achieved in Agriculture and Agro-based industry. In 1927, 66% of the raw materials used in industrial production belong to agriculture and 17% to textile (İlkin, 1971).

The Republic made the first agricultural census inspired by the Ottoman Empire in 1927. Considering the conditions of the period, it is understood that the 1927 Agricultural Census was conducted according to very modern methods (Saçlı, 2009). In today's modern agriculture statistics; Data such as "land use, enterprise structures, crop production, livestock and animal production, agricultural machinery, fertilizer and pesticide use" are taken as basis. Based on these data, governments determine their future agriculture policies, shape their support to agriculture, as well as imports and exports according to the country's food need and

surplus, or give incentives to increase production. When the 1927 agricultural census data are examined, it is seen that it was made by taking these principles and data into account with little differences. There is a great similarity between the 1927 Agricultural Census data and the 1993 Agriculture data in classification (Cillov, 1971). The number of provinces to be taken into account in agriculture data increased only due to the establishment of new provinces, and there was no change in the classification of Agricultural Regions.

Table 1: The classification agricultural regions used in agricultural statistics (1927-1993)

Regions	**1927**	**1993**
	Provinces	**Provinces**
I.	Eskişehir, Ankara, Bilecik, Yozgat, Bolu, Çankırı, Çorum, Kırşehir, Kütahya	Ankara, Bilecik, Bolu, Çankırı, Çorum, Eskişehir, **Kırıkkale**, Kırşehir, Kütahya, **Uşak**, Yozgat
II.	İzmir, Isparta, Aydın, Balıkesir, Burdur, Çanakkale, Denizli, Manisa, Muğla	Aydın, Balıkesir, Burdur, Çanakkale, Denizli, Isparta, İzmir, Manisa, Muğla
III.	Bursa, Edirne, İstanbul, Kırklareli, Kocaeli, Tekirdağ	Bursa, Edirne, İstanbul, Kırklareli, Kocaeli, **Sakarya**, Tekirdağ
IV.	Adana, Antalya, Gaziantep, **İçel, Mersin**, Maraş, **Cebeli Bereket**	Adana, Antalya, Gaziantep, **Hatay**, İçel, Kahramanmaraş
V.	Artvin, Erzurum, Erzincan, **Doğu Beyazıt (Ağrı)**, Kars	Ağrı, **Ardahan**, Artvin, Erzincan, Erzurum, **Iğdır**, Kars
VI.	Urfa, Bitlis, Diyarbakır, Hakkâri, Mardin, Siirt, Van	**Batman**, **Bingöl**, Bitlis, Hakkâri, Mardin, **Muş**, Siirt, Şanlıurfa, **Şırnak**, Van, Diyarbakır,
VII.	Trabzon, Ordu, Rize, Samsun, Sinop, Zonguldak, Giresun, Gümüşhane, Kastamonu	**Bartın**, **Bayburt**, Giresun, Gümüşhane, Kastamonu, Ordu, Rize, Samsun, Sinop, Trabzon, Zonguldak
VIII.	Amasya, Elazığ, Malatya, Sivas, Tokat, **Şebinkarahisar**	**Adıyaman**, Amasya, Elazığ, Malatya, Sivas, Tokat, Tunceli
IX.	Afyonkarahisar, Aksaray, Kayseri, Konya, Niğde	Afyonkarahisar, Aksaray, **Karaman**, Kayseri, Konya, Nevşehir, Niğde
Province number	63	76

Source: Turkish Agricultural Statistics

Figure 1: Agricultural regions in Turkey in 1927

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

Agriculture in Trakya region based 1927 Agricultural Statistics

1. Farmer population, farmer family number, Animal and land amount per family

According to the 1927 census; The population of Turkey is 13,517,385 and 391,497 of them live in Trakya region. The rate of Trakya population to the general population is 2.89%. The number of households in Trakya is 80.160, including Edirne: 31.910, Tekirdağ: 26.685 and Kırklareli: 21.565 (Miran, 2005). In this census, 47.79% of the population of Edirne, 51.54% of the population of Tekirdağ and 56.09% of the population of Kırklareli, and 51.80% of the average of Trakya identified themselves as having a profession (Table 2).

Table 1: The people related to Agriculture and farmer family population

Provinces	Population	Farmer population	Rate (%)	Farmer family number	The average person per family
Edirne	150.889	88.918	58,92	17.578	5,0
Tekirdağ	132.122	100.760	76.26	18.368	5,4
Kırklareli	108.486	86.108	79,37	15.910	5,4
Trakya	**391.497**	**275.786**	**71,51**	**51.856**	**5,2**
III. region	1.897.395	873.060	46,01	168.194	5,3
Turkey	13.517.385	9.145.008	67,65	1.751.239	5,2

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

The amount of land per household in the Trakya is 50.43 acres and its own zone (31.4) and Turkey average (22.32) is over. These amounts are 53.4 in Edirne; 63.7 in Tekirdağ and 34.2 in Kırklareli. While the amount of arable land per household in Edirne and Tekirdağ is above the Trakya average, but below in Kırklareli. The number of pairs of animals per household for each farmer family to cultivate their land is 1.47 in Edirne; 1.97 in Tekirdağ and 1.38 in Kırklareli (Table 3). Only Tekirdağ was able to get above the Trakya average, while the other two provinces remained below. According to these data, some farmer families do not even have a pair of animals to cultivate their land. This situation is due to the low number of animals distributed to the immigrants who came to Trakya in 1923-1927. Although the amount of land distributed in the villages vacated due to the Greeks who left Trakya is large, the number of animals to cultivate the land is small. The situation is different in the number of other animals. The falling number of animals to families in all three provinces and districts is above the average in Turkey.

Table 3: Farmer family number, Animal and land amount per family

Provinces	Farmer family number	The land amount per family (Da)	The number of farmer animal per family	The number of other animals per family (*)
Edirne	17.578	53,4	1,47	18,74
Tekirdağ	18.368	63,7	1,97	16,99
Kırklareli	15.910	34,2	1,38	19,86
Trakya	**51.856**	**50,43**	**1,60**	**18,53**
III. Region	168.194	31,4	1,86	12,65
Turkey	1.751.239	24,9	1,96	14,50

(*) Except poultry; *Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927*

2. The distribution of crops in the cultivated land

In the 1927 Agricultural Census, agricultural products cultivated in Trakya were grouped into three groups as cereals, pulses and industrial crops. The amount of pasture land in Trakya is 2,654,321 decares. In the majority of this land (92.93%) is cereals and the remaining part is reserved for legumes (1.4%) and industrial crops (5.7%). The average cereals in Trakya is above the average of Turkey and 3rd region but it is under in pulses and industrial crops (Table 4). The distribution of the planting areas, production and income per area of crops were given in tables based on provinces, 3rd region and Turkey below (Table 5, 6, 7 and 8)

Table 4: The distribution of crops in the cultivated land (da)

Provinces	**Cereals**	**Rate %**	**Pulses**	**Rate %**	**Industrial crops**	**Rate %**	**Turkey**
Edirne	832.834	88,6	20.432	2,2	86.038	9,2	939.299
Tekirdağ	1.142.924	97,6	3.568	0,3	24.577	2,1	1.171.029
Kırklareli	503.934	92,6	9.086	1,7	30.973	5,7	543.993
Trakya	**2.479.692**	**92,93**	**33.086**	**1,4**	**141.588**	**5,6**	**2.654.321**
III. region	4.741.789	89,8	160.624	3,0	380.523	7,2	5.282.936
Turkey	39.093.220	89,5	1.740.403	3,9	2.804.403	6,1	43.637.727

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

Table 5: Crop production (P) (Kg), The Values (V) (Lira), Seed yield (Da/Kg), Total income per famer family (Lira)

Crops		**Provinces**			**Trakya**	**III. region**	**Turkey**
		Edirne	**Tekirdağ**	**Kırklareli**			
Cereals	**P**	61.405.214	71.768.877	48.215.669	**181.389.760**	356.044.262	2.400.262.796
	V	4.752.337	6.378.523	3.483.146	**14.614.006**	33.401.518	234.508.943
	Kg/da	73,7	62,8	95.7	**77,4**	75,1	61,4
Pulses	**P**	1.290.273	326.887	564.106	**2.181.266**	11.324.111	100.222.773
	V	127.094	44.387	67.655	**239.136**	1.312.630	10.638.931
	Kg/da	63,1	91,6	62,1	**72,26**	70,5	57,6
Indus-	**P**	5.927.623	6.825.504	10.328.022	**23.081.149**	42.317.106	163.417.712
trial	**V**	854.346	752.508	391.617	**1.998.471**	11.853.130	91.784.833
crops	**Kg/da**	68,9	278,2	333,5	**226,8**	111,2	58,3
Total Income		5.733.777	7.175.418	3.942.418	**16.851.613**	46.607.278	336.932.707
Total Income per family		326,1	390,7	248,5	**321,76**	277,1	192,4

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

Table 6: Total cereal production and planted area (Da-Kg)

Crops	Da/ Kg	Provinces			Trakya	Turkey
		Edirne	Tekirdağ	Kırklareli		Turkey
Wheat	Da	318.093	746.605	154.203	**1.218.901**	22.383.714
	Kg	25.447.522	30.492.539	15.272.806	**71.212.867**	1.333.150.811
Barley	Da	198.701	116.399	119.912	**435.992**	10.103.598
	Kg	17.208.681	18.068.796	12.381.836	**47.659.313**	629.280.734
Oat	Da	18.742	74.788	34.947	**128.477**	1. 045.976
	Kg	976.285	6.231.360	2.781.875	**9.989.520**	59.187.862.
Rye	Da	147.800	46.946	106.021	**300.768**	1.746.638
	Kg	9.252.641	4.641.176	10.595.316	**24.489.133**	101.056.766
Einkorn	Da	16.769	77.027	16.819	**110.615**	834.737
	Kg	1.295.422	5.547.785	1.670.081	**8.513.288**	59.964.251
Rice	Da	1.309	202	1.101	**2.612**	110.282
	Kg	115.170	36.550	162.162	**313.882**	10.076.999
Sorghum	Da	8.783	3.531	2.418	**14.732**	1.119.140
	Kg	471.457	81.228	112.023	**664.708**	78.122.910
Corn	Da	122.637	77.426	68.513	**268.576**	1.749.135
	Kg	6.638.036	6.669.443	5.239.570	**18.547.049**	129.557.463

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

Table 7: Total pulse production and planted area (Da-Kg)

Provinces	Da / kg	Edirne	Tekirdağ	Kırklareli	Trakya	III. region	Turkey
Fava bean	Da	279	943	1.550	**2.772**	38.219	213.719
	Kg	49.581	103.887	122.956	**276.424**	4.712.137	19.656.346
Pea	Da	33	28	7	**68**	1.343	11.933
	Kg	4.194	8.913	703	**13.810**	114.165	478.580
Chickpea	Da	4.140	418	236	**4.794**	12.791	280.953
	Kg	384.825	49.445	27.736	**462.006**	847.374	12.271.747
Bean	Da	3.594	857	1.951	**6.402**	46.905	274.627
	Kg	93.044	46.842	78.640	**218.526**	1.478.399	11.633.062
Kidney bean	Da	693	21	570	**1.284**	2.434	44.867
	Kg	40.943	774	34.851	**76.568**	152.825	2.624.440
Lentil	Da	636	370	1.090	**2.096**	3.116	104.597
	Kg	35.485	24.810	14.464	**74.759**	111.959	4.614.542
Pumkin	Da	515	-	153	**668**	1.544	134.816
	Kg	34.464	-	13.643	**48.107**	102.293	9.638.538
Bitter vetch	Da	10.542	931	3.529	**15.002**	54.272	674.894
	Kg	644.737	92.216	271.113	**1.008.066**	3.814.959	39.305.518

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

Table 8: Total industrial crop production and planted area (Da-Kg)

Provinces	D-K	Edirne	Tekirdağ	Kırklareli	Trakya	III. region	Turkey
Potato	Da	431	11	558	**1.000**	19.785	135.803
	Kg	68.397	988	265.778	**335.173**	6.272.993	20.738.756
Beet	Da	9.459	6.114	10.374	**25.947**	26.532	86.776
	Kg	3.698.870	4.342.084	9.346.825	**17.387.779**	17.614.432	23.935.398
Onion	Da	2.642	2.448	1.250	**6.340**	19.238	94.390
	Kg	207.281	1.327.502	125.591	**1.660.374**	5.261.277	14.853.550
Garlic	Da	126	480	19	**625**	2.538	9.269
	Kg	11.611	136.878	3.029	**151.518**	363.627	944.421
Saffron	Da	17	-	-	**17**	50	767
	Kg	48	-	-	**48**	254	30.893
Cotton	Da	2.671	3.258	137	**6.066**	8.559	991.279
	Kg	43.364	362.934	3.520	**409.818**	456.906	38.905.066
Linseed	Da	2.961	3.255	183	**6.399**	24.879	65.343
	Kg	105.729	121.597	58.889	**177.215**	1.398.188	2.930.809
Sesame	Da	57.099	2.762	5.814	**65.675**	76.429	460.050
	Kg	944.459	50.628	89.857	**1.084.944**	1.301.953	10.961.169
Tobaco	Da	10.562	6.148	12.095	**28.805**	196.126	790.765
	Kg	846.608	432.054	383.287	**1.661.949**	9.535.371	47.531.635
Poppy	Da	65	61	543	**669**	6.387	169.662
	Kg	24	119	18	**161**	4.958	112.257
Total Seed kg		1.232	720	51.228	**53.180**	107.152	2.473.758

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

3. The Agricultural mechanization and animal power in agricultural production

According to the 1927 Census of Agriculture, there is 1,187,004 wooden plow (karasaban) in Turkey, and only 15 711 210 794 plows farming machinery is available. All of them are 1.413.509. According to the data, there is 1,751,231 family farmers in Turkey at that times, it does not have even one wooden plow per household version. The number of plows and plows per farmer family corresponds to 0.80 on average (Table 9). There are slight differences between regions. Almost all agricultural machinery equipments are in I. (Eskişehir, Ankara, Bilecik, Yozgat, Bolu, Çankırı, Çorum, Kırşehir, Kütahya) and IV. (Adana, Antalya, Gaziantep, İçel, Mersin, Maraş, Cebeli Bereket / Osmaniye) regions. The amount of agricultural machinery in other regions is almost negligible. Based on these numbers, it is clearly seen that even the use of plows by means of farm animals was very insufficient in Turkish agriculture during this period, sowing and planting was generally performed with wooden plow, and the mechanization was almost nonexistent.

The use of agricultural tools in Trakya region is not very different from Turkey. Although the number of falling agricultural tool as 1.25 in Trakya region to each farmer is more thank Turkey, the amount of land committed per household is more, the use per hectare of agricultural equipment remains below the average of Turkey except Kırklareli province (Table 10).

Table 9: The number and rates of agricultural tools and machines

Provinces	The total number of agricultural tools and machines	The number of agricultural machines (*)	The number of agricultural tools	The number per family	Number / Ha
Edirne	22.555	170	22.385	1,28	0,2
Tekirdağ	23.459	201	23.258	1,27	0,2
Kırklareli	19.184	94	19.090	1,20	0,3
Trakya	**65.198**	**465**	**64.733**	**1,25**	**0,23**
III. region	169.609	1.089	169.520	1,00	0,3
Turkey	1.413.509	15.711	1.412.798	0,80	0,3

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

Table 10: The number of animal power in agricultural production

Provinces	Ox	Water buffalo	Horse	Mule	Total	The number per family
Edirne	21.137	2.629	1867	218	25.851	1,47
Tekirdağ	27.604	4.195	3.994	267	36.060	1,96
Kırklareli	17.873	2.332	1.879	369	21.953	1,37
Trakya	**66.614**	**9.166**	**7.740**	**854**	**83.864**	**1,61**
III. region	204.193	74.826	30.214	3.732	312.965	1,86
Turkey	2.616.089	340.438	314.374	43.624	3.314.525	1,89

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

4. Animal production

In the transition from the Ottoman to the Republic, there was a significant decrease in animal husbandry (Anonymus, 2015). The main reason for this is the massacres caused by wars and losses in the country's territory, the decrease in the rangelands and epidemics. Due to all these reasons, the number of animals decreased by 58% during this period (Gül, 2015). The Republican administrators who wanted to prevent this decline had forbidden the slaughter of animals suitable for breeding and breeding and also developed the legislation to protect animal health. In addition, animal farms were established by the state to increase animal production and feed support was provided to farmers (Temel, 2010). As a result of the support given by the Republic to animal husbandry, gradual increases were observed. As a result, in 1927 the number of bovine animals reached 6,120,207 and the number of ovine animals reached 19,592 (Table 11). However, animal husbandry was done in the form of pasture fattening on the basis of pasture and winter, except for state farms and some businesses.

The number of livestock (Bovine-Ovine) in Trakya is 957,590, excluding those used for power. 738,548 of them are ovine and 218,742 bovine animals. There is no significant difference in the distribution of these in Trakya provinces. The number of animals in Trakya, Turkey constitutes 3,72's% of the general. However, the income from animal production is so higher when comparing to Turkey average (Table 11). Similarly, the poultry amounts is also higher in Trakya region when comparing with Turkey (Table 12).

Table 11: The values and the number of animal used in as power and animal production (*)

Provinces	Cattles	Sheep and goat	Animal power	Total	Rate %	The value of animals
Edirne	77.360	251.839	25.851	355.350	0,60	10.363.423
Tekirdağ	62.884	249.204	36.060	348.148	0,30	9.816.390
Kırklareli	78.498	237.505	21.953	337.956	0,40	9.678.920
Trakya	**218.742**	**738.548**	**83.864**	**1.041.454**	**0,43**	**29.858.733**
III. region	550.605	1.577.704	312.965	2.431.274	0,5	77.935.529
Turkey	6.120.207	19.592.384	3.314.525	29.027.116	0,7	637.315.717

(*) Except poultry

Table 12: Poultry production

Provinces	Chickens	Turkey	Goose - Duck	Total
Edirne	108.824	5.787	8.080	122.691
Tekirdağ	116.862	4.502	13.378	134.742
Kırklareli	108.403	2.793	5.631	116.827
Trakya	**334.089**	**13.082**	**27.089**	**374.260**
III. region	951.833	56.089	62.286	1.070.208
Turkey	7.231.345	270.801	266.413	7.768.559

Source: Turkish Agricultural Statistics ("Zirai Tahriri") in 1927

CONCLUSIONS

In the transition period of from Empire to Republic, the majority of Turkey's population is engaged in agriculture but it is much decreased comparing with today. The agricultural sector, which had undertaken important roles in the economic and social development of the country until the early 1980s, has gradually lost this mission in recent years. The rate of agriculture in employment decreased from 67% in 1927 to 50% in the 1980s, to 34% in the early 2000s, and to 25% today. The main reason for this decline is the increased mechanization in agriculture, as well as the emphasis on industrialization and service sectors. As a result of this, migration from village to city was started in the 1980s, the young population in the villages were gradually decreased, and abandoned villages began to appear in Anatolia and Trakya regions. Despite the increase in arable land amount, product variety and mechanization, Turkish agriculture has become unable to feed the country's population. Faced with a similar statement in animal husbandry in Turkey, was founded with cattle and poultry farms want to close this gap. A similar situation is experienced in Trakya. Due to the high cost of agricultural inputs, small farmer families have become unable to cultivate their lands, agricultural lands have been lost to family heirs and transferred to large enterprises.

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