PROCEEDINGS
OF
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WELCOME NOTES

You are welcome to our III. 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 again. 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. Due to COVID-19 situation, AGBIOL 2021 has organized fully on-line event which was one of the biggest online conferences in recent years in the world with 499 papers and 1133 authors with 333 oral and 166 e-poster presentations from 55 countries. Therefore, this great interest gave ambition to organizers to make it a periodical event then we decided to organize 3rd one in this year.

The Organizing Committee of AGBIOL 2021 considers the health, safety, and security of its conference attendees and community as its top priority. Due to 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 2021 has decided to move towards the organization of on-line again but with limited participation**.** There is a worldwide participation from 44 countries with 422 papers by contributing 1066 authors. Our AGBIOL 21 conference was organized with 288 oral, 134 e-poster presentations.

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.

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Head of the Organizing Committee

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CONTENT

REVIEW OF ESCHERICHIA COLI PATHOGENS AND FOOD-BORNE ANTIBIOTIC RESISTANCE TO PREVENTION

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ABSTRACT

Escherichia coli (*E. coli*) is part of the intestinal microbiota of all mammals and in this regard it is not surprising that it is widespread in the environment, and is one of the most common food pathogens. In animals, it is the cause of colibacillosis and colisepticemia, and in humans, alimentary intoxications and food poisoning are also significant. The foodborne pathogen *E. coli* most commonly causes gastroenteritis in humans. It is always native to the intestines of mammals, and the primary transmission is through food. A special strain is the enterotoxinogen *E. coli* (ETEC), and the most dangerous serovor is 0157: H7, the cause of hemorrhagic colitis or hemolytic-uremic syndrome in humans. In addition to the disease of consumers, another problem is antibiotic resistance (ABR), which reduces therapeutic options. Recent research detects resistant isolates, with the presence of ABR showing regional specificity and a growing prevalence of fluoroquinolones, which may have implications for human health. In order to prevent the spread of alimentary intoxications, it is important to apply the HACCP concept in food production, which is made possible by *Good Manufacturing Practice* (GMP) and *Good Hygiene Practice* (GHP). In order to prevent the spread of ABR, it is necessary to rationalize the consumption of antibiotics (AB) in agriculture, veterinary medicine and medicine. Monitoring is an important prerequisite for the adoption of appropriate measures and control, all in order to prevent the spread of ABR.

Key Words: *Escherichia coli*, Human and animal pathogen, Foodborne pathogens, Epidemiology, Antibiotic resistance, Prevention

INTRODUCTION

Escherichia coli (*E. coli*) is a commensal bacterium that immediately after birth of each mammal, colonizes the digestive tract, the colon and the distal part of the small intestine. It is a gram-negative, facultatively anaerobic, asporogenic, rod-shaped bacterium that performs a variety of physiological functions within the gut but can also cause opportunistic infections. We distinguish physiologically non-pathogenic, pathogenic and enterotoxic *E. coli* (ETEC) which is not a physiological resident of the digestive tract of humans and animals, but in humans it is the leading cause of one example of the spread of antibiotic resistance (ABR) across the food chain, which points to the need to monitor ABR of bacteria originating from animals, in order to prevent the spread of ABR in animals and humans. The greatest danger is the loss of effective antibiotics (AB) in the eradication of bacterial infections, which can become incurable diseases. To reduce evident risk, the *World Health Organization* (WHO) has built international protocols that provide guidance to physicians in choosing empirical AB therapy. What has been neglected is the local, irrational use of AB in animals, environmental pollution by the anthropogenic factor, and the possibility of the spread of ABR from alimentary intoxications resulting from

the consumption of infected food from *E. coli* of animal origin. When it comes animals and the environment to humans, via bacteria originating from animals. Awareness of the possibility of movement of genes across the genome of bacterial cells, to the healthy gut microbiota, the concentration of non-pathogenic *E. coli* is higher than that of the pathogen *E. coli*, and ETEC, verotoxinogenic *E. coli* (VTEC) or "*shiga like*" (STEC) is an evolved bacterium formed by the exchange of genetic material with *Shigella spp*., a process of horizontal/ lateral gene transfer. It is present in the digestive tract of domestic and wild animals, as a colonizer originating in the environment, but is often the cause of infections in young calves, lambs, pigs, sometimes carnivores. For E. *coli* to cause infections, it must be pathogenic, contain pathogenic elements such as adhesins, flagella or whip, capsule, endotoxins, exotoxins and other pyrogenic substances. Infections with ETEC in humans are caused by ingestion, ingestion through contaminated water, food or direct contact with animals, carriers of the infection. ETEC diseases in young animals are often lethal and peracute in nature, with susceptible animals often exposed to AB therapy from birth, with the aim of prophylactic and therapeutic effects. Given that domestic animals used for human consumption are often treated with AB, the spread of acquired ABR between bacteria originating from and from different species of living creatures is a growing danger. Given that meat and products of animal origin, often contaminated with animal *E. coli*, thermally unprocessed food, in addition to the risk of causing alimentary intoxication, may mediate the spread of ABR from *E. coli* of animal origin to the same bacterium by human origin, which is attributed to horizontal gene transfer. This is introduces us to the world of uncontrolled exchange of genetic material, because bacteria can exchange their genetic material with any bacterium they find nearby. Bacteria are less frequently susceptible to mutations, but this possibility is not left out, and ABR can result from bacterial damage, most often under the influence of AB, at the dose and timing of AB therapy. Bacteria mutants transmit mutated genes to offspring via vertical gene transfer, and by horizontal gene transfer, conjugation, transformation and transduction processes, the same genes can be transmitted to other bacteria. ABR of *E. coli* can arise from the development of various biochemical mechanisms of bacterial defense, which are related to the mechanisms of action of the AB used. The most common mechanism used by *E. coli* is enzymatic modification and modification and active efflux, which use all gram negative bacteria. Local monitoring of ABR of *E. coli* by food of animal origin is of great importance, because in this way we can act prophylactically to reduce the spread of ABR of bacteria of animal origin to bacteria by humans.

Taxonomy

E. coli is an enterobacteria belonging to the genus *Escherichia*, together with *E. albertii*, *E. hermannii*, *E. fergusonii*, *E. marmotae* (Table 1) (Euzéby, 2019). *E. adecarboxylata*, *E. blattae*, *E. vulneris* were once classified under the same genus *Escherichia*, and are now separated (Euzéby, 2019).

Table 1. Taxonomy of *E. coli* (Euzéby, 2019)

Kind	*E. coli*
Rod	*Escherichia*
Family	*Enterobacteriaceae*
Red	*Enterobacteriales*
Class	*γ-Proteobacteria*
Knee	*Proteobacteria*

General characteristics of *E. coli*

E. coli is a type of enterobacteria isolated in 1885 by pediatrician Theodor Escherich, after whom *E. coli* was named (Shulman et al., 2007). Like all other bacteria of the genus *Enterobacteriaceae, E. coli* are gram-negative, facultatively anaerobic bacteria, bacillus-shaped, rod-shaped, or rod-shaped. They are asporogenic. They move with the help of flagella or peritrich. Some also contain fimbriae, ie saws, with the help of which they are attached to the base. Strains that contain chicken are immobile. Some strains are considered more resistant to harmful noxa because they contain a capsule. The size of *E. coli* species is variable, usually ranging from 1-3h and 0.4-07 μm. It is characterized by fermentative and oxidative properties (Table 2) (Markey et al., 2013). *E. coli* is a bacterial species that grows successfully on a variety of substrates. Turbid liquid broth, grows successfully on both selective and non-selective media. It forms smooth S-type colonies, which can become rough if subcultured. Colonies may be mucous, spilled, or mucoid, which is characteristic of capsular strains of *E. coli* species. On blood agar, some species of *E. coli* are beta hemolytic, and on endo agar, some species of *E. coli* also form a metallic reflection (Markey et al., 2013).

Table 2. Biochemical characteristics of *E. coli* (Markey et al., 2013)

Biochemical analyzes	The result
Oxidase	+
Catalase	+
Nitrate and nitrite reduction	+
Indole	+
Methyl red	+
Ortonitrophenol	+
Glucose fermentation	+
Mannitol fermentation	+
Triple sugar	+
Voges Proskauer	-
Citrates	-
Fast urease	-
Phenylalanine	-
KCN	-
Gelatin	-
Hydrogen sulfide	-

Commensal and pathogenic *E. coli*

Commensal *E. coli* is an integral part of the saprophytic flora of the digestive tract of all mammals, along with the pathogen *E. coli*, which is present in small amounts in a healthy organism (Aerestrup et al., 2008). Commensal *E. coli* when found in its natural habitat, in physiological concentration, performs a number of physiological functions, of which protein synthesis, decomposition of nutrients, etc. It is generally harmless, while being tied to a natural habitat. The bacterium *E. coli* is often the cause of urogenital infections of carnivores, while in herbivores it more often causes diseases of the digestive tract. The problem occurs if the bacterium colonizes the urogenital tract, multiplies and causes an infection. In herbivores, as a result of excessive intake of rotten food containing a large amount of *E. coli* bacteria, which cannot be neutralized, indigestions such as rot of rumen content result. *E. coli* can also be an integral part of the flora of an unclean wound, and cause other infections in the body (Clermont et al., 2011). Commensal *E. coli* rarely causes opportunistic infections, while pathogenic *E. coli* is cited as a possible cause of extraintestinal infections. Some are caused by the avian pathogen (APEC), which has zoonotic potential, septicemic (SEPEC), uropathogenic (UPEC) and *E. coli* strains, which cause local infections, while intestinal infections are caused by the enteropathogenic *E. coli* (EPEC) (Quinn et al., 2011; Markey et al., 2013). Pathogenic intestinal serotypes include: ETEC, EPEC, causes of severe diarrhea in young mammals, enteroinvasive (EIEC), cause of dysentery, enterohemorrhagic (EHEC), cause of hemorrhagic colitis or hemolytic - uremic syndrome. The pathogen *E. coli* can cause infections of any organ in which it is found and in favorable conditions can cause: urethritis, cystitis, pyelonephritis, gastroenteritis, cholecystitis, cholangitis, appendicitis, peritonitis, meningitis, metritis, pyometra, mastitis, sepsis, sepsis, sepsis skin, soft tissues, and wounds (Yassin et al., 2017). In female carnivores, metritis, mastitis and pyometra, caused by UPEC, which is the dominant flora in the case of mixed infections, with the possibility of infection through the mother in the womb, congenitally and during sucking, are common. The presence of any pathogenic *E. coli* may represent one of the complications of others e.g. carnivorous viral diseases (Sykes, 2013). EPEC must be distinguished from ETEC, which is not a normal resident of the digestive tract of mammals and is a possible cause of death of neonatal pigs, calves, lambs, less often carnivores, and in humans is the cause of severe food poisoning. Different animals may be carriers of ETEC, which is often of environmental origin (Quinn et al., 2011; Rice et al., 2013).

Uropathogen E. coli, extraintestinal infections

UPEC is the cause of urogenital infections in mammals. Large and small ruminants are more resistant to urinary tract infections, while carnivores are more susceptible. Dogs are more receptive than cats (Saputra et al., 2017). In non-sterilized dogs and cats, purulent inflammation of the uterus with UPEC is also common (Bassessar et al., 2013). Complicated urinary tract infections are related to the existence of certain anomalies of the urogenital tract, other diseases, weakened immunity, etc. (Dissanayake et al., 2014; Saputra et al., 2017). Diseases of the urinary tract are also related to age, sexual activity. Younger individuals are more susceptible, and older ones are associated with asymptomatic bacteriuria, which do not require special treatment because they have no special etiological significance (Bedenić, 2005; European Association of Urology, 2006). In older women, prevention of urovaginal cononization can be prevented by the administration of topical estrogen (Hooton, 2000). Bacteria from the intestine are potentially uropathogenic and are often colonizers of the perineal region, the distal part of the urethra, but due to the decline in immunity, they lead to infections (Bedenić, 2005). Females are more susceptible to urinary tract infections than the male population of animals and humans. The proximity of the urethra to the vagina and rectum is the main precursor of diseases of the

urogenital tract (Škerk et al., 2006). In the male population, infections are most often associated with the generative age. UPEC is often the cause of acute and chronic prostatitis in carnivores and humans, which is explained by the decline in immunity due to a decrease in the concentration of zinc in the prostate and due to hormonal imbalance. These are most often ascending infections with urethral bacteria (Irekpita, 2017). Ascendants are associated with UPEC, which has the ability to adhere, adhere to the walls of the epithelium with the help of fimbriae, adhesins, whose receptors are located along the epithelium of the urogenital tract (Klemm, 1985). Diseases with UPEC are very common in humans and carnivores. The susceptibility factor is age, eventual castration, and intact individuals are more susceptible to infections. The greatest importance in the development of urinary tract infections is given to UPEC, which contains the antigen P of fimbriae and is the cause of acute pyelonephritis, sepsis. Certain types of mannose carbohydrates, oligosaccharides and glycoproteins, Tamm-Horsfall glycoprotein, are found in the urine of some individuals and have the ability to bind to S fimbriae and therefore *E. coli* loses its pathogenicity, which is considered nonspecific immunity (Klemm, 1985). Said carbohydrates do not have the ability to bind to P fimbriae UPEC, the causative agent of pyelonephritis and urosepsis (Conell et al., 1997). Recent research cites the efficacy of cranberry juice in the treatment of UPEC infections, due to the proven ability to reduce the binding of P fimbriae antigen to the epithelium of the urogenital tract (Stapleton et al., 2012).

Pyometra with uropathogen E. coli

In unsterilized dogs and cats, pyometra, a purulent inflammation of the uterus, is common. According to many authors, the most common causes of pyometra are microorganisms that make up the mixed flora of the vagina, with the dominance of UPEC. Pyometra is associated with luteal phase disorders, hormonal imbalance, progesterone dominance (Bassessar et al., 2013). The onset of the disease is related to older age, in bitches it occurs most often at the age of seven, but according to many authors, the occurrence of pyometra is not excluded in younger individuals. The incidence is more common in dogs than in cats. Pyometra can be open or closed. In the initial stage, the disease is often inert or the owner notices frequent urination, consumption of a larger amount of fluid, more frequent cleaning of the urogenital region. In the second case, the first symptoms appear only with the appearance of viremia, sepsis, peritonitis. At the first symptoms of the disease, the urgency of the response is important, due to the prevention of lethal outcome (Smith, 2006). Treatment options include conservative therapy, fluid replacement, electrolytes, antibiotics (AB), prostaglandins to increase uterine contractility, antiprogesterone. Surgical therapy is much safer, but it is necessary to bring the patient to the best possible state of health before surgical treatment. In open pyometra, the use of prostaglandins is indicated, in order to release pus, because AB does not act in organic detritus. This method of therapy requires a carefully considered clinical picture, due to the risk of uterine rupture, due to an excessive amount of pus. In closed pyometra, the use of antiprogesterone preparations is recommended, which help the decomposition of the corpus luteum and excrete pus. After excretion of purulent contents, AB is used (Table 3) (Quinn et al., 2011). Fluoroquinolines are most commonly used, and often, exclusively veterinary AB enrofloxacin (Bassessar et al., 2013).

***E. coli* pathogen, intestinal infections**

The enteropathogen *E. coli* (EPEC) in young dogs and cats causes various colibacillosis, especially in animals that have not received colostrum from the mother. In certain dog and cat species, pathogenic *E. coli*, enteroinvasive (EIEC) and adherently invasive *E. coli* (AIEC) have the ability to adhere and invade and can cause granulomatous colitis, chronic bowel disease,

with a characteristic increase in histiocyte count, eosinophil infiltration, manifest ulcers, proliferative malformations, with a clinical picture reminiscent of Crohn's disease in humans (McGavin and Zachary, 2008; Markey et al., 2013). Granulomatous colitis also occurs in cats in almost the same percentage as in dogs and affects individuals at the age of four. Infectious peritonitis and histoplasmosis are considered differentially diagnosed in cats. The final diagnosis is made on the basis of a biopsy of the altered parts of the intestinal tract (Simpson et al., 2006). Enrofloxacin is most often used in therapy, to which resistance often occurs, because the therapy lasts for eight weeks (Manfields et al., 2009). In dogs, various enteropathies can also occur with enterohemorrhagic *E. coli* (EHEC), which is a VTEC or STEC subgroup, which through severe exotoxins, verotoxins causes severe vascular damage and causes bloody diarrhea with frequently present hemolytic uremic syndrome, which includes clinical poisoning (Sykes, 2013). EPEC is also a cause of diarrhea in carnivores, more often in younger individuals. EPEC damages enterocyte microvilli and shows the greatest capacity in biofilm formation. Biofilm-coated bacteria cause chronic, persistent infections, due to their high resistance to antibiotics and the immune system of the attacked host. There is also gene-related biofilm production, gene transfer from pathogens to commensal *E. coli* (Schiebel et al., 2017). In untreated cases of the disease with the pathogen *E. coli*, they can contribute to various complications. In the case of viremia, it can reach various tissues, organs, and in some cases can cause death. In small and large ruminants, poultry pathogens and toxinogens, intestinal *E. coli* (ETEC) is usually the cause of gastrointestinal infections and these animals represent reservoirs of this infection (Yassin et al., 2017). Infections in young calves and suckling or weaned piglets pose a risk of death. Older individuals can carry pathogens, with the absence of health problems, but with the possibility of transmitting the infection to humans. In humans, they are the cause of food intoxications. Because *E. coli* is extremely resistant, it can be transmitted by direct contact with infected animals, but also indirectly through objects, the environment or infection through food, milk in which it can survive, resisting the influence of high temperatures during pasteurization, as well as low (also resistant to refrigerator temperature). It is also resistant to acidic media (contaminant of various sour salads). *E. coli* infections have recently been determined as zoonoses, where the main reservoirs of infection are different species of animals (Labro and Bryskier, 2014).

Enteropathogenic E. coli, tendency to create biofilm

The formation of a bacterial biofilm is a widespread phenomenon in the world of microorganisms and is an undesirable phenomenon, because it prevents the access of an effective drug to a bacterial prokaryotic cell, which a certain active substance should destroy. Formed sessile community of bacteria (gene series, adherence, microcolony formation, growth and reproduction, interconnection in sessile communities, maturation of biofilm, association of other bacteria, algae, fungi, etc., and separation from the original adherent substrate) represents favorable conditions for growth, development and safety against the destruction of bacteria through "artificial destruction mechanisms", such as the targeted use of AB, disinfectants, detergents to eradicate it, and the natural defense of the infested organism by bacteria, such as the action of killer cells or macrophages. EPEC, which damage enterocyte microvilli, show the greatest capacity in biofilm formation. Biofilm-coated bacteria cause chronic, persistent infections, due to their high resistance to AB and the immune system of the attacked host. There is also gene-related biofilm production, gene transfer from EPEC to *E. coli* commensal bacteria (Schiebel et al., 2017).

Table 3. Enterotoxins and entherotoxinogens *E. coli*

Therapeutic area in carnivores	Antibiotics
I Urogenital infections with UPEC	Cephalosporins, penicillins, fluoroquinolones, tatracyclines
II Skin diseases, wounds, abscesses, pyodermatitis	Cephalosporins, penicillins, fluoroquinolones, potentiated sulfonamides, lincosamides
III Respiratory diseases	Lincosamides, fluoroquinolones, cephalosporins, tetracyclines, penicillins
IV Periodontal diseases	Penicillins, cephalosporins, lincosamides, macrolides, quinolones
V Other	Penicillins, fluoroquinolones, cephalosporins

Intestinal diseases are most often caused by various ETEC, which are not the physiological microflora of mammals (Markey et al., 2013). They mainly represent alimentary intoxications in humans, and the route of transmission takes place directly / indirectly, through contaminated objects, through the environment and the like (Thorsteinsdottir et al., 2008; Szmolka and Nagy, 2013). The problem of ETEC is reflected in the strong virulence of pathogens and the creation of strong exotoxins, which bacteria produce during their lifetime and endotoxins, which are a problem in the period of convalescence, which are released after death (Croxen and Finlay, 2010; Markey et al., 2013). ETEC carriers are domestic and wild animals, but pathogenicity has only been demonstrated in domestic livestock (Table 4) (Markey et al., 2013). In humans, similar diseases are not common, they are associated with developing countries (Varga et al., 2012). If the disease occurs in humans, it is most often the animal EHEC subgroup ETEC, dangerous serovor 0157: H7, a possible contaminant of food of animal and plant origin (Marinculić et al., 2009). It is important to mention that food is a vector, and that ETEC is always derived from mammalian feces. Intestinal pathogens, derived from animals and transmitted to humans, through the food chain are: ETEC and EPEC, causes of severe diarrhea, EIEC, cause of dysentery, EHEC, cause of hemorrhagic colitis or hemolytic-uremic syndrome (Table 4,5) (Markey et al., 2013).

Basic knowledge of bacterial exotoxins

Exotoxins are the strongest known poisons, whose strength is expressed in the form of LD 50, and it represents the smallest amount of bacterial exotoxin, which would cause death in fifty percent of laboratory animals. The toxicity of the same is great, so that the exotoxin causes damage to the bacteria themselves, so that it does not secrete it. Exotoxins directly damage the host organism, with their pathogenicity. In addition to the direct impact on the host organism, the organism is also damaged by the immune response due to the presence of the pathogen and its products. The organism reacts with an inflammatory reaction, in a way that releases phagocytes, which in order to defend the organism from pathogens release enzymes and their own toxic products, which are without selectivity of action, and can cause damage to the host itself. In case the organism defends itself, the inflammatory cells withdraw. In addition to the described immune response, the entry of pathogenic bacteria into the body is a "foreign substance" or antigen, to which the body responds by producing antibodies, which is bacterial antigenicity (Nester et al., 2004). Bacterial exotoxin can also be ingested parenterally, through food, which contains a small amount of toxinogenic bacteria, which produce exotoxins in food. The exotoxin thus produced is subject to denaturation within the digestive tract of hydrochloric acid. This exotoxin is non-functional, but it is still an antigen for the host organism, ie its individual parts activate the immune system as a result of defense against a "foreign body", in the reaction already described. Some exotoxins are resistant to the acidic medium of the

stomach, small intestine and this ability allows them to have a harmful effect in the digestive tract, as is the case with bacterial enterotoxins, ingested through contaminated food. Exotoxins are toxins that can cause damage to the body before it reacts with an immune reaction (Nester et al., 2004).

Exotoxins

Exotoxins, which act on enterocytes, belong to enterotoxins. They cause damage to the digestive tract, with consequences in the form of osmotic diarrhea and vomitus. Components A and B of enterotoxins, act on epithelial cells by binding the B component of the exotoxin to specific receptors, located on the microvilli of epithelial cells of the small intestine. The active, enzymatic A component of the exotoxin transfers ADP ribose from nicotinamide-adenine nucleotide to G-protein, which becomes reversibly activated. The amounts of it exceed the physiological limits, and G-protein accumulates in intestinal cells, within cyclic-adenosine monophosphate. The described phenomenon causes constant secretion of fluid inside the intestine, rich in electrolytes and chlorine ions. Under normal conditions, a small amount of intestinal fluid is easily resorbed, and it is not a health problem. Under the above conditions, the intestines are unable to resorb, larger amounts of fluid mature per unit time, and severe osmotic diarrhea and acid-base imbalance occur (Nester et al., 2004).

Enterotoxinogen E. coli (LT and ST enterotoxin)

The pathogen ETEC during its lifetime, secretes exotoxins, or enterotoxins. They differ from other strains in their genetic material. ETEC contains a toxin gene. With the help of adhesin, it possesses adherence, the ability to attach to the epithelium of the intestinal tract (Nester et al., 2004). The same enzyme, together with the released enterotoxin, has the ability to encode genes on plasmids, which are transmitted by conjugation to another bacterium (sexually multiplying). ETEC causes diarrhea in pigs, goats, sheep, horses, dogs and humans. Enterotoxin causes the movement of water from the tissue into the intestinal lumen and leads to the appearance of osmotic diarrhea, the so-called passenger diarrhea. There are two exotoxins. Thermolabile LT (60 ° C/ 30 min.) And other thermostable ST (not destroyed at 100 ° C/ 30 min) (Prescot et al., 2005). The toxins cause the activity of adenyl cyclase, ie guanyl cyclase, under the action of which cAMP and cGMP are created, which stimulate secretion and at the same time block the resorption of ions from the intestinal lumen, which leads to symptoms of watery diarrhea, the so-called traveler's diarrhea (Todar, 2011). The infection and the disease itself are caused by exotoxins and result in diarrhea as with cholera toxin, with one toxin of the mentioned bacterium being identical to the cholera-causing enterotoxin (Nester et al., 2004; Kolenda et al., 2015).

Enterotoxinogenic strains of E. coli in calves

ETEC is the cause of severe diarrhea in calves from two to three days. According to many authors, infection per os, before birth, is reported as septicemia (Table 4) (Markey et al., 2013). Bacteria usually enter through the nasopharynx and lead to a clinical manifestation in the form of white diarrhea. The disease is sudden and, depending on the virulence of the causative agent, the strength of the exotoxin, can lead to collapse and death. There is a possibility of development and septicemia, with consequent focal processes, abscesses on parenchymal organs, consequent hypothermia, atony, etc. (Markey et al. 2013). Therapy is supportive, in the form of rehydration, electrolytes, preparations based on bicarbonate, vitamins, mineral complexes and basic in the form of mandatory use of AB (Table 6) (Quinn et al., 2011). Forty

days before calving, mothers are given high doses of vitamin A to strengthen the resistance of the intestinal mucosal epithelium. ETEC is a bacterium that is also a human pathogen, and it is transmitted from animals to humans, it is a zoonosis (Labro and Bryskier, 2014; Kolenda et al., 2015).

Table 4. Colibacillosis in calves and cattle (Markey et al., 2013)

Calves from 2-3 days of age	Cattle
Dysentery (diarrhea), enterotoxemia, septicemia or combined Often in the presence of pneumonia and polyarthritis	Often reservoirs of enterotoxinogenic *E. coli*, without the described pathogenesis
Supurative bronchopneumonia, as an accompanying infection of another specific causative agent	Mastitis, metritis

Enterotoxinogenic strains of E. coli in swine

ETEC in piglets is the cause of severe diarrhea in newborn piglets (Table 5) (Markey et al., 2013). Infection occurs by ingestion of the pathogen per os. Piglets can die in the first twelve hours of life. If they survive the first stage, in one to two days, watery diarrhea occurs, the animal loses weight. The disease is often lethal, which is associated with strong virulence and high susceptibility of the organism. Three weeks after weaning, a second phase occurs, often asymptomatic. After ten days, diarrhea occurs. If the animal overcomes the disease, it remains malnourished. Predisposing factors of the disease are age, poor zoohygienic conditions, intestinal pH 4-5. ETEC tends to multiply rapidly and produce exotoxins, resulting in a diarrheal form. If it penetrates the parenchymal organs, a septicemic form develops, which can occur in the first week. Therapy is supportive and antibiotic (Table 6) (Quin et al., 2011). Prophylaxis is immunization. Asymptomatic animals represent reservoirs of infection for humans (Labro and Bryskier, 2014).

Table 5. Colibacillosis in pigs (Markey et al., 2013)

I Piglets of 2-3 weeks	II Piglets from 6-14 weeks
Neonatal saprophytic diarrhea. Enterotoxic colibacillosis. Enteroaggregative *E. coli.* It rarely causes the disease, although it is common bowel colonizer. It causes persistent diarrhea, due to adhesions. Zoonotic character. It is usually accompanied pathogen of other diseases. Septicemic colibacillosis. Often caused mixed infections and occurs often as consequence of infection with ETEC. Septicemias cause arteritis, serositis, Meningitis, abscesses, cortical abscesses on kidneys, ophthalmitis, etc. Neonatal bronchopneumonia with *E. coli*, meconium aspiration syndrome.	Edema disease or enterotoxic colibacillosis, specific for pigs with hemolytic *E. coli*, without epithelial destruction. The best fed pigs get sick. Mastitis, metritis. Enteroinvasive colibacillosis caused by verotoxin, pathogenic enterotoxinogenic and hemolytic *E. coli*, with epithelial destruction and zoonotic potential. III Rejected Piglets Colibacillosis after weaning, an identical disease with swine edema. IV Gilts after pollination Mastitis-metritis-agalactia syndrome. V Sows after farrowing Koli mastitis.

Table 6. Most commonly prescribed antibiotics in bacterial infections of pigs and calves (Quinn et al., 2011)

Therapeutic area	Antibiotics
I - Calves, Diarrhea, colibacillosis	Polymyxins, fluoroquinolones, penicillins, aminoglycosides, cephalosporins
II – Pigs Diarrhea, colibacillosis and dysentery	Polymyxin, macrolides, fluoroquinolones, potentiated sulfonamides, pleuromutilin

Enterohemorrhagic E. coli (EHEC) VTEC subgroup

EHEC via verotoxin ("*shiga like*") causes severe mucosal damage and causes bloody colitis in calves, goats, sheep and humans. It is a zoonosis, which is transmitted from animals to humans through the food chain. The most well-known strain belongs to serovor 0157: H7 (Nguyen et al., 2012). In the target macroorganism, with a weakened immune system, it can lead to hemolytic ammonia syndrome, which includes hemolytic anemia with the possibility of thrombocytopenia purpura, where renal failure occurs, and if it reaches the CNS it causes neurological symptoms, where in 50% cases the disease ends in death (Todar, 2011). Fatal outcome occurs in 15% of cases. EHEC is ingested by the feco-oral route, most commonly through contaminated raw beef, chicken. EHEC, through its exotoxins, causes severe diseases with a dominant symptom of bloody diarrhea. Fortunately, the disease rarely occurs, although there is variation in its incidence, and in some parts of the United States, after *Salmonella*, it is the second most alimentary intoxication in humans (Nester et al., 2004; Kolenda et al., 2015).

Danger of food contamination from Enterohemorrhagic *E. coli* of animal origin

As already explained in the previous text, zoophilic EHECs have zoonotic potential, which means that they are potentially dangerous to human health. All food and water can be contaminated with fecal products, ie with any strain of *E. coli* species, from any mammal, with the most common animal EHEC, subgroup ETEC, especially serovor 0157: H7, which in the younger population can cause death. (Nester et al., 2004; Aarestrup et al., 2008; Marinculić et al., 2009). Such strains also resist the acidic environment of the stomach and a very small amount of it is able to lead to the manifestation of symptoms of food poisoning (Labro and Bryskier, 2014). Although EHEC in older domestic and wild animals is often an integral part of the gut microbiota, originating from the environment, monitoring data indicate that colibacillosis with EHEC rarely occurs in humans, with the exception of developing countries, and can be prevented by hygiene measures (Nester et al., 2004; Marinculić et al., 2009; Kolenda et al., 2015). The most important prevention measures are hand hygiene, hygienic food handling, consumption of thermally processed food of animal origin, pasteurized milk and dairy products, but also pasteurized fruit juices, consumption of peeled fruits and vegetables, because they may contain fecal products, which is most often attributed to pollution. groundwater with manure (Table 7) (Marinculić et al., 2009). It is important to note that the food used for human consumption does not naturally contain *E. coli*, it is always derived from feces, often animals that are slaughtered and whose products are used in human nutrition. It should be especially emphasized that it is necessary to avoid mixing thermally processed food with thermally unprocessed food, because in that case the possibility of infection opens up through thermally processed food. Symptoms of infection in people with EHEC include typical signs of food poisoning, with or without hyperthermia, with symptoms of vomitus and diarrhea with a special indication of bloody diarrhea caused by the action of exotoxins on enterocytes, and concomitant platelet inhibition, with consequent thrombocytopenia, resulting in lethal outcome. Rarely, the disease is asymptomatic. The disease is especially dangerous for younger children, who most often have hemolytic uremic syndrome caused by EHEC (Marinculić et al., 2009; Varga et al., 2012).

Epidemiological data of infection with the pathogen *E. coli*

70% -80% of cases of urinary tract infections are caused by UPEC, and the percentage of recurrent infections is important, which in the world population of women is 27% -44% (Norrby, 2003; Anderson et al., 2004). The prevalence of UPEC in acute prostatitis is 80% (Nickel, 2014). The percentage of enterobacteria in the etiology of intestinal diseases is 70% (Greene, 2006). Sepsis caused by enterobacteria is considered to be the most serious complication, with a high mortality rate (Borer et al., 2009; Martin, 2012). In the territory of Bosnia and Herzegovina, the percentage of mastitis caused by *E. coli* was recorded in the amount of 25% (Muftić et al., 2019). Bassessar et al., (2013) in their study report the presence of mixed flora in the vagina, with a predominance of *E. coli*, with a percentage of 41.9%. The bacterium *E. coli* is one of the causes of so-called nosocomial infections, where the percentage of *E. coli* is recorded at 9.3% (Magill et al., 2014). Shiga toxin, produced by ETEC, has been verified as a human pathogen, the cause of food poisoning. In food of meat origin, water, fruits and vegetables, the most common is the most dangerous serovor 0157: H7 (Marinculić et al., 2009). However, many authors state that the presence of ETEC in stuffed meat is in low values and that they rarely exceed the permissible limits, which would lead to symptoms of food poisoning in humans (Varga et al., 2012).

Table 7. Toxin infection prophylaxis with *E. coli* (Marinculić et al., 2009)

Prophylaxis of toxoinfection with *E. coli* in the household	Prophylaxis of toxin infection with *E. coli* at different stages of primary production
Hand hygiene Hygienic food handling Heat treatment of food Avoid cross-contamination on cutting boards Refrigerated storage Separation of raw and heat-treated food Peeling fruits and vegetables Mechanical cleaning, washing	Hand hygiene of all participants in production Hygienic food handling Pasteurization of milk, dairy products and fruit juices Avoiding cross-contamination on production, work surfaces Refrigerated storage Chlorination of water Mechanical cleaning and disinfection That is, the application of hygienic and sanitary standards in different stages of primary production, the application of the concept of HACCP system, which includes DHP and DPP

Antibiotics used in the treatment of infections caused by *E. coli*

Although AB does not act on *E. coli*-producing exotoxins and endotoxins, timely eradication of the bacteria causing the infection, along with supportive therapy, is crucial in preventing the effects on the organism of the attacked individual. We distinguish between bacteriostatics and bactericides (Kalenić et al., 2013). The action of bacteriostatics is reflected in the damage of sensitive bacteria, relying on the host immune system, and bactericides exhibit a bactericidal effect, causing bacterial death by different mechanisms of action (Table 8) (Quin et al., 2011). In the treatment of intestinal and extraintestinal diseases, various AB are used, which are also used in human medicine in the eradication of *E. coli* infections (Quin et al., 2013; Makovec et al., 2014). The most commonly used classes of AB in human and veterinary medicine in the treatment of *E. coli* infections are fluoroquinolones (Hopkins et al., 2005; Ma et al., 2009) (Table 6, 8) (Quinn et al., 2011). Fluoroquinolones in veterinary medicine are among the critically important AB for veterinary medicine - VCIA (OIE, 2019). Class AB, used in bacterial infections caused by *E. coli* are macrolides, with important representatives: erythromycin, azithromycin, spiramycin, tylosin, tilmicosin, trilatromycin, quinolones: sarafloxacin, difloxacin, floroquinolones, marbofloxacloxacin penicillins, cephalosporins of different generations, with indication of III and IV generation, which belong to VCIA, tetracyclines: chlortetracycline, tetracycline, doxycycline, oxytetracycline, aminoglycosides: gentamicin, neomycin, amikacin, kanamycin, streptomycin, apycinomycin, , glycopeptides and reserve antibiotics: vancomycin, teicoplanin, telavancin, polypeptides: bacitracin, polymyxin B, polymyxin E or colistin, which is the last defense in the treatment of infections with invasive gram - bacteria and less toxic sulfo preparations with trimethoprim (Makovec et al., 2014; OIE, 2019).

Table 8. Mechanisms of action of different classes of antibiotics (Quinn et al., 2011)

Antibiotic classes and sulfonamides	Mechanisms of action
β lactams, glycopeptides, polypeptides	I Effect on cell wall synthesis
Nitrofurans, tetracyclines, aminoglycosides, lincosamides, macrolides	II Effect on protein synthesis
Quinolones / fluoroquinolones, nitroimidazoles, aminocoumarins, rifamycins	III Effect on DNA synthesis
Sulfonamides and trimethoprim	IV Effects on metabolism

Effective and alternative therapy in E. coli eradication

Effective antibacterial therapy is the application of AB on the basis of a previously made antibiogram. The correct choice of drug includes knowledge of indications, contraindications, drug effects, main and side effects, toxicology, bioavailability of AB, as well as knowledge of the health status of the treated individual. Good clinical practice achieves a therapeutic effect, with minimal side effects. The applied dose of the drug should be in accordance with the age structure, weight, condition of the organs, especially taking into account the tissues and organs in which the drug is distributed (compartment models: central and peripheral component), where the process of biotransformation and elimination of the drug (clearance) and it is necessary to take into account the possibilities of hyperactivity of the organism (allergy) and possibly reported idiosyncrasy. Properly adjusted dose implies the optimal dose of the drug, which exhibits bactericidal action, while reducing side effects. Failure to comply with the correct drug concentration may result in the accumulation of antibiotics with consequent poisoning. The same is avoided by applying an appropriate dose, where resorption should not be more intense than the process of drug elimination (cumulation), nor elimination more intense than the process of resorption (reduction of drug effectiveness). Improperly applied dose of antibiotics can result in residues of the same in food of animal origin, which is used for human consumption (Mulalić et al., 2005). In contrast to the risk of accumulation, long-term administration of a lower dose of the drug, at irregular intervals, can lead to damage to bacteria, which are potential mutagens. The appearance of mutants is especially present with the use of various AB selective actions. During antibiotic therapy, monotherapy in accordance with the antibiogram should be practiced as often as possible, which is one of the measures to rationalize the use of AB. Emergency empirical therapy involves the selection of AB based on recent WHO recommendations or based on monitoring of local antibiotic resistance. Recently, forms of alternative therapies in the treatment of colic infections have been investigated. One option for treating *E. coli* infections is the use of antibiofilm peptides, which inhibit bacterial biofilm (Pletzer and Hancock, 2016). Another alternative therapy involves the use of bacteriophages, bacterial viruses. The use of phage also has side effects, because it creates a strong immune response, due to its own peptide coating, which the body recognizes as a foreign body. Phage use may represent supportive therapy (Nilsson, 2014). More recently, the effect of the probiotic strain *Lactobacillus rhamnosus* on *E. coli* has been investigated, where it has been shown that this species of *lactobacillus* inhibits biofilm formation, which can help prevent urogenital and intestinal infections (Petrova et al., 2016). In the treatment of *E. coli* infections, old AB colistin from the polymyxin group is returned to use, and less toxic carbapenems are introduced in humans, but they have not yet been approved for use in animals. The data, which indicate an increasing presence of resistance and reserve antibiotics, are worrying (Boyen, 2010; Meletis,

2016; Alonso et al., 2017; Bilić, 2018; EFSA, 2018). In recurrent urinary tract infections with UPEC, the biological activity of cranberries is also supported, as a form of supportive therapy with antibiotic therapy (Staptelon et al., 2012).

Errors in the application of antibiotic therapy in E. coli eradication

In empirically prescribed antibiotic therapy, the most common mistakes are reflected in the wrong choice of AB, inadequate dose, improper schedule of prescribed doses and irrational, excessive use of AB, continuous antibiotic treatment without considering the possibility of super infections, various side effects, ABR and other risks that long-term use of AB may cause within the body. Errors in the application of AB are possible due to ignorance of the virulence of the bacterium, the degree of pathogenicity of the bacterium that caused the infection, ignorance of the current data of ABR monitoring, or ignorance of the current results of ABR monitoring at the international and local level. Doctors, most often make a mistake in prescribing AB in any case of hyperthermia, not relying on the body's defense mechanisms. Prescribing AB in diseases of viral etiology, prophylactic measures in delaying surgical operations, especially festering wounds, since the action of AB is reduced in organic detritus, is also a mistake. A possible mistake is the use of AB based on the wrong identification of the cause, and the wrong interpretation of the antibiogram. Mistakes in the use of AB increase the risk of bacterial mutants, changes in the genetic material of the bacterium and ABR (Bagatin, 2000).

Hazard use of antibiotics in animals used for human nutrition

Intensive animal husbandry on farms is often associated with the irrational use of AB for the purpose of increasing growth. Animals have been treated with various AB for years since birth in order to show a prophylactic and therapeutic effect. In AB veterinary medicine, in addition to exhibiting a therapeutic effect, they may exhibit biostimulation effects, in domestic animals for food production, and may still be used. The use of biostimulators reduced the incidence of disease in animals and contributed to food safety in production. Recently, however, there is evidence to suggest that this type of prophylaxis carries a high risk of producing ABR in bacteria, especially those that are constantly present within the body of treated animals. In this regard, the European Union has banned the use of AB in animals as a biostimulator, as a measure of protection against the occurrence of ABR (Chantziaras et al., 2014; Hao et al., 2014). Prolonged, irrational use of AB may lead to the occurrence of ABR (Hendriksen et al., 2008). A special problem is opportunistic bacteria, such as *E. coli*, which according to many authors is listed as a reservoir of resistance genes, because it is constantly present in the body (Schierack et al., 2009). Animals, which are carriers of resistant *E. coli* and are used for human consumption, transmit resistance genes through the food chain through food contaminating bacteria to bacteria of human origin. The described phenomenon in the world of bacteria is attributed to horizontal / lateral gene transfer (Thorsteinsdottir et al., 2008). Increasing the resistance of *E. coli* strains increases the risk of possible loss of effective AB. The pathogen *E. coli* becomes more numerous and therefore more dangerous. Another danger of irrational application of AB is the danger of cumulation and manifestation of cumulative effects on the individual organism. Residues can be found in meat, milk, eggs and other products of animal origin. Harmful consequences are reflected in environmental pollution and harmful effects on animals and humans, who consume food and products of animal origin. The application of AB changes the composition of the microflora, leads to ABR, and a number of autoimmune diseases, allergic syndromes, etc. (Shankar et al., 2010). A third danger to public health is the consumption of heat-treated food, which contains AB residues. Many chemical structures

during heat treatment can become even more toxic and cause a number of harmful effects on the individual organism (Botsoglou and Fletouris, 2001; Mateu and Martin, 2008).

Disinfectants used in the application of hygienic and sanitary standards

For successful disinfection of various surfaces in food technology, it is very important to properly mechanical cleaning which will remove stubborn impurities and allow the disinfectant to work successfully. Certain disinfectants are effective even if mechanical cleaning is not applied, but much more disinfectant is needed to get the desired effect, because the disinfectant will be spent too much on the cleaning itself, instead of reducing the remaining population of undesirable pathogenic microorganisms. There are various disinfectants on the market, which show both the power of cleaning and the power of disinfecting the space, and some even deodorize. Some agents exhibit residual activity and remain active on the surface for longer. Some are corrosive, irritating to mucous membranes, and better in terms of biosafety do not have these side effects. Newer disinfectants are less toxic and do not pollute the environment. Known disinfectants are various surfactants, detergents: quaternary ammonium compounds or cationic surfactants, anions, nonionic surfactants, aldehydes, cresols, phenols, alcohols, halogen elements, among which the most famous and most effective is chlorine, then acids, bases, salts, etc. (Quinn et al., 2011).

Contamination of beef through different stages of primary production

Slaughter of domestic animals used for human consumption is the primary phase of processing, but also the most sensitive phase in terms of the possibility of contamination of raw meat through contaminated carcasses, because in this phase it separates clean from impure part. The carcasses are often contaminated with enterobacteria originating from the intestinal tract of the animals being slaughtered. If hygienic standards are not observed in the slaughter process, all other applications of GHP and GMP in the late stages of production are in vain. In order to reduce contamination, it is important to apply the HACCP concept enabled by the application of GHP and GMP. The application of DHP is very important during slaughter, in order to reduce the possibility of contamination of raw meat. It is important to prevent cross-contamination from production, work surfaces, to neutralize the contaminants present on raw meat, but the correct application of the HACCP system does not exclude contamination (Marković et al., 2012).

Antibiotic resistance *E. coli*

Basic knowledge of antibiotic resistance

Alexander Fleming warned about the possibility of ABR of bacteria in case of irrational use of AB in medicine, veterinary medicine and economy, which is true today, transmitted directly and through the food chain (Hogberg et al. 2010; Jelesić et al. 2011). In order to reduce the growth of resistant bacteria, it is necessary to monitor the occurrence of local ABR, especially pluripotent bacteria prone to multiresistance (Felmingham, 2002; Jelesić et al., 2011). Bacteria are of short life span, a variable genetic material depending on the use of certain AB during life (Aaerstrup et al., 2008; Szmolka and Nagy, 2013). Altered genetic material, which contains genes from ABR, is transmitted to offspring by vertical gene transfer, and to different bacteria by horizontal gene transfer (Boireau et al., 2017; Poirel et al., 2018). There are also biochemical mechanisms of bacterial ABR, which are consistent with the mechanisms of action of AB. Thanks to these mechanisms, bacteria can long have ABR and at a time of abstinence from AB

(Richardson, 2017). It is important to emphasize that horizontal transfer is also possible through animal bacteria, potential contaminants of food to bacteria originating from humans, most commonly via the food chain (Boireau et al., 2017; Yassin et al., 2017; Poirel et al., 2018).

Methods for generating resistance mediated by genes

ABR can be genetic, natural / intrinsic and acquired. Genetic ABR results from a mutation in the bacterial chromosome. Acquired ABR to once effective AB represents an unpredictable genetic change in a bacterium, whose ancestors are sensitive to a particular AB, which is ineffective in offspring. The genetic material of the bacterium can mutate, as a result of bacterial defense, adaptation, survival in the case of the use of certain AB (Boireau et al., 2017). Altered genetic material, transmitted to offspring via vertical gene transfer. Gene transfer from one bacterium to another, of the same or different species is also possible by horizontal gene transfer, via sex saws, mediated by plasmids, through the conjugation process, and gene transfer is possible after bacterial death, by the transformation process or by bacteriophage / bacterial virus, by the process transductions (Aaerstrup et al. 2008; Giedraitiene et al. 2011; Cantas et al. 2013). The described horizontal transfer of resistance genes is also possible from animal bacteria to human-derived bacteria via the food chain (Thorsteinsdottir et al., 2008; Szmolka and Nagy, 2013). Inborn ABR is a genetic characteristic of a particular bacterium (Giedraitiene et al., 2011; Neu, 1992).

Antibiotic resistance gene transfer processes, conjugation, transformation and transduction

Bacteria can alter inherited material through the exchange of DNA between microorganisms, through the mechanisms of gene recombination, or by the process of conjugation, transformation and transduction. This causes changes in bacterial inherited material and expression of new traits, in some cases ABR (Poirel et al., 2018). Conjugation is the process of transferring bacterial DNA from one bacterium to another, and this mechanism of gene recombination is evident in *E. coli* and other gram-negative bacteria (Pavlica, 2012). Transformation is the process of changing a bacterial's genetic material, which occurs when a bacterium adopts free DNA from the environment. This ability has certain species of *Streptococcus spp*., *Hemophilus influenza*, and certain species of *Neisseriae spp.* (Rice et al., 2003; Murray et al., 2003). Transduction is the process of recombination of a gene when a non-pathogenic bacterium, through lysogenic conversion, becomes a pathogen. The described phenomenon occurs when a bacterium virus (bacteriophage) attaches itself to bacterial saws and injects its DNA into it (transformation), "abducts, infects a bacterium" and uses its components to form new parts of the phage. In some cases, the reproductive cycle of the phage kills the bacterium and in other cases the bacterium survives. Then the virus incorporates its DNA into the bacteria's DNA. The new phage depends on the bacterium to replicate the new phage parts. When a new phage leaves the host by the lysis process, some also carry parts of bacterial DNA. If such a bacteriophage attaches to the "new bacterium" by the same originally described process, and the DNA thus created is incorporated into the DNA of the new bacterium, a new genetic type of bacterium may be formed. This process is called transduction. A bacterium becomes pathogenic when a bacterial virus enters the lysogenic cycle and embeds its genome within the bacterial chromosome, and it acquires the toxic properties of the virus (Dale and Park 2004).

Biochemical mechanisms leading to antibiotic resistance

ABR does not have to be related to the genetic and phenotypic changes of the bacterium, as bacteria may develop certain biochemical mechanisms, which are related to the mechanism of AB action to which the bacterium has acquired resistance (Aaerstrup et al., 2008; Szmolka and Nagy, 2013; Boireau et al., 2017). Biochemical mechanisms of ABR formation are enzymatic inactivation and modification, alteration of gyrase DNA and topoisomerase IV, target modification, alteration of ribosome structure, active efflux, alteration of metabolic pathway, and alteration of cell membrane permeability (Poirel et al., 2018).

Enzymatic modification

Bacterial enzymes have the ability to inactivate AB. This type of ABR has been reported with β lactam AB. There are more than three hundred created bacterial enzymes of β lactamase, which have the ability to cleave the links of the β lactam ring of AB, whose active penicillin nucleus is six amino penicillin acid, to which a five-membered thiazolidine ring is attached to the β lactam ring and inactivated. One of the enzymes produced by bacteria resistant to cephalosporins is serine β lactamase. The enzyme produced by resistant strains of carbapenems, which, in addition to other mechanisms of ABR to carbapenems, causes their inactivation is carbapenem hydrolyzing oxacillinase (OXA), responsible for the emergence of *Acinetobacter boumanni* resistance (Medić et al., 2011; Thomson et al., 2005; Shaikh et al., 2015).

Alteration of DNA gyrase and topoisomerase

The DNA is located in the dividing and unbundling regions in the form of super strands. The form is maintained by topoisomerase IV. Excess super strands remove gyrase DNA during replication. Following DNA replication, topoisomerase IV helps to separate daughter DNA (Ferguson et al., 2007; Hopkins et al., 2005). Alteration of DNA gyrase and topoisomerase IV is an acquired bacterial mechanism of ABR, which leads to antibiotic inefficiency, because gyrase DNA is responsible for maintaining DNA in the form of super-strands and eliminates excess super-strands during replication times. Bacteria develop this mechanism for AB, which exert their mechanism of action by inhibiting DNA synthesis and replication and acting on gyrase DNA (Khodrsky et al., 1995; Poirel et al., 2018).

Target modification (change of target enzyme)

This biochemical mechanism of ABR is evident in gram positive cocci, *Enterococcus* spp. ABR is based on the formation of a weakly binding bacterial protein from or modification of the target site of action of the AB. The β-lactam AB is usually bound to the binding protein. Bacteria produce a poorly binding protein, which causes catalysis of peptidoglycan synthesis. Reduced affinity to the target binding site leads to the emergence of ABR of the bacteria (Garrelts, 1996; Pitout et al., 2004).

Modification of ribosome structure

Alteration of the structure of ribosomes, or receptors, located on the ribosomes of a bacterial cell prevents the binding of AB to the target site of action, that is, to the 30S or 50S subunit of the ribosome. The inability to bind AB to the receptors of the 30S or 50S ribosome subunit is via the erythromycin methylase gene. This biochemical mechanism of ABR is developed by AB bacteria, which exert their mechanism of action by inhibiting protein synthesis, acting on

the subunits of ribosomal activity. These are bacteriostats of the nitrofuran, tetracycline, aminoglycoside, aminocoumarin, and rifamycin bactericides (Lambert, 2002; Quin et al., 2011).

Active efflux

Active efflux, as a mechanism of ABR, is used by many gram-negative bacteria. For this mechanism to work, the bacterium must have a functional metabolism, as it takes place with energy expenditure (Džidić et al., 2008). Bacteria that use active efflux, as a mechanism of ABR, achieve ABR in a way that eliminates the AB via a pump or by a subset of carrier proteins. (Lindgren et al., 2003).

Modification of metabolic pathway

The bacterium can undergo metabolic changes, develop new target sites by which it uses growth factor, that is, paraaminobenzoic acid. This kind of biochemical mechanism, bacteria develop to acquire ABR to trimethoprim and sulfonamides. The biochemical mechanism is based on a decrease in bacterial sensitivity and a decrease in the sensitivity and affinity of the enzyme dihydropteroate synthetase and dihydropteroate reductase (Jacoby and Munoz - Price, 2005).

Modification of cell membrane permeability

Hydro-soluble AB exert their mechanism of action on bacteria by passing through bacterial channels (porins). Bacteria alter the permeability of porin, and this bacterial phenomenon is most often relevant to gram negative bacteria. This type of resistance mechanism is acquired by *Pseudomonas aeruginosa* for all AB (Lambert, 2002; Ferguson et al., 2007; Sevic, 2007).

Antibiotic resistance monitoring data *E. coli* from animal producing food

A review of studies to date on the prevalence of ABR of *E. coli* in food-producing animals, in some cases, show variations and in some do not (Wasyl i sar., 2017). ABR is also present in *E. coli* isolates from food-producing animals such as cattle, pigs and poultry. ABR to β-lactam AB, penicillins (amoxicillin), 3rd generation cephalosporin (ceftiofur), aminoglycosides (streptomycin), tetracyclines (tetracycline), sulfonamides / diaminopyrimidine (sulfamethoxolin) trimethoprimacin (trimethoprimacin / trimethoprimacin / trimethoprimacin / trimethoprimolin). The maximum percentage of ABR to ceftiofur was recorded in 2010 at a percentage of approximately (22%), but in the following years the percentage decreased. The percentage of resistance to fluoroquinolones was (30%), with no significant differences between different animal species, with a slight decrease after 2009. The ABR for tetracyclines and amoxicillin was high (90%) and (40%), but decreased after 2010. Poultry resistance after 2009 was (84%) in 2009, (43%) in 2015, which was specifically recorded for tetracyclines. In Member States of the European Union (EU), the percentage of ABR of *E. coli* producing β-lactamase of a broad spectrum of activity originating from poultry varies between countries by (10%) and over (70%) (EFSA and ECDC; 2018). China is the largest consumer of AB in the world (Zhang et al., 2015). In a survey in China between 2004 and 2012, the percentage of *E. coli* resistance isolated from chickens, ducks, pigs and cows was evident. The highest percentage of resistance of *E. coli* isolates of all animals was recorded on tetracyclines, nalidixin acid, sulfamethoxazole, trimethoprim / sulfamethoxazole and ampicillin, and increasing resistance was also recorded for amikacin, aztreonam, ceftazidime, cefotaxime, ciprofloxin, ciprofloxin, aliprofloxin resistance was generally low. For the third generation of

cephalosporins: ceftazidime was (7.6%), ceftriaxone (17.1%), cefotaxime (14.8%). The lowest percentage of resistance was observed for amoxicillin-clavulanic acid in the amount of (3.4%) and ertapenem in the amount of (0.2%). The most multidrug resistant *E. coli* isolates were isolated from duck (100%), chickens (82.2%), pigs (82.3%), cows (21.3%). Duck- derived *E. coli* isolates have shown resistance to 6-8 AB, and those sensitive to 11 AB have been reported (Yassin et al., 2017). These ABR monitoring data are important for risk assessment as well as for evaluating the effectiveness of measures for the survival of effective AB. At the World Economic Forum, held in 2013, ABR was presented as one of the major global risks for humans and animals (Schrijver et al., 2018).

Methods to provide antibiotic resistance

E. coli can be cultivated on almost all laboratory media, liquid (broth), semi-solid and solid nutrient media, to which agar (agar) is added, such as MacConkey, blue agar (bromocresol-purpur) and blood agar (Maksimovic and Rifatbegovic, 2015). To differentiate *E. coli* from food, it is best to use Tryptone were x-glucuronide medium (TBX) (Gross and Rowe, 1985). A solid nutrient medium (agar) can also be treated with an antibiogram. The methods that are routinely used are: disc diffusion method, qualitative and dilution method, quantitative, which can determine the minimum antibiotic concentration (MIC) required to exert a therapeutic effect. For the MIC determinations, the E-test method is also used (Jorgensen and Turnidge, 2003; Mulić, 2013). In determining colistin sensitivity, disc diffusion method is not recommended, as it may be unreliable (Boyen et al., 2010). There are also innovative phenotypic tests to demonstrate the various mechanisms of E. coli resistance, most commonly the ESBL and beta-lactamase AmpC extended-spectrum β-lactamases, which inactivate newer generation III cephalosporins and β-lactam carbapenems, causing the loss of porin through which antibiotics enter in the bacterium, to exert a mechanism of action. Carbapenemase-producing bacteria are usually resistant to all existing antibiotics (Cornaglia et al., 1999; Walsh, 2008). The phenotypic tests also include a modified Hodge test, a combi combined with polymerase chain reaction (PCR) and PCR protocol detects carbapenemases with a high percentage sensitivity of 100% in gram negative bacilli. More recently, PCR methods for the detection of resistance genes, which encode various types of ESBL extended spectrum β lactamases and clones, have been in use (Lee et al., 2013; Osei Sekyere et al., 2015). PCR can also be used to determine the phylogenetic affiliation of an isolated bacterium, E. coli, which determines its pathogenicity (Clermont et al., 2000; Antão, 2010; Clermont et al., 2011). All E. Coli strains can be divided into four phylogenetic groups: A, B1, B2, and D, whose membership is demonstrated by PCR, detection of the yjaA and chuA genes and TspE4 DNA. C2. In this paper, a disc diffusion method was used to determine the sensitivity of *E. coli* isolates, which is a qualitative method. Disk diffusion method, also called the tablet method. The discs are small paper pills, lined with a specific concentration of antibiotics. "*In vitro*" antibiotic susceptibility testing is tested in such a way that the antibiotic concentration is appropriate to that achieved in the "*in vivo*" individual. According to the recommendations of the Institute for Clinical and Laboratory Standards and the European Commission for Antimicrobial Sensitivity Testing, different AB are used at different, well-defined concentrations. The second standard according to CLSI and EUCAST, includes standardized temperature, pressure, pH, incubation time. The substrate that meets the standard for performing the method is Mueller-Hinton agar (MHA), which allows the bacteria to reproduce without interruption. A certain pH (7.3) is slightly acidic and ideal for bacterial development, and altered standards can result in errors (Bronzwaer et al., 2002; Jorgensen and Turnidge, 2003). Meat extract, sheep blood or defibrinated horse blood is added, which is low in acidic pH and an ideal medium for bacterial development (Andrews, 2008). Starch is also added, which, along with meat, is a source of food

for bacteria that are seeded on agar in pure culture. The amount of agar is 17 g and the distilled water is 100 ml. It is sterilized in an autoclave at 121 ° C for 15 min before inoculation of the pure bacterial culture to avoid contamination. When pure bacterial culture is poured onto the substrate, paper disks are placed. Up to five commercially prepared fixed antibiotic discs are used, if the Petri cup is 100 mm in diameter and if it is 150 mm, 12 discs are used. Then incubation is carried out in a thermostat for 16-24 hours, at approx temperature of 37◦C, which is ideal for bacterial growth. AB from the disc diffuse through the substrate and create a zone of inhibition. A zone of inhibition is created around a disposed disk, in the form of a circular clearing, which is a reaction of the action of AB. Depending on the zone of inhibition created, we conclude whether the bacterium is sensitive (S), intermediate (I) or resistant (R) (Bedenić, 2005; Jorgensen and Turnidge, 2003).

CONCLUSION

Assessment of epidemiological data regarding *E. coli* infections has shown a significant prevalence of intestinal and extraintestinal infections in humans, indicating that *E. coli* infections are a significant public health problem. Alimentary intoxications in humans, caused by the pathogen *E. coli* are present worldwide, and toxoinfections are primarily transmitted by food of animal and plant origin, but also by non-chlorinated water. Since *E. coli* is commensal and is constantly present in the intestines of mammals, the prevalence in the environment is considerable, and prevention includes the application of high hygienic and sanitary standards when handling food, of animal and plant origin. In addition to the possible disease of consumers, another important problem is the antibiotic resistance of *E. coli*, which is widespread, since *E. coli* is constantly present in all mammals and its genetic material is variable and often changes under the influence of various antibiotics. Resistance as well as multidrug resistance of *E. coli* is on the rise and *E. coli* infections threaten to become incurable diseases. The key to preventing the spread of resistance is in the rational use of antibiotics in agriculture, veterinary medicine and medicine, because bacteria spread resistance genes among themselves, and resistance genes are also transmitted from food bacteria to bacteria of human origin. In the case of *E. coli* infection, the biocidal effects of natural preparations based on cranberries have recently been investigated, and the use of probiotic cultures that show efficacy and safety is recommended.

Abbreviations AB: Antibiotic; ABR: Antibiotic resistance; AIEC: Adherently invasive *E. coli*; APEC: Avian pathogen *E. coli*; CLSI: Institute for Clinical and Laboratory Standards: DNA: Deoxyribonucleic acid; E. coli: *Escherichia coli*; EPEC: Enteropathogen *E. coli*; ETEC: Enterotoxinogen *E. coli*; EIEC: Enteroinvasive *E. coli*; EHEC: Enterohemorrhagic *E. coli*; GMH: Good Hygiene Practice; GMP: Good Manufacturing Practice; HACCP: Hazard Analysis Critical Control Point; SEPEC: Septicemic *E. coli*; STEC/ VTEC: Shiga toxin *E. coli* or verotoxinogen *E. coli*; UPEC: Uropathogen *E. coli*.

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FOOD PATHOGEN OF BROILER CHICKEN MEAT CAMPYLOBACTER SPP. AND ANTIBIOTIC RESISTANCE

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ABSTRACT

Campylobacter spp. are the most common microbiological food contaminants. Consumption of such foods, most commonly chicken meat, causes disease in humans, and the species most common are *C. jejuni* and *C. coli*. Campylobacteriosis most often manifests itself in the form of gastroenterocolitis. The chronic form can result in more serious complications in the form of *Guillain-Barré syndrome* (GBS) and *Miller Fisher syndrome* (MFS) which cause partial or complete paralysis, chronic diseases and a decline in intestinal tract immunity. Another important aspect of the presence of *Campylobacter* spp. in food, in addition to the possible disease of consumers, there is antibiotic resistance (ABR), which consequently significantly reduces the available therapeutic options. Recent research detects resistant isolates from broiler production and carcasses, with the presence of resistance to certain antibiotics (AB) showing a certain regional specificity, as well as a growing prevalence of fluoroquinolones, which may have a significant implication on human health. *Campylobacter* spp. have the ability to accumulate resistance genes that are most commonly transmitted by horizontal plasmid transfer between competent bacteria. The most problematic biochemical mechanism of resistance in *C. jejuni* and *C. coli* is active efflux which confers resistance to various antibiotics. In order to prevent the spread of campylobacteriosis, it is important to apply the *Hazard Analysis Critical Control Point* (HACCP) concept in food production, which is enabled by *Good Manufacturing Practice* (GMP) and *Good Hygiene Practice* (GHP). In order to prevent the spread of ABR, it is necessary to rationalize the consumption of AB, especially in veterinary medicine. Monitoring is an important assumption that increases the awareness of participants in production and ensures the quality and safety of food.

Key Words: *Campylobacter spp.,* broiler meat production, sanitation efficacy, antibiotic resistance.

INTRODUCTION

Campylobacter spp. are microaerophilic, gram negative bacteria, of zoonotic potential, which are transmitted from animals to humans and cause campylobacteriosis. *Campylobacter* spp. are commensals of the intestinal tract of all warm-blooded animals. For public health, *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter fetus* and *Campylobacter upsaliensis* are of particular importance (Workman et al. 2005). Campylobacteriosis is a seasonal zoonosis, and the predilection site of bacteria is the epithelium of the intestinal and reproductive tract mucosa (Chukwu et al., 2019). Animals rarely contract the disease and most commonly occur in humans in the form of gastroenterocolitis. If they enter the bloodstream, various complications are possible (Markey et al., 2013). The transmission path is direct and indirect. Hazard represent different stages of primary production (fattening, slaughtering and primary processing of chicken meat) (Crim et al., 2014). The possibility of cross-contamination also poses a danger (Crim et al., 2014). Animal feed also plays a role in the spread of

campylobacteriosis (Kaakoush et al., 2015). *Campylobacter* spp. they are sensitive to different disinfectants, it is therefore important that hygiene and sanitary standards are respected at different stages of primary production, from farm to table (Crim et al., 2014). Laboratory detection is important for identifying foodborne pathogens (Nicholas, 2005). Treatment is supportive, with or without antibiotic (AB) administration. Antibiotic resistance (ABR) has been linked to the irrational use of antibiotics in industry, veterinary medicine and medicine (Wieczorek and Osek, 2013). In order to prevent the spread of ABR, it is necessary to rationalize the consumption of AB (Garcia-Migura et al., 2014). The genetic material of bacteria can be mutated (induction) and ABR genes can be transferred between the same and different bacteria, including those with different phylogenetic affiliations. Bacteria also acquire biochemical mechanisms of ABR: enzymatic inactivation and modification, alteration of DNA gyrase and topoisomerase IV, target modification, alteration of ribosome structure, active efflux, alteration of metabolic pathway, alteration of cell membrane permeability (Wieczorek and Osek, 2013). Species of the genus *Campylobacter* spp. have the ability to rapidly acquire ABR genes, and monitoring data indicate the presence of resistant isolates (Yamada et al., 2019). The *Agency for Food Safety* (EFSA) has introduced monitoring of ABR of food bacteria, but we still do not have regulations in BiH to regulate continuous monitoring and control.

Genaral features Campylobacter spp. from food

Taxonomy

Campylobacter spp. by taxonomic hierarchy belong to the genus *Campylobacter* spp., the family *Campylobacteraceae*, the order *Campylobacterales*, the class *Epsilonproteobacteria*, and the knee *Proteobacteria* (Vandamme and De Ley, 1991; He, 2001). *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter lari*, *Campylobacter hyointestinalis* and *Campylobacter sputorum* are the best known species with zoonotic potential. Genus *Campylobacter spp*. belong to many other species, subspecies, which are still being explored. The development of molecular biology has also revealed more difficult species and subspecies, such as: *Campylobacter concisus*, *Campylobacter upsaliensis* and *Campylobacter ureolyticus* (On, 2001; Sandberg et al., 2002). oday, the authors also mention 30 species, which were isolated in the genus *Campylobacter* spp. (Markey et al., 2013; Chlebicz and Śliżewska, 2018).

Morphological and physiological characteristics

Campylobacter spp. first mentioned by Sebald and Veron in 1963. They were discovered as far back as 1880. by the pediatrician Theodor Escherich, who first described them in 1887. and in 1909. classified them under the names *Vibrio fetus* and *Vibrio bubulus* (Sebald and Veron, 1963; Markey et al., 2013). *Campylobacter* spp. belong to potential zoonoses, diseases transmitted from animals to humans. The same commensals of the intestinal tract of different hosts, domestic, wild animals, mollusks, and the most important vectors are poultry: chicken, duck, goose, turkey, then domestic livestock: cattle, sheep, pigs, pets: dogs and cats, wild animals: most common wild birds, then oysters and shellfish (Krstulović and Šolić, 1997; Brown et al., 2004; Workman et al., 2005). All raw foods of animal origin contain *Campylobacter* spp., so it is important to respect hygienic and sanitary standards in different stages of primary production of broiler, chicken meat, but it is impossible to completely eliminate the pathogen (Ivanović, 2004). They are sensitive to most disinfectants, acids and salts. Formalin and Sodium hypochlorite in a dose of 0.5%, within five minutes, exhibits a germicidal effect on *Campylobacter* spp. They are resistant to low temperatures, but most species are inactivated at a temperature of -12◦C. Some species also survive at -70◦C. They are

sensitive to high temperatures and drying (Ivanović, 2004). Optimal temperature for growth and development of thermophilic *Campylobacter* spp. it ranges from 30◦C to 45.5 ° C (Marriott and Gravani, 2006; Chukwu et al., 2019). *Campylobacter* spp. are small bacteria, spirally curved, S-shaped, spreading seagull wings or rod-shaped. Gram-negative, asporogenic, motile bacteria, which move in a spiral with the help of a polar flagella. Length 0.5-5 μm, diameter 0.2-0.5 μm. *Campylobacter* spp. are oxidase positive, urease, gelatin and indole negative, do not ferment carbohydrates, but most reduce nitrate to nitrite. The exceptions are *Campylobacter gracilis*, which is immobile and oxidase negative and *Campylobacter showae*, which possesses multiple flagella (Facciolà et al., 2017). Species differentiation was based on phenotype, cultivation temperature, and biochemical tests (Table 1) (Markey et al., 2013).

Table 1. Differentiation of species of the genus *Campylobacter spp*. (Markey et al., 2013)

Campylobacter spp.	Catalase	5◦C	2◦C	1% glycine	3,5% NaCl	H2S
C. fetus subsp. venerealis	+	+	-	-	-	-
C. fetus subsp. fetus	+	+	-	+	-	+
C. jejuni subsp. jejuni	+	-	+	+	-	+
C . lari	+	-	+	+	-	+
C. coli	+	-	+	+	-	+
C. hyointestinalis	+	+	+	+	-	+
C. mucosalis	-	-	+	+	-	+
C. sputorum biovar Sputorum	-	-	+	+	+	+

(+) = 90% and more strains positive (–) = 90% and more strains negative

Campylobacter jejuni, *Campylobacter coli* and *Campylobacter lari* are physiologically, morphologically and biochemically similar, so additional tests are performed in laboratory diagnostics, with the help of which we perform differentiation (Table 2) (Markey et al., 2013).

Table 2. Biochemical properties of *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* (Markey et al., 2013)

Biochemical tests	***C. jejuni***	***C. coli***	***C. lari***
Test: oxidase	+	+	+
Test: catalase	+	+	+
Sensitivity to nalidixic acid	S	S	R
Test: hydrolysis of hippurate 1% Na-hippurate = benzoic acid + glycine	+	-	-

(+) = 90% and more strains positive
(–) = 90% and more strains negative

S – Sensitive
R – Resistant

Campylobacter jejuni and *Campylobacter coli*, because of their similarity, were once classified under one subspecies. Both are variable morphologies, but are most commonly S-shaped. *Campylobacter coli* is more resistant to the presence of air than *Campylobacter jejuni*. Both move rapidly through the epithelium of the intestinal tract, due to the viscous medium. To differentiate these two subspecies, hippurate hydrolysis assays are in use, which are more

sensitive today (Murray et al., 2002). *Campylobacter lari* is also similar to the species described above and can very easily be confused with *Campylobacter jejuni* or *Campylobacter coli*. To identify and differentiate these three species, it is necessary to additionally perform a sensitivity test to nalidixic acid. *Campylobacter jejuni* and *Campylobacter coli* are naturally sensitive to nalidixic acid and *Campylobacter lari* is naturally resistant to the same (Markey et al., 2013). A problem in the identification of these three species is the spread of acquired resistance to nalidixic acid in certain species of *Campylobacter jejuni* and *Campylobacter coli* (Ristić et al., 2009). Another problem in the identification is the presence of new biovars within the species *Campylobacter lari*, which are also urease positive and sensitive to nalidixic acid (Endtz et al., 1997). Genus *Helicobacter spp*. and *Campylobacter spp*. they are also similar. Because of their similarity, they were once classified under one genus. To differentiate the mentioned genera, a urease test is used today, which is in the genus *Campylobacter spp*. negative and in the genus *Helicobacter spp*. positive (Markey et al., 2013). *Campylobacter spp*. they grow best in the presence of a small concentration of oxygen in the amount of 5-7% and carbon dioxide of 5-10%, which they need for cellular metabolism, because they are microaerophiles. *Campylobacter coli* is more resistant to the presence of air than *Campylobacter jejuni*. Due to age or the presence of oxygen radicals, colonies of *Campylobacter spp*. can become cocoid and then difficult to subculture. The appearance of colonies is variable and depends on the species *Campylobacter spp*. and used nutrient media for cultivation. Colonies are not hemolytic (Table 3) (Quinn et al., 2011).

Table 3. Morphology of *Campylobacter spp*. colonies (Quinn et al., 2011)

Campylobacter spp.	Colony morphology
Campylobacter jejuni	Mostly small, flat, gray, watery in appearance.
Campylobacter coli	Mostly small, round, shiny and white.
Campylobacter fetus subsp. fetus, Campylobacter fetus subsp. venerealis.	Mostly small, round, smooth, transparent, and some slimy, rough and slightly pigmented.
Campylobacter upsaliensis	Older than 48 hours, they become spotty.
Substrate type	Colony morphology
Karmali agar	Flat, gray, moist and spilled.
Skirrow agar	Gray and brown.
Modifikovani agar sa ugljenom-cefoperazonom-deoksiholatom (mCCDA)	They are mostly small, flat, grayish white, spilled, shiny and watery in appearance.

Etiology, Epizootiology

Campylobacteriosis is a zoonosis, which means that it is transmitted from animals to humans (Sahin et al. 2008). In humans, it causes campylobacteriosis with symptoms of acute food poisoning (Markey et al., 2013). Certain species of the genus *Campylobacter spp*. in humans they cause alimentary intoxications, as does *Salmonella spp*., but are less well known (EFSA and ECDC, 2012). *Campylobacter jejuni* is considered to be the most common cause of intestinal campylobacteriosis, with a percentage of 95-98% of cases and *Campylobacter coli* in 2-5% of cases. Extraintestinal diseases are also possible (Allos, 2001; Vučković and Plečko, 2013; Wieczorek and Osek, 2013). For small *Campylobacter jejuni*, an infectious dose of 10^5 bacteria (a drop of contaminated meat juice) is bound, a much smaller dose than the infectious dose of *Salmonella spp*. (Tambur et al., 2009). Immediately after *Campylobacter jejuni*, *Campylobacter upsaliensis*, also a bacterium of zoonotic potential, originating from the intestinal tract of pets, dogs and cats, is associated with cases of gastroenterocolitis (Cvetnić, 2002; Mohammed, 2010). The most sensitive are kittens and puppies, housed in shelters and

who have not received a sufficient antibody titer from their mother. Acceptable adults are those who are exposed to stress, poor zoohygienic conditions and an improper diet (Marks et al., 2011). In humans and animals, intestinal and extraintestinal campylobacteriosis can be caused by *Campylobacter fetus* and its subtypes. The sources of the disease are secretions and excretions of the genitals, aborted fetus, envelopes and contaminated food. The infection is also spread by coitus (Kalenić, 2005; Allos et al., 2014). It is thought that the general population is regularly exposed to *Campylobacter fetus*, but that it is rarely isolated from food due to the unsuitability of selective media for its isolation (Wagenaar et al., 2014). Humans become infected through direct contact with poultry, livestock or dogs, cats and indirectly, through contaminated, non-chlorinated water, unpasteurized milk, insufficiently heat-treated meat (Marriott and Gravani, 2006; Tambur et al., 2009; Wieczorek and Osek , 2013). Transmission from infected person to person is very rare (Huang et al., 2005). *Campylobacter spp*. are commensal, intestinal bacteria, which can enter the bloodstream through the intestinal wall and cause a number of different infections (Chukwu et al., 2019). Complications are rare, with the exception of patients with immunocompromising diseases, such as HIV and various autoimmune diseases (Kalenić et al., 2013; Markey et al., 2013). Young children are especially susceptible (Balen-Topić et al., 2007; Trošelj-Vukić and Cekinović, 2010).

Primary and secondary food contamination

Primary contamination of food for human consumption most commonly originates from the feces of poultry and domestic livestock (Markey et al., 2013). In cattle, it is not uncommon for bacteria to reach the mammary gland, so unpasteurized milk from infected cows is dangerous to consume. Unpasteurized eggs are also a source of infection, if they come from an infected animal. All food of animal origin can also be subject to secondary contamination, which means that it can be subsequently contaminated with *Campylobacter spp*. The danger is also the cross-contamination of roasted meat with raw meat, which is possible on stumps where processing is done, on tables, and the carriers can be insects and rodents with their fecal products and urine. Food can also be contaminated by germ carriers (HAH, 2015/16). Food contaminated with *Campylobacter spp*. it does not lose its organoleptic properties, therefore laboratory detection of the causative agent is extremely important. Fodder, which can be contaminated with *Campylobacter spp*., through the feces of poultry, domestic livestock or smallpox, rodents, insects and birds, also plays an important role in the spread of campylobacteriosis (Nicholas, 2005). Food trafficking has enabled the dissemination of pathogens worldwide and campylobacteriosis is today a global, public health problem (Kaakoush et al., 2015). Although campylobacteriosis most commonly occurs as a sporadic toxin infection, epidemics have been described worldwide, most commonly related to the consumption of contaminated animal food or drinking water (Table 4). Hazard is also represented by different stages of primary production (fattening, slaughter and primary processing of chicken meat), so it is important to respect hygienic and sanitary standards in different stages of primary production, from farm to table (Crim et al., 2014). It is extremely important to respect the concepts of production control, which include *Hazard Analysis Critical Control Point* – HACCP, *Longitudinal Integrated Safety Assurance* – LISA, *Good Manufacturing Practice* – GMP, *Quality, Safety, Acceptability* – QSA, *Specific Pathogen Free* – SPF etc. (Nedić, 1991; da Cruz, Cenci and Maia, 2006). Each country should have a strategic plan and strict biosecurity measures to prevent contamination of production facilities with *Campylobacter spp*. (Facciolà et al., 2017). Regular monitoring, computerization of the system, as well as cooperation of all participants in the primary production of broiler and chicken meat are indispensable in the control of the mentioned measures. The evaluation of the veterinary health system represents protection against financial risk, health protection, quality, safety and consumer satisfaction.

Table 4. Campylobacteriosis associated with the consumption of contaminated food and water

Area	Cases / year	Food and water	Source
USA	12 in 1984	Milk products	(Silva et al., 2011)
Australia	78 in 1995	Cucumber	(Kirk et al., 1997)
Spain	79 in 1998	Custard	(Silva et al., 2011)
Australia	11 in 2005	Chicken	(Silva et al., 2011)
Denmark	79 in 2005	Chicken salad	(Silva et al., 2011)
Spain	81 in 2005	Custard	(Jiménez et al., 2005)
Scotland	86 in 2005	Chicken liver pate	(Forbes et al., 2009)
USA	68 in 2007	Cheese	(CDC, 2009)
Norway	105 in 2007	Unchlorinated tap water	(Silva et al., 2011)
USA	5 in 2008	Unpasteurized milk	(Silva et al., 2011)
USA	98 in 2008	Peas	(Gardner et al., 2011)
Greece	37 in 2009	Unchlorinated tap water	(Silva et al., 2011)
United Kingdom	24 in 2010	Chicken liver pate	(Silva et al., 2011)

Campylobacteriosis epidemics are most commonly associated with the consumption of unchlorinated drinking water, contaminated animal feed, most commonly based on chicken meat, unpasteurized eggs, unpasteurized milk and dairy products, as well as various sauces made from fresh eggs, milk, sugar and sweet cream. Animal food is most commonly contaminated with *Campylobacter spp*. and therefore it is very important to reduce primary and secondary contamination of food and not consume it unless it is decontaminated by potential pathogens, using appropriate, hygienic methods (Silva et al., 2011; Markey et al., 2013). Plant foods can be primarily and secondarily contaminated with various thermophilic species of *Campylobacter spp*., most commonly through bird feces or contaminated irrigation water (Gardner et al., 2011; Facciolà et al., 2017). Cucumber poisoning is also often associated with cross-contamination with raw meat, through contaminated cutting boards or salaries for serving food (Kirk et al., 1997).

Epidemiological data

Campylobacteriosis is a seasonal zoonosis, occurring predominantly in the warmer seasons, in the spring and summer seasons (Marriott and Gravani, 2006; Chukwu et al., 2019). According to many authors, in recent years, there has been an increase in the incidence of campylobacteriosis, with special reference to developed and developing countries (Sahin et al., 2012; Kaakoush et al., 2015). Campylobacteriosis in the United States (USA) is the leading alimentary intoxication, a toxin infection transmitted by contaminated food, where toxoinfections with *Salmonella spp*. are in second place (Table 5) (EFSA and ECDC, 2008-2019). Food-borne food poisoning is not the leading enteritis in Africa, Asia, the Middle East, where intestinal infections are most common, associated with poor hygiene habits, poor living conditions, most often in the poor, children and those with poor immune systems (Vukelic et al., 2003). In underdeveloped countries, one billion people become ill with intestinal infections annually (Trošelj-Vukić and Cekinović, 2010). However, Bartkowiak-Higgo et al., (2006) in a pilot study in South Africa, which related to the percentages of carcass contamination, originating from poultry carcasses with *Campylobacter jejuni* and *Campylobacter coli*, stated that the percentage of skin and liver contamination of poultry was (24%), and intestinal contamination in the amount of (28%), indicating an increased risk of infection for consumers, either through contaminated chicken meat or through cross-contamination with other foods. In the countries of South America and Europe, there is an increase in the percentage of

toxoinfections, the causative agents of gastroenterocolitis, which are transmitted by contaminated food, most often of animal origin (Hugas et al., 2009). Hauri et al., (2013), in their retrospective study, from 2005 to 2011, reported an increase in the incidence rates of campylobacteriosis in Germany. Jore et al., (2010), report data on the decline in the incidence of campylobacteriosis in Sweden and Denmark, in the period from 2001 to 2007. According to epidemiological data from the World Health Organization (WHO), campylobacteriosis is a seasonal disease in Croatia as well. Children and people with weakened immunity are most susceptible to campylobacteriosis (Balen-Topić et al., 2007; Trošelj-Vukić and Cekinović, 2010).

Table 5. Epidemiological data of human campylobacteriosis, on an annual basis (EFSA and ECDC, 2008 - 2019)

Area	Number of patients	Source
Europe	150 332 in 2002	(EFSA and ECDC, 2008)
Europe	139 581 in 2003	(EFSA and ECDC, 2008)
Europe	183 479 in 2004	(EFSA and ECDC, 2008)
Europe	195 426 in 2005	(EFSA and ECDC, 2008)
Europe	175 561 in 2006	(EFSA and ECDC, 2009)
Europe	200 507 in 2007	(EFSA and ECDC, 2009)
Europe	190 566 in 2008	(EFSA and ECDC, 2010)
Europe	198 252 in 2009	(EFSA and ECDC, 2011)
Europe	212 064 in 2010	(EFSA and ECDC, 2012)
Europe	220 209 in 2011	(EFSA and ECDC, 2013)
Europe	214 779 in 2013	(EFSA and ECDC, 2015)
Europe	236 851 in 2014	(EFSA and ECDC, 2015)
Europe	229 213 in 2015	(EFSA and ECDC, 2016)
Europe	246 307 in 2016	(EFSA and ECDC, 2017)
Europe	246 158 in 2017	(EFSA and ECDC, 2018)
Europe	246 571 in 2018	(EFSA and ECDC, 2019)

Since 2005, a growth trend of campylobacteriosis has been recorded in Europe, which ranked the leading alimentary intoxication salmonellosis in second place (EFSA and ECDC, 2008). Campylobacteriosis is also evident in the United States, where out of 250 000 000 to 350 000 000 cases of enteritis annually, 22-30% is due to alimentary intoxications (Mead et al., 1999).

Pathogenesis and mechanisms of pathogenicity

Campilobacter spp. enter the body "*per os*", and the predilection site of action is the epithelium of the mucous membrane of the colon and reproductive tract. Pathogenicity is related to adherence, motility, chemotaxis (directed movement), translocation (passage of infection through the epithelium to other organs), ability to invade (reproduction in the epithelium), biofilm, toxicity, which is manifested "*post mortem*" through endotoxin and the ability to resist host immunity (Quin et al., 2011; Bolton, 2015; Kaakoush et al., 2015). In order to exert a mechanism of action, they must overcome the cellular and humoral immunity of the host (Begovac et al., 2006). The digestive tract also possesses non-immune mechanisms. To cause disease, *Campylobacter spp*. they must pass barriers in the oral cavity in which bactericidal mucosa rich in lysosomes, immunoglobulins, lactoferrin, peroxidase and lipoproteins is secreted. They must then survive the bactericidal action of gastric acid in the stomach and the mild bactericidal action of the same in the small intestine, which acts synergistically with bile

salts (Guerrant, Lohr, & Williams, 1986; Begovac et al., 2006). If bacteria enter the stomach with more sugary, fatty, and high-protein foods such as meat, milk, and dairy products, such foods serve as a buffer and survive (Tambur et al., 2009). Due to the ability of chemotaxis, with the help of flagellas, they move quickly and directed to the small intestine, where bactericidal, polypeptide defensins are also obstructed by them. They then survive the small intestine peristalsis, mix with the mucosa, which is a chemoattractant, and move toward the colon in a directed way (Said and Kaunitz, 2016; Wilson, Wiens, & Smith, 2013). Adhesion to the colonic epithelium was enabled with the help of the enzyme adhesin (Vučković and Plečko, 2013). The most important adhesins are considered to be (O) antigen, which belongs to endotoxins, and capsular (S) antigen, which is characteristic of *Campylobacter fetus*. It interferes with the adhesion of the Cd3 component of complement and prevents bacterial phagocytosis. This antigen is also responsible for the occurrence of bacteremia (Kalenić 2005; Quinn et al., 2011). With the help of the mucinase enzyme, the bacteria penetrate deeper parts of the epithelium and exert a destructive effect on the enterocytes. Enterocyte destruction is mediated by endotoxins, lipopolysaccharides of the bacterial cell wall. Endotoxin is a pyrogenic substance, which damages the endothelium and leads to endoxic shock (Quinn et al., 2011). Invasion occurs with the help of protein pathogenicity, and virulence factors are the strength of enterotoxins (Markey et al., 2013). Edematous changes on the mucosa of the affected intestines are also possible, as well as the appearance of numerous ulcers (Allos et al., 2014). The problem of infection is the non-recognition of structural proteins by antibody receptors, which results in a delayed reaction of the organism, because there is no release of cytokines (Vučković and Abram, 2009). Pathogenesis depends on the causative agent, the diseased individual, age, sex, immunity and site of action (Table 6) (Quinn et al., 2011).

Table 6. Pathogenesis of campylobacteriosis depending on type and site of action *Campylobacter spp*. (Quinn et al., 2011)

The causative agent	Pathogenesis
Intestinal and extraintestinal campylobacteriosis	I
Campylobacter jejuni subsp. jejuni	Via enterotoxin.
Campylobacter coli,	Via enterotoxin.
Campylobacter lari,	Via enterotoxin.
Campylobacter hyoilei,	Via enterotoxin.
Campylobacter mucosalis,	Via enterotoxin.
Campylobacter upsaliensis.	Via enterotoxin.
Extraintestinal campylobacteriosis	II
Campylobacter fetus subsp. venerealis, *Campylobacter fetus subsp. fetus.*	In males, a local infection of the foreskin can occur, without a tendency to spread to deeper parts of the reproductive system, because it is a commensal bacterium. In this regard, antibody production is absent. No self-cleaning. Breeders are carriers of the infection.

Campylobacter fetus subsp. venerealis, *Campylobacter fetus subsp. fetus.*	In females, vaginal epithelial cells invade, with a tendency to spread to deeper parts of the reproductive system, such as fallopian tubes, uterine horns, and uterine cotyledons. It is not a commensal bacterium. Antigens lead to antibody activation. The process of self-cleansing is activated, but it is still a persistent infection, which can last up to 10 months, due to the pathogenic ability of bacteria to resist immunity. Infertility is related to embryo death and resorption of the fetus.

Cellular and humoral immunity

Humoral immunity has a more important role in reducing the frequency of campylobacteriosis, as well as preventing the development of a more severe clinical picture. The clinical picture is of lower intensity in individuals with an adequate titer of specific antibodies, immunoglobulin IgA, IgG, which are evidence of antigen exposure to campylobacter bacteria. It is believed that the most important driver of humoral immunity is the antigen flagellin, from which the flagella of *Campylobacter spp* are built (Vučković and Abram, 2009). Based on epidemiological data, a higher frequency of campylobacteriosis is evident in developed countries, compared to the frequency of its occurrence in underdeveloped and developing countries (Sahin et al., 2012; Kaakoush et al., 2015). Epidemiological monitoring data also indicate a reduced incidence of campylobacteriosis in older individuals compared to young children, which confirms the fact that humoral immunity plays a significant role in the incidence of campylobacteriosis (Balen-Topić et al. 2007; Trošelj-Vukic and Cekinović, 2010). Residents in developed countries, as well as young children, do not have specific antibodies, given their infrequent exposure to bacteria of the genus Campylobacter spp., Relative to population exposure in regions with high levels of drinking water pollution from *Campylobacte spp*. often present specific secretory and serum antibodies, or IgA immunoglobulins, which are produced on the mucosa of the intestinal and respiratory tract. IgM immunoglobulins are also important, which are the first to react to the presence of infection. They are especially important in patients with reduced or complete blockade of IgA production, where production of IgM immunoglobulin is a compensatory defense mechanism (Vuckovic and Abram, 2009). In cellular immunity, the production of specific T – lymphocytes, the so – called "killer cells", whose specific receptors have the ability to recognize infected and damaged cells, which can be infected, and kill them. On the other hand, with the help of receptor inhibition, T – lymphocytes protect healthy tissue from potential damage. Cellular immunity is particularly important in immunocompromising patients, which refers to patients with the presence of a more dangerous, underlying disease that suppresses the activity of humoral immunity, HIV, chronic infection and malignancy. If campylobacteriosis is overcome, immunity is insecure. Re – disease is possible, but in milder form of the disease (Vuckovic and Abram, 2009; Markey et al., 2013).

Non-immune mechanism of colon defense

The mechanism of defense of the colon is also the probiotic flora, which provides a number of benefits to the immune system. Maintains homeostasis, prevents colonization of pathogens, bacteria, parasites, pathogenic fungi and viruses, "transitory bacteria" from food, neutralizes toxins, neutralizes heavy metals, protects against chemicals, deactivates carcinogens, participates in food digestion and nutrient absorption, protects mucous membranes intestine from undigested food particles, maintains the health of enterocytes (Ljungh and Wadstrom, 2006). In their study by Saint-Cyr et al., (2016), it was reported that lactobacilli used in drinking

chickens reduce colonization of the gut with *Campylobacter jejuni*. The probiotic flora, in a healthy colon, is mostly composed of bacteria of the genera *Lactobacillus* and *Bifidobacterium* (Ljungh and Wadstrom, 2006). A thick layer of positive bacteria coats the walls of the colon. Positive bacteria are producers of a number of substances of bactericidal, fungicidal and virucidal effect, but are more sensitive to AB than pathogenic species, which are in a much smaller intestine in the preserved gut microbiota (Zhang et al., 2017). The probiotic flora releases free fatty acids and is responsible for maintaining the optimal pH of the intestine, which prevents the colonization of pathogens. Food, which is taken into the body, plays a major role in regulating the pH of the intestine and can act as a prebiotic and probiotic (Fijan, 2014). Intestinal microflora disorder is most often conditioned by the use of AB, primary disease, stress, anything that contributes to the decline in immunity (Shi, 2017). The claim that we are what we eat is actually thought to be what we absorb from the gut. In the case of disruption of the intestinal microflora, inflammation of the intestinal wall and weakening of the resorption of nutrients from food can occur, which can consequently cause a lack of nutrients. The intestinal microflora participates in the digestion of proteins, ferments carbohydrates, breaks down fats, fibers and produces transporters, which carry nutrients, vitamins, minerals and water through the intestinal wall into the bloodstream. If "bad bacteria" are prevalent in the microflora, enterocytes do not produce food degrading enzymes (Duncan et al., 2007). Without healthy gut microflora, dietary fiber from fruits and vegetables cannot be digested. Neither gluten nor other cereal proteins, legumes or milk protein casein can be digested. Incompletely broken down proteins enter the bloodstream, which can result in the appearance of various health problems and contribute to the development of autoimmune diseases such as celiac disease, Crohn's disease, ulcerative colitis, etc. (Lebwohl et al., 2015).

Probiotic bacteria in food

Foods of dairy origin, which are subject to fermentation, can contain beneficial and pathogenic bacteria. Lactic acid bacteria, in addition to a number of positive functions, which they perform in the intestines of mammals, are also responsible for the fermentation of milk and the preparation of various dairy products, but also sour salads, which are used for human consumption. Consumption of such foods contributes to human health and helps maintain proper intestinal microflora (FAO and WHO, 2001). Reducing the number of lactic acid bacteria in favor of pathogens, contributes to reducing the quality of dairy products. Probiotics belong to the genus *Lactobacillus* and *Bifidobacterium*, with their species and subspecies, and pathogenic bacteria of fermentative food, usually belong to the genus *Escherichia*, *Campylobacter*, *Bacillus*, *Proteus* and *Lysteria*. *Lactobacillus acidophilus* is part of the intestinal microbiota of all mammals and is one of the best known species of *Lactobacillus spp*. Starter culture with *Lactobacillus spp*., is in the retail production chain and is widely used for dairy products (Ljungh and Wadstrom, 2006; 2014). With the help of *Lactobacillus spp*. milk is used to make fermentative products, yoghurt, sour milk and other lactic acid products (Fabre-Gea et al., 2000). In addition to the synthesis of lactic acid, which inhibits pathogens, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus bavaricus* and *Lactobacillus curvatus*, have the ability to synthesize bactericins, which damage the membrane of pathogenic bacteria *Bacillus spp*., *Enterococcus spp*., *Staphylococcus spp*., *Clostridium spp*., *Listeria monocytogenes* (Federal register, 1988). The effect of bacteriocins on food pathogens is still being investigated, and only nisin has been accepted, to be used in the preparation of dairy products (Federal register, 1988; Cotter et al., 2013). Bactericins are commonly used as preservatives in the food and cosmetics industries (Perez, Zendo, & Sonomoto, 2014). Many authors also state that bactericins, derived from Lactobacillus salivarius, can inhibit the growth of gram-positive and gram-negative bacteria, especially *Campylobacter spp*. (Messaoudi et al.,

2012). In their study by Wang et al., (2014), they came to the conclusion that *Lactobacillus plantarum* inhibits the growth of *Campylobacter jejuni* from food and these *Lactobacillus spp*. is recommended for use in food. Microorganisms from food of dairy origin go through three phases, the lag phase, the dormancy phase and the extinction phase, which determine the quality of the dairy product. Some *Lactobacillus spp*. thay can also have a negative impact on food and cause food spoilage (Marriot and Gravani, 2006).

Chemical and biological preservatives in food of meat origin

Meat and meat products, due to their protein and other nutritional composition, are liable to deteriorate at temperatures above 5◦C. Spoilage is caused by the multiplication of microorganisms, from which no type of food of animal origin is completely free, but through various stages of primary production, from farm to table, efforts are made to reduce the number of microorganisms to a minimum. In order to inhibit their growth, and at the same time preserve the sensory properties of food, as well as the nutritional and nutritional values of meat and meat products, various methods are used in primary production, but also the use of high temperature and a number of additives in meat products and export meat, which are used to prolong the life of the consumable product, that is, to inhibit the growth of food microorganisms (Lawrie and Ledward, 2006). Various additives are used, such as: preservatives, antioxidants, stabilizers, emulsifiers, flavor enhancers, volume, acidity regulators, dyes, foaming and anti-foaming agents, substances that absorb excess moisture, substances used to treat flour , carriers, sweeteners, various gases, which suppress excess gas when packaging various products of meat origin. Chemical preservatives, which are approved for use in various stages of production, processing, transport and storage of food, are nitrites and nitrates (potassium nitrite and sodium, E249 and E250), which are most often used in canning finished meat products, ie meat processing. Nitrites should provide a product free of pathogens, which will last longer, which will preserve sensory quality and nutritional value, and which will be safe for the health of consumers. Many authors cite the harmfulness of nitrite application to consumer health.The harmful effects of nitrite are manifested when nitrites enter the body with a larger amount of protein food, resulting in toxic N-nitroso compounds. In recent times, new solutions have been increasingly explored, which would enable "new food", free of pathogens, with preserved sensory properties and nutritional values, but without harmful chemical additives, which contribute to the deterioration of consumer health. Many years ago, people used food the most, which is subject to fermentation. Today, attempts are being made to improve the system of food quality and safety, by preserving meat products with bacteriocins, products of probiotic bacteria, which have good preservative properties and contribute to health protection and consumer satisfaction (Perez, Zendo and Sonomoto, 2014).

CAMPYLOBACTERIOSIS IN HUMANS AND ANIMALS

Campylobacteriosis is zoonosis, a toxin infection that most commonly causes symptoms of acute gastroenterocolitis in humans, most commonly occurring for 1 to 7 days. In the case of prolonged campylobacteriosis, zoonosis can last up to 14 days, and a maximum of up to 21 days (Begovac et al., 2006). In humans, it most commonly causes intestinal toxoinfection with *Campylobacter jejuni* and *Campylobacter coli*, which predominantly originate from the poultry digestive tract (Saint-Cyr et al., 2016). In the case of extraintestinal infections, they are usually associated with *Campylobacter fetus*, which is associated with reproductive disorders. Infection may or may not occur. Foods of animal origin are often contaminated with pathogenic bacteria, and morbidity is conditioned by bacterial counts, virulence, pathogenicity mechanisms and host susceptibility, which is highly dependent on immune and non-immune defense mechanisms.

Three species of thermophilic *Campylobacter spp*. are the most pathogenic for humans: *Campylobacter jejuni, Campylobacter coli, Campylobacter fetus* and their subspecies (Table 7) (Markey et al., 2013)

Table 7. Disease and host of thermophilic *Campylobacter spp*. (Markey et al., 2013)

The causative agent	Disease and host
Campylobacter jejuni	Poultry is considered to be the most important vector of zoonosis. Manifestation: gastroenterocolitis. Rare: multifocal liver lesions, hepatitis. Sheep: miscarriage. Dogs: predominantly enteritis.
Campylobacter coli	The most common reservoirs of infection are pigs. Manifestation in humans: predominantly enterocolitis.
Campylobacter fetus subsp. fetus	Cattle, goats, sheep, pigs, horses and humans: intestinal and reproductive campylobacteriosis.
Campylobacter fetus subsp. venerealis	Embryo death and reversible infertility.
Campylobacter upsaliensis	It can cause enterocolitis in dogs and humans.
Campylobacter lari	The most common reservoirs of infection are seagulls and wild birds. Manifestation in humans: predominantly enterocolitis.

Campylobacter jejuni

Campylobacter jejuni is considered to be the most common cause of campylobacteriosis in humans. Campylobacteriosis most often occurs through the consumption of contaminated food (Wieczorek and Osek, 2013). Poultry meat is considered the main reservoir of infection. In humans it causes mostly intestinal diseases with symptoms of diarrhea, often hemorrhagic diarrhea, fever and abdominal pain. It can also cause bacteremia. Complications are possible in the form of arthritis and Guillan-Barré syndrome, which is considered to be the most severe consequence of campylobacteriosis, with symptoms of a neurological disorder. It is thought to be an autoimmune disorder of the peripheral nervous system, with the production of antibodies in the myelin sheath of peripheral nerves (Varnam and Evans, 1996; Hadden and Gregson, 2001). Overcoming campylobacteriosis can contribute to the development of irritable bowel syndrome. The occurrence of clonicism is also possible in humans (Jansen et al., 2008). In sheep, it can also lead to reproductive disorders, with abortion symptoms (Vuckovic and Plecko 2013). Chickens rarely get sick, but can be infected through contaminated water, food, or through a contaminated environment. The infection in them is inert, but the flesh of the infected is significant in the transmission of the infection to humans. The carcasses of the infected can also be contaminated during slaughter. The occurrence of campylobacteriosis in poultry is rare, but if it occurs it can also be manifested by the appearance of multifocal lesions on the liver (Markey et al., 2013).

Campylobacter coli

Campylobacteriosis in humans can also be caused by *Campylobacter coli*, but less frequently. Although poultry is considered the main reservoir of *Campylobacter spp*., this species is less frequently isolated in poultry compared to *Campylobacter jejuni*. The path of transmission, from animal to human, is direct and indirect. Pigs are considered to be the largest reservoir of infection, but important vectors are: poultry, domestic livestock, pets (Workman, Mathison and Lavoie, 2005). It is a predominantly intestinal disease, with all signs of alimentary intoxication.

It is most often a toxoinfection with the appearance of a specific infectious syndrome, which includes fever, abdominal pain, diarrhea and often hemorrhagic diarrhea (Markey et al., 2013).

Campylobacter fetus

Campylobacter fetus primarily causes reproductive, but also intestinal and systemic diseases, to which cattle, sheep, goats, pigs, horses and humans are particularly susceptible. It most commonly causes infertility, miscarriages, and prolonged estrus. *Campylobacter fetus* and its strains have also been associated with systemic campylobacteriosis, especially in neonates (Table 8) (Quinn et al., 2011). In case of septicemia, they can also cause enterocolitis, as well as diseases of various organs and organ systems (Kalenić, 2005; Allos et al., 2014). In cows and heifers, 4-7 days after infection, vaginitis, cervicitis, mucous membrane appear and the uterus is often slightly edematous. The highest concentration of pathogens is present in the placenta and therefore the most common symptom of reproductive campylobacteriosis is abortion, which occurs in the third semester of pregnancy. In bulls it can cause the appearance of inflammatory changes on the genitals, which occur in the form of hyperemia, erosion, and edema (Markey et al., 2013). Complications are: bacteremia, septicemia, meningitis, neurological symptoms, endocarditis, peritonitis, thrombophlebitis, urinary tract infection, reactive arthritis, irritable bowel syndrome, Guillain Barre syndrome, Reiter's syndrome etc. Complications are associated with immunocompromising patients with some other disease (Kalenić, 2005; Chukwu et al., 2019).

Campylobacter upsaliensis

Campylobacter upsaliensis predominantly causes enterocolitis in pets, such as dogs and cats (Workman, Mathison and Lavoie, 2005). These are commensals of the intestinal tract of healthy dogs and cats, in which an intestinal, diarrheal form of the disease develops, most often due to a decline in immunity or the presence of some other, underlying disease. In dogs and cats with diarrhea caused by *Campylobacter upsaliensis*, other pathogenic bacteria are usually detected. It is often isolated in pets with infectious viral infections and parasitic invasions. It is important to mention, that *Campylobacter upsaliensis* has zoonotic potential and that dog and cat owners are at greatest risk of developing this type of campylobacteriosis (Cvetnić, 2002; Quinn et al., 2011).

Campylobacter lari

Campylobacter lari causes similar alimentary intoxication as other thermophilic *Campylobacter jejuni* and *Campylobacter coli*. It is a bacterium of zoonotic potential, which can cause alimentary intoxication in humans, most often via contaminated mediterranean food, most commonly through raw mussels and molluscs. Shellfish are thought to be the most commonly contaminated with *Campylobacter lari*. The main reservoirs of infection are seagulls and wild birds, as *Campylobacter lari* mostly colonizes the intestines of seagulls and birds (Facciolà et al., 2017). Dissemination of pathogens is enabled through contaminated surface waters, seas, lakes, most commonly through various species of wild birds (Endtz et al., 1997; Markey et al., 2013).

MEDICAL IMPORTANCE OF CAMPILOBACTERIOSIS

Campylobacteriosis can be intestinal and extraintestinal (Table 8) (Quinn et al., 2011). In humans, intestinal campylobacteriosis most commonly occurs, most commonly in the form of

gastroenterocolitis, characterized by sudden disease, vomitus, infrequently hemorrhagic diarrhea, and possible concomitant manifestations such as hyperthermia, dehydration, and gastric colic. Enteroxins can also lead to a septic form of campylobacteriosis. The septic form of campylobacteriosis most commonly occurs in young children in developed countries, which is related to the absence of antibody titers (Balen-Topic et al., 2007; Troselj-Vukic and Cekinovic, 2010). The septic form is of great importance in medicine, because the consequences can be lethal. Campylobacteriosis can also occur in a subclinical form, with the absence of clinical manifestations of the disease, and the presence of bacteria in the feces (Jansen et al., 2008). It is characteristic of adult individuals, usually in underdeveloped and developing countries (Vuckovic and Abram, 2009). The fourth medically significant form is the prolonged form, with changes in clinical manifestations in the form of diarrhea and constipation. In the chronic form, progressive weight loss is also possible. Non-specific symptoms, such as conjunctivitis, pharyngitis, may also occur and sterility is possible in the case of reproductive campylobacteriosis (Markey et al., 2013). Prolonged, chronic campylobacteriosis can result in reactive arthritis, irritable bowel syndrome, Guillain Barre syndrome, and Reiter syndrome (Kalenic, 2005; Chukwu et al., 2019).

Table 8. Intestinal and extraintestinal campylobacteriosis (Quinn et al., 2011)

The host	The causative agent	The disease
All warm-blooded animals, birds and humans	*Campylobacter jejuni subsp. jejuni*	Systemic campylobacteriosis
Humans	*Campylobacter fetus subsp. fetus*	Systemic campylobacteriosis
Cattle and sheep	*Campylobacter fetus subsp. fetus*	Reproductive disorders (abortions)
Cattle	*Campylobacter fetus subsp. venerealis*	Reproductive disorders (abortions)
Pigs	*Campylobacter hyoilei*	Intestinal disorders (proliferative enterocolitis)
Pigs	*Campylobacter mucosalis*	Intestinal disorders (proliferative enterocolitis)
Cattle and Pigs	*Campylobacter hyointestinalis*	Intestinal disorders (proliferative enterocolitis)
Humans	*Campylobacter jejuni subsp. jejuni, Campylobacter fetus subsp. fetus, Campylobacter coli, Campylobacter lari.*	Intestinal disorder (diarrhea)
Humans and pets	*Campylobacter upsaliensis*	Intestinal disorders (diarrhea)

GUILLIAN BARRÈ AND MILLER FISHER SYNDROME

Guillian Barrè syndrome (GBS) is an autoimmune, acute inflammation of the peripheral nerve myelin membrane with partial or complete muscle paralysis. GBS is thought to occur most likely as a consequence of bacterial or viral infection and most often as a consequence of chronic campylobacteriosis with *Campylobacter jejuni* other bacterial, viral infections and less frequently after surgery, organ transplantation, vaccination, isotretinoin treatment and others with neo autoimmune diseases (Meena, Khadilkar, & Murthy 2011; Murthy, 2017). The

pathogenesis is related to the recognition of myelin as a foreign body (antigen) by the antibody (immunoglobulin) of the patient's humoral immune system. Meta antibodies are gangliosides GM1 and glycolipids GD1b of myelin membrane of nerves, which are similar to antigens of lipopolysaccharide envelope of bacteria and viruses. GBS occurs as acute inflammatory demyelinating polyneuropathy (AIDP) and in more severe, less frequent form, such as: Miller Fisher syndrome (MFS), acute motor axonal neuropathy (AMAN), acute motor and sensory axonal neuropathy (AMSAN), pharyngeal-cervical-brachial or brachial paraparetic form of GBS (Murthy, 2017). The clinical picture is variable. It mostly occurs in the form of paralysis of the extremities of the legs, arms, and facial muscles. The accompanying symptoms are: weakness, tingling, muscle pain, the appearance of so-called "rubber feet", neck pain, spine. Bladder, bowel, and bowel innervation may be impaired, depression of the respiratory center, which is a consequence of CNS nerve involvement, may also result in death (Meena, Khadilkar and Murthy, 2011; Murthy, 2017). Variations of GBS syndrome are: MFS ocular syndrome (ophthalmoplegia, ataxia and arefelexia), AMAN (targets are Ranvier nodal gangliosides), AMSAN (severe nerve damage), pharyngeal-cervical-brachial weakness and paraparetic form (Yuki, 2012); . The diagnosis is made two weeks after the onset of the first clinical symptoms. It can be placed on the basis of lumbar puncture and electromyoneurography (Meena, Khadilkar and Murthy 2011). Therapy is supportive: physical therapy, corticosteroids, respirator, anticoagulants, diuresis, nonsteroidal anti-inflammatory drugs, and immunomodulatory: plasmapheresis and immunoglobulins (Meena, Khadilkar and Murthy 2011). The prognosis may be good or poor, depending on the age structure of the patient (elderly: poor prognosis), the clinical picture, the titer of antibodies produced (GM1) and axonal damage (Meena, Khadilkar and Murthy 2011).

PROPHYLAXES AND CAMPILOBACTERIOSIS CONTROL MEASURES

Prevention measures are: hand hygiene, hygienic handling of food, consumption of heat-treated food, consumption of food immediately after heat treatment, storage of food in refrigerators, pasteurization of milk, dairy products, fruit juices, consumption of peeled fruits and vegetables, due to the possibility of groundwater contamination with standing water, which must contain *Campylobacter spp*. It is important to know that food used for human consumption does not naturally contain *Campylobacter spp*. Bacteria originate from faeces, most commonly animals that are slaughtered and whose products are used in human consumption and it is therefore important that hygiene and sanitary standards are respected at different stages of primary production (Facciolà et al., 2017). It should be emphasized that the mixing of thermally processed foods with thermally untreated foods, which are often the source of various food contaminants and in this connection, creates the possibility of cross – contamination (Marinculić et al. 2009; Varga et al. 2012). All of the above indicates the importance of hygiene, health status of workers, as well as the importance of proper application of hygienic and sanitary standards in different stages of primary production, from farm to table. In primary production, it is important that sanitation is done by trained workers, who will perform proper cleaning and disinfection of plants, production equipment, production work surfaces and it is very important that the implementation of hygienic and sanitary measures is constantly monitored and controlled (Facciolà et al., 2017). In primary food production, several aspects of the sanitation process are often mentioned, which include pre – washing, foam washing, thorough cleaning and control, rinsing of impurities, sanitation using cationic surfactants, then surfactants, which are chemical acids or bases, disinfectants, which are also acids or bases then important halogen elements chlorine, iodine, etc. (Šubaric, Babic and Achkar, 2012). In order to preserve product quality, nutritional value, sensory properties, food authenticity, prolong shelf life and ensure safe food, it is very important to apply the HACCP system at different stages of primary

production, which allows early detection of risks in production, which would could affect product quality and safety (Bilska and Kowalski, 2014; Koprivnjak, 2014). The implementation of the HACCP system is an obligation of all EU Member States. In order to enable the functioning of the HACCP system it is extremely important to apply good hygiene practice (GHP), but also good manufacturing practice (GMP), which unites all phases of primary food production (Bilska and Kowalski, 2014).

CLEANERS AND ANTIMICROBIAL ACTION

Before applying any disinfectant, it is extremely important to mechanically clean the area we want to disinfect, because the accompanying feature of many disinfectants is inactivation in the presence of organic detritus or organic matter. Mechanical cleaning can be done manually (foam, spray, fogging), which is a cheaper option or with the help of automatic or semi-automatic process of mechanical cleaning – *Cleaning in place* – CIP, which is connected to the inside of the production device and external cleaning system – *Cleaning out of place* – COP. CIP is used for cleaning of machines, internal pipelines, which are difficult to access. Hot water, which is of appropriate temperature and pressure, is used in combination with appropriate disinfectant (Karahmet, Toroman and Hamidović, 2017). In CIP internal cleaning, the most commonly used are chlorine-based agents (Šubarić, Babić, and Ačkar, 2012). For cleaning disassembled equipment, COP cleaning is used. Cleaning is performed with the help of a powerful pump, through the phases of washing, rinsing and squeezing, with an average duration of about 40 minutes (Karahmet, Toroman and Hamidović, 2017). The combination of several methods of mechanical cleaning improves the efficiency of mechanical washing and disinfection, ie sanitation (Šubarić, Babić and Ačkar, 2012). Inadequate mechanical cleaning, food residues on machines and space, the presence of bacterial sessile communities or biofilm, as well as inadequate concentration of disinfectant, can result in reduced or complete suppression of the effectiveness of sanitation, propulsion machines in different stages of primary production (Müller et al., 2014). Disinfection is a process, which is applied after proper mechanical cleaning, and represents the destruction of residual microorganisms, to a concentration at which they cannot cause disease. Mechanical cleaning together with the disinfection process is sanitation (Marriott, 1997). Sanitation removes microorganisms from equipment, work surfaces, production facilities and ensures safe production, which contributes to the protection of consumer health. Sanitation is also a legally prescribed measure, enabling hygienic safety and product quality (Scheffler, 2009).

Detergents and disinfectants

Detergents or surfactants are surfactants, which have the ability to break down lipids with the help of polarized molecules. Some act on the basis of ion exchange, taking away the ion of the bacterial cell, and the same receives the ion from the detergent. The hydrophilic molecule has a strong affinity for water, and the hydrophobic one dissolves in lipids, causing emulsification. Since the cell membrane of a microorganism is made up of lipids, detergents cause it to burst and the cell becomes susceptible to enzymes, coenzymes and metabolic products. As a result of increased membrane permeability, detergents also exhibit germicidal activity. We divide them into cationic, anionic, nonionic and amphoteric surfactants, and chemically these are: acids, bases and salts (Castro Burbarelli et al., 2017). There are different disinfectants, which are used in the sanitation process, which use different mechanisms of action (Table 9) (Quinn et al., 2011).

Table 9. Mechanisms of action of different disinfectants (Quinn et al., 2011)

Disinfectants	Mechanisms of action
Cationic (quaternary ammonium compounds), anionic, nonionic and amphoteric surfactants.	I They damage the bacterial cell with the help of surface activity.
Various disinfectants: acids and bases.	II They hydrolyze internal cell structures.
Peroxide halogen element hydrogen peroxide (H2O2), halogen elements: fluorine (F), chlorine (Cl), bromine (Br), iodine (I), astatine (At), acetic acid and potassium permanganate.	III They damage the bacterial cell by the oxidation process.
Various alcohols, phenolic compounds and aldehydes (formaldehyde).	IV They coagulate bacterial proteins.

Cationic surfactants

Cationic surfactants are widely used in sanitation. Germicidal effect is exerted on gram positive and gram negative bacteria, with some authors citing activity against certain fungi, viruses and protozoa (Carmona-Ribeiro and de Melo Carrasco, 2013; Olorode, Bamigbola and Ogba, 2015). They are reduced by the presence of anionic surfactants and organic detritus. They are not combined with anionic surfactants (soaps), but it is possible to use them before applying cationic surfactants. Combination with alcohol is preferable as they are potentiated (Quinn et al., 2011). Cationic surfactants possess cleansing power and can be used as disinfectants (Müller et al., 2013). They are relatively non-toxic, non-corrosive, fast-acting, clean, emulsifying fats and often have a pleasant smell. The disadvantages are antagonism with soaps and the formation of a surface film, which is only externally germicidal, while the microorganisms below are free to vegetate. To prevent undesirable occurrences, they are used in combination with alcohols (Carmona – Ribeiro and de Melo Carrasco, 2013). More important representatives are benzalkonium chloride, bezalkonium bromide, benzododecinium bromide, benzododecinium chloride, cetrimonium bromide, cetrimide, benzethonium chloride and others. Cetrimide if used in 10% concentration with chlorine hexidine, can serve as a disinfectant for hands, suits, aprons and in the decontamination of areas contaminated with *Campylobacter spp*. as well as with other causes of alimentary intoxications (Olorode, Bamigbola and Ogba, 2015; Battersby et al., 2017).

Anionic surfactants

Anionic surfactants are the oldest cleaning agents. These include potassium, sodium and toilet soap, alkylbenzenesulfonates, alkanesulfonates, alkylsulphates alkyl ethersulphates. Potassium is commonly used in medicine and sodium soap in everyday life. Scented substances are also added to toilet soaps. In addition to mechanical cleaning, they also have a weaker germicidal effect on gram-positive and acid-resistant bacteria, because they create an alkaline environment. For potentiation of antiseptic action they are combined with antiseptics, such as: hexachlorophene, borax, sulfur etc. They are more effective than other surfactants, they are weakly toxic, and the disadvantage is inactivation in hard water (Douglas et al., 1999; Madunić – Čačić, 2008).

Nonionic surfactants

They are not subject to dissociation. Solubility binds to functional groups, with a strong affinity for water. They have a role in disinfection. They are mixed with cationic surfactants (quaternary ammonium compounds), which potentiates their action. Nonionic surfactants are not toxic, but their breakdown products are potential contaminants of food and the environment. Nonionic surfactants include: alcohol ethoxylates, alkyl phenol ethoxylates, alkanol fatty acid amides, alkyl amine oxides, n – methylglucamides and alkyl polyglycosides (Zoller and Sosis, 2008).

Amphoteric surfactants

Amphoteric surfactants include ampholytic and amorphous soaps. These are odorless powders that mix with water. They are subject to dissociation, giving anions, which clean and cations, which exhibit a bactericidal effect. They can act both as acids and as bases, which represents their amphoteric, dual action. They are also used for ambient disinfection, especially in the meat and dairy industry, fruit juice industry, etc. They wash well, clean, and often add ingredients that smell (deodorize) and are very toxic. They are often used to disinfect hands or instruments. Betaines, alkylbetaines, sulfobetaines, imidazole compounds are in frequent use (Stanga, 2010).

Acids and bases in sanitation

In food production, depending on the type of impurity, we use different cleaning and disinfecting agents. Organic impurities, such as biofilm, fats, blood, are most successfully cleaned with bases, with the exception of proteins, which are equally successfully cleaned with acids. For the cleaning of impurities of inorganic origin, such as scale, rust, salt and sugar, acids are the best choice (Marriott, 1997; Scheffler, 2009). Alkaline agents for mechanical cleaning are characterized by cleaning, saponification and emulsification properties, prevention of scale formation, and in a concentration of 5%, they have a germicidal effect on spores (Kanegsberg and Kanegsberg, 2011). Alkaline agents are successfully used in the meat industry, because they are the best pure fats from meat. In general, most bacteria cannot survive in a highly alkaline environment, hence the bases and bactericides. Germicidal activity is related to the concentration of the alkaline agent. They are difficult to wash, so they are often combined with oxidizing agents. The main representatives of the bases are sodium and potassium bases (Šubarić, Babić and Ačkar, 2012). Bases as disinfectants can be divided into weak, medium and strong (Marriott, 1997). An example of a weak base is sodium bicarbonate, which is characterized by extremely mild action, poorly cleansing, alkalizing, or has the ability to soften water. Sodium carbonate is an example of a medium – strong base, which has slightly better cleaning properties. On the other hand, strong bases, such as sodium hydroxide (caustic soda), have the power to clean and disinfect a room. The disadvantages of strong bases are toxicity, corrosivity and strong corrosive properties (Marriott and Gravani, 2006). The use of strong bases requires adequate protection in the form of gloves, protective masks, goggles, protective clothing, which should be made of high quality, solid material, to protect the mucous membranes and skin from the potential for damage (Šubarić, Babić and Ačkar, 2012). Acids are also characterized by high hazard because they possess corrosive properties. Acid handling also requires appropriate equipment. They are especially dangerous if they are combined with chlorine – based products, because they create a deadly gas. They must not be mixed with bases either, so they must be stored with great care. The main representatives of acids, which are widely used in disinfection, are nitrate, phosphorus and fluoride. Used in higher concentration, they can also lead to death, due to the strong corrosive power to the epithelium of exposed mucous membranes (Šubarić, Babić and Ačkar, 2012).

Phenols

Phenols are industrially obtained from coal tar and chlorine benzene, which is also the oldest way of producing this compound. They are strong bactericidal agents, they also act on Mycobacterium tuberculosis, but they have a weak effect on fungal spores. They are used in the disinfection of farms, because they act in the presence of organic matter. The main representative is carboxylic acid, which shows activity at a concentration of 1% (Todorević, 2003; Quinn et al., 2011).

The halogen element chlorine

Chlorine compounds include hypochlorites, liquid chlorine, chlorine amines, which are used in households, water disinfection, food production, etc. For disinfection of water, they are used in the gaseous state, and as chlorinated lime for disinfection of spaces, barns, garbage dumps (Gagić et al., 2013). Advantages of chlorine compounds are: wide range of action, low cost, speed of initial action, poor surface retention, and disadvantages: corrosivity, inhibition in the presence of organic detritus, high temperature and high pH and inability to mix with acids (Cramer, 2006; Marriott and Gravani , 2006). The germicidal effect on many microorganisms, including various types of fungi, is manifested by the action on deoxyribonucleic acid (DNA). Hypochlorites are formed by adding lime, whereby chlorinated lime is formed (Gagić et al., 2013). Important representatives of chlorine compounds are: sodium hypochlorite, unstable calcium hypochlorite, stable caporite and hypochlorite acid (Marriott and Gravani, 2006; Gagić et al., 2013).

Halogen peroxide element hydrogen peroxide

Oxidizing agents have a wide range of effects on various microorganisms. They show bactericidal action on gram positive, gram negative bacteria, show moderate activity on mycobacteria, act on endospores and fungal spores, then on viruses, especially those with a protein coat. They stand out in particular by acting on anaerobes (Quinn et al., 2011). It works best at acidic pH and lower temperatures; as high temperatures cause oxidizing agents to evaporate. Although they have a wide range of action, they are also quite weak for application on larger surfaces, so it is used in sterile packaging of products, in a concentration of 40%. The main representative of halogen peroxides is hydrogen peroxide (Šubarić, Babić and Ačkar, 2012).

MOST COMMONLY USED DISINFECTANTS IN DIFFERENT PHASES OF PRIMARY CHICKEN MEAT PRODUCTION

In the process of sanitation, it is necessary to choose the appropriate means in the appropriate concentration, in order to show germicidal activity. When choosing a disinfectant, toxicity, corrosivity, remaining on the substrate (residual activity), activity in the presence of organic matter and the price of the product should be taken into account (Table 10) (Wakenell, 2005).

Table 10. Most commonly used disinfectants in different stages of primary production (fattening, slaughter, primary processing) of broiler, chicken meat (Wakenell, 2005; Quinn et al., 2011)

Disinfectant	Advantages and disadvantages
Halogen element chlorine	Germicidal acts on many microorganisms, but is the best bactericide. It leaves no residue but is toxic, corrosive and inhibited by organic detritus. In the gaseous state, it is used for the disinfection of water, and in the form of chlorine lime, calcium hypochlorite, it is used for sanitation. It's low prices.
Iodine	Bactericide, fungicide and virocide. It has no residual activity. It lacks toxicity, moderate corrosivity and inhibition in the presence of organic detritus.
Phenols	Bactericides, fungicides, virocides, but not sporocides. Slight residual activity, not inhibited by organic detritus. Potentiated by detergents. Disadvantages: toxicity, corrosion, high cost, strong odor and ecological unacceptability.
Cationic surfactants	They work better on gram positive bacteria. Bactericides, fungicides, weaker virocides. Medium residual activities, non-toxic, non-corrosive and odorless. They are inactivated by hard water, alkalis, soaps, organic detritus.
Cresols	Bactericides, fungicides, virocides and sporocides. They leave residues. Slightly toxic and low cost, but corrosive and moderately inactivated by organic detritus.
Aldehyde	Bactericide, fungicide, virocide. With potassium permanganate it is used for fumigation of eggs. Disinfection of the space is achieved in a concentration of 1-2%. It leaves no residue, but is toxic, corrosive, pungent and inhibited by organic detritus. Odor neutralization, done with the help of 25% ammonia.

CLEANERS AND ANTIMICROBIAL ACTION

Before the discovery of AB, antiseptics and disinfectants were widely used in all branches of medicine and were one of the basic treatment agents. After the discovery of AB, antiseptics and disinfectants were suppressed by more effective AB, which possess high selectivity of action, with minimal toxicity. Today, it is impossible to imagine life without AB and ABR is increasingly present in the world of microorganisms. The danger of acquiring ABR was also warned by Alexander Fleming (Hogberg, Heddini and Cars, 2010). In order to prolong the effectiveness of AB, efforts are being made to increase the use of atiseptics and disinfectants and to rationalize the consumption of AB, which are among the best selling drugs in the world (Kalenić, 2000). Recently, new disinfectants have been discovered, which do not have toxic properties and are widely used in sanitation processes, in various stages of primary production of broiler and chicken meat. These are agents based on stabilized, liquid chloride dioxide, which have a broad spectrum of action, and without the negative characteristics of chlorine compounds, such as toxicity and corrosivity (Hadžiabdić et al., 2013). However, irrational use of any antimicrobial agent, especially if applied in low concentrations, can result in the development of induced resistance of microorganisms (Quinn et al., 2011). Bacteria can mutate (induced mutation) and create different mechanisms of resistance and "outwit" the mechanisms of action of even disinfectants, although this process is quite long (Cantas et al., 2013; Pidot et al., 2018). Mutated bacteria have the ability to spread genes, which encode ABR, by vertical and horizontal gene transfer (Cantas et al., 2013). Bacteria acquire resistance to antiseptics and disinfectants most slowly, but resistance has been reported in *Campylobacter spp*, *Escherichia coli spp.*, *Listeria monocytogenes*, *Staphylococcus spp*. and *Pseudomonas aeruginosa* (Randall et al., 2003; Quinn et al., 2011). The use of antiseptics and disinfectants still has an advantage over the use of AB, in order to prevent the spread of ABR. Aseptic environment can also be achieved by physical methods, UV radiation or the use of high hydrostatic pressure, but often

with chemical methods (Bechstein et al., 2019). In the future, in order to provide an aseptic environment, in order to prolong the effectiveness of the action of available agents in the fight against microorganisms (Hogberg, Heddini and Cars, 2010). Today, the use of various biological biocides, such as pine oil, to which bacteria do not develop resistance, is also being considered (Quinn et al., 2011).

ANTIMICROBIAL RESISTANCE TO DISINFECTANTS

Recently, a number of certain bacteria have been recorded, which show tolerance to various disinfectants. Resistance occurs when applying an inappropriate concentration, applying too diluted disinfectant, which helps to create induced mutations and spread resistance. The risk of resistance is also increased by the use of disinfectants, which show inhibition of action in the presence of organic matter. Therefore, it is important that when disinfecting a heavily polluted area, with an abundance of organic detritus, proper mechanical cleaning is taken into account, which would reduce the inactivation of the disinfectant. Mechanical cleaning is also very important, in order to eliminate possible biofilms of various bacteria, because the mentioned formation shows resistance to all available antimicrobial agents. Limiting the use of disinfectants, which are less effective in organic detritus, is also considered one of the measures, in preventing the spread of antimicrobial resistance (AMR). Special attention is required for disinfection of premises, work surfaces, equipment, etc., where microorganisms have been previously isolated, which have shown a certain tolerance to certain disinfectants, including *Campylobacter spp*. and many other gram negative bacteria. The choice of disinfectant, in this case, depends on the data of disinfectant resistance monitoring and selection in accordance with the recommendations. It is also believed that the spread of AMR to disinfectants increases the risk of cross-ABR, because the mechanisms of AMR to disinfectants often coincide with the mechanisms of ABR. In gram negative bacteria, AMR to disinfectants is more common, because they can develop biochemical mechanisms of resistance, such as changes in cell membrane permeability, which is reduced permeability of bacterial porins to disinfectants, which is also known in ABR to different classes of AB. Also, disinfectants can potentiate the formation of bacterial endospores, which are resistant to disinfectants. Bacteria can also use the efflux pump, as a biochemical mechanism of AMR to disinfectants, which is also known as the mechanism of ABR (Quinn et al., 2011).

MICROBIOLOGICAL DIAGNOSTICS

For the isolation of *Campylobacter spp*. from food in use are selective and highly selective substrates. To improve selectivity, AB are added: vancomycin, cefoperazone, amphotericin B, polymyxin B, etc. The most commonly used selective media are Karmali, Skirrow, Preston, Bolton, mCCDA, CAT, CAMPY-CEFEX and highly selective, factory agars: CampyFood ID® (bioMérieux, France) and Brilliance CampyCount® (Oxoid, UK) (Murray et al. 2003; Habib, Uyttendaele and De Zutter, 2011). Incubation is in microaerophilic conditions, in pots with gas-pack bags. It lasts for two hours, at a temperature of 32 ◦C, and 40 to 42 hours, at a temperature of 42◦C (Markey et al., 2013). Identification of isolates is achieved by culture, microscopic and biochemical tests. Thermostable lipopolysaccharide (O), thermolabile capsular, flagellar, and surface (S) antigens can be used for serotyping. Serological methods are not used routinely because they are demanding, but are used for epidemiological purposes (Murray et al., 2002). Commercial tests, such as latex agglutination, can be used to detect fimbrial antigens. Enterotoxins can be detected by enzyme – linked immunosorbent assay (ELISA) (Penner et al., 1983; Markey et al., 2013). Among the molecular methods in use is polymerase chain reaction

(PCR), which can detect genes that encode enterotoxins and various pathogenic factors (Markey et al., 2013). The advantages of PCR application are high sensitivity, specificity and speed. The disadvantages are the indistinguishability of dead from living cells and the inhibition of the PCR reaction by inhibitors from food, water, feces, the environment and therefore it is not the gold standard in the detection of Campylobacter spp. from similar samples (Mikulić et al., 2017). RAPD and rep-PCR, can be used for epidemiological purposes, for typing the species Campylobacter spp. (Petković, 2013). Isolation of Campylobacter spp. from food, is done in accordance with international standards for business quality management and food safety. They were established by the Codex Alimentarius, by the Food and Agriculture Organization – FAO and the World Health Organization – WHO and under the auspices of the United Nations – UN, all for the purpose of ensuring safe, healthy and wholesome food (Lukač-Havranek, 1998).

TREATMENT OF CAMPYLOBACTERIOSIS

If it is the most common form of campylobacteriosis, is intestinal disease, it is usually a disease that has mild or severe symptoms of food poisoning. Younger children have a more severe clinical picture, and in older individuals it sometimes proceeds inversely or the disease is limited to symptoms of acute poisoning (Vučković and Abram, 2009). Depending on the clinical picture of the disease, specific drug therapy is prescribed. In case of a more severe form of the disease, which is accompanied by the appearance of general infectious syndrome, severe dehydration, specific therapy, rehydration solutions, vitamin-mineral preparations, infusion with specific immunoglobulins (IgM) can be given and AB are prescribed (Vučković and Abram, 2009; Allos et al., 2014). If it is a milder form, in 25% of cases, within five days, there is a spontaneous recovery, without permanent consequences for the body (Vučković and Abram, 2009). The most commonly used AB in human campylobacteriosis are AB from the macrolide class: erythromycin or from the fluoroquinolone class: ciprofloxacin. Fluoroquinolone is also used in animals: marbofloxacin (Quinn et al., 2011). In case of the presence of certain cardiovascular diseases, erythromycin is not prescribed, azithromycin is recommended. Ciprofloxacin is contraindicated in children, due to the possibility of permanent damage to articular cartilage (Talsma et al., 1999). An alternative choice is tetracyclines (Wieczorek and Osek, 2013). In poultry campylobacteriosis, AB from the class of aminoglycosides are most often prescribed: dihydrostreptomycin sulfate, which is placed in food in poultry (Markey et al., 2013). *Campylobacter spp*. are also sensitive to certain penicillins: amoxicillin, ticarcillin with clavulanic acid and carbapenems: cilestatin or imipenem. They are usually resistant to other penicillins and cephalosporins. Sensitivity to sulfonamides and nitroimidazoles is variable. In the septic form of campylobacteriosis, aminoglycosides may also be prescribed: gentamicin, lincosamides: chloramphenicol, clindamycin, or carbapenems: cilestatin or imipenem (Quinn et al., 2011; Allos et al., 2014).

ANTIBIOTICS AND MECHANISMS OF ACTION

Narrow-spectrum penicillin was discovered in 1928, and in 1935, sulfonamides were discovered. In 1943, the first broad – spectrum, aminoglycoside AB streptomycin was discovered (Božinović, 1995; Bedenić, 2005; Fejzuli et al., 2018). AB are selective because they do not damage host cells. Bactericides induce bacterial death and bacteriostatics damage the bacterium. AB exhibit different mechanisms of action and different spectrum of action (Table 11) (Quinn et al., 2011).

Table 11. Mechanisms and spectrum of action of different classes of AB (Quinn et al., 2011)

Antibiotic classes and sulfonamides	Mechanisms and spectrum of action
β lactams, glycopeptides	Inhibition of cell wall synthesis. Bactericidal action.
Polypeptides	Inhibition of cytoplasmic membrane function. Bacteriostatic action.
Nitrofurans, tetracyclines, macrolides, aminoglycosides, lincosamides.	Inhibition of protein synthesis. Bacteriostatic and bactericidal action.
Quinolones / fluoroquinolones, nitroimidazoles, aminocoumarins, rifamycins.	Inhibition of nucleic acid synthesis. Bactericidal and bacteriostatic action.
Sulfonamides with trimethoprim.	Inhibition of the metabolic pathway. Bacteriostatic action.

Bactericidal AB, which inhibit cell wall synthesis are β lactams: penicillins, amoxicillin, amoxicillin with clavulanic acid, ampicillin, ampicillin sulbactam, cephalosporins of four generations, reserve carbapenems: carbapenem, imipenem. Reserve glycopeptides: vancomycin, teicoplanin, telavancin, antitumor AB belomycin. Vancomycin acts on gram positive bacteria and is used in pseudomembranous colitis, due to its efficacy in eradication of *Clostridium difficile* and Methicillin resistant *Staphylococcus aureus*, and has a weak effect on *Streptococcus spp*. Polypeptides inhibit the function of the cytoplasmic membrane and belong to bactericides. Topical polypeptides include bacitracin, which is an integral part of creams, ointments, sprays, and acts on gram negative bacteria including *Pseudomonas aeruginosa*, and does not act on gram – positive bacteria and *Proteus spp*. Systemic polypeptides are: polymyxin B, polymyxin E or colistin, which are the last line of defense against invasive gram negative bacteria. AB whose mechanism of action is based on inhibition of protein synthesis, blocking the 30S subunit of bacterial ribosomes, while bacteriostatics are nitrofurans: nitrofurantoin. Then tetracycline antibiotics: tetracycline, doxycycline. The same mechanism of action is manifested by bactericidal aminoglycosides: gentamicin, reserve amikacin, kanamycin, neomycin, etc. AB that act by inhibiting protein synthesis, blocking the 50S subunit of bacterial ribosomes, and belong to bacteriostatics and bactericides are lincosamides. Important representatives are the bacteriostatic clindamycin and generally the bactericide lincomycin, then AB from the class of macrolides: azithromycin, erythromycin, clarithromycin, etc., which have the best resorption in the lungs, but can also be used in infections of other organs. AB that affect the synthesis and replication of DNA, whose mechanism of action is based on the prevention of DNA synthesis by influencing DNA gyrase responsible for the placement of DNA in the form of super threads, while exhibiting bactericidal action are quinolones and fluoroquinolones: ciprofloxacin, norfloxacin and marbofloxacin. AB which use the same mechanism of action are aminocoumarins: novobiocin and bacteriostatics rifamycins: rifampicin or rifampin. Sulfonamides and trimethoprim act by inhibiting the metabolic pathways of bacteria, preventing the synthesis of folic acid, because they are competitive antagonists of paraaminobenzoic acid. They exhibit a bacteriostatic effect and are often combined with trimethoprim, because they have a potentiated effect (Quinn et al., 2011).

Mistakes in the application of antibiotic therapy

Wrong choice of AB, wrong dose, irrational use, continuous treatment (without considering the consequences, in the form of super infections, side effects, ABR, and other risks of their use), prescribing AB in any case of hyperthermia, prescribing AB for viral diseases, prophylaxis in the postponement of surgical works, etc. A possible mistake is the use of AB based on the

wrong identification of the cause and the wrong interpretation of the antibiogram. Mistakes increase the risk of creating bacterial mutants (with a change in genetic material) and ABR (Bagatin, 2000).

Effective and alternative therapy of campylobacteriosis

The correct choice of AB includes knowledge of: indications, contraindications, drug effects, main and side effects, toxicology, AB bioavailability and health status of the treated individual. Good clinical practice is achieved therapeutically with the reduction of side effects. The dose of AB is related to the age structure, weight, tissue and organ conditions in which the drug is distributed (compartment models: central and peripheral component), where the process of biotransformation and elimination of AB (clearance) takes place and it is necessary to take into account possible hyperactivity organism (allergy) and reported idiosyncrasy. Properly adjusted dose involves the administration of an optimal, therapeutic dose of AB, while reducing side or side effects. Failure to observe the correct concentration can result in accumulation, with consequent poisoning. Resorption should not be more intense than the drug elimination process, nor should elimination be more intense than the resorption process, as the efficacy of the drug is reduced. Improper use of AB in animals can result in AB residues in food of animal origin, which is used in human nutrition (Mulalić et al., 2005). In contrast to the risk of accumulation, long-term administration of a lower dose of AB at irregular intervals can cause damage to bacteria, which are potential mutagens. When prescribing AB therapy, targeted therapy should be resorted to in accordance with the established antibiogram, and experiential or empirical therapy should be avoided. Recently, alternative treatments for campylobacteriosis have been investigated, and in chickens, the use of *Lactobacillus spp*. has been shown to prevent colonization of the intestinal tract of chickens with the pathogenic bacterium *Campylobacter jejuni* (Saint-Cyr et al., 2016).

ANTIBIOTIC RESISTANCE *CAMPYLOBACTER* SPP.

Gene-mediated antibiotic resistance

Bacteria possess the ability to rapidly change genetic material in such a way that the genetic material undergoes spontaneous mutation during DNA division (to cause a defect in the genetic material) or induced, which can occur using various chemical agents, such as AB. AB can induce DNA damage to bacteria and such genetically modified bacteria, through vertical gene transfer, from parents to offspring, transmit mutated genes (Giedraitiene, 2011). Mutants appear, which are resistant to certain AB. Mutations are not common in bacteria (Wilson et al., 2009). ABR in most cases is acquired by horizontal gene transfer, from the donor bacterium to the recipient or recipient bacterium, by conjugation, transformation or transduction processes, and mediated by plasmids, transposons and genetic cassettes (Džidić et al., 2008). Horizontal gene transfer is a phenomenon, which was recently discovered and is now known, that genes can walk along the genome of a bacterial cell and transfer, among other things, ABR genes to other competent bacteria, regardless of strain or origin, which includes the transfer of ABR genes, animal bacteria to human bacteria and vice versa (Aaerstrup et al., 2008; Pérez-Boto et al., 2014). ABR genes are located within plasmids. The processes of horizontal gene transfer are: conjugation, transformation and transduction (Pavlica, 2012). Bacteria do not possess gene selection selection, but take the genes of any bacteria found in their vicinity. The exchange of ABR genes takes place with the help of sex saws, mediated by plasmids, transposons and integrans, most often by the process of conjugation (Wieczorek and Osek, 2013; Allos et al., 2014). Gene transfer is also possible by transformation, where after the death of a bacterium,

by the process of lysis, DNA is released and transferred to another bacterium by means of a plasmid or active transfer. This possibility is possessed by several bacteria: *Neisseria gonorrhoeae*, *Streptococcus spp*., *Haemophilus influenzae* (Murray et al., 2003). The third process is transduction, mediated by bacteriophages, which inject their DNA inside the bacterium. Through the process of transduction, a new genetic strain of the bacterium can emerge, but if the bacterium survives, it also transfers such newly formed DNA to another bacterium (Dale and Park, 2004). International trade in beef and poultry has contributed to the spread of bacteria and ABR (Alonso et al., 2017).

Antibiotic resistance mediated by biochemical mechanisms

The development of biochemical mechanisms is related to the action of AB (Table 12) (Quinn et al., 2011; Markey et al., 2013). Enzyme modification is the ability of bacteria to secrete beta lactamases, which cleave the beta lactam ring of β lactam AB. 1300 β-lactamase enzymes are known (Bush, 2013). Carbapenem hydrolyzing oxalinase (OXA) is responsible for carpapenem inactivation (Medić et al., 2011). Multidrug-resistant bacteria secrete various β-lactamases, cephalosporinases, β-lactamases of broad and extended spectrum (Bedenić, 2005). A change in DNA gyrase and topoisomerase is a change in the target site of action of AB, which act on DNA gyrase, and these include quinolones and fluoroquinolones (Allos et al., 2014). They belong to the category of important AB for veterinary medicine – *Veterinary Critically Important Antimicrobial Agents* – VCIA (OIE, 2019). *Campylobacter spp*. they acquire ABR to fluoroquinolones faster than macrolides (Yan et al., 2006). Target modification or change of the target enzyme is most often developed by gram positive coca, on AB that act by preventing the synthesis of bacterial protein. The protein has a high affinity for AB, and ABR is based on the formation of a weakly binding protein or modification of the target binding site (Garrelts, 1996; Allos et al., 2014). Alteration of the ribosome structure is an alteration of receptors, which are located on bacterial ribosomes and thus prevents the binding of AB, which act on the 30S and 50S subunit of the ribosome (Nissen et al., 2000; Allos et al., 2014). This mechanism is acquired by bacteria through methylase genes, responsible for ABR to erythromycin (Nissen et al., 2000; Lambert, 2002). Active efflux is used by gram negative bacteria, which have a functional metabolism (Džidić et al., 2008). Ejection of AB from the bacterium takes place via a pump or carrier protein, with energy expenditure (Wieczorek and Osek, 2013). CmeABC is a multiple pump, which enables *Campylobacter spp*. to acquire ABR to a large number of AB (Lin et al., 2003). Alteration of the metabolic pathway is a mechanism of bacterial defense, where they develop target sites, through which they use growth factor (paraaminobenzoic acid). This mechanism is used by bacteria to acquire ABR to sulfonamides and trimethoprim (Jacoby and Munoz-Price, 2005). Alteration of cell membrane permeability is a mechanism, which leads to reduced permeability of bacterial porins (Sević, 2017). Alteration of cell membrane permeability is developed by gram negative bacteria on water-soluble AB and carbapenems. This mechanism is acquired by *Pseudomonas aeruginosa* on all AB (Ferguson, Cahill and Quilty, 2007).

Tabela 12. Antibiotic resistance mediated by biochemical mechanisms to various antibiotics (Quinn i sar., 2011; Markey i sar., 2013)

Biochemical mechanisms of resistance
Enzyme modification:
β lactams: penicillins, amoxicillin, amoxicillin with clavulanic acid, ampicillin, ampicillin sulbactam, cephalosporins I, II, III and IV generations, reserve carbapenems: carbapenem, imipenem, meropenem, ertapenem. Glycopeptides: vancomycin, teicoplanin, telavancin, antitumor antibiotic belomycin. Polypeptides: polymyxin B, polymyxin E or colistin, bacitracin.
II Change in DNA gyrase and topoisomerase IV:
Quinolones and fluoroquinolones: ciprofloxacin, norfloxacin, marbofloxacin. Aminocoumarins: novobiocin. Rifamycins: rifampicin or rifampin.
III Target modification or change of target enzyme i IV Modification of ribosome structure:
Nitrofurans: nitrofurantoin. Tetracyclines: tetracycline, doxycycline. Aminoglycosides: gentamicin, reserve amikacin, kanamycin, neomycin, etc. Lincosamides: clindamycin, lincomycin. Macrolides: azithromycin, erythromycin, clarithromycin, etc.
V Active efflux (pump or protein carrier):
To all antibiotic classes.
VI Change in metabolic pathway:
Sulfonamides and trimethoprim.
VII Change in cell membrane permeability:
To all antibiotic classes.

PROBLEMS OF ANTIBIOTIC RESISTANCE

ABR is considered a global, public health problem, as AB are the most commonly used drugs (Kalenić, 2000; Markey et al., 2013). In order to reduce the spread of ABR, it is necessary to rationalize the consumption of AB in veterinary medicine, economy and medicine (Wieczorek and Osek, 2013; Garcia-Migura et al., 2014). Data from the monitoring of the use of AB indicate that 50% of the detected AB in the world are used in order to show a prophylactic and therapeutic effect in domestic animals. In veterinary medicine, AB can also have biostimulation effects. The use of biostimulators reduces the frequency of morbidity, and contributes to food safety in production. More recently, however, based on evidence suggesting a risk of spreading ABR, the European Union has banned the use of AB as biostimulants (Chantziaras et al., 2014; Hao et al., 2014). It is important to mention that AB change the composition of the microflora of each part of the body in which it is resorbed, increasing the risk of spreading ABR, autoimmune diseases, allergic syndromes, etc. (Shankar et al., 2010). The problem with the use of broad-spectrum AB is the selection of resistant bacteria, which show induced mutations, caused by variation in hereditary material (genome). ABR genes, which are located in bacterial cell plasmids, can be transmitted to offspring by vertical transfer and to various bacteria, via sex saws (conjugation), by horizontal gene transfer (Aaerstrup et al., 2008; Cantas et al., 2013). Irrational use of antibiotics potentiates the reproduction of selected bacteria (Kalenić et al.,

2013). They change their hereditary material through the exchange of DNA and between different microorganisms, including those of different phylogenetic affiliation and origin from different animals or humans. The phenomenon is called horizontal gene transfer, which takes place asexually, mediated by plasmids, transposons, and gene cassettes, and by conjugation, transformation, and transduction processes (Markey et al., 2013). By accepting DNA from different bacteria, by horizontal gene transfer, a new genetic strain of the bacterium can be obtained (Giedraitiene et al., 2011). Due to the consumption of various AB during life, bacteria, especially commensals, acquire ABR genes or develop resistant mutants, all for the purpose of bacterial survival and maintenance of homeostasis in the intestinal tract (Markey et al., 2013). Horizontal transfer of ABR genes is thought to be most prevalent in *Campylobacter spp*. (Thomrongsuwannakij, Blackall and Chansiripornchai, 2017). *Campylobacter spp*. is problematic in terms of creating ABR, because it is constantly present in the intestinal tract of almost all warm-blooded animals (Shankar et al., 2010). When we talk about *Campylobacter spp*., a food contaminant, it is most often a bacterium originating from poultry and livestock (Kaakoush et al., 2015). Food contaminants, in addition to the danger of causing alimentary intoxications in humans, can spread ABR genes by horizontal gene transfer to bacteria of human origin (Markey et al., 2013; Garcia-Migura et al., 2014). International trade in beef and poultry has contributed to the spread of ABR mediated by plasmids and other extended-spectrum β-lactamases, such as CTX-M-14 and clones, often responsible for ABR and to carbapenems. Carbapenemase-producing bacteria are usually resistant to all existing AB (Cornaglia et al., 1999; Walsh, 2008). ABR genes can also be transmitted to nonpathogenic bacteria (Ammor and Mayo, 2007). Bacteria are short-lived, variable genetic material, which changes frequently (acquired resistance) (Aarestrup, Wegener and Collignon, 2008). ABR can be innate and acquired. Congenital ABR is a genetic characteristic of a particular bacterium. It occurs due to a mutation in the bacterial genome or due to the acquisition of a new DNK, creating a new mechanism of resistance (Davey et al., 1992; Neu, 1992; Giedraitiene et al., 2011). Acquired ABR, which changes uncontrollably, is a problem for public health (Garcia-Migura et al., 2014).It represents an unpredictable genetic change, where bacteria acquire resistance to AB effective in ancestors, ineffective in offspring. ABR also occurs as a result of the already described biochemical mechanisms, which are not related to changes in genetic or phenotypic characteristics. Biochemical mechanisms allow bacteria to possess ABR even during abstinence from AB (Richardson, 2017). In order to reduce the spread of ABR, it is necessary to rationalize the consumption of AB in veterinary medicine, economy and medicine (Garcia-Migura et al., 2014). As a measure to prevent the spread of ABR, the use of AB based on antibiograms is recommended (Kaakoush et al., 2015).The problem of adherence to the mentioned measures is represented by infections, which require the urgency of therapy where doctors resort to the application of experiential therapy. As a control measure, regular monitoring of ABR is recommended. As an important measure, international protocols have been developed, with AB of choice. The problem of protocol validity is reflected in the change in the genetic material of bacteria, which brings with it a change in ABR. AB can also show the effects of accumulation in the body. Residues can be found in milk, eggs and other products of animal origin. Thermally processed food, which contains AB residues, is also a danger to public health. Many chemical structures, after heat treatment, can become more toxic and cause a number of detrimental effects on consumer health (Botsoglou and Fletouris, 2001; Mateu and Martin, 2008).

METHODS TO PROVE ANTIBIOTIC RESISTANCE

Routine laboratory methods and molecular methods are used to prove ABR. Routine methods are divided into quantitative and qualitative. Quantitative methods also determine the minimum inhibitory concentration (MIC), which represents the highest dilution of AB, which inhibits

bacterial growth (Markey et al., 2013). MIC is also determined by E-test (Quinn et al., 2011). By qualitative methods, we cannot determine the minimum inhibitory concentration of AB, but qualitative methods are in wider use. Quantitative methods are broth dilution, agar dilution, and the qualitative method is the disk diffusion method (Markey et al., 2013). We determine the ABR by the disk diffusion method or the tablet method, by placing paper tablets, coated with a precisely determined concentration of AB, on the substrate with the tested culture. AB diffuse through the substrate creating around themselves a so-called zone of inhibition or a zone of AB saturation, within which no isolate sensitive to the test AB grows. Through the middle of the disc, the diameter of the created zone of inhibition is measured, which is expressed in millimeters. It is measured using a standardized method, with the help of which we conclude whether the tested bacterium is resistant (R) to the tested AB. Otherwise it shows sensitivity (S) or moderate sensitivity (I) (EUCAST, 2017b; CLSI, 2018). It is very important not to deviate from standard recommendations in terms of incubation conditions, temperature, atmosphere, incubation, bacterial inoculum size, medium composition, content, age and storage of AB, inoculation methods, because changes in standards significantly affect antibiogram results. It is important to mention that depending on the type and strain of bacteria, variations in the degree of ABR are also present (Quinn et al., 2011). Reading and interpretation of antibiogram results is performed based on the recommendations of the *European Committee on Antimicrobial Susceptibility Testing* and the *Clinical and Laboratory Standards Institute* (EUCAST, 2017a; EUCAST, 2017b; CLSI, 2018). ABR indicates that the AB under study is ineffective, sensitivity suggests efficacy, and moderate sensitivity that the AB is effective, only if administered, at the maximum dose (Quinn et al., 2011; Markey et al., 2013). Of the molecular methods, PCR is used, which encodes various types of beta lactamases, extended-spectrum β-lactamases (ESBLs), and clones (Lee et al., 2013; Osei Sekyere et al., 2015). Various phenotypic tests are also used, which prove ESBL and β-lactamases AmpC, and they include modified Hodge test, combined disk test, synergism test, biochemical tests and spectrophotometry (Lee et al., 2013; Osei Sekyere et al., 2015).

MONITORING OF ABR *CAMPYLOBACTER SPP*. AND THE INFLUENCE OF SANITATION ON THE CONTAMINATION OF CHICKEN MEAT

Alagić et al. (2016) in their research in BiH, which refers to the frequency of contamination of chicken bodies sampled at the slaughter line after the evisceration phase of *Campylobacter* spp., state the data of contamination of chicken carcasses with 27.4% (23/84), most often in chicken breasts %), less in the visceral cavity (15.5%) and least in chicken liver samples (9.5%). In the highest percentage, contamination with *C. jejuni* was recorded (91.9%), and mild contamination with *C. coli* (8.1%). The results suggest the importance of microbiological control of chicken meat in BiH. **Bartkowiak-Higgo** et al. (2006) in a pilot study in South Africa, which related to the percentages of carcass contamination originating from poultry carcasses with *C. jejuni* and *C. coli*, stated that the percentage of skin and liver contamination of poultry was (24%) and the contamination intestines in the amount of (28%), indicating an increased risk of infection for consumers, through contaminated chicken meat or through cross-contamination. **Bechstein** et al. (2019) in a prospective study in Germany, which refers to the effect of laurin arginate on reducing contamination of raw minced meat and broiler carcass with *C. jejuni*, state that the use of this disinfectant, without the application of hydrostatic pressure, has no significant effect on reducing contamination *C. jejuni*. **Cokal** et al. (2009) in a study in Turkey, reported high percentages of chicken meat contamination from six slaughterhouses with *Campylobacter* spp. (*C. jejuni*: 97/240, 40.4%; *C. coli*: 29/240, 12.1%) and the percentages of antibiotic resistance of the same isolates with the highest observed percentage to nalidixic acid, tetracyclines, ciprofloxacin and enrofloxacin (*C. jejuni*: 79.4%, 76.3%, 74.2%, 15.5%). *C. coli* isolates

showed antibiotic resistance in the same percentage to nalidixic acid and ciprofloxacin (*C. coli*: 65.5%). Multiresistance to tetracyclines, nalidixic acid, ciprofloxacin was found in both isolates, and to enrofloxacin in *C. coli* isolates (*C. jejuni*: 48.5%; *C. coli*: 51.7%). The **European Food Safety Authority** (EFSA) and the **European Center for Disease Prevention and Control** - ECDC (2008-2019) report that campylobacteriosis has been on the rise in Europe since 2005., which has not been recorded in any other alimentary epidemic and that campylobacteriosis is the leading alimentary intoxication, with salmonellosis in second place. **Hauri** et al. (2013) in their retrospective study, in the period from 2005 to 2011, recorded an increase in the prevalence of campylobacteriosis in Germany. **Jore** et al. (2010) report data on the decline in the prevalence of campylobacteriosis in Sweden and Denmark, in the period from 2001 to 2007. **Kaakoush** et al. (2015) report data on the growth of campylobacteriosis prevalence in Sweden, Denmark, and the United States. **Khan** et al. (2018) in a prospective study in India, found that the highest percentage of *C. jejuni* contamination was present in broiler carcasses (38.6%) and chicken intestines (24%), and the lowest percentage of contamination was recorded on cutting boards and knives (14.0%). Antibiotic resistance was found in 97% of *C. jejuni* isolates, and the highest percentage was recorded for cafalotine (81.1%) and tetracyclines (59.4%). Lower percentages of antibiotic resistance were evident for nalidixic acid, ciprofloxacin, erythromycin, gentamicin, and azithromycin (6.9–8.9%). **Nair** et al. (2014) in a study in the state of Mississippi, state, that lauric arginate reduces the contamination of chicken meat with *C. jejuni*. **Šimunović** et al. (2020) state that the highest percentages of resistance of thermophilic species of the genus *Campylobacter* are evident on macrolides and fluoroquinolones. **Thomrongsuwannakij, Blackall and Chansiripornchai** (2017) in a study in Thailand, in the period from 2014 to 2015, examined the percentages of antibiotic resistance of *C. jejuni* and *C. coli* isolates originating from broilers, monitored through two production chains and found the presence of most multidrug-resistant isolates (*C. jejuni*: 100%; *C. coli*: 98.9%). The highest antibiotic resistance was found to enrofloxacin, followed by tetracyclines, trimethoprim-sulfamethoxazole and doxycycline (*C. jejuni*: 100%, 55.6%, 36.1%, 50%; *C. coli*: 98.9%, 97, 9%, 81.9%, 79.8%). **Wagner** et al. (2003) from Germany in their 1998–2001 study examined the presence of antimicrobial resistance in 144 isolates of thermophilic *C. jejuni*, tested by routine laboratory tests in patients prior to antimicrobial therapy. A high percentage of resistance was recorded to ciprofloxacin (45.1%). Wagle et al. (2017) in a study in Arkansas (USA) report a positive effect of the disinfectant phytophenolic compound β-resorcic acid on the decrease in contamination of raw skins and chicken meat with *C. jejuni*, after cultivation.

CONCLUSION

A review of the literature concludes that the thermophilic *Campylobacter* spp. a significant public health problem worldwide. The route of transmission of campylobacteriosis is indirect and direct. The most important reservoir of infection is chicken food. Due to the constant presence of *Campylobacter* spp. in the intestinal tract, this is a significant food pathogen due to its large prevalence in the environment. *Campylobacter* spp. are sensitive to almost all known disinfectants. Another public health problem is antibiotic resistance, which is widespread in the world and present in various percentages to fluoroquinolones, which belong to clinically important antibiotic classes and are most often used in the treatment of campylobacteriosis.

Abbreviations AB: Antibiotic; ABR: Antibiotic resistance; AMR: Antimicrobial resistance; Campylobacter spp.: *Campylobacter species*; CLSI: Clinical and Laboratory Standards Institute; ECDC: European Centre for Disease Prevention and Control; EFSA: European Food Safety Authority; ESBL: Extended spectrum beta-lactamases; EUCAST: European Committee

on Antimicrobial Susceptibility Testing; FAO: Food and Agriculture Organization; GHP: Good Hygiene Practice; GMP: Good Manufacture Practice; GBS: Guillain–Barré syndrome; HACCP: Hazard Analysis Critical Control Point; MFS: Miller Fisher syndrome; VCIA: Veterinary Critically Important Antimicrobial Agents; WHO: World Health Organization.

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SYNTHESIS OF PLANT-MEDIATED SILVER NANOPARTICLES USING *MOMORDICA CHARANTIA* EXTRACT: EVALUATION OF ANTIOXIDANT ACTIVITY AND SURVIVAL RATE ON FRUITF LIES

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ABSTRACT

The nanoparticle synthesis by the biological method is starting a new era in nanotechnology research. Silver nanoparticles are generally synthesized by chemical and physical methods which are highly toxic and flammable. This study related to an environmentally friendly process of biosynthesis of the silver nanoparticle using *Momordica charantia* fruit, and their innate antioxidant and catalytic activities. Characterization of the AgNPs was done by UV-Visible Spectroscopy (UV-VIS), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FT-IR). AgNO3 (5 mM) was allowed to react with an aqueous extract of *M. charantia* fruit. Antioxidant activity and survival rate assays were conducted by exposing *Drosophila melanogaster* larvae to particle concentrations at 50, 100, and 150 ppm of AgNPs. UV-VIS spectra show an absorption peak between 420 and 430 nm. The SEM images showed the size distribution of the nanoparticles and the average size was found to be 11-18 nm. Results of these analyses confirmed the formation of silver nanoparticles. Upon exposure to silver nanoparticles sized 11-18 nm, fruit fly survival rate did not exhibit a statistically significant control. However, AgNPs treatment also produced a significant increase in total antioxidant status as compared to control ($p<0.05$). Results indicate a protective role of AgNPs for oxidative stress. Silver nanoparticles so synthesized in this study are a simple, easy, and effective technique of nanoparticle production and synthesis of plant-mediated silver nanoparticles using *M. charantia* fruit extract that provides AgNPs are not toxic to fruit flies.

KEYWORDS: Green Synthesis, Silver Nanoparticles, *Momordica charantia*, *Drosophila melanogaster*, Antioxidant Activity, Survival Rate

INTRODUCTION

Nanoparticles exhibit unique characteristics based on the structure and size, effectively with a wide range of applications, and thus provides a useful platform to explore various interdisciplinary areas (Templeton et al., 2000). In particular, silver nanoparticles (AgNPs) have been extensively exploited due to their excellent physical, chemical, and antimicrobial properties and thus applied in catalysis, bio-sensing, food industry, photography, optics, electronics, imaging, and medicine (Jain et al., 2007; Jain et al., 2008). Conventional nanoparticle synthesis approaches use highly toxic chemicals that cause toxic side effects upon administration. Therefore, an alternative method is needed to overcome these toxic effects. A green synthesis is an approach that not only ensures the safety and efficacy of the created nanoparticles but also enables the availability of cheaper, non-toxic nanoparticles (Roy et al., 2013).

Since noble metal nanoparticles such as gold, silver, and platinum nanoparticles are widely used in human contact regions, there is an increasing need to develop environmentally friendly nanoparticle synthesis processes where no toxic chemicals are used (Song and Kim, 2009). As an alternative to chemical and physical methods, it has been suggested that nanoparticle synthesis is eco-friendly by using biological methods using microorganisms (Klaus et al., 1999; Konishi et al., 2007; Nair and Pradeep, 2002), enzymes (Wilner et al., 2006), and plant or plant extracts (Shankar et al., 2004). *Momordica charantia* L. is a well-known medicinal plant belonging to the Cucurbitaceae family found in tropical and subtropical regions of the world. It has shown antibacterial, anticancerous, antileukemic, antiprotozoal, antitumoral, antiviral, antiparasitic, antifungal, antiobesity, antiulcer, hypoglycemia, and immune-stimulant activities (Alam et al., 2009; Gupta et al., 2010; Agrawal and Beohar, 2010; Santos et al., 2012). It has been used by natural health practitioners for diabetes, cancer, high cholesterol, viral infections, and bacterial infections (Grover and Yadav, 2004; Beloin et al., 2005). The main constituents of *M. charantia* responsible for the medicinal properties are triterpenes, proteins, steroids, alkaloids, and other phenolic compounds (Budrat and Shotipruk, 2008; Saeed et al., 2021).
In this study, we have synthesized silver nanoparticles by a simple, fast, green, and cost-effective method using a popular medicinal plant *M. charantia*. Survival rate and an antioxidant assay of synthesized AgNPs has evaluated on the fruit fly *Drosophila melanogaster*, developed as an in vivo model organism for toxicology studies (in particular the field of nanotoxicity), in the presence of *M. charantia* extract was also studied.

MATERIALS AND METHODS

Preparation of plant extract

M. charantia fruit extract was used to prepare silver nanoparticles based on cost-effectiveness, ease of availability, and its medicinal property. The healthy fruits of *Momordica charantia* (Cucurbitaceae) were collected from Eskişehir, Turkey in May 2018, and the taxonomic identification was made by Dr. Ali Kandemir. They were surface cleaned with running tap water to remove debris and other contaminated organic contents, followed by double distilled water and air-dried at room temperature for a week. Dried powdered *Momordica charantia* fruits (5g) were mixed with 100 mL distilled water then the solutions were kept for continuous heating at 80°C for 1 hour at room temperature with frequent shaking. After that, the extracts were filtered by using Whatmann No1 filter paper. The extracts were collected and stored at 4°C for further use.

Green synthesis of silver nanoparticles

Silver nitrate (AgNO3, >99% pure, CAS NO:7761-88-8) was purchased from Merck, Türkiye. 100 mL, 1 mM solution of silver nitrate was prepared in an Erlenmeyer flask. Then 1, 2, 3, 4, and 5 mL of fruit extract were added separately to 10 mL of silver nitrate solution keeping its concentration at 1 mM. Silver nanoparticles were also synthesized by varying concentrations of AgNO3 (1 mM- 5 mM) keeping extract concentration constant (1 mL). 10 mL of the aqueous fruit extract of *Momordica charantia* were added into 90 mL of an aqueous solution of 1 mM Silver nitrate. The mixtures were exposed to a range of controlled temperatures for 24h. The colorless reaction mixtures changed to brown color resulting in AgNPs formation. After incubation the silver nanoparticles were isolated and concentrated by repeated (3- times) centrifugation of the reaction mixture at 10,000×*g* for 10 min. The supernatants were replaced by distilled each time and suspensions stored at 4°C for further use as a lyophilized powder.

Characterization of synthesized silver nanoparticles

The optical measurements were confirmed by using UV-visible spectroscopy (Perkin Elmer, Lambda 35) and scanned the spectra between 400- 700 nm at the resolutions of 1 nm. The structural morphological characteristics were observed under a scanning electron microscope (SEM) fitted with an Energy Dispersive Spectrophotometer (EDS) System (FEI-Quanta FEG 450) at a magnification 120,000X operated at an accelerating voltage of 30 kV. The elemental analysis was carried out under an X-ray diffraction (XRD) analyzer (Panalytical, Empyrean). The powder XRD spectrum of the sample was taken to characterize the crystal structure and purity of Mcf-AgNPs. Fourier transform infrared (FTIR) spectra were recorded on Thermo Scientific, Nicolet 6700 FTIR Spectrophotometer was used to identify the oxide forms and the biomaterials on the surface of the NP. The FTIR spectrums were taken in the mid-IR region of 400-4000 cm^{-1}.

Antioxidant Assay

Total antioxidant status (TAS) and total oxidant status (TOS) were measured by using commercial kits of Rel Assay (Rel Assay Kit Diagnostics, Turkey) developed by Erel (2005). Trolox, a water-soluble analog of vitamin E for TAS, and hydrogen peroxide for TOS were used as a calibrator. Results were expressed as mmol Trolox Equiv/lt and μmol H2O2 Equiv/lt, respectively. The percentage expression of the ratio of TOS level to TAS level was calculated as OSI. Results were expressed as an "arbitrary unit" (AU) and calculated according to the following formula: OSI = TOS (μmol H2O2 Equiv/lt) / TAS, mmol Trolox Equiv. / lt × 10.

Assessment of survival rate

The fruit aqueous extract of the plant (Mcf-AgNPs) was prepared at the following concentrations (ppm): 50, 100, and 150 in distilled water. *Drosophila melanogaster* Oregon wild-type strain was used for the survival rate experiments. 1- 3 days-old virgin fruit fly individuals were transferred to fresh media as 5 ♀♀ X 5 ♂♂ and kept at 25 ± 1°C and 40-60% relative humidity for 3 days. At the end of the third day, the parents were removed from the media to collect the larvae. Later, distilled water was placed in a clean petri dish and a piece was taken from the media containing the 3rd instar larvae (72 ± 4h) to separate the larvae from the distilled water. 100 larvae were counted for control and application groups and treated in vials with Standart *Drosophila* Medium (SDM) with the plant extract concentrations until imago emergence. During this process, all experimental groups were checked every day. Adult flies were recorded twice a day by making a distinction between male and female. All experiments were conducted in triplicate.

Data analysis

Results were presented as mean ± standard deviation (SD) or percentages, when applicable. To be able to determine the statistical significance of the results, Duncan's one-way range test was applied. Data were analyzed using SPSS (ver. 22 for WindowsTM) software.

RESULTS

Characterization of synthesized silver nanoparticles

The characterization of Mcf-AgNPs has been done by XRD, SEM equipped with EDX, FT-IR, and UV-Vis. After the addition of 1 mM $AgNO_3$ solution to the aqueous extracts, the color of the compositions changed to dark brown color. It showed an absorbance peak around 420-430 nm for all samples (Figure 1).

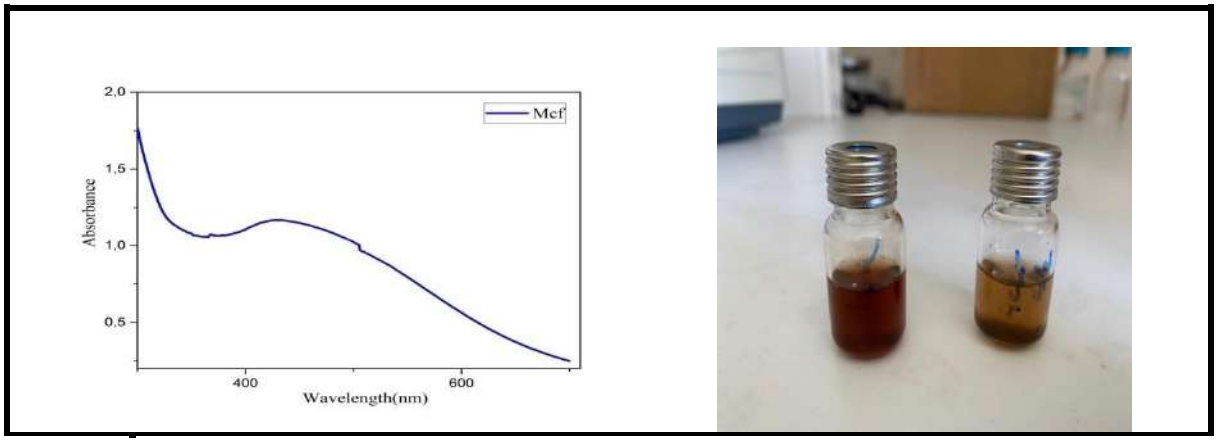

Figure 1: UV/Visible absorption spectrum of synthesized silver nanoparticles from the extract of *Momordica charantia* fruit

The elemental analysis was carried out under energy-dispersive X-ray spectroscopy (Panalytical, Empyrean). The powder XRD spectrum of the sample was taken to characterize the crystal structure and purity of Mcf-AgNPs. The results are shown in Figure 2. Diffraction peaks corresponding to the impurity were not found in the XRD patterns, confirming the high purity of the product. Also, the fact that these peaks are sharp and narrow shows that the crystallization of Mcf-AgNPs is quite good.

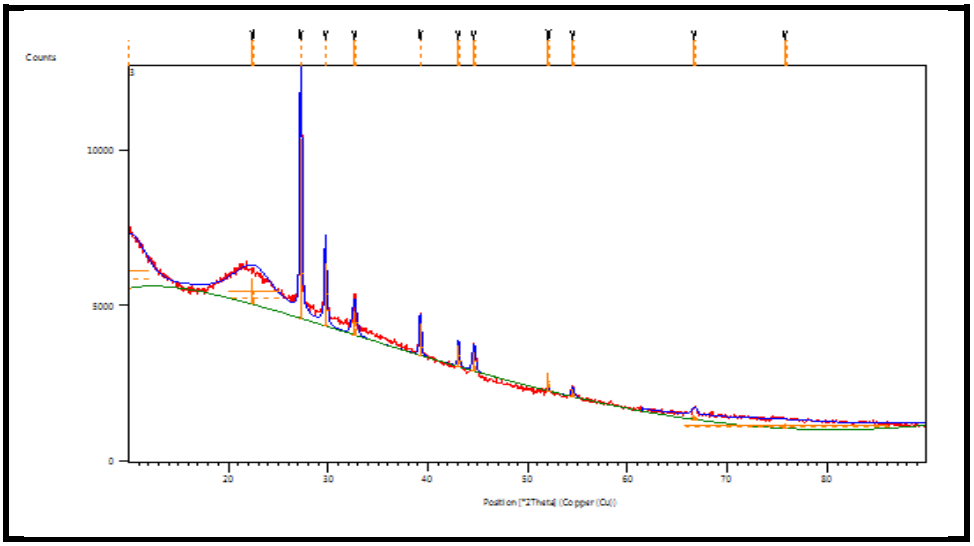

Figure2. XRD patterns of Mcf-AgNPs

Silver nanoparticles obtained from the aqueous extract of *Momordica charantia* fruit were characterized from the SEM micrograph, it is evident that AgNPs were spherical and were poly-dispersed. AgNPs range from 11.24 nm to 20.73 nm occasional agglomeration of the AgNPs has been observed. The energy dispersive spectra of the samples obtained from the SEM-EDS analysis are clearly shown in Figure 3.

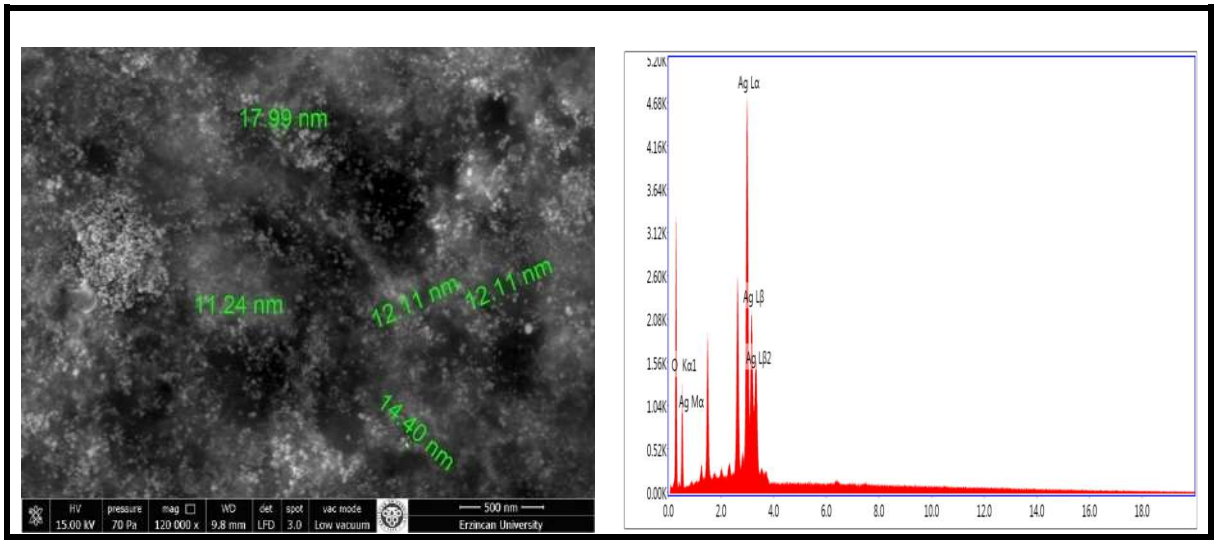

Figure 3. SEM and EDAX analysis of synthesized silver nanoparticles from the aqueous extract of *Momordica charantia* fruit

The FTIR spectrum of Mcf-AgNPs was obtained from Thermo Scientific, Nicolet 6700 in ATR mode at 400-4000 cm-1. Figure 4 shows the characteristic bands of the extract. These characteristic bands predict that in the extract there are structures as proteins, polysaccharides/sugars, and phenolic compounds, mainly flavonoids.

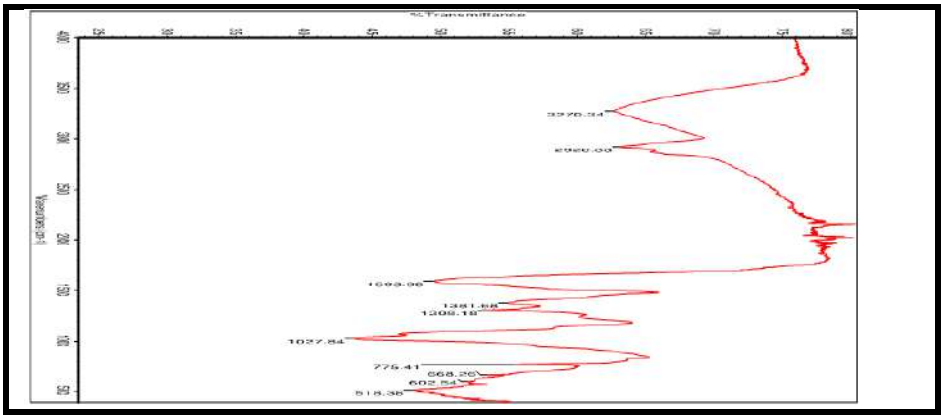

Figure4. FTIR spectra of Mcf-AgNPs

Antioxidant Assay

Total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI) values of adult *D. melanogaster* were examined to determine oxidative parameters by following the Mcf-AgNPs applications to the transheterozigot larvae. We determined that in all AgNPs treated groups, the TOS value was higher and the TAS value was lower than in the control groups (Table 1). Measured levels of TOS and TAS in Mcf-AgNPs applied groups were statistically different from the control group ($p<0.05$). Furthermore, the high value of OSI, obtained by dividing the total oxidant status to total antioxidant status, indicated the presence of toxic effects (Table 1).

Table 1. Oxidative parameters data obtained from *D. Melanogaster* for experimental groups with the Mcf-AgNPs

Experimental groups	TOS (µmol H_2O_2 Equiv. L^{-1})	TAS (mmol Trolox Eqvui. L^{-1})	OSI (AU)
Control	6.21 ± 0.02^{a}	0.93 ± 0.08^{a}	6.67 ± 0.25^{a}
50 ppm	5.30 ± 0.18^{b}	1.21 ± 0.05^{b}	4.38 ± 3.6^{b}
100 ppm	4.47 ± 0.26^{c}	1.48 ± 1.01^{c}	3.02 ± 0.25^{c}
150 ppm	3.12 ± 0.11^{d}	1.97 ± 0.04^{d}	1.58 ± 2.75^{d}

[a-d]: The letters in the same column showed significantly different at the 0.05 level.

Survival rate determination

The data obtained as a result of the experiments to examine the effect of Mcf-AgNPs aqueous extract on the survival rate are shown in Table 2. According to the results obtained, the survival rate in the larvae treated with 50, 100, and 150ppm Mcf- AgNPs aqueous extracts increased compared to the control.

Table 2. The effects of Mcf-AgNPs aqueous extracts on the survival rate of *D. melanogaster*

Treatment concentration	The mean survival rate ±standarderror		
	♀♀ Population	♂♂ Population	Total Population
Control	39 ± 1.15^{a}	35 ± 0.57^{a}	74 ± 0.57^{a}
50 ppm	41 ± 0.57^{b}	39 ± 1.05^{b}	80 ± 1.73^{b}
100 ppm	46 ± 1.02^{c}	43 ± 1.01^{c}	89 ± 3.46^{c}
150 ppm	47 ± 0.18^{c}	45 ± 0.21^{c}	92 ± 3.46^{d}

[a-d]: The letters in the same column showed significantly different at the 0.05 level.

DISCUSSION AND CONCLUSION

The nanoparticles have a smaller size and hence a larger surface area to volume ratio in comparison with the bulk made up of larger molecules. This makes nanoparticles more chemically reactive. Our results confirmed the successful conversion of inert or less active Mcf extract to highly active Mcf-AgNPs via bioreduction; with enhanced biological properties. Structural information of the formed Mcf-AgNPs was obtained by SEM, EDX, Uv-Vis, XRD, and FTIR analysis. *M. charantia* fruits contain a variety of flavonoids and phenolic compounds which may involve the biosynthesis of AgNPs and act as a reducing agent for the reduction of

Ag+ to Ag^0. Phenolic compounds possess carboxyl and hydroxyl groups, which are capable to bind to metal (Harborne, 1964). Flavonoids can also directly remove molecular species of active oxygen. Their antioxidant activity resides mainly in their capability to provide electron or hydrogen atoms (Ahmad et al., 2010). Biomedical applications of AgNPs synthesized from plant extracts have been reported (Burdusel et al., 2018). Bitter gourd (*Momordica charantia* L.) has long been regarded as a food and medicinal plant. AgNPs alter the permeability of the cell by penetrating the membrane, which produces the exit of intracellular material and therefore cell death (Qing et al., 2018).

In conclusion, the popular medicinal plant *M. charantia* extract for the synthesis of AgNPs is a simple, low-cost, and eco-friendly approach. This study synthesized silver nanoparticles by a simple, fast, green, and cost-effective method using a popular medicinal plant M. charantia. The nanoparticles are characterized by using several UV-vis., FTIR, XRD, SEM, and EDAX. Silver nanoparticles show good protective properties. Therefore, M. charantia functionalized silver nanoparticles can be effectively used as a powerful antioxidant. Nanoparticles also exhibit remarkable antioxidant properties. Due to these properties, synthesized nanoparticles have remarkable applications in the biomedical field.

Based on the results, we conclude that silver nanoparticles have a potential application in a broad range of industries, mainly food and medicine.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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CLINICAL UTILITY OF TUMOR MARKERS IN MANAGEMENT OF CANCER

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ABSTRACT

The basic aspect of cancer treatment is prevention through early identification and diagnosis. Patient identification is done with laboratory tests specific to the type of cancer. The diagnosis is made based on the respective tumor markers. The aim of the study is to monitor the alteration of tumour markers after chemotherapy. This is a prospective study performed during the period 2014-2016 with 107 patients diagnosed with Ca mamal, uterus and ovaries in the hospital of Vlora district who also were treated with chemotherapy. The study included 107 patients with Ca, with a mean age of 57.1 (± 8.13) years and ranging from 38 to 70 years of age. By comparing the median values of hematobiokemic and tumor parameters after the 1st and 2nd cycle of chemotherapy, the statistically significant difference was found only for SGOT which showed decrease after the second cycle. Concerning the types of Ca, significant decrease of CA 15.3 was observed in all three types of Ca following the second cycle of chemotherapy. Significant decrease of CA 125 was observed in all three types of Ca following the second cycle of chemotherapy ($p < 0.01$). The values of the three tumor markers are higher in metastatic patients, with statistically significant change in metastasis-free patients ($p < 0.01$). The values of hematobiochemical parameters and tumor markers are important in identifying the course of therapy as well as predicting malignant conditions.

Key words: cancer, hematobiochemical parameters, tumor markers

INTRODUCTION

The basic aspect of cancer treatment is prevention through early identification and diagnosis. Patient identification is done with laboratory tests specific to the type of cancer. The diagnosis is made based on the respective tumor markers. Of the millions of new cases of cancer worldwide and deaths associated with cancer, a large number of them were breast and gynecological tumors (Molina R et al., 2005). Some tumor markers are fundamental to the workflow in diagnosis, control of therapy and the monitoring of advanced gynecological diseases (Sturgeon CM et al 2010). The biomarker should be absent in healthy people as well as in good conditions and it is released exclusively from specific tumor cells (Duffy MJ et al 2013). Tumor markers are soluble glycoproteins that are found in the blood, urine, or tissues of patients with certain types of cancer. They are typically produced by tumor cells, but in some cases they may be produced by the body in response to malignancy or to certain benign conditions. Tumor markers are not elevated in all cancer patients, particularly patients with early-stage cancer. The various tumor markers differ in their usefulness for screening, diagnosis, prognosis, assessing therapeutic response, and detecting recurrence. Normalization of tumor marker values may indicate cure despite radiographic evidence of persistent disease. In this situation, residual tumor is frequently nonviable. Sometimes, tumor marker values may rise after effective treatment (due to cell lysis), but the increase may not portend treatment failure. The temporary increase of CA15-3 that is affected by chemotherapy and followed by

the decrease of CA15-3 (CA15-3 increases and decreases) may result in inappropriate early discontinuation or chemotherapy change. The vast majority of ovarian tumors are of epithelial origin and the 125 carbohydrate antigen (CA 125) is the most important marker of tumor. Increased levels depend on the histological type and stage of the disease (Sölétormos G et al 2016). Though it is sensitive in the early stages, CA 125 has high sensitivity and specificity in early dictation of the disease, especially in women and premenopause period (Castrillon DH et al 2002). Furthermore, some factors may cause high levels of CA 125, such as ethnicity, pregnancy, age, premenopausal postmenopausal period and menstrual cycle (Pauler DK et al 2001). In breast cancer, a combination of carcinoembryonic antigen (CEA) and CA 15-3 has prognostic potential in a preoperative environment (Huh JW et al 2010). While CEA and CA 15-3 are recommended for monitoring the therapy and early detection of disease recurrences they are also recommended for early diagnosis or screening due to their high sensitivity. In cervical cancer, the guidelines of the National Academy of Clinical Biochemistry (NACB) discuss (among the markers of other tumors such as the CEA and CA 125 squamous cell carcinoma antigen (SSCA) for predicting prognosis and preoperative prediction of metastases (Colombo N et al 2016) CEA has prognostic significance in colorectal cancer (Thirunavukarasu P et al 2011) but in cases where the origin of an uterine tumor is unclear, panel tumor markers are recommended including CEA (Zur B et al 2012, Haas M et al., 2013). The aim of the study is to monitor the alteration of tumour markers after chemotherapy.

MATERIAL AND METHODS

This is a prospective study performed during the period 2014-2016 with 107 patients diagnosed with Ca mamal, uterus and ovaries in the hospital of Vlora district who also were treated with chemotherapy. For all patients, laboratory tests: biochemical, hematologic, tumor markers: CEA (mg / l) CA15.3 (U / ml) CA 125 (U / ml) were performed after the first and second cycle of chemotherapy. Patients were compared to a non-Ca group control group for comparing the values of tumor markers between the two groups and for determining cut-off values and predictive parameters of tumor markers for Ca. The reported data are expressed as mean ± standard deviation (SD). Wilcoxon test was used to compare the hematobiochemic and tumor markers between the 1^{st} and 2^{nd} cycle of chemotherapy. The receiver operating curve (ROC) curves for determining cut-off values and predictive parameters of tumor markers for Ca. A p-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The study included 107 patients with Ca, with a mean age of 57.1 (± 8.13) years and ranging from 38 to 70 years of age. By comparing the median values of hemato-biochemical and tumor parameters after the 1st and 2nd cycle of chemotherapy, the statistically significant difference was found only for SGOT which showed decrease after the second cycle. Concerning the types of Ca, significant decrease of CA 15.3 was observed in all three types of Ca following the second cycle of chemotherapy. Significant decrease of CA 125 was observed in all three types of Ca following the second cycle of chemotherapy ($p<0.01$). The values of the three tumor markers are higher in metastatic patients, with statistically significant change in metastasis-free patients ($p <0.01$) (fig. 1).

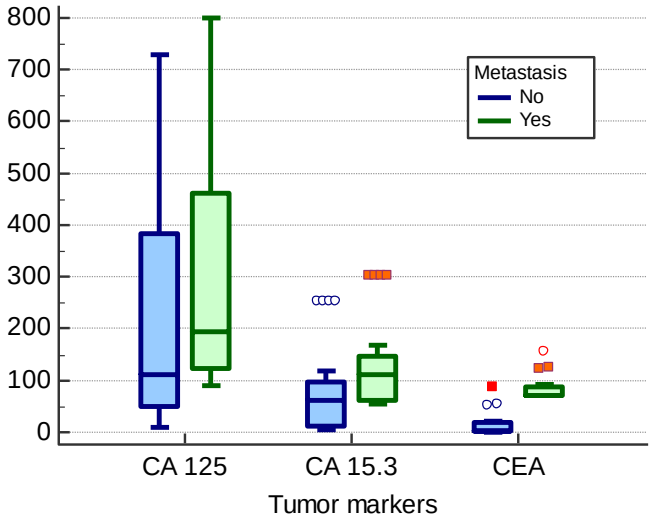

Figure 1. Mean values of tumor markers according to the presence of metastases

No statistically significant difference was found between the ROC curves of the three tumor markers for the determination of Ca (fig. 2).

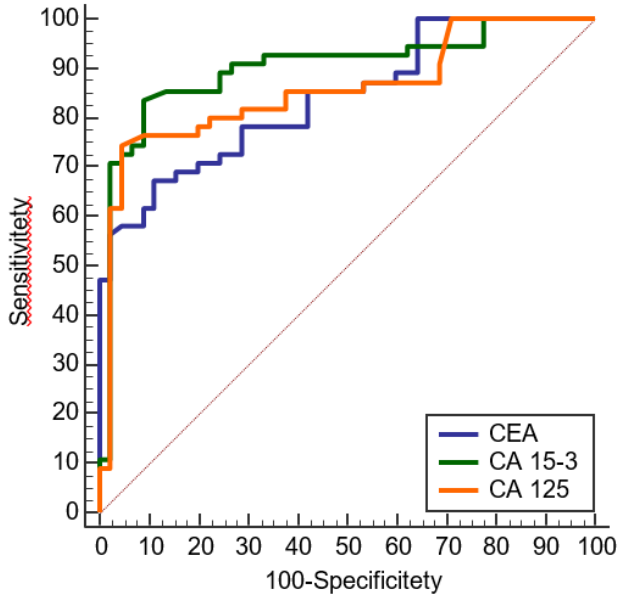

Figure 2. Comparison of ROC curves of tumor markers for prediction of Ca

In predicting malignancy in ovarian tumor patients, besides history taking and physical examination, the use of tumor markers as a part of evaluation is also important.

Patients receiving first line chemotherapy for ovarian cancer are usually offered a minimum of 5 courses of chemotherapy. Unless there is evidence of clinical progression the first three courses will almost certainly be administered. If there is then evidence of inadequate response or progression when the patient attends for her fourth or subsequent course, there could be a change of therapy. A serial rise of tumor markers of 25% over three samples has been shown to indicate progression. Many doctors would consider a lesser rise or slight fall indicates poor response. It is essential that any decision is based on a baseline result and at least two further

marker results with the second or subsequent marker results confirming the trend. To summarise: Obvious clinical improvement: continue planned therapy. Obvious clinical progression: change therapy. If patients not in above groups: Marker response (at least downward trend): continue planned therapy. Marker progression (>25% rise) chnage therapy. Our findings correspond to current guidelines such as NACB that recommend CA 15-3 in breast cancer - although not for diagnosis but for advanced disease monitoring and postoperative surveillance. (Haas M, et al 2013). Although the CEA application is still being discussed, various studies have shown its importance in, for example, anticipating and early detection of disease progression and metastasis (Stieber P et al 2015). While in the breast cancer analysis the our results show the high clinical performance of CA 15-3. In ovarian cancer, the best diagnostic performance was achieved for CA 125. These results are in line with current recommendations, suggesting that CA 125 is of major importance in therapeutic monitoring, differential diagnosis for legal measures, recurrence and prognosis (Sölétormos G , et al 2016). Serum Cancer Biomarker Cancer Antigen 125 (CA125) is proposed as an adjunct to non-invasive procedures in patients with advanced disease (Shao Y et al 2015; Wu SG et al 2014; Wang G et al 2014). However, challenges remain on how to determine values in CA125 concentrations that allow an optimal interpretation that is vital for early diagnosis of tumor growth.

CONCLUSIONS

The values of hematobiokimic parameters and tumor markers are important in identifying the course of therapy as well as predicting malignant conditions. Accurate determination of serum tumor marker levels is crucial, as their impact on diagnosis, prognosis, and therapy monitoring has been shown for many types of tumors.

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INVESTIGATION OF THE USE OF FOOD GRADE SANITATION AGENTS IN PREVENTING THE FORMATION OF Salmonella TYPHIMURIUM BIOFILM STRUCTURES

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ABSTRACT

In this study, the effectiveness of sanitizing agents that are allowed to be used in food production processes at certain rates in preventing the formation of Salmonella biofilm structures, which are one of the main causes of infections on food production surfaces and especially skin-transmitted medical materials, have been determined. Preliminary studies were carried out to determine effective preparation formulations by examining whether these agents create a synergetic effect in their combined use, as well as their capacity to prevent individual biofilms. Antimicrobial agents used in trials and their concentrations was chosen to be; nisin (1, 5 and 10 mg / mL), ciprofloxacin (0.25, 0.5 and 5 µg / mL), alpha amylase (0.5, 1 and 5 mg / mL), proteinase K (0.2, 0.4 and 4 mg / mL), CTAB (05, 1 and 10 mg / mL) EDTA (1, 10, 50 mM), SDS (5, 10 and 100 mg / mL) and Tween 80 (5, 10 and 100 mg / ml). The combination prepared in the lowest concentrations of nisin and ciprofloxacin (nisin 10 mg / mL, and ciprofloxacin 5 µg / mL), which was found to have more effective antibiofilm capacity than other antimicrobial agents with their individual use, was determined the most effective synergetic antibiofilm mixture. The possibilities of using this mixture with the food industry and medical fields will be determined by the preparation and testing of large scale preparations of the aforementioned combinations. For this reason, industrial scale production and trial studies are planned for the continuation of the studies.

Key Words: S. typhimurium, biofilm, nisin, ciprofloxacin

INTRODUCTION

The phenotypic structures, which are formed by embedding the bacterial populations into the extracellular polymeric matrix that they produce as a result of their attachment to interfaces, each other or a substrate, differ in terms of reproduction rate and genetic expression, are called biofilms (Donlan and Costerton 2002). For many years, bacteria were thought to be sessile (planktonic) and non-social creatures. However, on the contrary, when bacteria living in nature are exposed to stress conditions such as pH, nutrient deficiency, temperature, and gradients in oxygen levels, they can adhere to a suitable surface and produce an extracellular matrix and form a biofilm. Thanks to this structure, it creates a resistant life form by taking it under chemical and mechanical protection against environmental stresses, antibiotics, and host immune response in a symbiotic relationship with bacteria from both its own kind and other species (Nadell et al. 2008). This resistance due to the biofilm structure complicates the treatment in biofilm infections. While the biofilm can form in very different environments, even

the simplest biofilm has complex dynamics. Heterogeneous and compartmentalized microenvironments (10 - 100 μms) within the biofilm develop a system that locally regulates microbial activity, intercellular signaling and metabolic exchange, thereby regulating cellular and social behavior, which is an important factor for increasing tolerance and persistence (Shi and Zhu 2009, Thallinger et al. 2013).

Members of the *Salmonella* genus have the ability to form biofilms on biotic and abiotic surfaces in their natural life cycle (Prouty and Gunn 2003, Ledeboer and Jones 2005). These bacteria are one of the main microbial problems in the food industry, as they are one of the most important pathogens causing foodborne illness (Hohmann 2001). The formation of *Salmonella* biofilms on abiotic surfaces also causes major problems for the food processing industry (Joseph et al. 2001, Prouty and Gunn 2003). It is estimated that non-typhoid salmonellosis affects 1.4 million people each year in the United States alone, and 95% of these infections are caused by foodborne bacteria. Salmonellosis outbreaks have been associated with a wide range of fresh products such as parsley, fennel, melon, celery stalk, unpasteurized orange juice, in developed countries, as well as ready-to-eat food products (Hohmann, 2001).

Since biofilm cells exhibit a phenotype resistant to environmental stresses, antibiotics and disinfectants, these structures are extremely difficult to remove in the food industry. Therefore, much effort is invested in developing new strategies to interfere with biofilm formation. Srey et al. (2013) tried traditional biofilm control strategies, including sodium hypochlorite, hydrogen peroxide, ozone and peracetic acid, used in the food industries to maintain hygiene efficiently and provide greater effectiveness against biofilm structures and found certain levels of efficacy. Giaouris et al. (2014) also examined in detail some new pathogen biofilm control methods such as essential oils and bacteriophages and prepared effective preparations. Besides, microbial metabolite molecules such as N-acylhomoserine lactone (AHL), autoinducer-2 (Al-2) and c-di-GMP have been introduced as an alternative way to prevent biofilm formation (Park et al. 2015, Wang et al. 2013). However, serious doubts have arisen in the light of recent studies about the efficacy, safety and applicability of these approaches. As a result, many approaches studied under laboratory conditions have been found to be ineffective or risky for human health and the environment in real food processing processes. Because, these control strategies have well-known disadvantages such as the inability of control agents to penetrate the biofilm structures due to the limitation of the permeability provided by EPS in the biofilm structure, the possible toxicity of the residues of the agents used, the induction of genetic exchange between different bacteria and the promotion of resistance to disinfectants. Therefore, new approaches to control biofilm structures in the food industry; focuses on the development of food-grade combinations based on the synergetic effect of agents (Mizan et al. 2015).

MATERIALS AND METHOD

Bacterial Strains and Growth Conditions

Dam and seqA mutants and wild-type S. Typhimurium 14028 strain were obtained from the Prokaryotic Genetics culture collection. Strains were grown in Luria Bertani Broth (LB) broth at 37 °C at a shaking speed of 200 rpm for 18 hours. Since our mutant strain contains chloramphenicol gene cassette in terms of dam gene, chloramphenicol antibiotic (20 μg mL-1) was added to the breeding media. Bacteria samples used in the biofilm experiment were grown in salt-free LB Broth (LB-NaCl) medium, at 37 °C and 20 °C under static conditions. During

the study period, all cultures were stored at -20 °C and 80 °C by adding 15% glycerol to sterile microcentrifuge tubes.

Determination of the Efficacy of Antibiofilm Agents on Biofilm Formation

To determine the amount of biofilm produced by S. Typhimurium strains (wild type, Δdam and ΔseqA) on the polystyrene surface, Woodward et al. (2000) proposed method was used. In order to measure the biofilm formation capacity on the polystyrene surface in the presence of biosurfactants and bioenzymes, the above-mentioned steps were followed after adding 30 µL (Table 2.1) of bioenzymes or biosurfactants prepared at different concentrations to the wells containing the medium, in accordance with the experimental design (Wang et al. 2016)**.**

Table 1. Antimicrobial agents and their concentrations

Biosurfactants	CTAB (0,5 mg/ml - 1 mg/ml – 10 mg/ml)
	SDS (5 mg/ml - 10 mg/ml – 100 mg/ml)
	Tween-80 (5 mg/ml - 10 mg/ml – 100 mg/ml)
	EDTA (1mM – 10 mM – 50 mM)
Bioenzymes	CIP (0,25 µg/ml - 0,5 µg/ml - 5 µg/ml)
	Proteinase-K (0,2 mg/ml - 0,4 mg/ml - 4 mg/ml)
	Alpha-amylase (0,5 mg/ml - 1 mg/ml - 5 mg/ml)
	Nisin (1 mg/ml - 5 mg/ml - 10 mg/ml)

In the study, after calculating the cut-off OD (ODc, limit, threshold) over the negative control, it was evaluated for biofilm production. The results were evaluated as "non-producer", "weak", "moderate" and "strong" in terms of biofilm level produced according to "cut off" values (Stepanovic et al. 2000, Vestby et al. 2009). The biofilm production capacities of the biofilm producer strains are expressed by the transformations of the limit values expressed below (Table 2.3).

Table 2. Cut off conversions used in the evaluation of biofilm production capacities

OD ≤ ODc	Non producer
ODc < OD ≤ 2xODc	weak
2xODc < OD ≤ 4xODc	moderate
4xODc < OD	strong

Statistical Methods

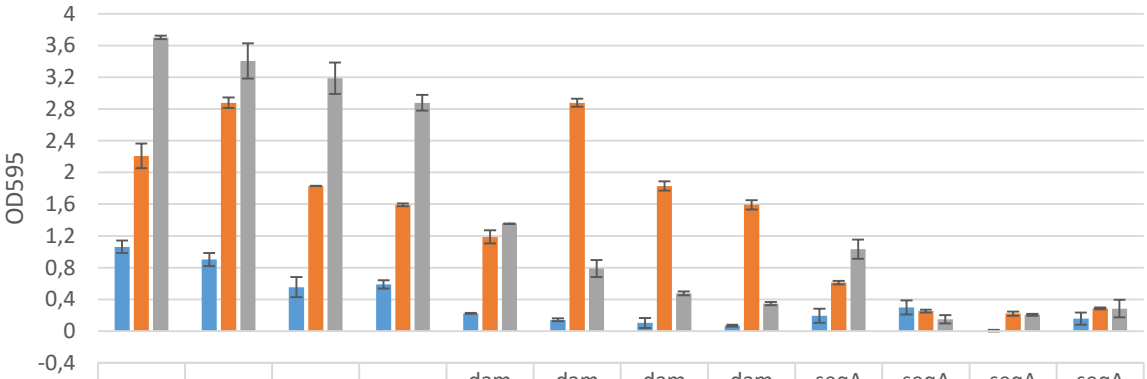
Statistical analysis was performed using GraphPad Prism 7.0 software. The data shown in the figures are the mean ± standard error (SEM) from repeated samples of 3-8 different experiments. Statistical analysis was performed with Bonferroni's post-hoc test, two-tailed Student's t-test or one-way ANOVA. P values <0.05 were considered significant.

RESULTS

Determination of the Effects of Nisin, Bioenzymes and Biosurfactants on Biofilm Formation on Polystyrene Surface

Nisin

The most effective nisin concentration was found to be 10 mg/mL in wild-type strain, 1 mg/mL in *dam* mutant and 5 mg/mL in *seqA* mutant (Figure 1.).

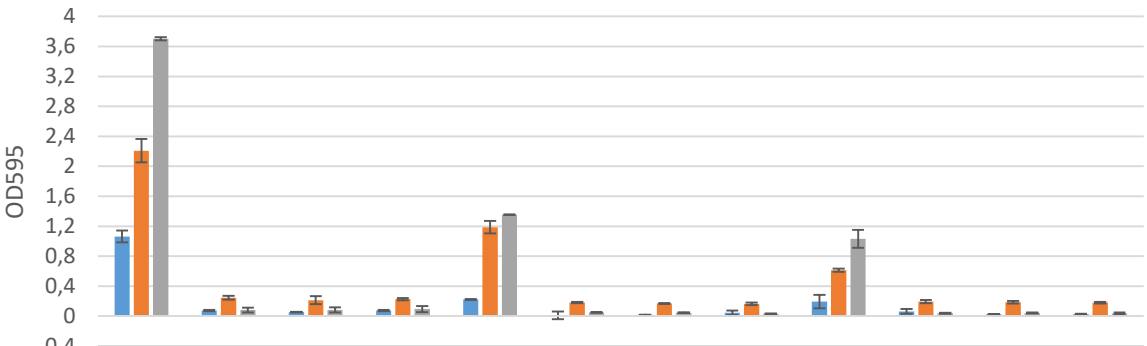


	WT (C)	WT (1)	WT (5)	WT (10)	dam (C)	dam (1)	dam (5)	dam (10)	seqA (C)	seqA (1)	seqA (5)	seqA (10)
24 h	1,063	0,902	0,554	0,59	0,223	0,143	0,104	0,067	0,194	0,299	0,007	0,157
48 h	2,208	2,878	1,829	1,593	1,187	2,878	1,829	1,593	0,612	0,253	0,22	0,287
72 h	3,702	3,405	3,189	2,879	1,354	0,79	0,475	0,347	1,032	0,149	0,206	0,283

Figure 1. Effect of nisin (1 mg/mL, 5 mg/mL and 10 mg/mL) on biofilm formation in S. Typhimurium 14028 wild-type strain and its seqA and dam mutants

Ciprofloxacin (CIP)

All ciprofloxacin concentrations tested showed maximum efficacy in wild-type strain and mutants (Fig. 2.).

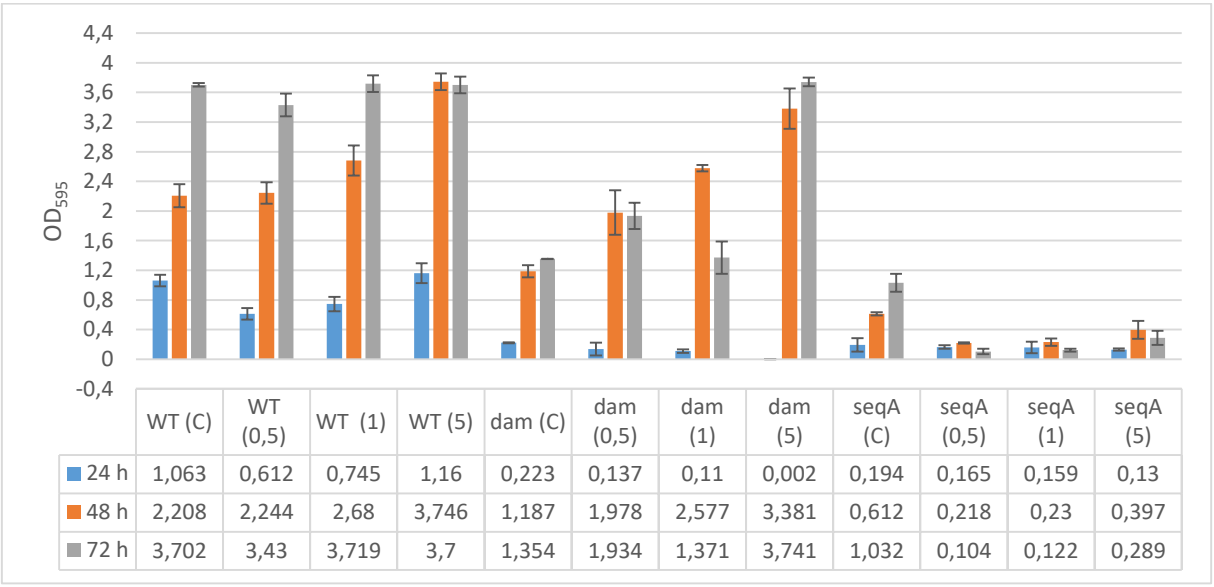


	WT (C)	WT (0,25)	WT (0,5)	WT (5)	dam (C)	dam (0,25)	dam (0,5)	dam (5)	seqA (C)	seqA (0,25)	seqA (0,5)	seqA (5)
24 h	1,063	0,074	0,051	0,074	0,223	0,011	0,018	0,043	0,194	0,062	0,026	0,027
48 h	2,208	0,245	0,213	0,227	1,187	0,181	0,167	0,164	0,612	0,195	0,185	0,179
72 h	3,702	0,082	0,081	0,094	1,354	0,049	0,046	0,032	1,032	0,038	0,042	0,036

Figure 2**.** Effect of ciproflaxacin (0.25 µg/µL, 0.5 µg/µL and 5 µg/µL) on biofilm formation in *S.* Typhimurium 14028 wild-type strain and its *seqA* and *dam* mutants

Alpha amylase

This enzyme did not inhibit biofilm production in wild-type strains and mutants, and induced biofilm formation in wild strain and *dam* mutant at a concentration of 5 mg/mL. (Fig. 3.).

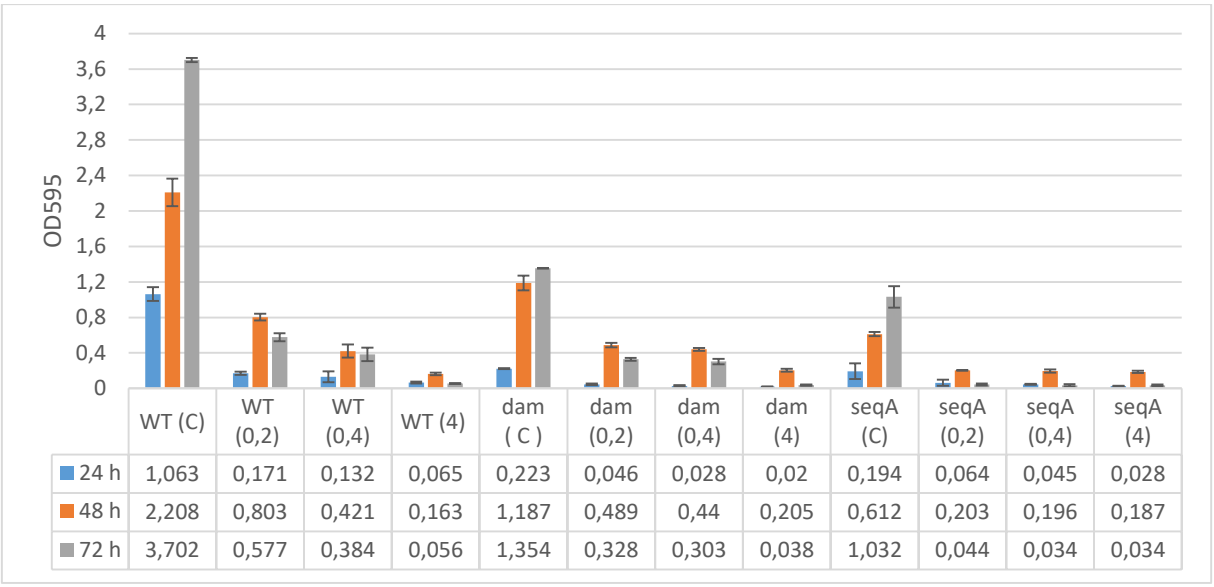


	WT (C)	WT (0,5)	WT (1)	WT (5)	dam (C)	dam (0,5)	dam (1)	dam (5)	seqA (C)	seqA (0,5)	seqA (1)	seqA (5)
24 h	1,063	0,612	0,745	1,16	0,223	0,137	0,11	0,002	0,194	0,165	0,159	0,13
48 h	2,208	2,244	2,68	3,746	1,187	1,978	2,577	3,381	0,612	0,218	0,23	0,397
72 h	3,702	3,43	3,719	3,7	1,354	1,934	1,371	3,741	1,032	0,104	0,122	0,289

Figure 3. Effect of alpha amylase (0.5 mg/mL, 1 mg/mL and 5 mg/mL) on biofilm formation in S. Typhimurium 14028 wild-type strain and its seqA and dam mutants.

Proteinase-K

The concentration of active agent in wild-type strain and dam mutant was determined as 4 mg/mL. In the seqA mutant, this ratio was found to be 0.2 mg/mL (Figure 4.).

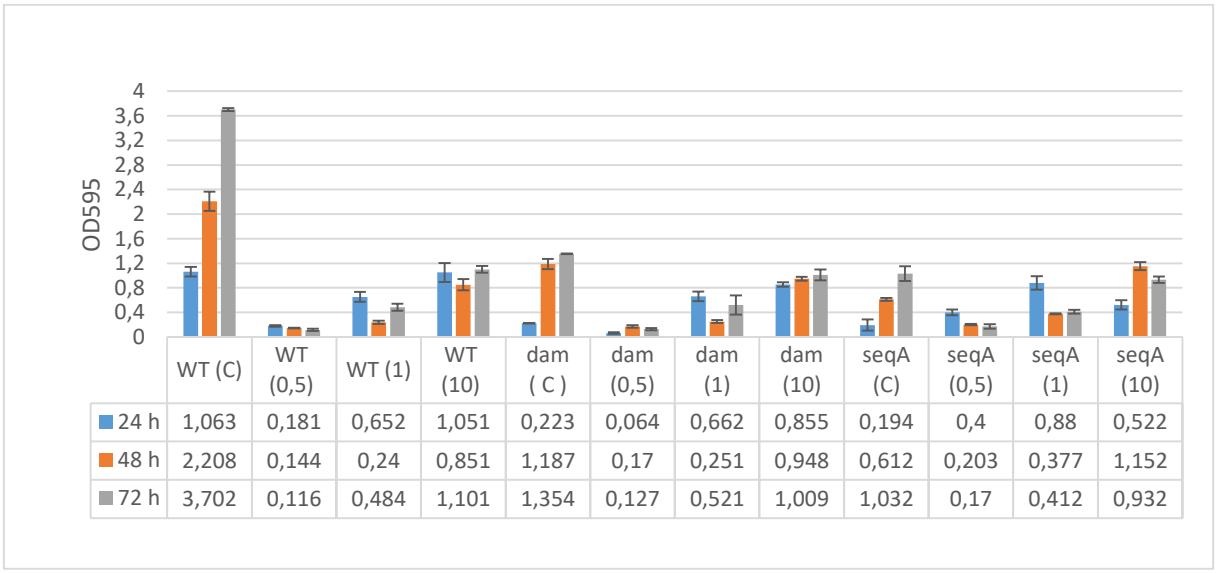


	WT (C)	WT (0,2)	WT (0,4)	WT (4)	dam (C)	dam (0,2)	dam (0,4)	dam (4)	seqA (C)	seqA (0,2)	seqA (0,4)	seqA (4)
24 h	1,063	0,171	0,132	0,065	0,223	0,046	0,028	0,02	0,194	0,064	0,045	0,028
48 h	2,208	0,803	0,421	0,163	1,187	0,489	0,44	0,205	0,612	0,203	0,196	0,187
72 h	3,702	0,577	0,384	0,056	1,354	0,328	0,303	0,038	1,032	0,044	0,034	0,034

Figure 4. Effect of proteinase K (0.2 mg/mL, 0.4 mg/mL, and 4 mg/mL) on biofilm formation in S. Typhimurium 14028 wild-type strain and its seqA and dam mutants.

CTAB

The most effective CTAB concentration was observed to be 0.5 mg/mL in all strains (Figure 5.).

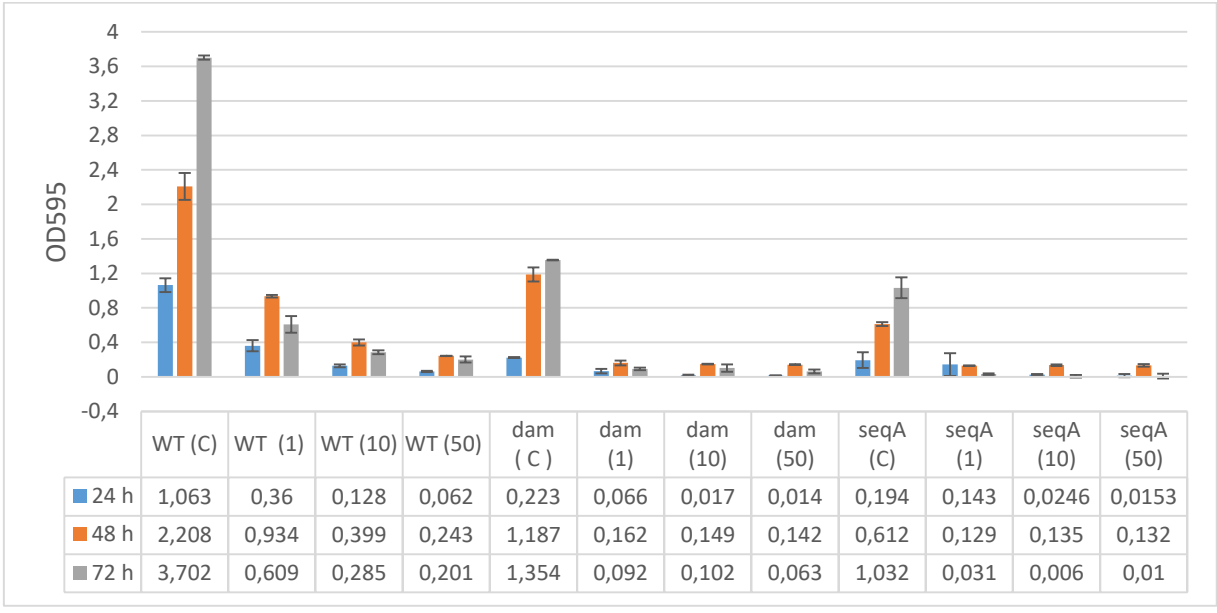


	WT (C)	WT (0,5)	WT (1)	WT (10)	dam (C)	dam (0,5)	dam (1)	dam (10)	seqA (C)	seqA (0,5)	seqA (1)	seqA (10)
24 h	1,063	0,181	0,652	1,051	0,223	0,064	0,662	0,855	0,194	0,4	0,88	0,522
48 h	2,208	0,144	0,24	0,851	1,187	0,17	0,251	0,948	0,612	0,203	0,377	1,152
72 h	3,702	0,116	0,484	1,101	1,354	0,127	0,521	1,009	1,032	0,17	0,412	0,932

Figure 5. Effect of CTAB (0.5 mg/mL, 1 mg/mL, and 10 mg/mL) on biofilm formation in *S.* Typhimurium 14028 wild-type strain and its *seqA* and *dam* mutants.

EDTA

While the most effective EDTA concentration was 50 mM in wild-type strain, this ratio was determined as 1 mM in *dam* and *seqA* mutants (Figure 6).

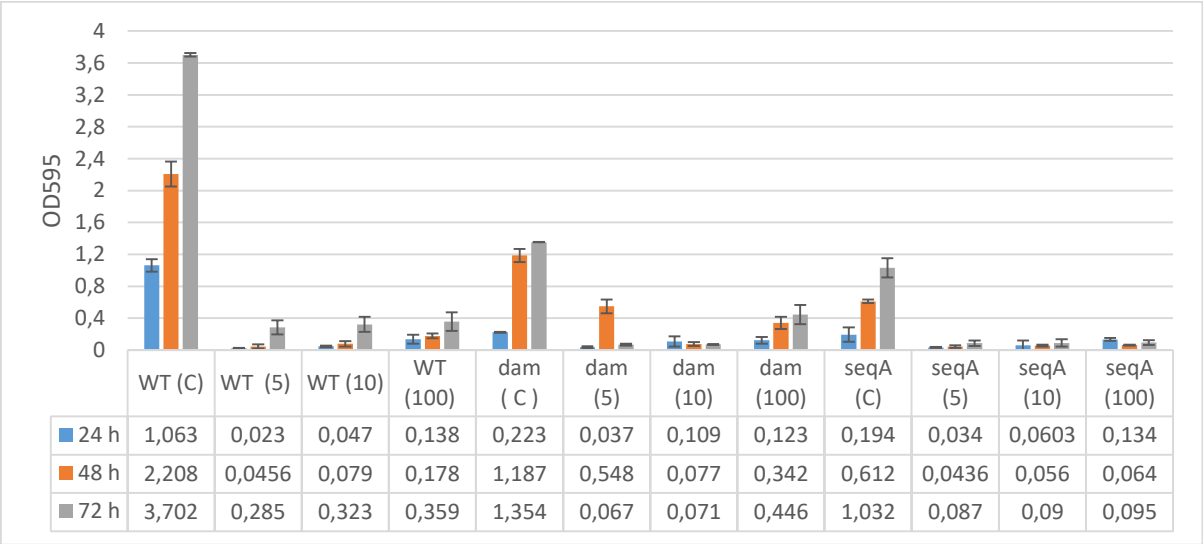


	WT (C)	WT (1)	WT (10)	WT (50)	dam (C)	dam (1)	dam (10)	dam (50)	seqA (C)	seqA (1)	seqA (10)	seqA (50)
24 h	1,063	0,36	0,128	0,062	0,223	0,066	0,017	0,014	0,194	0,143	0,0246	0,0153
48 h	2,208	0,934	0,399	0,243	1,187	0,162	0,149	0,142	0,612	0,129	0,135	0,132
72 h	3,702	0,609	0,285	0,201	1,354	0,092	0,102	0,063	1,032	0,031	0,006	0,01

Figure 6. Effect of EDTA (1mM, 10mM and 50mM) on biofilm formation in S. Typhimurium 14028 wild-type strain and its seqA and dam mutants

SDS

SDS showed the most effective antibiofilm activity in wild-type strain and *seqA* mutant at a concentration of 5mg/mL in dam mutant and 10mg/mL in *dam* mutant (Figure 7).

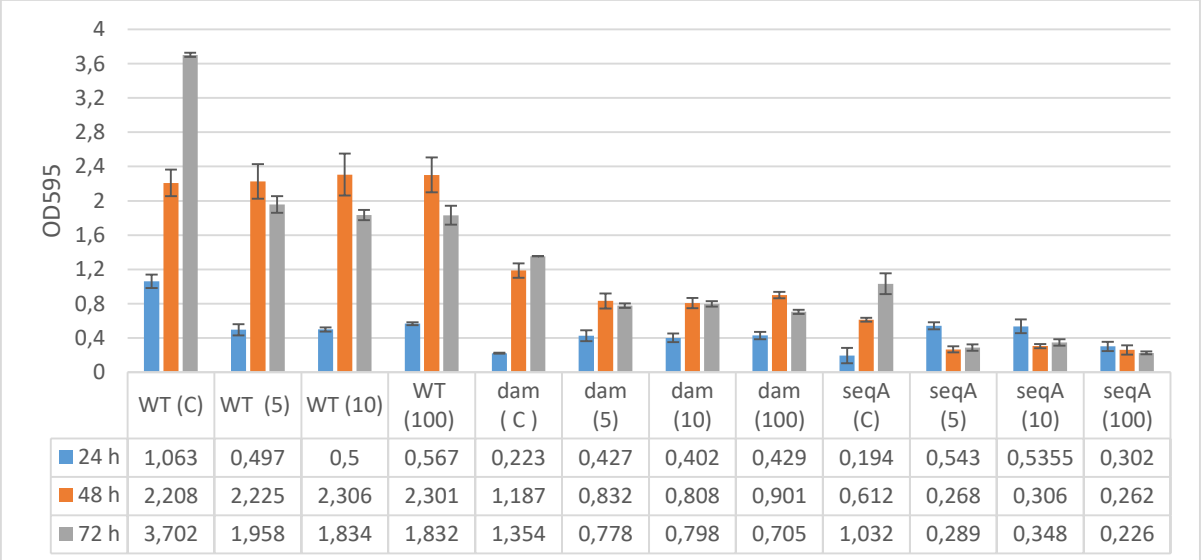


	WT (C)	WT (5)	WT (10)	WT (100)	dam (C)	dam (5)	dam (10)	dam (100)	seqA (C)	seqA (5)	seqA (10)	seqA (100)
24 h	1,063	0,023	0,047	0,138	0,223	0,037	0,109	0,123	0,194	0,034	0,0603	0,134
48 h	2,208	0,0456	0,079	0,178	1,187	0,548	0,077	0,342	0,612	0,0436	0,056	0,064
72 h	3,702	0,285	0,323	0,359	1,354	0,067	0,071	0,446	1,032	0,087	0,09	0,095

Figure 7. Effect of SDS on biofilm formation in 5 mg/ml (10 mg/mL and 100 mg/mL) *S. typhimurium* 14028 wild-type strain and its *seqA* and *dam* mutants

Tween-80

It was found to have maximum antibiofilm activity at a concentration of 5 mg/ml in the Tween 80 wild-type strain and *dam* mutant, and at a concentration of 100 mg/mL in the *seqA* mutant (Figure 8.).



	WT (C)	WT (5)	WT (10)	WT (100)	dam (C)	dam (5)	dam (10)	dam (100)	seqA (C)	seqA (5)	seqA (10)	seqA (100)
24 h	1,063	0,497	0,5	0,567	0,223	0,427	0,402	0,429	0,194	0,543	0,5355	0,302
48 h	2,208	2,225	2,306	2,301	1,187	0,832	0,808	0,901	0,612	0,268	0,306	0,262
72 h	3,702	1,958	1,834	1,832	1,354	0,778	0,798	0,705	1,032	0,289	0,348	0,226

Figure 8. Effect of Tween-80 (5 mg/mL, 10 mg/mL and 100 mg/mL) on biofilm formation in S. Typhimurium 14028 wild-type strain and its seqA and dam mutants.

Combination trials with nisin and other antimicrobial agents

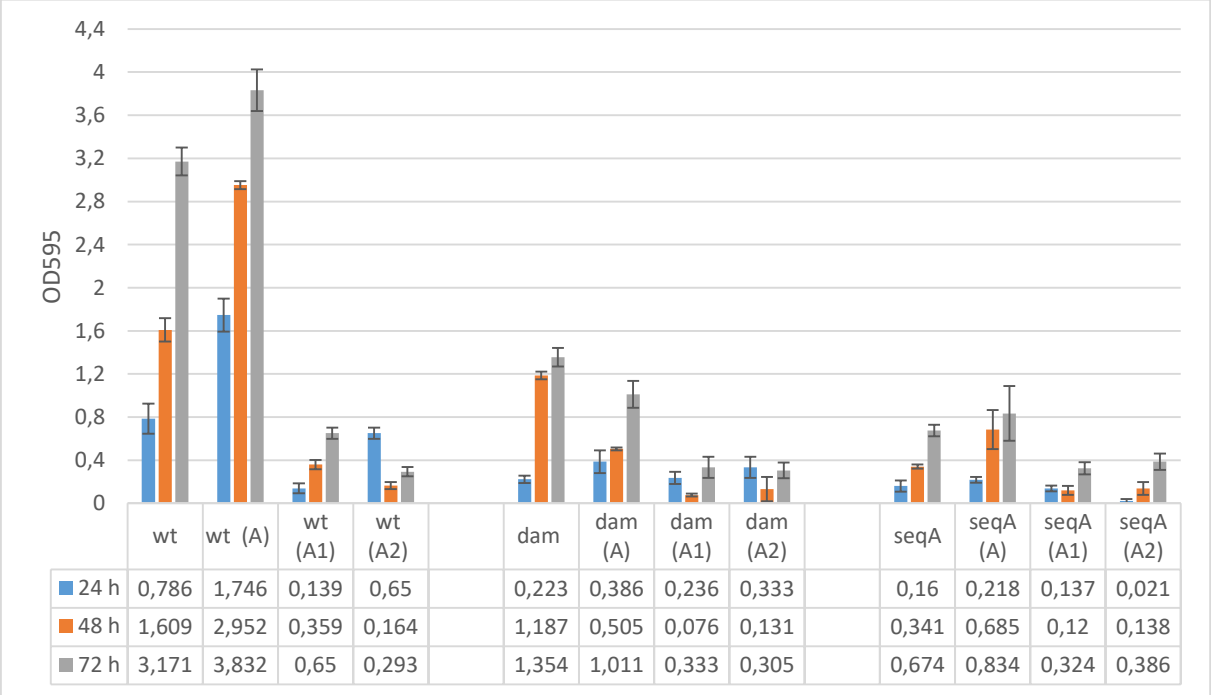
In order to increase the effectiveness of nisin bacteriocin, which is active in Gram-positive bacteria, on the biofilm structures produced by Gram-negative Salmonella strains, this

bacteriocin was used in combination with antimicrobial agents prepared at different concentrations (Table 3.).

Table 3. Antimicrobial agents used in combination trials with nisin and their concentrations

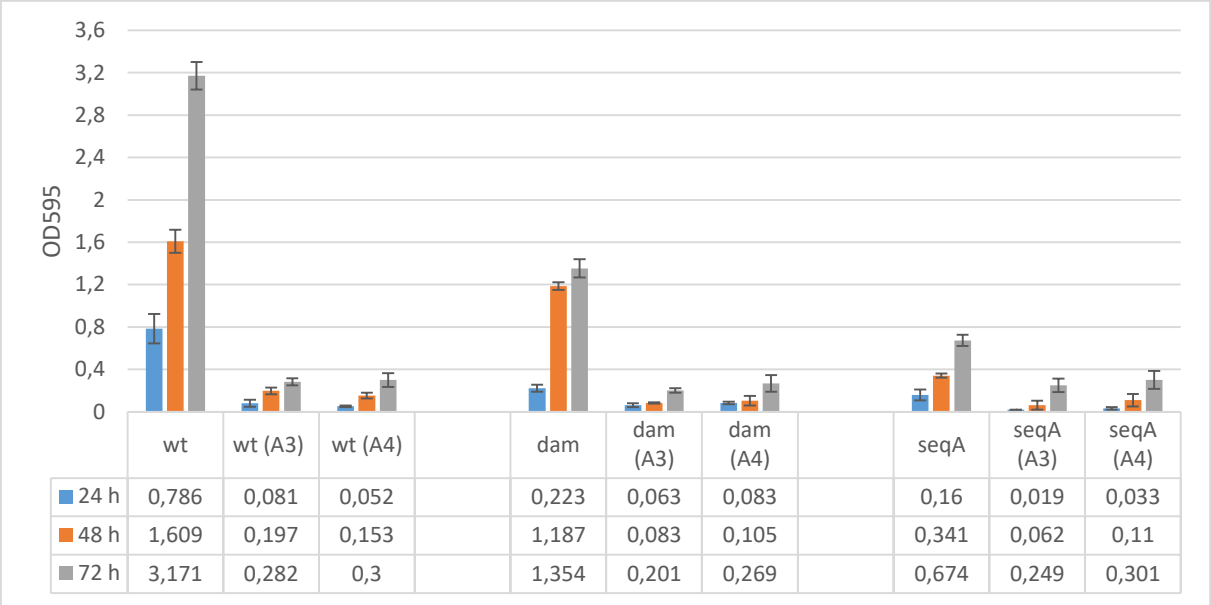
Nisin 10 mg/ml+ EDTA 1 mM	A
Nisin 10 mg/ml + EDTA 10 mM	A1
Nisin 10 mg/ml + EDTA 50 mM	A2
Nisin 1 mg/ml + EDTA 50 mM	A3
Nisin 5 mg/ml + EDTA 50 mM	A4
Nisin 10 mg/ml+ CIP 5 µg/mL	B
Nisin 1 mg/ml + EDTA 10 mM + CIP 5 µg/mL	C

All combinations tried showed effective antibiofilm activity. Among these combinations, the most effective statistically was determined as the B combination (Figure 10., 11. and 12.).



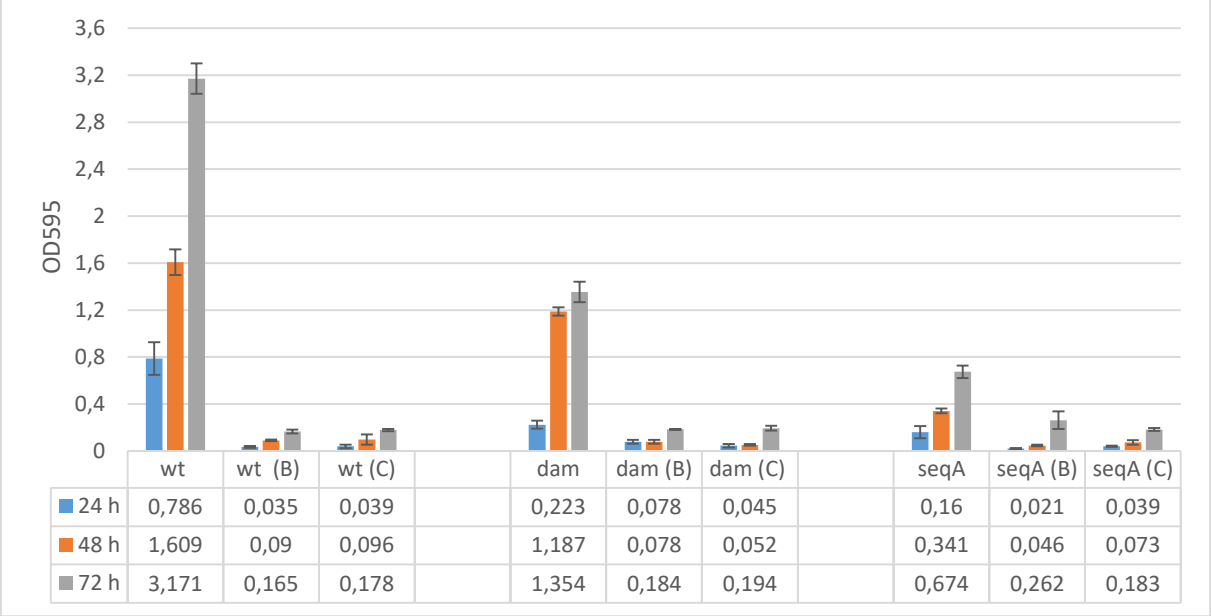
	wt	wt (A)	wt (A1)	wt (A2)	dam	dam (A)	dam (A1)	dam (A2)	seqA	seqA (A)	seqA (A1)	seqA (A2)
24 h	0,786	1,746	0,139	0,65	0,223	0,386	0,236	0,333	0,16	0,218	0,137	0,021
48 h	1,609	2,952	0,359	0,164	1,187	0,505	0,076	0,131	0,341	0,685	0,12	0,138
72 h	3,171	3,832	0,65	0,293	1,354	1,011	0,333	0,305	0,674	0,834	0,324	0,386

Figure 10. The effect of preparations prepared in A, A1 and A2 combinations (Table 3.) on biofilm formation of *S.* Typhimurium 14028 wild-type strain, *seqA* and *dam* mutants



	wt	wt (A3)	wt (A4)	dam	dam (A3)	dam (A4)	seqA	seqA (A3)	seqA (A4)
24 h	0,786	0,081	0,052	0,223	0,063	0,083	0,16	0,019	0,033
48 h	1,609	0,197	0,153	1,187	0,083	0,105	0,341	0,062	0,11
72 h	3,171	0,282	0,3	1,354	0,201	0,269	0,674	0,249	0,301

Figure 11. The effect of preparations prepared in A3 and A4 combinations (Table 3.) on biofilm formation of S. Typhimurium 14028 wild-type strain, *seqA* and *dam* mutants



	wt	wt (B)	wt (C)	dam	dam (B)	dam (C)	seqA	seqA (B)	seqA (C)
24 h	0,786	0,035	0,039	0,223	0,078	0,045	0,16	0,021	0,039
48 h	1,609	0,09	0,096	1,187	0,078	0,052	0,341	0,046	0,073
72 h	3,171	0,165	0,178	1,354	0,184	0,194	0,674	0,262	0,183

Figure 12. The effect of preparations prepared in combinations B and C (Table 3.) on biofilm formation of *S.* Typhimurium 14028 wild-type strain, *seqA* and *dam* mutants

DISCUSSION AND CONCLUSION

Identification and preparation of food-grade antibiofilm agents are of great importance for the food industry (Park et al. 2015, Wang et al. 2013). As a result of the use of 10 mg/mL nisin, maximum biofilm activity was reached at 72 hours in wild-type strain and its *dam* and *seqA* mutants. Ciprofloxacin, like nisin, showed its highest efficiency at the end of 72 hours. The ability to inhibit biofilm activity of up to 97% is a very high value. With the addition of alpha amylase, the inhibition efficiency of up to 70% in the wild-type strain and its *seqA* mutant at the end of 72 h showed the opposite situation in the *dam* mutant. In the samples added 5 mg/mL α-amylase, an increase of 176.3% was observed in the

amount of biofilm produced by the mutant in question. Proteinase K, like ciprofloxacin, showed a High antibiofilm activity at all trial times and at all concentrations tried in the *S.* Typhimurim wild-type strain and mutants. The activity in question reached its highest levels at the 72nd Hour. CTAB had a high antibiofilm activity in the wild strain at all concentrations tested, but failed to maintain this activity in mutants. Antibiofilm activities observed in mutants were lower than in wild type strains. An increase in the amount of biofilm was determined at some concentrations at 24 and 48 hours. EDTA showed High antibiofilm activity at all tested concentrations and all strains. Similar activities were also defined for SDS. Tween 80 showed antibiofilm activity at 72 hours in all tested strains, albeit lower than other antimicrobials. However, the effect was not stable at other incubation times and concentrations. Nisin 10 mg/ml + CIP 5 μg/mL mixture was determined as the most effective antibiofilm combination. These concentrations are the concentrations that do not cause any harmful effects in terms of environmental and consumer health. Because higher ratios of these concentrations are used in food preservation and treatment with these agents one by one (Shi and Zhu 2009, Wang et al. 2016). The combinations developed in this study show potential for effective inhibition of *Salmonella* biofilms.

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ECOLOGICAL SURVEY OF MOST IMPORTANT BIOCENOSIS OF ALGAE AND SEAGRASSES OF SAZANI ISLAND, ALBANIA

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ABSTRACT

Marine and coastal habitats of Sazani Island are characterized by important communities and species, including rare and endangered species at national and international levels. The present paper reveals the results of a benthic survey on most important biocenosis of algae and seagrasses carried out along the coasts of Sazani Island. The presence of three species of regional and international importance, namely associations with the alga *Lithophyllum byssoides*, the seagrasses *Posidonia oceanica* and *Zoostera noltei* has been considered of a high relevance. Cover has been evaluated for algae and seagrasses, while for the meadow of *Posidonia oceanica* the upper and lower depth limits have also been recorded, as well as shoot density. Findings of this study highlight the urgent need for conservation measures to protect the area, to implement medium and long-term monitoring and to improve its conservation state.

Key words: *Posidonia oceanica* meadows, MPA Karaburun-Sazan, Adriatic Sea.

INTRODUCTION

Marine Protected Area Karaburun - Sazan is located in Vlora region, southwestern Albania. The existing information on marine benthos of Sazani Island is very scarce and has mainly been provided under several rapid and sporadic assessments of coastal benthic communities of Vlora Bay, as well as within the framework of proclamation of the Marine Protected Area Karaburun - Sazan (after Kashta & Beqiraj 2010). The present study has been focused on the most important biocenosis of algae and seagrasses along the coasts of Sazani Island. Quantitative assessments have been done on populations of the red alga *Lithophyllum byssoides*, the seagrasses *Posidonia oceanica* and *Zoostera noltei*, as well as the assessment of their ecological and environmental state. These species are very important for marine ecosystems, especially as bio-constructors and habitat formers, as well as shelter, reproduction and nursery areas for a high diversity of marine organisms, especially for invertebrates and fish. The importance of this study and assessment of these species and habitats should be considered of a high relevance, because they play an important role for marine ecosystem and biodiversity, and they are also related to the definition of priorities for conserving marine ecosystems in general and especially marine and coastal protected areas.

MATERIAL AND METHODS

Sazani Island is 4.8 km long, 2 km wide and has a surface of 5.7 km^2. Its eastern coast, in general, is shallow and with an open coastline, while its western coast is steep, with high rocks, cracks, immediate depth from the coastline and fragmented by small bays. (Figure 1).
The benthic survey has been carried out in four sites along the rocky coast of Sazani Island during 2012 - 2014 (Figure 2).

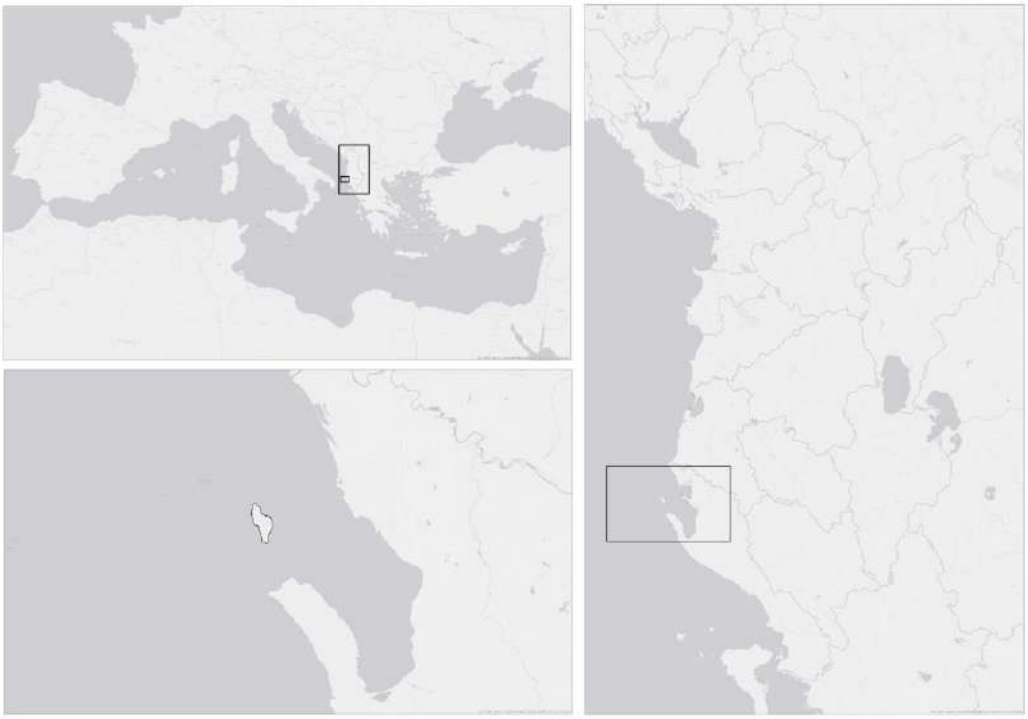

Figure 1. Map of Albania, highlighting the position of Sazani Island

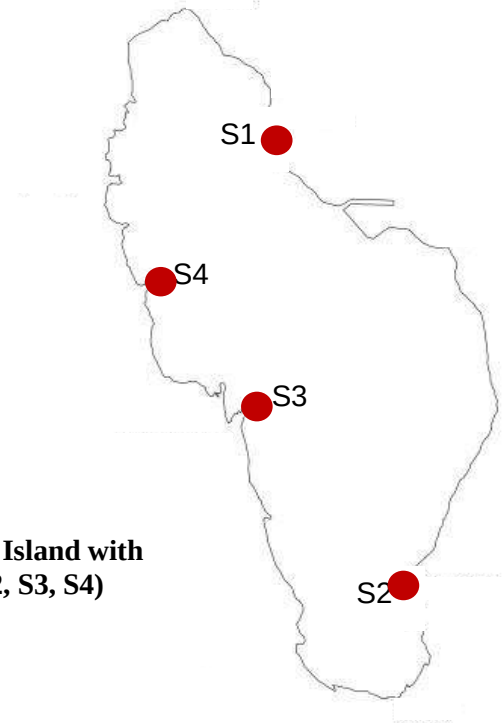

Figure 2. Map of Sazani Island with sampling sites (S1, S2, S3, S4)

The two sites on the western coast (S3 and S4) are highly exposed to the waves, facing the open sea, while the two sites on the eastern coast (S1 and S2) are more sheltered, facing the mainland. The survey was conducted by snorkeling and scuba diving, respectively in mid-littoral and infralittoral, focused on the most widespread biocenosis of algae and seagrasses, aiming to assess their ecological and environmental state.
The methods used for this survey and assessment were referring to Schlieper (1976), Cattaneo et al. (1978), Drago et al. (1980).
Cover (in percentage) has been evaluated for algae and seagrasses in all sampling sites. In the meadow of *Posidonia oceanica* the upper and lower depth limits have been recorded, as well as shoot density.
Species of special importance in international and regional scale have been defined by referring to some important international conventions on biodiversity conservation and some other more specific documents relevant for the Mediterranean Sea, such as the IUCN Red List, the SPAMI list, the Annex II of Barcelona Protocol, the Bern Convention and the Habitats Directive 92/43/ EEC of 21.5.1992.

RESULTS AND DISCUSSION

During this benthic survey three biocenosis of seaweed and seagrass of regional and international importance were analyzed and assessed. These species are the red alga *Lithophyllum byssoides* and the marine phanerogams *Posidonia oceanica* and *Zostera noltei.* These species have been considered as especially important, as they have been included in some of the most important international conventions, in the IUCN Red List, as well as in the SPAMI list.

The red alga *Lithophyllum byssoides* is a calcareous algae that is a good biological indicator of superficial pollution and fluctuant sea levels. It is a characteristic species of Western Mediterranean and Adriatic Sea that grows slightly above mean sea level, in small caves, corridors and along cliffs. In this area it has created small cushions (hemispheric concretions) and rarely builds rims, usually known as “trottoirs”. It creates shelter for a high number of macroinvertebrate and algae species, as well as for small and juvenile fishes. This alga has an almost regular distribution along the western coast of Sazani Island.

Figure 3. Sampling site 3 with the presence of *Lithophylum byssoides*

In this study it has been recorded with a cover of 50% in the sampling site 3 (figure 1), while it had a very small cover in the sampling site 4, and it was missing on the eastern coast.

Especially in the southern part of the island, a number of *L. byssoides* rims were in very poor condition. In contrast, in the northern part, the upper side of the rims showed in better conditions. (Blanfunè et al., 2016).

This irregular distribution and the absence of *L. byssoides* on the eastern part of the Island is a consequence of human activity and its negative impacts in this area. This is mainly related to uncontrolled and expanding urbanization in Vlora Bay during the three last decades, increased maritime traffic, uncontrolled and huge touristic pressure, as well as unproper environmental management of the studied marine and coastal area. Additional possible causes of regression of *L. byssoides* might be related to trampling, sea surface pollution and the world- wide rise in sea-level (Verlaque, 2010; Faivre et al., 2013; Thibaut et al., 2013).

In the present study *Posidonia* meadow has been recorded on the eastern coast of the Island only. Patches of *Posidonia* were present on the western coast, but on rocks in deeper water, as the western coast of Sazani is very steep, even reaching 30 m depth almost immediately below the coastline in some areas. On the eastern side the *Posidonia* meadow had a limited extension, with the upper limit of 4.5 m and the lower depth of 16 m. Its cover was relatively high, from 50% to 80%, while its average density varied from low to medium (from 150 shoots/m^2 to 520 shoots/m^2). Although this meadow seemed to be under degradation, it was still a good shelter for a high species number and high abundance of benthic macroinvertebrates. The seagrass *Posidonia oceanica*, an endemic species of the Mediterranean Sea, has been considered as a species and habitat, too, of special importance, and included in the lists of the Annex II of Barcelona Protocol, the Bern Convention and the Habitats Directive 92/43 / EEC of 21.5.1992. This species has also been included in the IUCN Red List, as a globally threatened species, with the status LC (least concern), as well as in the list of important habitats in the Mediterranean after SPAMI. The meadows of *P. oceanica* have been considered as the most important marine habitat in the Mediterranean, because of their multiple values for marine biodiversity and water quality.

Figure 4. The seagrass *Posidonia oceanica*

Zostera noltei is a species known by the common name dwarf eelgrass. This species creates a habitat with special importance, because of the main role in stabilizing sediments and reducing wave energy. Seagrass beds of *Zostera* are highly productive and form the basis of important coastal ecosystems. In this study *Zostera* has been recorded on the south-eastern part of Sazani Island, near the sampling site 1. It was missing on other sites of Sazani Island. In a general consideration, the reason of the absence of *Zostera* could be related to the sensitivity to eutrophication, direct human impact and reduce of water quality, as well as being affected by shading (Van Lent et al. 1991). Zostera has been shown to have potential as an indicator of estuarine and coastal system health. This species is listed in the Rio Declaration (http://www.unep.org/Documents.Multilingual/Default.asp?documentid=78&articleid=1163) as diverse habitats in need of protection and monitoring. Although *Zostera noltei* populations are declining slowly, the IUCN Red List of Threatened Species lists it as being of "least concern".

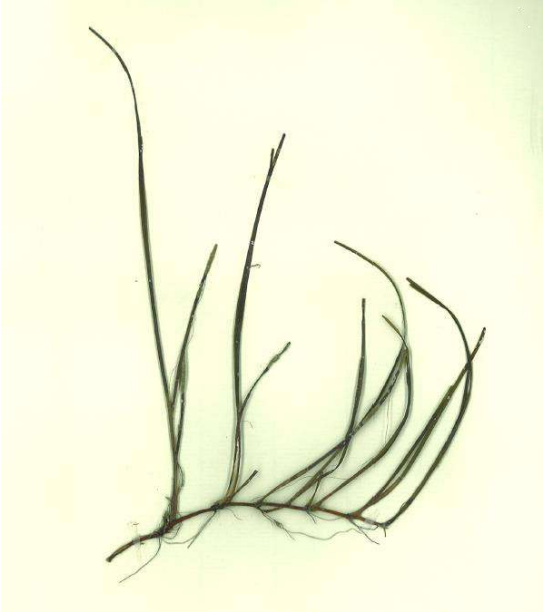

Figure 5. The dwarf eelgrass *Zostera noltei*

The presence of marine benthic habitats and biocenosis of national, international and regional importance on Sazani Island shows the importance of this area for marine biodiversity in the Adriatic scale and larger. This area is also important in a larger bio-geographic point of view, because of its position on the eastern side of the Otranto canal, as a transitional area between the Adriatic Sea and Ionian Sea and a migration corridor for the species expanding their distribution from the central Mediterranean towards north Mediterranean.

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MOLLUSCS FROM SHALLOW COASTAL HABITATS OF SAZANI ISLAND, ALBANIA

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ABSTRACT

The coastal and marine area of Sazani Island is part of the Marine National Park Karaburun-Sazan, proclaimed as such in 2010. Existing data on benthos and especially molluscs of this area is very limited, mainly based on sporadic assessments, or short surveys during other assessments that have been carried out for Vlora Bay. This paper gives data on species composition of molluscs' community and a general assessment of its quantitative characteristics, seasonal variations and stability in the studied area. A total of 24 molluscs' species were recorded, of which 21 are gastropods, 2 are bivalves and 1 polyplacophora. 9 species recorded in this study are in the existing Red List of Flora and Fauna of Albania, with different threat status. It's worthy to highlight the presence of the bivalve *Lithophaga lithophaga*, the date mussel, a species of international concern that is included in the lists of protected species of Bern Convention and CITES. Quantitative assessments revealed that seasonal variations were relatively high, in both species number and abundance. The presence of endangered species shows the importance of this area at national and regional level. Molluscs have important ecological roles, as well as economic importance in the region, but habitat degradation along with overexploitation may threaten natural resources in the Sazani Island marine and coastal area.

Key words: gastropods, bivalves, rocky coast, MPA Karaburun-Sazan, Adriatic Sea, Albania.

INTRODUCTION

This study has been carried out in shallow rocky coasts of the Sazani Island including two sampling sites, on the eastern part and two other ones on the western part of the Island. Molluscs of Sazani Island are very poorly known and studied. Most of few existing information is very general and has been provided under several rapid and sporadic assessments of coastal benthic communities of Vlora bay, as well as within the framework of declaration of the Marine Protected Area Sazan-Karaburun (after Kashta & Beqiraj 2009). The difference in species composition, quantitative characteristics and degree of stability of the molluscs' community is evident between eastern and western coast of the island. The environmental and ecological situation of these populations, especially on the eastern part of the island, better reflects the impact from human activities in the whole area.

Considering the limited knowledge of the animal biodiversity in the intertidal and shallow subtidal marine ecosystem along the Albanian coast, the main aim of this study was to make a preliminary assessment of the species composition, abundance and environmental state of the molluscs' population of these areas.

MATERIALS AND METHOD

Samples of benthic macroinvertebrates have been taken in four sampling sites, of which two on the western coast and two on the eastern coast of the island (Table 1).

Table 1. Site number, location and coordinates of sampling sites in Sazani Island.

Site number	Location	Coordinates
S1	East coast	N 40°30'25.17"; E 19°16'34.04"
S2	East coast	N 40°28'47.26"; E 19°17'12.01"
S3	West coast	N 40°29'26.80"; E 19°16'28.58"
S3	West coast	N 40°29'53.89"; E 19°16'2.50"

Three sampling transects, at a distance 50 m from each – other have been selected in each of the four sampling sites. Benthic samples have been taken during spring and early autumn in shallow water including the supralittoral, mediolittoral and upper limit of infralittoral.
The samples were taken through standard methods for benthic sampling in hard bottoms, within a frame 50 x 50 cm for the quantitative assessment, after the methods of Schlieper (1976), Cattaneo et al. (1978), Drago et al. (1980) and Zenetos et al. 2000 (Fig.1).
In each transect has been taken 6 frame samples of which 3 in supralittoral and 3 in medio and 3 in upper infralittoral. There have been taken 18 samples for each site and 72 samples in total. It has evaluated the species composition in each site, abundance of each species in each sample, average abundance of each species in each site.

Figure 1: The frame 50 x 50 cm used during sampling for the quantitative assessment

RESULTS

During the sampling period a total of 24 Mollusca' species have been recorded in the study area, representing the largest number of total species identified during the whole sampling period. Mollusca, is represented by 3 classes Gastropoda, Polyplachophora and Bivalvia, respectively: (Figure.2)

Figure 2: Some of Mollusca 'species recorded in the study area of Sazani Island

Gastropoda class with presence of 21 species part of Patellidae family (3 species), Trochida family (9), Littorinidae family (1), Columbellidae family (1), Buccinidae (2), Muricidae (1), Cerithiidae (2), Aplysiidae (1), Vermetidae (1), Polyplachophora class, represented by Chitonidae family (1 specie); Bivalvia class, represented by Mytilidae family (2 species).
The species are found in 4 sampling sites in general during spring season, but also some species part of Patellidae, Trochidae and Littorinidae family, are well represented even during autumn season. This can be explained by the ability of these species to withstand more water-level fluctuations on coastlines, and temporarily stay outside of the water compared to other species. It is worthy to note that 9 or 38% of the total number of mollusca' species recorded in Sazani Island have been included in the Red List of Flora and Fauna of Albania 2013 (stated by the Ministry of Environment of Albania) (Table 2).

Table 2: Mollusca' species recorded in Sazani Island have been included in the Red List of Flora and Fauna of Albania 2013

Taxa	Status
Gastropoda	
***Patella caerulea* Linnaeus, 1758**	VU A1c
***Patella rustica* Linnaeus, 1758**	VU A1c
***Gibbula adriatica* Philippi, 1844**	LR nt
***Phorcus articulates* (*Monodonta articulata)* Lamarck, 1822**	LR nt
***Osilinus (Monodonta) turbinatus* (Born, 1778)**	VU A2b
***Pollia dorbignyi (Cantharus dorignyi)* (Payraudeau, 1826)**	DD
***Stramonita haemastoma* (Linnaeus, 1767)**	VU D2
Bivalvia	
***Mytilus galloprovincialis* Lamarck, 1819**	VU A1c
***Lithophaga lithophaga* (Linnaeus, 1758)**	VU A1a

The date mussel Lithophaga lithophagha, (Fig. 3) one of the priority species for conservation in the Mediterranean, has become very rare along the Albanian coast, which is also the case of Sazani Island. In the present study the date mussel was found in one sampling site only, site nr 1. It was found in spring and autumn, with a relatively low abundance. The lowest species number was recorded in the sampling site IV in both seasons.

Figure 3: The date mussel *Lithophaga lithophaga*

It has been recorded a relatively low species diversity, comparing with other rocky coasts of the Adriatic Sea in Albania, especially with Vlora Bay (referring publications of Beqiraj & Kasemi 2006); Kasemi et al. (2008, 2012, 2013, 2015); Kasemi et al. (2012);

One reason for this low diversity might be related to the high exposure of the coast, especially on the western side of the island. Urban and tourist developments in Vlora Bay have continuously impacted the island and its benthic communities, especially on the eastern side of the island, also affecting the Karaburun – Sazan marine protected area.
Although the predominance of gastropods regarding species number has been higher related with other groups' species, their abundance has been low, showing in this way the degradation of environmental quality of Sazani coasts. The highest abundance for gastropods has been recorded in autumn. This might be related to lower competition between species (because of lower number of species in this season). In both seasons it has been recorded the predominance of patellid and trochid gastropods.

The seasonal variations were relatively high in both species number and abundance. The difference in species composition, quantitative characteristics and degree of stability of the Mollusca population is evident between eastern and western coast of the island.

CONCLUSIONS

24 Mollusca' species have been recorded in the study area of Sazani Island, with a high dominance of gastropods.

Abundance was low for most of the recorded species. Although predominating, the gastropods had a low density.

The highest seasonal difference in species number has been recorded for the gastropods and bivalves.

9 species of mollusca recorded in the studied area are endangered on a national scale.

The low species richness and low abundance seems to be mainly related to the high exposure of the coast, especially on the western side of the island, as well as the impacts from urban and tourist developments in Vlora Bay.

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ANNEX I

List of the Mollusca 'species recorded for each sampling site

TAXA	I	II	III	IV
Mollusca				
Gastropoda				
Patellidae				
Patella caerulea Linnaeus, 1758	*	*	*	*
Patella rustica Linnaeus, 1758	*	*		
Patella rustica (Patella lusitanica) Linnaeus, 1758				
Trochidae				
Gibbula umbilicaris (Linnaeus, 1758)		*		
Gibbula adriatica Philippi, 1844		*		
Gibbula rarilineata (Michaud, 1829)	*			
Gibbula adansonii (Payraudeau, 1826)		*		
Gibbula turbinoides (Deshayes, 1835)	*			
Gibbula sp. Risso, 1826		*		
Phorcus articulates (*Monodonta articulata)* Lamarck, 1822	*	*	*	
Phorcus richardi (Gibbula richardi) (Payraudeau, 1826)	*			
Osilinus (Monodonta) turbinatus (Born, 1778)	*	*	*	*
Littorinidae				
Melarhaphe neritoides(Littorina neritoides) Linnaeus, 1758	*	*	*	*
Columbellidae				
Columbella rustica (Linnaeus, 1758)	*		*	
Buccinidae				
Pisania striata (*Pisania maculosa*) Gmelin, 1791	*	*	*	
Pollia dorbignyi (Cantharus dorignyi) (Payraudeau, 1826)	*			
Muricidae				
Stramonita haemastoma (Linnaeus, 1767)		*		
Cerithiidae				
Cerithium vulgatum Bruguière, 1792	*			
Bittium reticulatum (da Costa, 1778)	*			
Aplysiidae				
Aplysia fasciata Poiret, 1789	*			
Vermetidae				
Vermetus.sp. Daudin, 1800	*	*		
Polyplacophora				
Chitonidae				
Chiton (Rhyssoplax) olivaceus Spengler, 1797		*		
Bivalvia				
Mytilidae				
Mytilus galloprovincialis Lamarck, 1819	*		*	*
Lithophaga lithophaga (Linnaeus, 1758)	*			

DETERMINATION OF ENTERIC METHANE EMISSIONS AMOUNT FROM SHEEP PRODUCTION BETWEEN 2004-2020 IN TURKEY

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ABSTRACT

Animal production is one of the most important sources of methane (CH_4) emissions in agriculture. Especially, ruminant livestock animals account for the majority of global anthropogenic methane emissions. The main sources of methane emissions from livestock animals are microbial fermentation (80%) and manure (20%). Methane is a serious greenhouse gas with 25 times the effect of carbon dioxide (CO_2). Therefore, its impact on global warming cannot be ignored. Sheep production is common in Turkey as it requires less capital and investment, creates labour capital in the region where it is made, adapts quickly to climate conditions and is a preferred source of animal protein. According to the Turkish Statistical Institute (TUIK) data, the sheep population has increased by 66% in the last 17 years. In this study, enteric methane emissions from sheep production in Turkey in the last seventeen years were determined by the Tier-2 method determined by the Intergovernmental Panel on Climate Change (IPCC). Methane emission factor (EF) was determined on average 11,6 kg CH_4/head/year, and the gross energy was determined 33,3 MJ/day. Accordingly, enteric methane emissions from sheep breeding have increased by 72,9% since 2004, and it has been calculated that 517 kilotonnes of CH_4 emissions were realized in 2020. It is predicted that the increase in emissions will continue depending on the increase in sheep numbers for the following years. For this reason, effective precaution should be taken on a farms basis or nationwide to reduce enteric methane emissions. Scientists need to focus more on this issue.

Keywords: Enteric fermentation, Methane, Sheep, Tier-2, Turkey.

INTRODUCTION

Livestock production has important in agricultural activities. Small ruminant production provides cultural and ecological benefits and multi-functionality with products such as meat, milk, wool, hair, leather, etc. (Semerci and Celik, 2016). Because there is constant living activity (manure generation, rumination, respiration, etc.) in animal production, gaseous emissions into the atmosphere are constantly occurring. Especially cattle and small ruminants that ruminant stomach are a severe source of methane gas. The main sources of greenhouse gas emissions from sheep and goat farming are methane (CH_4), which is formed due to manure decompose and enteric fermentation. (Opio et al., 2013). Methane emissions are produced by anaerobic fermentation of manure and enteric fermentation by bacteria called methanogens (McDonald et al., 1995; Bell et al., 2012). Ruminant animals that produce red meat release more methane emissions than monogastric animals that produce white meat. Therefore, red meat production is more significant than white meat production in terms of methane gas (Jones et al., 2014). Higher the enteric methane emissions as the lower the digestibility of feed in animals (Yaylı and Kılıc, 2020). Methane gas (CH_4) has a greater capacity to absorb heat by 25 times the effect of carbon dioxide gas. Therefore, excessive methane emission of methane gas into

the atmosphere and its ability to retain heat is a dangerous greenhouse gas that significantly impacts global warming.

The Intergovernmental Panel on Climate Change has published a guide on estimating and calculating emissions from livestock. From a general estimate to a modelling approach at the national level, Tier 1-2-3 methods were developed. (IPCC, 2006)

The current paper is aimed to determine the amount of enteric methane (CH_4) emissions from sheep farming using the Tier-2 method between 2004-2020 in Turkey.

MATERIAL AND METHODS

This study aimed to determine the amount of CH_4 gas emissions resulting from enteric fermentation caused by sheep breeding in Turkey between 2004 and 2020. The amount of methane emissions resulting from enteric fermentation was calculated by the Tier-2 method developed by the Intergovernmental Panel on Climate Change.

Although the sheep population has fluctuated in some periods since 2004, it has generally increased (Table 1). This increase is expected to continue in the coming years.

Table 1. Sheep number in Turkey between 2004-2020 year (head) (TUIK, 2021)

Year	Sheep (lamb to 1 year)	Sheep (older than 1 year)
2004	7 588 483	17 612 672
2005	7 434 032	17 870 293
2006	7 581 104	18 035 808
2007	7 937 711	17 524 582
2008	7 165 557	16 809 034
2009	6 236 192	15 513 316
2010	5 912 642	17 177 049
2011	6 473 761	18 557 804
2012	6 670 707	20 754 526
2013	6 762 870	22 521 377
2014	7 622 468	23 517 776
2015	7 190 059	24 317 875
2016	7 002 468	23 981 465
2017	6 192 426	27 485 210
2018	6 602 810	28 592 162
2019	7 230 214	30 045 836
2020	8 607 924	33 232 161

The energy equations required for enteric fermentation and activities used for this study are given in Table 2 as Equation-I-II-III. In the study, the sheep population's situation is confined in the barn was taken into account. Data such as weight averages (birth weights, weaning weights, slaughter weights), annual wool production, number of animals for animal categories were obtained from case studies or related statistical databases.

Table 2. Tier-2 equations about CH_4 emission derived from enteric fermentation

$GE = \{[(NE_m + NE_a + NE_l + NE_{work} + NE_p) / REM] + [(NE_g + NE_{wool}) / REG]\} / DE$ (I)
GE = gross energy (MJ / day) NE_m = net energy required by the animal for maintenance (MJ/day) NE_a = net energy for animal activity (MJ/day) NE_l = net energy for lactation (MJ/day) NE_{work} = net energy for work (MJ/day) NE_p = net energy required for pregnancy (MJ/day) NE_g = net energy needed for growth (MJ/day) NE_{wool} = net energy required to produce wool, (MJ/day) REM = ratio of net energy available in a diet for maintenance to digestible energy REG = ratio of net energy available for growth in a diet to digestible energy consumed DE = digestibility of feed expressed as a fraction of gross energy (digestible energy/gross energy)
$EF=[GE*(Ym/100)]*365/55.65$ (II) EF = emission factor for entetric fermantation (kg CH_4/head/yr) GE = gross energy intake (MJ/day) Y_m = methane conversion factor, per cent of gross energy in feed converted to methane The factor 55.65 (MJ/kg CH_4) is the energy content of methane
Emissions = Number of animals *EF (III) Emissions=methane emissions from enteric fermantation (kg CH_4/yr)

RESULTS AND DISCUSSION

In this study, daily required gross energy (GE) amounts and enteric methane emission factors (EF) were calculated according to sheep's daily performance and feeding status. The daily maintenance energy (Ne_m) is required to keep the animal in equilibrium per sheep (Jurgen, 1988), activity energy (NE_a) needed to reach water, feed, and barn, growth energy required for weight gain (NE_g)(NRC, 1996), the energy required for lactation (NE_l) (AFRC, 1990), the average daily net energy (NE_{wool}) required for one-year wool production and the energy required for pregnancy (NE_p) are given in Table 3. The average daily energy needs of one-year-old and younger sheep is 34,3 MJ/day, and the daily energy requirement for sheep older than one year is 32,6 MJ/day.

Table 3. Daily gross energy for sheep in this present study (MJ/day)

Metabolic Functions	MJ/day
Net energy required by the animal for maintenance (Ne_m)	4,9
Net energy for animal activity (NE_a)	0,402
Net energy needed for growth (NE_g)	0,07
Net energy for lactation (NE_l)	1,17
Net energy required to produce wool (NE_{wool})	0,132
Net energy required for pregnancy ($NE_{pregnancy}$)	0,38
Gross energy (GE)	33,3

The enteric methane emission factor was developed for the produced CH_4 emission per animal for a year. This study calculated that 10,1 kg of CH_4 gas emissions per year per sheep is less than 1-year-old, while 13,2 kg of enteric CH_4 per year for sheep older than one-year-old. It has been monitored that enteric methane emissions produce fewer methane emissions than older sheep, as young sheep use the energy they receive from the feed for their development. Since the metabolic activities of older sheep are slow and they have completed their development, they give the energy that they can not use from the feed as more density methane emission to the atmosphere. Table 4 shows the enteric methane emissions generated by Turkey in the last seventeen years according to sheep categories. Even if it has followed a fluctuating course from time to time in the last twenty years, it is generally seen that it has an increasing trend.

Table 4. Enteric emission amount of sheep between 2004 and 2020 in Turkey (tonne CH_4/year)

	Sheep (lamb to 1 year)	**Sheep (older than 1 year)**
2004	76 783	222 220
2005	75 220	225 541
2006	76 709	227 708
2007	80 317	221 470
2008	72 504	212 456
2009	63 100	198 004
2010	59 826	219 224
2011	65 504	237 468
2012	67 497	266 273
2013	68 429	289 798
2014	77 127	301 624
2015	72 752	312 927
2016	70 854	308 455
2017	62 657	353 558
2018	66 810	370 437
2019	73 158	389 843
2020	87 098	429 994

In Turkey, 517 kilotons of enteric methane were released into the atmosphere from approximately 42 million sheep number in 2020 (Figure 1). Calculations could not be made as TUIK has not yet published the sheep population for 2021. However, as the number of sheep is predicted to increase in the following years, enteric methane emissions are also expected to increase.

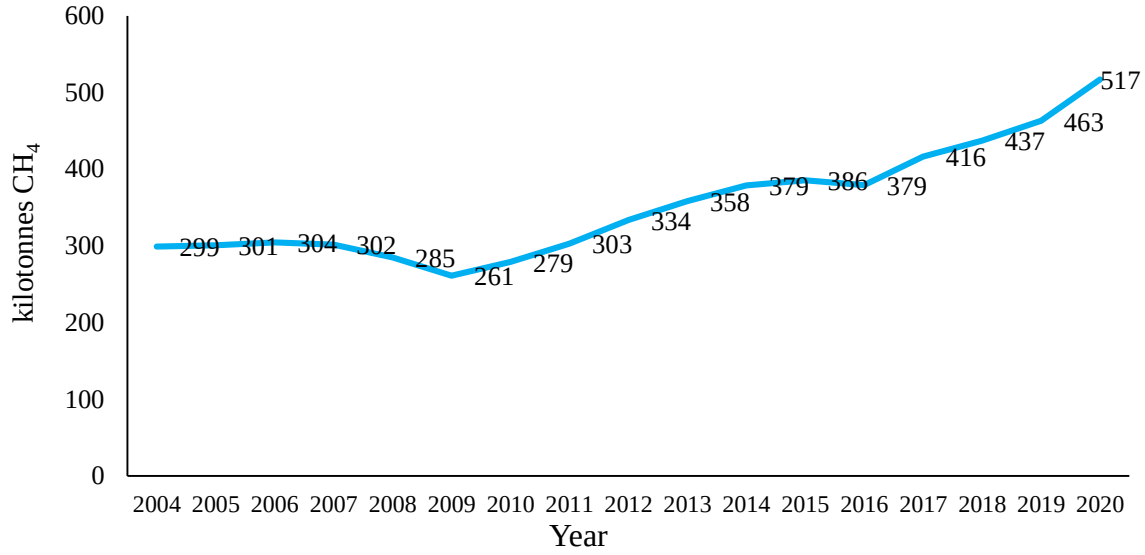

Figure 1. Total enteric methane emissions from sheep production between 2004-2020 in Turkey (kilotonnes/year)

CONCLUSIONS

The increase in the number of animals in livestock breeding provides with it an increase in methane emission rates. Mainly, CH_4 emissions resulting from enteric fermentation of ruminant animals cause yield loss, and this causes economic loss. Changes to be made in the contents of feed rations are an influential factor in the formation of CH_4 emissions. It is stated that the enteric methane formation of the fats used as an energy source in the rations of ruminant animals is reduced (Dohme et al., 2000; McGinn et al., 2004; Arslan ve Celebi, 2017). In addition, it is using concentrate feed instead of roughage hold down enteric CH_4 formation in animals. Reducing the number of animals and increasing productivity by breeding productive breeds with low methane production is another reduction strategy that can reduce methane formation (Naqvi and Sejian, 2011).

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POLLUTION LOADS OF MANURE FROM LIVESTOCK PRODUCTION IN BURSA REGION

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ABSTRACT

One of the serious problems in livestock farms is manure. It hasn't correct manure management, control and storage, manure causes environmental problems such as global warming, acidification and eutrophication. It is important to determine the pollution potential of manure for livestock operations, region and national wide, monitoring them and determining their effect. This study aimed to determine the potential of manure-related pollution caused by livestock in Bursa. For 2019, pollution occurred from poultry (laying hens and broiler) 395027,2 tons/year, sheep and goat 276312,5 tons/year and cattle (dairy cattle, beef and calves) 3200018,5 tons/year. For 2020,pollution potential was calculated 407475,4 tons/year pollution from poultry (laying hens and broiler), 278142,3 tons/year from sheep and goat and 3088401,1 tons/year from cattle (dairy cattle, beef cattle and calves). Using the relevant coefficients, the total pollutant dispersed loads from farm animals were obtained as 78210,9, 22098,2 and 3236,1 tons/ton live animal weight for BOD, TN and TP for 2020, respectively. For 2019, it was calculated as 80135,1, 22653 and 3337,5 ton/ton live animal weight for BOD, TN and TP, respectively. Since the pollution from livestock operation is not a point source pollutant, its harmful effects are quite large. In this study, attention is paid to the pollution dimensions, and the damages it can cause and the precautions that can be taken are emphasized.

Keywords: Global warming, Livestock, Manure, Non-point source pollution

INTRODUCTION

Livestock production is essential in terms of supply people's food and protein needs. The amount of pollution that arises with the increase in the need for consumption due to the population and the increase in the production in livestock farms is also increasing. The biggest problem is manure in animal production. If there is no systematic manure storage, operation or application order in the farm, manure causes serious risks for the environment.

Livestock farms cause non-point source pollution contrary to industrial and urban pollution sources (Karaman, 2005). Therefore, it is complicated to determine, monitor and control the extent of pollution. Particularly, the leaching of animal wastes to the surface and underground waters, water leaking from agricultural areas and lands where manure is stored are the main sources of water pollution (Can, 2021). Animal manures are rich in terms of content because they contain nutrients such as nitrogen (N), phosphorus (P), potassium (K) and sulphur (S).

In the study, it was aimed to determine the pollution dimension by calculating the amount of manure from livestock production in Bursa region in 2019 and 2020.

MATERIALS AND METHODS

Bursa, where the study was conducted, has seventeen districts. The population of livestock (broiler, layer hen, sheep, goat, calves, dairy and beef) for 2019 and 2020 by districts was obtained from the Turkish Statistical Institution (TUIK, 2021)(Table 1).

Table 1. Livestock numbers in Bursa region between 2019-2020

	2020						
	Broyler	**Layer hen**	**Sheep**	**Goat**	**Calves**	**Dairy**	**Beef**
Büyükorhan	0	7 122	14 150	8 980	502	3 415	502
Gemlik	0	51 161	5 897	3 847	771	1 462	771
Gürsu	0	355	6 081	2 802	1 124	1 037	1 124
Harmancık	0	1 436	8 808	1 514	662	1 216	662
Karacabey	2 031 120	3 504 567	102 257	8 271	4 732	24 116	4 732
Keles	0	12 717	20 244	3 150	556	2 143	556
Kestel	0	3 469	16 975	2 977	1 390	2 265	1 390
Mudanya	122 356	1 080 410	13 005	1 110	767	3 710	767
Mustafakemalpaşa	1 411 855	230 998	74 475	4 865	7 269	26 528	7 269
Nilüfer	15 475	76 630	32 006	842	7 121	7 933	7 121
Orhaneli	0	73 385	26 260	8 170	2 663	6 632	2 663
Orhangazi	78 000	94 929	14 023	5 350	7 221	5 893	7 221
Osmangazi	0	73 800	25 963	4 078	1 022	3 597	1 022
Yenişehir	1 444 973	406 455	67 890	8 725	12 842	25 360	12 842
Yıldırım	0	6 133	1 298	96	560	1 093	560
İnegöl	651 866	24 599	61 490	4 867	2 383	10 559	2 383
İznik	329 650	1 538	13 645	13 134	596	1 628	596
TOPLAM	**6 085 295**	**5 649 704**	**504 467**	**82 778**	**52 181**	**128 587**	**52 181**
	2019						
	Broyler	**Layer hen**	**Sheep**	**Goat**	**Calves**	**Dairy**	**Beef**
Büyükorhan	0	7 552	19 828	12 350	2 123	6 103	1 505
Gemlik	0	68 235	5 868	2 586	445	1 284	405
Gürsu	0	289	5 423	4 214	293	824	969
Harmancık	0	1 529	8 905	2 043	520	1 257	748
Karacabey	667 289	4 399 281	104 124	8 795	14 891	30 103	4 781
Keles	0	19 471	19 416	4 030	1 075	2 186	728
Kestel	0	3 369	15 374	1 339	1 390	2 347	1 275
Mudanya	0	1 367 527	12 295	1 890	1 390	3 812	712
Mustafakemalpaşa	955 471	310 538	74 330	7 360	11 001	28 954	8 190
Nilüfer	64 165	166 165	29 948	1 755	2515	5 846	3 511
Orhaneli	0	90 000	25 520	8 435	3 175	6 317	2 805
Orhangazi	0	134 127	9 841	3 351	1 169	5 281	3 735

Osmangazi	0	104 250	21 617	3 501	1 094	3 138	976
Yenişehir	422 845	916 784	69 241	8 850	11 553	24 250	12 724
Yıldırım	0	4 768	1 240	120	305	1 054	527
İnegöl	119 523	224 616	58 706	5 094	4 844	11 012	2 671
İznik	133 183	7 339	12 918	9 218	801	1 785	472
TOPLAM	**2 362 476**	**7 825 840**	**494 594**	**84 931**	**58 584**	**135 553**	**46 734**

The average manure amount produced by livestock per day was obtained using ASABE standards' values. Sheep, goat, laying hen, broiler, dairy, beef and calves produce 1.08, 2.62, 0.12, 0.08, 55.04, 20.88 and 5.64 kg/head/day respectively (ASAE, 2003).

Manure amounts of the Bursa region were calculated that considered these values. While calculating the non-point pollution load size from livestock, pollution coefficients for biochemical oxygen demand (BOD), total nitrogen (TN) and total phosphorus (TP) related to the relevant pollutants from the literature were used (Table 2) (Andreadakis et al., 2007). The coefficients are used to represent the daily production rates of animal waste according to the weight of the animals. Livestock weights were calculated based on the standards published by ASABE. Body weight values considered are 27, 64, 1.8, 0.9, 640, 360, and 91 kg for sheep, goats, laying hens, broiler, dairy, beef and calves, respectively (ASAE, 2003).

Table 2. Pollution coefficients by livestock (kg/ton live weight/day)

kg/ton live weight/day	BOD	TN	TP
Sheep	1.67	0.41	0.007
Goat	1.67	0.41	0.007
Poultry	1.53	0.33	0.22
Cows	1.5	0.45	0.05
BOD: Biochemical oxygen demand, TN: total nitrogen, TP: total phosphorus			

RESULTS AND DISCUSSIONS

In the Bursa region, there have been changes in the amount of manure in livestock production in 2020 compared to the previous year, depending on the number of livestock (Table 3 and Table 4). While the number of broiler, sheep and beef increased, the amount of manure also increased; in laying hens, goats, calves, and dairy, the amount of manure decreased with a decrease in the number of animals.

Table 3. Livestock manure amounts of Bursa region in 2020 (kg/day)

	2020						
	Broiler	Layer hen	Sheep	Goat	Calves	Dairy	Beef
Büyükorhan	0	820	15282	23564	2832	187962	10482
Gemlik	0	5894	6369	10095	4350	80468	16098
Gürsu	0	41	6567	7352	6342	57076	23469
Harmancık	0	165	9513	3973	3735	66929	13823
Karacabey	155381	403726	110438	21703	26698	1327345	98804
Keles	0	1465	21864	8266	3137	117951	11609
Kestel	0	400	18333	7812	7842	124666	29023
Mudanya	9360	124463	14045	2913	4327	204198	16015
Mustafakemal paşa	108007	26611	80433	12766	41012	1460101	151777
Nilüfer	1184	8828	34566	2209	40177	436632	148686
Orhaneli	0	8454	28361	21438	15025	365025	55603
Orhangazi	5967	10936	15145	14038	40741	324351	150774
Osmangazi	0	8502	28040	10701	5766	197979	21339
Yenişehir	110540	46824	73321	22894	72455	1395814	268141
Yıldırım	0	707	1402	252	3160	60159	11693
İnegöl	49868	2834	66409	12771	13445	581167	49757
İznik	25218	177	14737	34464	3363	89605	12444

Table 4. Livestock manure production for 2019 (kg/day)

	2019						
	Broiler	Layer hen	Sheep	Goat	Calves	Dairy	Beef
Büyükorhan	0	870	21414	32406	11978	335909	31424
Gemlik	0	7861	6337	6786	2511	70671	8456
Gürsu	0	33	5857	11058	1653	45353	20233
Harmancık	0	176	9617	5361	2934	69185	15618
Karacabey	51048	506797	112454	23078	84015	1656869	99827
Keles	0	2243	20969	10575	6065	120317	15201
Kestel	0	388	16604	3514	7842	129179	26622
Mudanya	0	157539	13279	4959	7842	209812	14867
Mustafakemalpaşa	73094	35774	80276	19313	62068	1593628	171007
Nilüfer	4909	19142	32344	4605	14190	321764	73310
Orhaneli	0	10368	27562	22133	17913	347688	58568
Orhangazi	0	15451	10628	8793	6595	290666	77987
Osmangazi	0	12010	23346	9187	6172	172716	20379
Yenişehir	32348	105614	74780	23222	65182	1334720	265677
Yıldırım	0	549	1339	315	1721	58012	11004
İnegöl	9144	25876	63402	13367	27330	606100	55770
İznik	10188	845	13951	24188	4519	98246	9855

For 2019, 3871358 tons/year of manure was calculated from 11 008 712 animals, and 3774019 tons/year from a total of 12 555 193 animals in 2020 in Bursa region. Since the amount of manure produced per animal is high, the largest production share belongs to cattle (Figure 1).

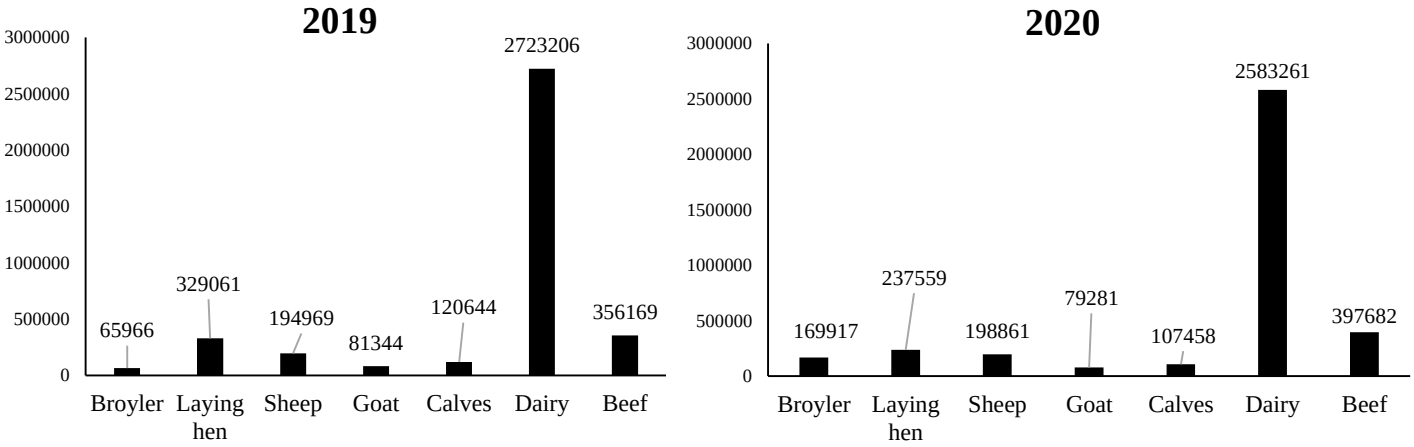

Figure 1. 2019-2020 Bursa region livestock manure production amount (tons/year)

Pollutant loads from manure for laying hens, broilers, sheep, goats, calves, dairy and beef using the related pollution coefficients are given in Table 5. For 2019, total pollutant loads originating from livestock are 80135, 22653 and 3337.5 tons/ton bodyweight.year for BOD, TN, TP, respectively. For 2020, it is 78211, 22098 and 3236 tons/ton bodyweight.year for BOD, TN, TP, respectively.

Table 5. Pollutant load from animal manure in Bursa region 2019-2020 (ton/ton live weight/year)

2019			
	BOD	**TN**	**TP**
Poultry	9054	1953	1302
Cattle	59628	17888	1988
Ovine	11453	2811	48
2020			
	BOD	**TN**	**TP**
Poultry	8738	1885	1256
Cattle	57942	17383	1931
Ovine	11531	2831	48

CONCLUSIONS

Manure from livestock production causes major environmental problems if not managed properly. Monitoring and control of diffuse pollution can be more difficult when pollutants in manure leak into soil and water. Today, the increasing severity of global warming and the rapidly decreasing water resources require more serious and comprehensive measures to be taken in this regard. In farm, it is necessary to store manure in storage and closed area and the warehouse floor must be well insulated. Biogas production and composting are important to reduce the environmental impact of wastes. In addition, in order to prevent the leakage of wastes into surface and underground waters, livestock farms should be at least 500 m away from urban areas, at least 300 m from lakes and water sources, at least 100 m from irrigation and drainage channels, and at least 30 m from sanitary installations that provide water (Parlakay et al., 2015). Scientists also need to focus on this issue in more detail.

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USING OF HAWTHORN AS ROOTSTOCK IN LOQUAT CULTIVATION

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ABSTRACT

This research was carried out in 2019-2021 years in Hatay, Turkey**.** The aim of the research is to illuminate the possibilities of using of hawthorn (*Crataegus* spp) as rootstock in loquat *(Eriobotrya japonica Lindl*.) growing. For this aim, cv. Hafif Çukurgöbek (HÇG) was budded on the hawthorn rootstock at different 5 dates (8th February, 18th May, 7th August, and 31st October 2019; and 6th May, 2020) in 2019-2020 years with the chip budding. Also, it was budded on the same rootstock 24th June, 2020 with shield budding. The ratios of bud-take successes were determined after 45 days of the budding operations. After 60 days, the ratio of bud-sprout was recorded. In addition, the bud shoot length and bud shoot diameter and also rootstock trunk diameter in all plants were assigned in the study. The trial was planned in a completely randomized design with 6 replications and 10 plants per replication. Differences among means were analyzed by the Tukey's HSD method using SAS program. The highest values of bud take and sprouting rates (respectively, 47.6 % and 70 %) were taken from budding done on 7th August, 2019. This was followed by 31st October, 2019 with 41.67 % bud take and with 21.43%. bud sprout. The lowest budding success rate (10.58 %) was taken from the buddings which were done on 8th February, 2019. Buddings done on 18th May 2019 yielded the higher values of bud shoot length and diameter. In the buddings made in 2020 year, the highest budding success was obtained from the "T" budding made on June 24 (68.20%). The results of this study show that hawthorn can be used in loquat cultivation as rootstock.

Key words: *Eriobotrya japonica Lindl., Crataegus* spp., grafting, vegetative growth

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, there is not enough research done about this matter. Only three studies (Jamil et al., 2012; Polat, 2020, Polat, 2021) 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 a 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. In another study, cv. Hafif Çukurgöbek (HÇG) was budded on the hawthorn rootstocks with the chip budding method on different dates during 2018 and 2019 years (Polat, 2021). The researcher stated that was achieved between 10.8% and 46% budding success in the trial, and according to preliminary results of the study, the hawthorn can be used as rootstock for loquats.

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 study was conducted during two growing sessions 2019 and 2020 in Antakya, Hatay, Turkey. Hafif Çukurgöbek cultivar was budded on the hawthorn rootstocks (Figure 1a) on 8th February, 18th May, 7th August, 31st October 2019 and 6th May, 2020 with the chip budding method in the field conditions. Also, it was budded on the same rootstock 24th June, 2020 with shield budding. The trial was planned in a completely randomized design with 6 replications and 10 plants per replicate. The percent of bud-take successes were recorded after 45 days of the budding operations. In the bud-take, the top of the rootstock was cut from 10 cm above the budding point in order to sprout of the budding (Figure 1 b). After 15 days from this cutting, the ratio of bud-sprout was recorded.

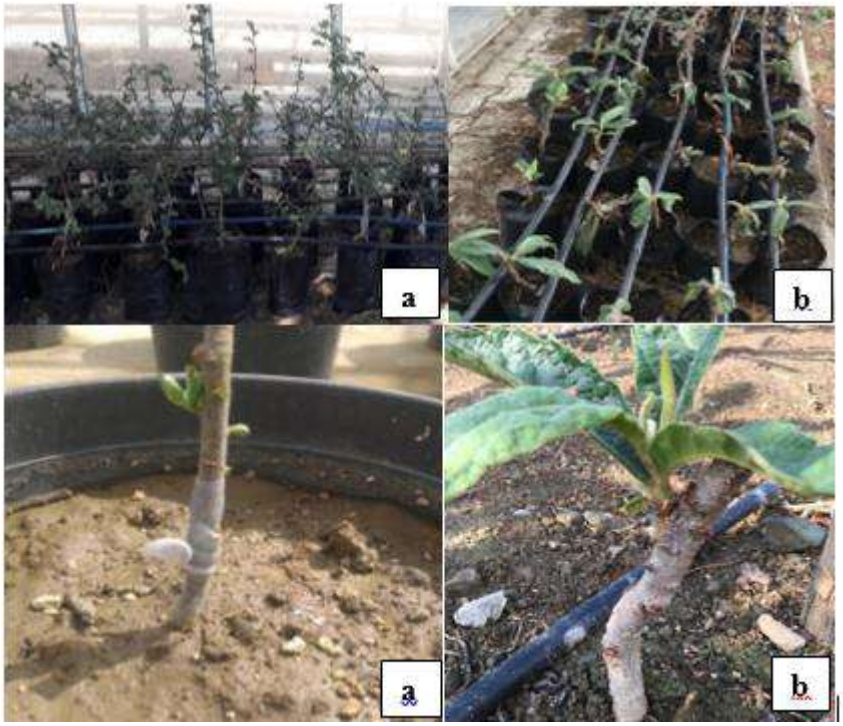

Figure 1. Budding(a) and bud sprouting(b) of loquats on the hawthorn rootstocks

In addition, the bud shoot length and bud shoot diameter and also rootstock trunk diameter in all plants were determined in four different times on 24 December, 2019 and 8 February, 2020, 21 August, 2020, and 15 March, 2021. The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. The data of the trial were analyses according to the completely randomized designed (Steel and Torrie, 1980) using SAS (2005). Differences among means were analyzed by the Tukey's HSD method at p=0.01.

RESULT AND DISCUSSION

Budding success

The success rates of budding made in different periods are presented in Table 1.

Table 1. The bud take rates of chip budding made in different periods

Budding periods	Bud take rates **(%)**	Bud sprout rates **(%)**
08/02/2019	10.58 d	66.66 b
18/05/2019	38.23 c	15.38 e
07/08/2019	47.62 a	70.00 a
31/10/2019	41.67 b	26.66 d
06/05/2020	6.86 d	42.80 c
Mean(%)	28.99	44.30

(x)Means followed by different capital letters are for rootstocks and budding dates and indicate significant difference by Tukey's test at 0.01.

In the chip buddings, the highest bud take (47.6 %) and sprouting (70 %) rates were determined on 7th August, 2019. This was followed by 31st October, 2019 with 41.67 % bud take and bud sprout (% 20). The lowest budding success rate (6.86 %) was taken from the buddings which were done on 6th May, 2020. The differences between the budding success rates of periods were found significant at P<0.01. However, in this study, the highest budding success was obtained from the "T" budding made on June 24, 2020, with 68.2 % bud take and with 100 % bud sprout. The budding success rates obtained in our study were lower than values of Jamil et al. (2012), but higher than that of Polat (2020).

Vegetative trait

The graft shoot length, scion and rootstock trunk diameter are given in Table 2.

Table 2**.** The vegetative growth of "HÇG" loquat cultivar on hawthorn rootstock in different dates

Measuring dates	Stock diameter (mm)	Graft shoot diameter (mm)	Graft shoot length (cm)
24 Dec. 2019	15,0	5,4	12,6
8 Feb. 2020	15,2	5,5	14,6
21 Aug. 2020	16,1	6,1	22,8
15 Mar. 2021	17,3	6,8	57,7

From the measurements made on different dates, it was determined that the hawthorn plant is a very slow growing rootstock. As a matter of fact, as can be seen from Table 2, while rootstock and scion trunk diameters and graft shoot length were measured as 15.04 mm, 5.38 mm and 12.60 cm, respectively, in December 2019, these values were measured as 17.25 mm, 6.85 mm and 57.68 cm, respectively, in 2021.

CONCLUSION

In loquat cultivation, the most effective method for reducing of vegetative growth is the use of dwarfing rootstocks such as quince (*Cydonia oblonga)* and hawthorn *(Crataegus spp).* The use of dwarfing rootstocks 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 aim of the research is to illuminate the possibilities of using of hawthorn (*Crataegus* spp) as rootstock in loquat (*Eriobotrya japonica* Lindl.) growing. As result, preliminary data obtained from this research have shown that can use of hawthorn as dwarf rootstock in loquat cultivation. However, researches need to be continued, especially to increase budding success rates on hawthorn rootstocks and also research should be continued to determine the effects on fruit quality using this rootstock.

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INVESTIGATION OF PROPOLIS AS A NATURAL ANTIBACTERIAL SUBSTANCE

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ABSTRACT

Propolis is a natural product that is collected from plants by honeybees and mixed with wax and used for many purposes in the hive. It has been shown by many scientific studies that propolis has been used in the treatment of various diseases in traditional medicine for many years and has biological activities such as antibacterial, antitumor, antioxidant and anti-inflammatory. Hence, the aim of the present study was to investigate the antibacterial activity of extracts and essential oils of propolis collected from various regions of Algeria against food-borne and clinically test microorganisms including *Micrococcus luteus* NRLL B-4375, *Enterecoccus faecalis* ATCC 29212, *Staphylacoccus epidermis* ATCC 11228 and *Bacillus megaterium* ATCC 11175. The antibacterial activity of the propolis extracts and essential oils were evaluated using disc diffusion method. The results showed that all propolis extracts and essential oils exhibited antibacterial activity against the tested microorganisms with inhibition zones varied from 6.41±0.20 mm to 21.66±0.57 mm. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the samples were determined by microdilution-broth method. The MIC and MBC values were in the range of 0.5-2 µg/µl and 0.5-8 µg/µl. Therefore, propolis extracts and essential oils from various regions of Algeria have potential to be used as a natural additive for food and pharmaceutical industries.

Keywords: Propolis, antibacterial, bactericidal, methanolic extract, essential oils

INTRODUCTION

Propolis is a partially digested bee product mixed with wax obtained by honey bees (*Apis mellifera* L.) by adding salivary enzymes (β-glucosidase) to the resin they collect from sprouts, leaves, buds and bark cracks in trees and plants (da Silva et al., 2018; Segueni et al., 2017). Honey and other bee products, which are functional food products, have come to the fore in recent years with the importance given to beekeeping. It is stated in Chinese inscriptions and written sources in Egypt that beekeeping products were used in the treatment of diseases before and after Christ (Sangeeta et al., 2018). The antibacterial, antioxidant and anticarcinogenic properties of propolis are due to the chemical components it contains (Viuda-Martos et al., 2008). The chemical component content of propolis varies depending on the local flora in the area where it is collected (Bankova et al., 2001).

Among the most important chemical components of propolis are flavonoids, aromatic acids, terpenoids (diterpenoid acids and triterpenoids), fatty acids, esters, phenols, aldehydes,

ketones. Phenolic compounds are often the main components (Chen et al., 2008; Popova et al., 2010). Some of these compounds (flavonoids, terpenoids, aromatic acids and their esters) are responsible for biological activities (Barros et al., 2007; Yang et al., 2015). One of the most researched activities of propolis is its antimicrobial activity. Many scientific studies have proved the effect of propolis extracts and essential oils on various bacteria, fungi, viruses and other microorganisms (Sforcin et al., 2000; Hegazi et al., 2000; Kujumgiev et al., 1993)

The studies made contribute to different sectors such as medicine, food, cosmetics and pharmaceuticals. Propolis, which is widely used in traditional medicine, has lost its importance with the use of synthetic drugs in modern medicine. However, in the last 20 years, the tendency towards the use of natural drugs has increased as the side effects of synthetic drugs have emerged and disease agents have become resistant to these drugs (Kutluca et al., 2006).

Besides being a therapeutic substance due to its pharmacological properties, propolis is also a functional food due to the presence of bioactive compounds in its extracts (Medić-Šarić et al. 2009). The Turkish Food Codex also describes these extracts as "Food Supplements" (Communiqué No: 2013/49). Although its source is plants, it is in animal food class as it is processed by bees and mixed with their own secretions.

Micrococcus luteus is a Gram-positive, spherical and saprophytic bacterium. They can be found in many different environments such as soil, water, animals and dairy products. It is thought to be an opportunistic pathogen (Murray and Holt, 2001). Enterococci, which are found as normal flora elements in the human intestine, mouth, vagina, urethra and biliary tract; Although they have low virulence, they are increasingly being detected as a factor in hospital infections and community-acquired infections (Aguş et al., 2006; Ekşi et al., 2008). In recent years, attention has been focused on enterococci, not only because they cause nosocomial infections and they are being detected more frequently in community-acquired infections, but also because they have gained significant and increasing resistance to many antibiotics (Erbek et al., 2002). *Staphylococcus epidermis* is a normal flora bacterium found commensally on the surfaces of the human body in contact with the external environment, especially the skin (Otto, 2009). *Bacillus megaterium* is aerobic, Gram positive, this species has a wide range of uses in the laboratory environment (Erol, 2007)

The aim of this study is to evaluate the *in vitro* antibacterial activity of propolis from Algeria against *M. luteus* NRLL B-4375, *E. faecalis* ATCC 29212, *S. epidermis* ATCC 11228 and *B. megaterium* ATCC 11175, which are important food-borne and clinical test microorganisms.

MATERIAL AND METHOD

Test Microorganisms

In vitro antibacterial activity of propolis extracts and essential oils were tested against four microorganisms (*Micrococcus luteus* NRLL B-4375, *Enterecoccus faecalis* ATCC 29212, *Staphylacoccus epidermis* ATCC 11228 and *Bacillus megaterium* ATCC 11175). Bacterial strains were cultured at 37ºC in Nutrient broth/agar mediums.

Propolis Samples

Propolis samples were collected from *Apis mellifera* hives located at different geographical regions of Northeast Algeria (Collo, El harrouch, Taref, Konstantin, Setif, Mila, Batna, Oum el Bouaghi) (Table 1).

Table 1. Collection regions of propolis samples

Samples name	Samples	Collection Region	City
1	Methanolic extract	Menia	Constantine
2	Methanolic extract	Grarem	Mila
3	Methanolic extract	Collo	Skikda
4	Methanolic extract	Mestaoua & Chelala mountains	Batna
5	Methanolic extract	El Harrouch	Skikda
6	Methanolic extract	Bouteldja	Taref
7	Aqueous fraction of methanolic extract	Babor	Sétif
8	Methanolic extract	Babor	Sétif
9	Methanolic extract	Oum el Bouaghi	Oum el Bouaghi
M	Essential oil	Menia	Constantine
H	Essential oil	El Harrouch	Skikda
C	Essential oil	Collo	Skikda
B	Essential oil	Mestaoua & Chelala mountains	Batna

Preparation of Propolis Extracts and Essential Oils

The collected propolis samples were pulverized after separation of impurities. 20 g of powdered propolis was extracted three times with 200 ml of hydroalcoholic solution (80% MeOH, 20% distilled water) for 72 h. After the extraction, the obtained extracts were filtered, evaporated and then kept at 4°C under dry conditions until use. The propolis essential oils were obtained by hydrodistillation of crude powdered propolis (100 g) using a Clevenger type apparatus for 3 h. The obtained oils were dried over anhydrous sodium sulphate and stored at 4°C. Prior to determine the antibacterial activity, propolis extracts and essential oils (10 mg) were dissolved in 1 ml of Dimethyl sulphoxide (DMSO) to obtain a final concentration of 10 µg/µl. Then, the obtained solutions were sterilized by 0.45 µm Millipore filters.

Determination of Antibacterial Activity

Disc diffusion assay

The disc diffusion method was used to determine the antibacterial activity of propolis extracts and essential oils. The culture suspensions were adjusted by comparing with 0.5 McFarland. Then, a volume of 100 µl of suspension was spread on agar plates. Thereafter, sterile 6 mm diameter filter discs (Whatman paper no 3) were placed on the inoculated plates and impregnated with 15 µl (150 µg/disc) of propolis extracts and essential oils. The plates were kept at 4°C for 1 h to enable prediffusion of propolis samples into the agar. The inoculated plates were then incubated at 37°C for 24 h for bacterial strains. The results were obtained by measuring the diameter of growth inhibition zone surrounding the discs and expressed in mm.

Microdilution assay

The two-fold microdilution method was used to determine the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of propolis extracts and essential oils. The propolis extracts and essential oils were added to each growth medium to obtain a final concentration of 16 µg/µl and diluted to 8, 4, 2, 1, 0.5 and 0.25 µg/µl in tubes. Then, the content

of the tubes was mixed and they were incubated at appropriate temperatures for 24 h for bacterial strains. The MIC value was defined as the lowest concentration of propolis extracts and essential oils, which inhibited bacterial growth. MBC was determined by spot dropping from each clear tube on solid growth medium and incubating for 24 h at appropriate temperature. The lowest concentration that did not show bacterial growth was defined as the MBC value. The results are expressed as µg/µl.

RESULTS AND DISCUSSION

Propolis and especially its extracts are antibacterial (Yadav et al., 2012), antiviral, antifungal, antioxidant (Piccinelli et al., 2013), anti-inflammatory (Moura et al., 2009), anticarcinogenic (Wang et al., 2016), it has been used for a long time for the prevention and treatment of various diseases due to its wide biological activities such as allergic, anti-diabetic (Kang et al., 2010), cytostatic, hepatoprotective effect, photoprotective effect, immunizing and numbing (de Castro et al. 2012; Nakajima et al., 2009). Propolis varies considerably according to the climate, season, geographical region, and collection time and source plant (Akpınar, 2020). Therefore, in this study we investigated the antibacterial activities of propolis extracts and essential oils from various regions of Algeria, against some test microorganisms, by two methods: disc diffusion and microdilution assays. The results of disc diffusion revealed the ability of propolis extracts and essential oils to inhibit the growth of all tested microorganisms with inhibition zone diameters ranged from 6.41±0.20 mm to 21.66±0.57 mm.

Table 2. Antibacterial activity of propolis extracts and essential oils

Samples	Inhibition zone diameter (mm)			
	M. luteus	***E. faecalis***	***S. epidermis***	***B. megaterium***
1	14.07±0.53	9.50±0.45	16.55±1.61	14.89±1.37
2	13.30±0.32	8.60±0.27	18.0±0.21	14.04±0.75
3	13.73±1.52	9.21±0.18	17.15±1.27	15.75±0.16
4	12.68±1.31	8.33±0.14	18.20±0.22	15.26±0.08
5	13.99±0.23	9.25±0.26	18.45±0.04	13.93±1.16
6	14.98±0.18	9.31±0.15	18.72±0.29	17.57±0.33
7	16.73±0.18	10.83±0.01	17.34±0.08	21.66±0.57
8	8.36±0.67	6.62±0.03	8.55±0.18	8.01±0.07
9	7.28±0.35	9.21±0.03	18.94±0.51	17.59±0.41
B	9.42±0.35	9.36±0.39	8.41±0.27	10.01±1.27
C	8.53±0.11	8.31±0.28	13.91±0.13	9.57±0.60
H	6.79±0.30	8.70±0.01	12.40±0.12	7.78±0.12
M	6.41±0.20	7.77±0.30	13.62±0.71	6.81±0.11

The antibacterial activity assay indicated that the highest inhibition effect was exhibited by Sample 7 against *B. megaterium* (21.66±0.57 mm) and Sample 9 against *S. epidermis* (18.94±0.5 mm). Sample 8 and sample 9, however, was the less active against *E. faecalis* and *M. luteus* with inhibition zone diameter values of 6.62±0.03 mm and 7.28±0.35 mm. Among propolis essential oils, Sample C and M were more active against *S. epidermis* 13.91±0.13 mm and 13.62±0.71 mm, while Sample H and M showed the lowest activity against *M. luteus*. The micro-dilution assay showed that among all propolis extracts and fatty acids, Sample 7 was the most effective extract with MIC value of 0.5 µg/µl against the *S. epidermis* (Table 3). The MBC values varied between 0.5 µg/µl and 8 µg/µl. The lowest MBC value was recorded by Sample

7 (0.5 µg/µl) against *M. luteus* and *S. epidermis* (Table 4). MBC values of the fatty acid samples (B, C, H, M) varied from 2 µg/µl to 8 µg/µl. The lowest MBC value was recorded by Sample B (2 µg/µl) against *M. luteus* and *S. epidermis* (Table 4).

Table 3. MIC values of propolis methanolic extracts and essential oils

	MIC (µg/µl)			
Samples	***M. luteus***	***E. faecalis***	***S. epidermis***	***B. megaterium***
1	2	2	2	2
2	2	2	2	2
3	2	2	1	2
4	2	2	2	2
5	2	2	1	2
6	2	2	1	2
7	1	2	0.5	1
8	2	2	2	2
9	1	2	1	2
B	2	2	2	2
C	2	2	2	2
H	2	2	2	2
M	2	2	2	2

Table 4. MBC values of propolis methanolic extracts and essential oils

	MBC (µg/µl)			
Samples	***M. luteus***	***E. faecalis***	***S. epidermis***	***B. megaterium***
1	2	2	4	4
2	2	2	2	4
3	2	2	1	4
4	2	2	2	4
5	2	2	1	2
6	2	4	1	2
7	0.5	4	0.5	1
8	4	8	2	4
9	1	4	1	4
B	2	4	2	4
C	4	8	4	4
H	4	8	4	4
M	4	8	4	4

Antimicrobials are usually considered as bactericidal if the MBC/MIC ratio is ≤4 and bacteriostatic if the MBC/MIC ratio is >4 (Krishnan et al., 2010; Hazen, 1998). The ratios obtained for all the test microorganisms were below or equal to 4 which indicated that all propolis extracts and essential oils were bactericidal against all tested microorganisms (Table 5).

Table 5. MBC/MIC ratios values of propolis methanolic extracts and essential oils

	MBC/MIC			
Samples	***M. luteus***	***E. faecalis***	***S. epidermis***	***B. megaterium***
1	1	1	2	2
2	1	1	1	2
3	1	1	1	2
4	1	1	1	2
5	1	1	1	1
6	1	2	1	1
7	0.5	2	1	1
8	2	4	1	2
9	1	2	1	2
B	1	2	1	2
C	2	4	2	2
H	2	4	2	2
M	2	4	2	2

In a study conducted by Przybyłek et al. (2019), ethanolic extracts of propolis from Brazil showed antimicrobial activity with MIC values 631 mg/ml, 258 mg/ml ,825 mg/ml against *Enterococcus* spp., *M. luteus, S. epidermidis,* respectively. They indicated that the ethanolic extracts of propolis from Turkey showed antimicrobial activity with MIC values of 19 mg/ml, 11 mg/ml, 20 mg/ml against *Enterococcus* spp., *M. luteus*, *S. epidermidis,* respectively.

Mohdaly et al. (2015) indicated that propolis methanol extracts were found to have antimicrobial activity effects on four different food-borne pathogens. As a result of the study, MIC concentration was between 0.20 mg/ml and 1.10 mg/ml, while MBC concentration varied between 0.25 mg/ml and 1.25 mg/ml. In another study, disc diffusion and MIC assays were used to determine antimicrobial activities of propolis ethanol extract on *S. epidermidis*. The inhibition zone diameter and MIC value was recorded as 25 mm and 0.05 mg/ml (Popova et al., 2009).

CONCLUSIONS

In our study, the antibacterial effect of propolis extracts and essential oils were evaluated for the first time against *Micrococcus luteus* NRLL B-4375, *Enterecoccus faecalis* ATCC 29212, *Staphylacoccus epidermis* ATCC 11228 and *Bacillus megaterium* ATCC 11175. Our results showed that the propolis methanolic extracts and fatty acids possess broad-spectrum antibacterial activity on food-borne and clinical origin pathogens, suggesting that propolis may be used in food and pharmaceutical industry.

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INVESTIGATING THE POTENTIAL USE OF Pistacia terebinthus FRUIT EXTRACTS TOGETHER WITH PROBIOTIC CANDIDATE LACTIC ACID BACTERIA

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ABSTRACT

The beneficial effects of probiotics on health have been known for many years. Lactic acid bacteria are the most commonly used probiotics. *Pistacia terebinthus* is a plant that has been used in traditional medicine since ancient times. In this study, it was aimed to determine the potential of the use of probiotic candidate various *Streptococcus thermophilus* strains belongs to lactic acid bacteria (LAB) together with the *P. terebinthus* extracts as a source of natural antimicrobial substances in various industry such as pharmaceutical and food. The antimicrobial effects of *P. terebinthus* fruit extracts prepared with different solvents (acetone, ethanol, methanol, dichloromethane (DCM), hexane, water) on 8 different *S. thermophilus* strains were investigated. Disc diffusion method was used to determine the antimicrobial activity of the extracts. The minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of the extracts were assayed using the micro-dilution method. In the current study, 3 different concentrations of the extracts (5 µl, 10 µl, 20 µl) were tested on 7 different human milk originated and one commercial strains of *S. thermophilus*. Among all concentrations studied, the water extract showed no inhibition zone on *S. thermophilus* MAS-1, MAS-2, MAS-4, MAS-5, MAS-7 and *S. thermophilus* RSKK 667. The highest inhibition zone for 5 µl, 10 µl and 20 µl concentrations of extracts was determined in the methanol extract on *S. thermophilus* MAS-8 as 25.04 mm, 26.03 mm and 29.46 mm, respectively. MIC and MBC values were in the range of 0.62-40 mg/ml. The results of the present study revealed the possible use of probiotic candidate lactic acid bacteria together with the plant extracts as effective natural preservatives in various industries.

Key words: Terebinth, Antibacterial, *Streptococcus thermophilus*, Probiotic

INTRODUCTION

Probiotics are live microorganisms that provide health benefits to the host when administered in adequate amounts (Hill et al., 2014). Probiotics prevent the growth of pathogenic microorganisms by lowering the intestinal pH and producing hydrogen peroxide, organic acids and bacteriocin, which are considered toxic substances for pathogenic microorganisms (Williams, 2010). Lactic acid bacteria (LAB) are the most popular group of probiotic microorganisms. LAB are non-pathogenic Gram-positive bacteria in the gastrointestinal tract of humans and animals, urogenital tracts and human milk (Anandharaj and Sivasankari, 2014; Gotteland, 2014). The potential benefits of LAB for human health include stimulating the immune system, balancing the intestinal flora, reducing serum cholesterol and the risk of tumors (Ayeni et al., 2011). LAB can also extend the shelf life of foods. Their non-pathogenic and non-toxic nature allows many LAB applications to improve safety and quality in food industry (Moller et. al., 2021). *S. thermophilus*, a Gram-positive bacterium belonging to LAB, is considered safe and can survive in the gastrointestinal tract (Elli et al., 2007). In

addition to having important features for the food industry such as organoleptic, technological and nutritional advantages, it also has beneficial effects such as improving human health (Leroy et al., 2004). *S. thermophilus* can improve intestinal microflora and prevent diarrhea caused by antibiotic use. It can also reduce lactose intolerance and the risks of cancers, ulcers, inflammation, stimulate the immune system, and can be used to treat some atopic dermatitis (Bojrab, 2002). Also, many *S. thermophilus* strains with many of the probiotic properties are desired for the food industry as starter cultures (Guarner et al., 2005). *P. terebinthus* (terebinth), perennial plant belongs to Anacardiaceae family, is widely grown western and southern areas of Turkey, where it is called "menengiç" in Turkish (Bozorgi et al., 2013). In traditional medicine, *P. terebinthus* fruits were used internally or externally to treat several diseases such as gastralgia, stomach ache, diarrheic, throat infections, asthma, cough, rheumatism, and eczema (Matthaus and Ozcan 2006)**.** Terebinth is also one of the plant species rich in oil, protein and dietary fibers and has been known for its unique taste and aromatic properties since ancient times. For this reason, different parts of the *P. terebinthus* tree are used in various parts of the world as a medicinal and aromatic plant (Karakaş and Certel, 2004). In the presented study, the effects of the *P. terebinthus* fruit extracts (ethanol, methanol, hexane, dichloromethane (DCM), acetone and water) on probiotic candidate human milk originated and one commercial *S. thermophilus* strains were investigated to determine the potential of combined use of the extracts and LAB in the food and health industries.

MATERIAL AND METHOD

Test Microorganisms

S. thermophilus MAS-1, *S. thermophilus* MAS-2, *S. thermophilus* MAS-4, *S. thermophilus* MAS-5, *S. thermophilus* MAS-6, *S. thermophilus* MAS-7, *S. thermophilus* MAS-8 and *S. thermophilus* RSKK 667 were used as test microorganisms.

Preparation of Extracts

The *P. terebinthus* fruit samples obtained from a herbalist in Adıyaman were air-dried and then powdered. 30 ml of solvent (ethanol, methanol, hexane, DCM, acetone, water) was separately added into 20 g of the powdered sample. The extraction was done by using a sonicator device (Hielscher) on ice for 10 minutes in 3 repetitions. After the extraction process, the solvent is allowed to evaporate. Then, the crude extract was stored at 4 °C under dry conditions until they were used.

Determination of Antimicrobial Activity

Disc diffusion assay

The test microorganisms were growth in M17 broth medium and then their concentrations were adjusted to the 0.5 McFarland standard in saline buffer. After inoculation of the bacteria onto solid agar, the steril discs were placed on solid media in 3 repetitions. 5 µl, 10 µl and 20 µl of *P. terebinthus* fruit extracts were dropped onto the discs. The Petri dishes were left to incubate for 24 h at 37°C. At the end of the incubation, inhibition zone diameters around the discs were measured and recorded.

Determination of Minimal Inhibition Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The tube containing the growth medium and *P. terebinthus* extract was diluted and then the adjusted bacterial culture (McFarland 0.5) was added to each tube. The tubes were incubated for 24 h at 37°C. After incubation, the concentration observed no-growth was determined as

the MIC value of the extracts. Then, the samples in the tubes were inoculated onto the solid medium by using spot-dropping method. The inoculated Petri dishes were again incubated at 37 °C for 24 h. The concentration at which no growth observed at the end of the incubation period was recorded as the MBC value.

RESULT AND DISCUSSION

The effects of different concentrations of the extracts prepared with various solvents of *P. terebinthus* fruits on probiotic candidate *S. thermophilus* strains isolated from human milk were investigated. The disc diffusion assay results with 5 µl of the extract dropped is given in Table 1. The water extract did not inhibit the growth of any *S. thermophilus* strains (Figure 1). In addition, the acetone and hexane extracts did not showed any inhibition zones on S. *thermophilus* MAS-6. The highest inhibition zone was measured as 25.04 mm with the methanol extract on *S. thermophilus* MAS-8. The disc diffusion assay results dropped 10 µl of extract are presented in Table 2. The water extract did not show inhibition zones on the test bacteria, except for *S. thermophilus* MAS-6 and *S. thermophilus* MAS-8. The highest inhibition zone was determined as 26.67 mm in the ethanol extract on *S. thermophilus* MAS-8. The disc diffusion assay results with 20 µl dropped extract are given in Table 3. The results indicated that the water extract did not show any inhibition zones on tested bacteria, except for *S. thermophilus* MAS-8 and *S. thermophilus* MAS-6 similar to the results of 10 µl dropped extract. The highest inhibition zone in methanol extract was determined as 29.46 mm on *S. thermophilus* MAS-8. MIC values of the extracts against *S. thermophilus* strains were ranged from 0.62 mg/ml to 40 mg/ml. MBC values were determined between 5 mg/ml and 40 mg/ml. These data indicated that the extracts had high MIC and MBC values on *S. thermophilus* strains. Therefore, appropriate concentrations of the extracts together with *S. thermophilus* strains with probiotic potential can be used as natural preservatives as alternatives to synthetics in the food and pharmaceutical industries.

Table 1. Disc diffusion assay results of 5 µl dropped extracts

Test Microorganisms	**Inhibition zone diameter (mm)**					
	Acetone	**Ethanol**	**DCM**	**Methanol**	**Hexane**	**Water**
S. thermophilus MAS-1	11.36± 0.16	12.4± 0.15	15.08± 0.09	13.86± 0.12	11.43± 0.07	-
S. thermophilus MAS-2	12.08± 0.82	13.71± 0.43	8.61± 0.20	15.75± 0.33	11.89± 0.22	-
S. thermophilus MAS-4	14.47± 0.39	15.04± 0.55	13.54± 0.007	11.95± 0.10	14.08± 1.23	-
S. thermophilus MAS-5	8.07± 0.01	9.77± 1.06	7.72± 0.04	9.15± 0.66	9.21± 0.43	-
***S. thermophilus* MAS-6**	-	12.59± 1.18	10.68± 1.46	20.41± 0.80	-	-
***S. thermophilus* MAS-7**	10.73± 0.36	13.33± 0.19	15.32± 0.07	16.00± 0.01	12.82± 0.02	-
***S. thermophilus* MAS-8**	12.21± 0.66	22.85± 0.81	9.53± 0.26	25.04± 1.05	13.35± 1.26	-
***S. thermophilus* RSKK 667**	7.82± 0.02	10.77± 0.14	6.68± 0.65	7.68± 0.11	7.15± 0.08	-

Table 2. Disc diffusion assay results of 10 µl dropped extracts

Test Microorganisms	Inhibition zone diameter (mm)					
	Acetone	**Ethanol**	**DCM**	**Methanol**	**Hexane**	**Water**
S. thermophilus* *MAS-1	12.49± 0.05	12.54± 0.03	15.39± 0.12	16.79± 0.007	14.9± 0.56	-
S. thermophilus* *MAS-2	12.17± 0.23	17.93± 1.79	9.99± 0.70	15.31± 0.52	16.03± 0.31	-
S. thermophilus* *MAS-4	18.03± 0.83	18.01± 0.08	15.27± 0.74	19.27± 0.007	15.90± 0.62	-
S. thermophilus* *MAS-5	9.21± 0.10	10.18± 0.27	8.47± 0.12	12.15± 0.01	13.56± 0.26	-
***S. thermophilus* MAS-6**	11.03± 0.73	16.44± 0.71	12.73± 0.67	25.96± 0.52	8.97± 0.48	6.35± 0.04
***S. thermophilus* MAS-7**	11.32± 0.37	16.51± 0.40	18.74± 0.34	16.79± 0.23	13.82± 0.16	-
***S. thermophilus* MAS-8**	18.63± 1.76	26.67± 0.96	23.53± 1.47	26.03± 0.19	18.43± 1.60	8.21± 0.12
***S. thermophilus* RSKK 667**	9.71± 0.30	11.25± 0.07	8.68± 0.14	10.12± 0.10	9.63± 0.18	-

Table 3. Disc diffusion assay results of 20 µl dropped extracts

Test Microorganisms	Inhibition zone diameter (mm)					
	Acetone	**Ethanol**	**DCM**	**Methanol**	**Hexane**	**Water**
S. thermophilus* *MAS-1	14.23± 0.58	13.85± 0.35	15.82± 0.01	17.96± 0.12	15.34± 0.40	-
S. thermophilus* *MAS-2	18.85± 0.88	21.42± 0.29	13.68± 0.83	20.47± 0.17	19.50± 0.02	-
S. thermophilus* *MAS-4	21.11± 0.14	20.27± 0.38	18.13± 0.55	21.18± 0.35	18.95± 0.63	-
S. thermophilus* *MAS-5	12.48± 0.23	14.88± 0.63	11.33± 0.30	13.8± 0.90	23.18± 0.70	-
***S. thermophilus* MAS-6**	14.93± 0.34	20.0± 1.40	18.0± 0.26	27.77± 1.20	12.10± 0.45	10.20± 0.04
***S. thermophilus* MAS-7**	14.35± 0.49	20.54± 0.47	20.03± 0.92	20.10± 0.47	15.51± 1.34	-
***S. thermophilus* MAS-8**	24.51± 0.50	26.34± 2.43	27.67± 0.19	29.46± 1.40	23.36± 0.75	11.54± 1.45
***S. thermophilus* RSKK 667**	12.38± 0.02	13.34± 0.24	10.27± 0.38	13.22± 1.13	11.71± 1.30	-

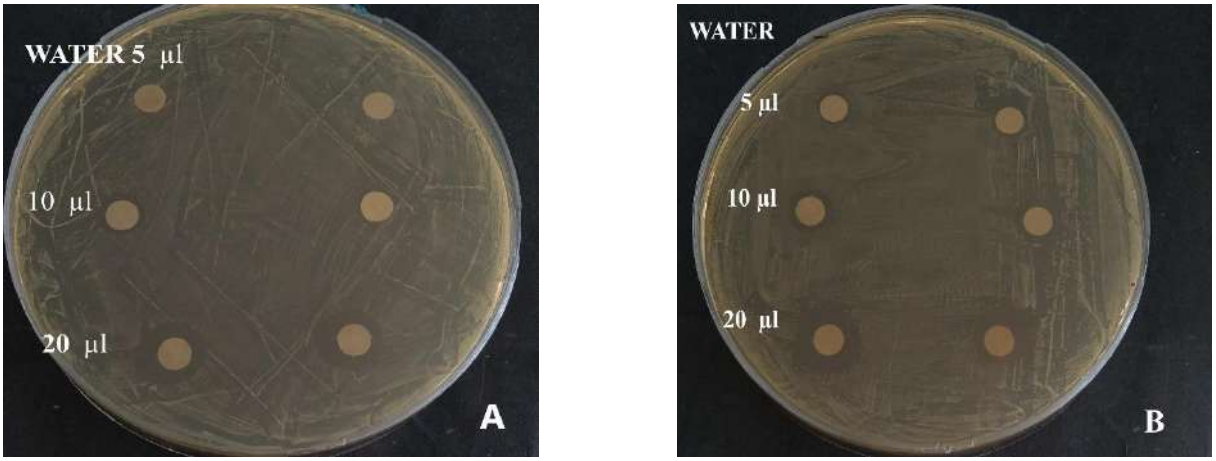

Figure 1. (A). Disc diffusion assay results of various concentrations of water extract on *S. thermophilus* MAS-6, (**B)**. Disc diffusion assay results of various concentrations of water extract on *S. thermophilus* MAS-8

Table 4. MIC values of *P. terebinthus* fruit extracts on *S. thermophilus* strains

Test Microorganisms	**MIC (µg/µl)**					
	Acetone	**Ethanol**	**DCM**	**Methanol**	**Hexane**	**Water**
***S. thermophilus* MAS-1**	2.5	1.25	5	2.5	5	2.5
***S. thermophilus* MAS-2**	2.5	1.25	5	2.5	5	10
***S. thermophilus* MAS-4**	40	10	40	40	40	20
***S. thermophilus* MAS-5**	2.5	2.5	10	5	5	10
***S. thermophilus* MAS-6**	40	40	40	40	40	40
***S. thermophilus* MAS-7**	5	2.5	10	0.62	5	20
***S. thermophilus* MAS-8**	40	40	40	40	40	40
***S. thermophilus* RSKK 667**	20	40	20	40	20	20

Table 5. MBC values of *P. terebinthus* fruit extracts on *S. thermophilus* strains

Test Microorganisms	MBC (µg/µl)					
	Acetone	**Ethanol**	**DCM**	**Methanol**	**Hexane**	**Water**
S. thermophilus MAS-1	40	5	40	10	40	40
S. thermophilus MAS-2	40	5	40	10	40	40
S. thermophilus MAS-4	40	10	20	40	40	20
S. thermophilus MAS-5	40	20	40	5	40	40
***S. thermophilus* MAS-6**	40	40	40	40	40	40
***S. thermophilus* MAS-7**	40	5	40	10	40	40
***S. thermophilus* MAS-8**	40	40	40	40	40	40
***S. thermophilus* RSKK 667**	40	40	40	40	40	40

CONCLUSION

Probiotics and plants are important natural additives for various industries. Plants are gaining importance as natural sources of antimicrobial substances and probiotic candidate LAB, with their beneficial effects on health, in both food and pharmaceutical fields, in terms of eliminating the side effects of synthetic origin agents today. The present study indicated that *P. terebinthus* fruit extracts together with *S. thermophilus* strains may have potential to be used as natural additives for various industries.

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DETERMINATION OF SUN PROTECTION FACTOR AND ANTIBACTERIAL ACTIVITY OF VARIOUS OLIVE EXTRACTS

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ABSTRACT

The globally accepted standard for evaluating Ultraviolet (UV) protection by a sunscreen formulation is the determination of sun protection factor (SPF). In this study, the in vitro SPF values and antibacterial activity of olive fruit and leaf extracts prepared with different solvents (water, acetone and ethyl acetate) from Ayvalık Yağlık variety grown in Izmir were investigated. The SPF values of the extracts were measured spectrophotometrically at 290-320 nm wavelengths of UV-B ultraviolet rays reaching the earth. Antibacterial activity was investigated against some food and clinical pathogens (*Salmonella enteritidis* RSKK 171, *Bacillus cereus* RSKK 863, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853). The antibacterial activity of olive fruit and leaf extracts were evaluated using disc diffusion assay. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the extracts were determined by micro-dilution method. The SPF values of the extracts were varied between 0.84 and 15.35. The highest SPF value (15.35) determined indicates 93% of the UV blocked percentage. The antimicrobial activity assay results showed that all olive fruit and leaf extracts exhibited antibacterial activity against the tested microorganisms with inhibition zones varied from 8.9±0.2 mm to 19.4±0.1 mm. The MIC and MBC values were in the range of 5-20 mg/ml and 5-80 mg/ml. Therefore, olive fruit and leaf extracts have potential to be used as a natural additive for cosmetics, pharmaceutical and food industries.

Keywords: Ayvalık Yağlık, fruit, leaf, sun protection factor (SPF), bactericidal, extracts

INTRODUCTION

Ultraviolet (UV) light is electromagnetic radiation with a wavelength in the range of 10-400 nm between visible light and x-rays. UV rays are the invisible part of the electromagnetic spectrum that reaches the earth from the sun (Joux et al., 1999; Matallana-Surget et al., 2008). Three types of ultraviolet radiation of the sun are at a level that can harm people. These rays are UV-A, UV-B and UV-C. Terrestrial radiation called sunlight is 8% UVA and 1% UV-B (Coohill and Sagripanti, 2009). UV-A wavelength is in the range of 320-400 nm and is the UV light that is little filtered by the earth's atmosphere and is the most incoming to the earth's surface. UV-B is one of the UV rays reaching the earth's surface, although most of it is filtered by the atmosphere, with a wavelength of 290-320 nm. UV-C wavelength is in the range of 200-290 nm and is the last ultraviolet light that falls on the earth in small amounts from the sun. UV-C reacts with the oxygen atoms in the earth's atmosphere and turns the oxygen into ozone. It is ultraviolet light that almost never reaches the earth's surface due to its reaction with oxygen atoms (Dutra et al., 2004). In the literature, it was determined that UV-A disrupts the antigen presenting cell (APC) activity of epidermal cells and consequently causes immune suppression.

Suppressed immunity has shown to contribute to the growth of skin cancer (Stoebner et al., 2007). In addition, the effects of UV-A radiation occur when exposed for a long time, causing decreased skin elasticity, increased wrinkles and reactive oxygen production, which leads to acute and chronic changes in the skin (Fonseca and Rafaela 2016). UV-B rays can cause acute changes such as pigmentation and sunburn, suppression of the immune system and chronic changes such as photocarcinogenesis (De Buys et al., 2000). Sunscreen products should be used to protect against the harmful effects of UV-A and UV-B. Sunscreen agents have a sun protection factor (SPF) value which is defined as the ratio of the minimal erythemal dose over the sunscreen agent (Algaba and Riva, 2006). UV rays (A-B) cause the release of toxic radicals for skin cells and cause skin aging (Matsumura and Ananthaswamy, 2004). It has been determined that sunscreen agents provide significant protection against epidermal antigen-presenting cell (APC) activity caused by UV-A and UV-B (Stoebner et al., 2007). Plant extracts obtained by various methods can prevent the acceleration of transcription factors in skin cells caused by UV rays (Rodrigues et al., 2015). Also, natural antioxidants are used in sunscreens to increase ray protection, as they can prevent damage caused by free radicals produced by the sun's rays (Chaudhuri, 2005). The biological source of phenolic compounds with antioxidant properties is fruits and vegetables. In this study, the SPF values was investigated in vitro with various extracts prepared from the fruit and leaves of Ayvalık Yağlık olive oil variety, which has antioxidant properties and contains rich phenolic compounds. The olive (*Olea europaea* L.) is one of the most important fruit tree of the Mediterranean countries, where it covers an area of 8 million hectares, constituting approximately 98% of the world's crop (Guinda et al., 2004). Besides the great economic and social importance of the olive crop, its by-products also show potential benefits as well as the olive fruit. The main product of the olive tree consists of olives, olive oil and olive leaves as a by-product. The phenolic compounds found in olive and its leaves have been reported to have antioxidant, antifungal, antibacterial, hypocholesterolemic, cardioprotective effects (Ferreira et al., 2007). In addition, phenolics such as p-hydroxybenzoic, vanillic, caffeic, protocatechuic, syringic and p-coumaric acids, oleuropein, quercetin, tyrosol, hydroxytyrosol, elenolic acid in olive fruit leaf have antimicrobial activity (Sousa et al., 2006, Veer et al., 1957). Today, pathogens of food and clinical origin cause various diseases. 10% of Salmonellosis cases are caused by *Salmonella enteritidis*. The only human pathogen to contaminate chicken eggs is *S. enteritidis* (Soerjadi-Liem and Cumming 1984; Caldwell et al., 1995). *Bacillus cereus* and *Staphylococcus aureus* are common on soil and vegetation. Some strains have been reported to cause food spoilage and food poisoning in humans (Mengeloğlu et al., 2011; Braga et al., 2005; Sonenshein et al., 2001). *Pseudomonas aeruginosa* causes 10% to 20% of nosocomial infections. It is especially common in patients with burn wounds, cystic fibrosis, acute leukemia, organ transplantation and intravenous drug addiction (Gerald et al., 1983). The aim of this study was investigation of SPF values and antibacterial activity against food-borne and clinical pathogens of various extracts (water, acetone and ethyl acetate) from the fruit and leaves of the Ayvalık Yağlık variety to determine their usage potential as natural additives in various industries.

MATERIAL AND METHOD

Plant Material

Ayvalık Yağlık variety olive fruit and leaf samples were obtained from Izmir Olive Research Institute (Turkey) in August 2019.

Preparation of Olive Fruit and Leaf Extracts

Olive fruit and leaf were ground after air-dried. In extraction, 10 g powdered plant material was extracted with 30 ml of water, acetone and ethyl acetate in a sonicator device (Hielscher) on ice in 3 repetitions for 10 minutes. After extraction, the solvents were evaporated. Then, the extracts were kept at 4°C under dry conditions until they were used.

Test Microorganisms

The food-borne and clinical origin test microorganisms (*S. enteritidis* RSKK 171, *B. cereus* RSKK 863, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853) were used in the study. All experiments were carried out with 24 h active cultures of the test microorganisms.

Determination of SPF

For determination of SPF of olive fruit and leaf water extracts, 0.006 g of the extracts was weighed. The weighed extract was then vortexed with 3 ml of ethanol (96%) until homogenized. The homogeneous mixture obtained was measured in 3 repetitions in a spectrophotometer (Beckman Coulter) at 5 nm intervals in the wavelength range of 290-320 nm. The obtained values were calculated using the Mansur equation (Mansur et al., 1986).

The solar protection factor (SPF) used in this study was determined using the following equation proposed by Mansur et al. (1986).

$$\mathbf{SPF = CF \times} \sum_{290}^{320} \boldsymbol{EE}(\boldsymbol{\lambda}) \times \boldsymbol{I}(\boldsymbol{\lambda}) \times \boldsymbol{Abs}\ (\boldsymbol{\lambda}) \quad (1)$$

CF= Correction factor (= 10);

EE(λ) = Erythemetogenic effect radiation wavelength (λ);

I (λ) = Intensity of sunlight at wavelength (λ);

Abs (λ) = Absorbance of extracts at wavelength (λ).

Determination of Antibacterial Activity

Disc Diffusion Assay

Disc diffusion assay was used to determine the antimicrobial activities of olive and fruit extracts (water, acetone and ethyl acetate). *S. enteritidis* RSKK 171, *B. cereus* RSKK 863, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 were growth in Nutrient broth/agar at 37°C. The active cultures of the test microorganisms were washed twice with physiological saline. The concentrations of the culture were adjusted to 0.5 McFarland and then they were spread onto agar medium. After sterile discs were placed on the Petri dishes, 20 µl (4000 µg/disc) of olive fruit and leaf extracts were dropped onto the discs. Petri dishes were incubated for 24 h at 37°C. At the end of the incubation period, the zones around the discs were measured with calipers and recorded. All experiments were done in duplicate.

Determination of minimal inhibition (MIC) and bactericidal (MBC) concentrations with micro-dilution method

MIC and MBC values of the extracts were determined against test microorganisms with micro-dilution method. Test microorganisms were added to each tube containing extract and medium at a concentration of 0.5 McFarland. Then, the tubes containing the mixture were

incubated for 24 h at 37°C. After incubation, the non-growth concentration in the broth tubes was recorded as MIC values. The samples from the tubes were spot dropped on specific agar medium and incubated at 37°C for 24 h. At the end of the incubation period, the extract concentrations preventing the growth of bacteria on solid media were evaluated as MBC values.

RESULTS AND DISCUSSION

The relationship between the radiation intensity at each wavelength and the erythemetogenic effect is given in Table 1. SPF values for olive fruit and leaf extracts were recorded between 0.84-15.35 (Table 2). The highest and lowest SPF effect was determined for the olive leaf acetone (OLA) and the olive fruit water (OFW) extracts. According to Table 3, the highest SPF value (15.35) of OLA extract was found to be 93% of UV blocked (Imam et al., 2015).

Table 1. The relationship between radiation intensity at each wavelength and erythemetogenic effect

λ (nm)	**EE (λ)x I(λ)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	**1.0000**

Table 2. SPF values of Ayvalık Yağlık variety olive fruit and leaf extract

Extracts	**SPF value**
OFW	0.84±0
OLW	2.37±0.04
OFA	1.42±0
OLA	15.35±0.33
OFE	1.00±0
OLE	2.21±0

OFW: Olive fruit water, OLW: Olive leaf water, OFA: Olive fruit acetone, OLA: Olive leaf acetone, OFE: Olive fruit ethyl acetate, OLE: Olive leaf ethyl acetate

Table 3. Percentage of UV blocked by SPF value (Imam et al., 2015)

SPF	Percentage of UV Blocked
2	50
4	75
5	80
10	90
15	93
25	96

In a study, the spectra of the wastewater obtained from the olive mill and the olive (water and ethanol) extracts at 220-400 nm wavelengths were evaluated. Obtained SPF values were determined in the range of 4.3-5.6 (Galanakis et al., 2018). Gönülalan (2017) determined the sun protection potential of methanol extracts of *Mentha piperita*, *Salviaofficinalis, Achillea millefolium* and *A. filipendulina* plants in the range of 290 nm-320 nm. Fang and Bhandari (2010) suggested that natural phenols should be evaluated as sunscreen agents. Olive fruit and leaf can be used as an alternative plant with sun protection feature with its rich phenol variety. In our study, it was determined that olive fruit and leaf have SPF protection potential.

The antibacterial activity of olive fruit and leaf extracts was determined by disc diffusion and micro-dilution methods. The results of disc diffusion revealed the ability of olive fruit and leaf extracts to inhibit the growth of all the tested microorganisms with inhibition zone diameters ranging from 8.9±0.20 mm to 19.4±0.10 mm. Regarding antibacterial activity, the highest inhibition effect was exhibited by OFW against *P. aeruginosa* ATCC 27853 (19.4±0.10 mm). The MIC and MBC values for *P. aeruginosa* ATCC 27853 were determined as 5 mg/ml (Table 4). Among acetone extracts, the highest inhibition activity was recorded on *S. aureus* ATCC 25923 (18.7±0) for OLA extract. MIC and MBC values for *S. aurues* ATCC 25923 were determined as 5 mg/ml (Table 5). OFE and OLE extracts have nearly inhibition zone diameter for *S. aureus* ATCC 25923 (17.78±0.93 mm and 16.80±0.20 mm). MIC and MBC values were determined at similar concentrations from *S. aureus* ATCC 25923 (5 mg/ml) (Table 6).

Table 4. Antimicrobial activity of Ayvalık Yağlık variety olive fruit and leaf water extracts

Test Microorganisms	Inhibition zone diameter (mm)		MIC (mg/ml)		MBC (mg/ml)	
	OFW	OLW	OFW	OLW	OFW	OLW
S. enteritidis RSKK 171	11.0±0.90	9.6±0.40	20	20	20	20
B. cereus RSKK 863	13.2±0.10	12.4±0.10	20	20	80	80
S. aureus ATCC 25923	18.9±0.30	17.0±0.50	10	10	10	10
P. aeruginosa ATCC 27853	19.4±1.00	18.5±1.20	5	5	5	5

Table 5. Antimicrobial activity of Ayvalık Yağlık variety olive fruit and leaf acetone extracts

Test Microorganisms	Inhibition zone diameter (mm)		MIC (mg/ml)		MBC (mg/ml)	
	OFA	OLA	OFA	OLA	OFA	OLA
S. enteritidis RSKK 171	8.9±0.20	12.1±0.30	20	20	80	80
B. cereus RSKK 863	9.9±0	12.0±1.40	20	20	80	80
S. aureus ATCC 25923	16.7±0.10	18.7±0	10	10	10	10
P. aeruginosa ATCC 27853	16.6±1.30	17.8±1.00	5	5	5	5

Table 6. Antimicrobial activity of Ayvalık Yağlık variety olive fruit and leaf ethyl acetate extracts

Test Microorganisms	Inhibition zone diameter (mm)		MIC (mg/ml)		MBC (mg/ml)	
	OFE	OLE	OFE	OLE	OFE	OLE
S. enteritidis RSKK 171	9.30±0.33	9.7±0.10	20	20	40	40
B. cereus RSKK 863	10.44±0.22	9.6±0.60	20	20	80	80
S. aureus ATCC 25923	17.78±0.93	16.80±0.20	10	10	10	10
P. aeruginosa ATCC 27853	16.44±0.04	16.4±1.30	5	5	5	5

Antimicrobials are generally considered as bactericidal if the MBC/MIC ratio is ≤4 and bacteriostatic if the MBC/MIC equation is >4 (Krishnan et al., 2010; Hazen and Kevin, 1998). The ratios obtained for all the test microorganisms were below or equal to 4 which indicated that all olive fruit and leaf extracts were bactericidal against all tested microorganisms (Table 7).

Tablo 7. MBC/MIC ratios of olive fruit and leaf extracts

Test Microorganisms	MBC/MIC					
	OFW	OLW	OFA	OLA	OFE	OLE
S. enteritidis RSKK 171	1	1	4	4	2	2
B. cereus RSKK 863	4	4	4	4	4	4
S. aureus ATCC 25923	1	1	1	1	1	1
P. aeruginosa ATCC 27853	1	1	1	1	1	1

Korukluoglu et al. (2010) obtained olive leaf samples from the Trilye region of Mudanya, Turkey. Olive leaf samples were extracted with various solvents (ethyl alcohol, diethyl ether,

acetone and water) using the soxhlet method. Olive leaf extracts investigated their antibacterial activities on 6 Gram-negative bacteria and 11 Gram-positive bacteria determinetion MIC and MBC values. All the tested bacterial strains presented varying degrees of antibacterial activity to the extracts of olive leaves. However, the water extract had no antimicrobial activity against any of the test bacteria in their study. In the present study, OFW and OLW extracts showed antibacterial activity against the tested bacteria.

CONCLUSION

The SPF values and antibacterial activity of olive fruit and leaf extracts of Ayvalık Yağlık variety prepared with different solvents (water, acetone and ethyl acetate) were investigated. SPF values of the extracts were determined to be high. In addition, it has been observed that olive fruit and leaf extracts have antibacterial activity against some food-borne and clinical pathogens. It has been determined that olive fruit and leaf extracts may have the potential to be used as natural sunscreen ingredients in cosmetics and as an antibacterial additive in the food industries. It has been foreseen that the fruit and by-products of olive, which is produced in our country and in the world, can be evaluated in various industries.

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INVESTIGATION OF THE USAGE POTENTIAL OF OLIVE AND JUJUBE EXTRACTS AS NATURAL ALTERNATIVES TO CHEMICAL CONTAINING SUN CREAM

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ABSTRACT

Artificial sunscreens are of interest to protect from photoaging, sun-induced skin burns and carcinogenic effects. However, the efficacy of the ingredients of chemical and artificial sunscreen creams is of concern due to their photostability, safety, toxicity, and also the damage they cause to marine ecosystems. Nowadays, many plant extracts used as UV blocking agents are considered as alternatives due to the side effects of chemical additives. The fruits of *Olea europaea* L. (Olive) and *Ziziphus jujuba* (Jujube) are used all over the world as both herbal medicine and food for their health benefits. In the study, sun protection factors (SPF) values of olive and jujube fruit extracts were determined spectrophotometrically *in-vitro*. The extracts were prepared using hot water bath (HWB) and sonicator devices (SN). The olive extracts were obtained in ethyl acetate and acetone solvents using with HWB. The extracts of the jujube were obtained using HWB and SN methods with using hexane solvent. Among the extracts, the highest SPF value was determined as 17.53 in olive fruit acetone extract and the lowest SPF was recorded as 2.21 in the olive fruit ethyl acetate extract. It was recorded as 6.36 for the jujube fruit hexane extract (HWB) and 8.57 for the jujube fruit hexane extract (SN). The results showed that the olive and jujube fruit extracts could have potential use as a natural alternative to chemical containing sunscreen creams.

Keywords: *Olea europaea*, *Zizyphus jujuba*, ultraviolet radiation, hot water bath, sonicator, solar protection factor (SPF)

INTRODUCTION

Jujube and olive have been used for centuries for health purposes. Jujube (*Zizyphus jujuba* Mill.), a plant of Chinese origin, is natural spread to Russia, India, North Africa, Southern Europe, the Middle East and Anatolia (Reichl, 1991). Jujube has been traditionally used for various purposes since ancient times. In a study, galactose, rhamnose, glucorinic acid, mannose carbohydrates were determined in jujube fruit and it was determined that jujube tea is antipyretic, pain and stress reducer (Lee et al., 2003, Williams et al., 2004). The extracts and pure active chemical compounds of jujube can be used in foods, pharmaceutical products and as natural antioxidants, antibiotics, anticancer agents and preservatives (Al-Saeedi et al., 2017). Jujube fruits contain abundant nutrients such as carbohydrates, protein, fat, minerals, vitamins (especially vitamin C) and phenolics (Li et al. 2007). It is a very popular fruit due to its high vitamin and phenolic compounds value. The olive (*Olea europaea* L.) is a member of the Oleaceae family and has a wide distribution in the world (Arslan-Karaboğa et al., 2017). It is

reported that the homeland of olives is Upper Mesopotamia, which includes South Asia and the Southeastern Anatolia Region (Owen, et al., 2000). Olive is an evergreen plant and cultivated in Mediterranean climate. In our country, olive cultivation has spread in the Marmara, Aegean, Mediterranean, Southeastern Anatolia and Black Sea (Pala, et al., 2001). The cultivation of olive and its by-products in such a wide area increases both its economic and social value. Studies have reported that olive fruit and leaves have hypoglycemic, hypercholesterolemic, antihypertensive, antibacterial and antioxidant and cardioprotective effects (Gonzalez et al., 1992; Hansen et al., 1996; Upadhyay et al., 2010; Aliabadi et al., 2012; Nora et al., 2012; Aytul, 2010). Phenolic compounds such as oleuropein, hydroxytyrosole, verbascoside, apigenin 7-glucoside and luteloin 7-glucoside identified in olive fruit (Gürbüz and Öğüt, 2018). Extracts of olive tree by-products can be used in the cosmetic, medicine, pharmaceutical and food industries as they contain important antioxidants such as phenolic compounds and can prevent oxidative degradation (Jemai et al., 2009; Bouaziz et al., 2010).

Flavonoids are a class of natural products available in fruits, vegetables, and potables; synthesized by plants; and exhibiting many important effects such as protection against pathogens and ultraviolet radiation B (UV-B) radiation (de Cooman and ark., 1998). It is divided into three regions as UV-A (320-400 nm), UV-B (290-320 nm) and ultraviolet radiation C (UV-C) (200-290 nm). 6% of the total ultraviolet radiation (UV) radiation reaching the earth's surface is UV-B and 94% is ultraviolet radiation A (UV-A) (Allen and Bain., 1994).

Sunscreens protect the skin by minimizing the harmful effects of harmful UV rays from the sun. About 90% of skin cancer cases are associated with exposure to the sun's harmful UV rays. The UV radiation irradiating the earth is absorbed by the ozone layer. As a result, UVA and UVB reach the earth. UVA reaches the earth and contributes to premature skin aging and skin cancer, while UVB causes sunburn (Shanbhag et al., 2019). The main purpose of sunscreens is to protect the skin from UVA and UVB rays and preserve the skin's moisture content and its own natural oils that can be lost when exposed to sunlight (Kale et al., 2011). In addition, UVB radiation can cause pigmentation and burn-like changes (DeBuys et al., 2000).

The effectiveness of a sunscreen is usually expressed by its SPF value, which is the ratio of the UV energy required to produce the minimum erythema dose (MED) in the protected skin to the unprotected skin (Mbanga and et al. 2015). Recent research has revealed that applying most synthetic sunscreens to the skin produces undesirable effects in the short or long term. Therefore, there is a worldwide need for effective and safe UV filters, especially of natural origin (Ahmady et al., 2020). In this study, SPF values of jujube and olive fruit extracts investigated the potential use as a natural additive in sunscreens for cosmetics industry.

MATERIAL AND METHOD

Supply of Fruit Samples

Jujube and olive fruit samples were obtained from Hatay and Izmir Olive Research Institute, respectively.

Preparation of Extracts

The supplied olive and jujube fruits were dried and then ground with a Waring Blender. The ground jujube fruits were extracted separately in a hexane solvent in a hot water bath (at 69°C for 36 hours) and in a SN device. The ground olive fruits were separately in acetone and ethyl acetate solvents in a HWB. The ground jujube fruits were extracted using the hexane

solvent with SN on ice. After filtered with Whatman paper, the solvents were allowed to evaporate. The crude extracts were stored under dry conditions at 4 °C.

Determination of Sun Protection Factor of Extracts

The SPF values of the prepared dry extracts were determined in vitro. The extracts were adjusted to a concentration of 2 mg/ml in ethanol (96%). The samples were then measured in the spectrophotometer at 5 nm intervals in the range of 290-320 nm. The results were recorded by substituting the values in the Mansur equation.

Sun Protection Factor (SPF) spectrophotometric = CF x $\sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs\,(\lambda)$ **(1)**

CF=Correction factor (=10), **EE(λ)=**Erythmogenic effect of radiation with wavelength (λ), **Abs (λ)=**spectrophotometric absorbance values at wavelength λ. The values of **EE**×I [13] are constant.

Table 1. The relation between radiation intensity at each wavelength and erythemetogenic effect

λ (nm)	**EE (λ)x I(λ)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	**1.0000**

RESULTS AND DISCUSSION

Jujube and olive fruits were extracted using the hot water bath and sonicator device. The SPF value of the crude extracts was investigated. The spectrometric values read in the range of 290-320 nm in the spectrophotometer were replaced in the Mansur equation and the results were recorded. The results are shown in Table 2. The highest SPF value was recorded as 17.53 in olive acetone HWB fruit extract. The lowest value was recorded as 2.21 in the ethyl acetate HWB fruit of olive.

Table 2. Sun Protection Factors of Jujube and Olive Fruit Extracts

Extracts	**SPF Value**
Jujube Hexane HWB fruit	6.36±0.01
Jujube Hexane SN fruit	8.57±0.05
Olive Acetone HWB fruit	17.53±0.09
Olive Ethyl acetate HWB fruit	2.21±0

In a study conducted by Sutar and Chaudhari (2020), SPF evaluation of the aqueous and methanolic extracts of *Aloe barbadensis miller* and *Cocos nucifera* was performed by UV spectroscopic method. The methanolic extract of *Cocos nucifera* was found to have an SPF of 1.305 and 3.207 at concentrations of 200µg/ml and 400µg/ml, respectively. The aqueous extract of *Aloe barbadensis miller* was found to have an SPF of 0.148 and 0.082 at concentrations of 200µg/ml and 400µg/ml, respectively. The methanolic extract of *Aloe barbadensis miller* was found to have an SPF of 0.604 and 1.961 at concentrations of 200µg/ml and 400µg/ml, respectively (Sutar and Chaudhari, 2020). In another study, the SPF value of some vegetable oils was determined. As a result of the study, the highest SPF value was recorded as 7.54 in olive oil, and the lowest SPF value was recorded as 0.24 in rose oil (Kaur and Saraf, 2010).

CONCLUSIONS

Nowadays, due to the side effects of chemical containing sunscreens, a tendency towards natural cream formulations has been raised. It has been determined that jujube and olive fruits extracts can be a natural alternative to chemical formulations for cosmetics industry.

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DETERMINATION OF SOME CHARACTERISTICS OF MIXTURES OF GELEMEN CLOVER (Trifolium meneghinianum Clem.) AND SOME ANNUAL RYEGRASS (Lolium multiflorum L.)

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The aim of this study is to determine the mixing ratio, plant height and hay yields in the Gelemen Clover + annual ryegrass mixture to be grown in Samsun conditions and to investigate the possibilities of placing especially one-year forage crops in the rotation systems to be applied. In the study, Ilkadım and Kocayaşar varieties, which are annual ryegrass varieties, and Yörem55 varieties, which are Gelemen Clover varieties, were used. The experiment was carried out in the Çarşamba district trial area of the Black Sea Agricultural Research Institute in the 2020-2021 growing season. In the research; 3 lean sowing processes, 100% for Gelemen clover (GC), Annual Ryegrass (ARGİ) 100%, Annual ryegrass (ARGK) 100%; There are 7 different mixtures of one-year grass (ARGİ) and 7 different mixtures with One-year Grass (ARGK). The study was planned to have a total number of 17 procedures. Mixing ratios are GC 80% + ARGİ 20%, GC 70% + ARGİ 30%, GC 60% + ARGİ 40%, GC 50% + ARGİ 50%, GC 40% + ARGİ 60%, GC 30% + ARGİ 70%, GC 20% + ARGİ 80%), GC 80% + ARGK 20%, GC 70% + ARGK 30%, GC 60% + ARGK 40%, GC 50% + ARGK 50%, GC 40% + ARGK 60%, GC 30% +ARGK 70%, GC 20% + ARGK 80%. The hay harvest was made during the 50% flowering period of the Gelemen clover.

The plant heights in the study were determined as 63.96 – 97.00 cm in the Gelemen clover, 71.53 – 126.63 cm in the annual grass cultivar İlkadım, and between 115.96 – 123.63 cm in the Kocayaşar cultivar. The highest fresh hay yield was determined in 4066 kg/da and 20% GC + 80% ARGI mixture, while the lowest wet grass yield was determined in 50% GC + 50% ARGK mixture (2227.7 kg/da). Dry hay yield varied between 478.4 – 911.5 kg/da. The highest hay yield was obtained from the mixture of 20% GC + 80% ARGI.

Key Words: Gelemen Clover, Annual Ryegrass, Hay Yield, Mixture Ratio

INTRODUCTION

Soil and climate conditions exhibit great diversity in Turkey and such diversity potentially allows farmers to cultivate several forage crops of those grown worldwide. However, the actual number of cultivated forage crops is quite low in Turkey. Therefore, there is a quite high deficit in forage supply in the country. lntercropped cultivation of legumes and grasses may provide higher yields and a more stable forage source rich in carbohydrate and protein for livestock feding (Hatipoglu et al. 2005). However, to have higher yields and more stable herbage from legumes + grasses in intercropping systems, the mixture rates of each component should be

well-adjusted (Shoaib et al. 2016). For the Samsun region, Gelemen clover (*Trifolium meneghinianum* Clem.) and annual ryegrass (*Lolium multiflorum* L.) are recommended as alternative crops to meet the forage deficit. One of the most important forage crops is annual ryegrass (AR) (*Lolium multiflorum* Lam.), which is a cool-season grass that is suitable for quality herbage production on account of its rich protein, minerals, and water-soluble carbohydrate content (Kusvuran et al. 2014). Gelemen clover (PC) (*Trifolium meneghinianum* Clem.) and annual ryegrass (*Lolium multiflorum* L.) can be grown for hay production until the end of May on land left uncultivated after the harvest of summer crops in October, without detriment to the main crop. The present study was conducted to determine the mixing ratio, plant height and hay yields in the Gelemen Clover + annual ryegrass mixture to be grown in Samsun conditions and to investigate the possibilities of placing especially one-year forage crops in the rotation systems to be applied.

MATERIALS AND METHODS

The experiment was carried out in the Çarşamba district trial area of the Black Sea Agricultural Research Institute in the 2020-2021 growing season. In the study, Ilkadım and Kocayaşar varieties, which are annual ryegrass varieties, and Yörem55 varieties, which are Gelemen Clover varieties were used. In the research; 3 lean sowing processes, 100% for Gelemen clover (GC), Annual Ryegrass (ARGİ) 100%, Annual ryegrass (ARGK) 100%; There are 7 different mixtures of one-year grass (ARGİ) and 7 different mixtures with One-year Grass (ARGK). The study was planned to have a total number of 17 procedures. Mixing ratios are GC 80% + ARGİ 20%, GC 70% + ARGİ 30%, GC 60% + ARGİ 40%, GC 50% + ARGİ 50%, GC 40% + ARGİ 60%, GC 30% + ARGİ 70%, GC 20% + ARGİ 80%), GC 80% + ARGK 20%, GC 70% + ARGK 30%, GC 60% + ARGK 40%, GC 50% + ARGK 50%, GC 40% + ARGK 60%, GC 30% +ARGK 70%, GC 20% + ARGK 80%. The hay harvest was made during the 50% flowering period of the Gelemen clover. The experimental soils had clay texture (54.75% clay), were neutral in pH (6.86), and medium in organic matter (2.93%). During the vegetation period (from November to May), total precipitation was 369.9 mm and mean temperature was 9.4 °C. Experiments were conducted using a randomized complete block design. Data were subjected to statistical analyses with SPSS 17.0 software. The differences amongst the mean values were calculated according to DUNCAN test (Gülümser et. l., 2013)

RESULTS AND DISCUSSION

Fresh hay yields, dry hay yields, plant height are provided in Table 1. Significant differences observed traitments between fresh hay yield, dry hay yield and plant height.

Table 1. The result obtained from Gelemen Clover and annual ryegrasses (İlkadım and Kocayaşar cultıvar)

Mixtures	Fresh Hay Yield* (kg/da)	Dry Hay Yield* (kg/da)	Gelemen Clover Dry Hay Yield*	Ryegrass Hay Yield*	Other Families Hay Yield*	Gelemen Clover Plant Height* (cm)	Ryegrass Plant Height (cm)
20GC+80ARGİ	4066.0a	911.5a	133.8bc	770.3ab	25.2b	64.0b	123.9
30GC+70ARGİ	3821.5**ab**	784.7ac	127.7bc	620.4ac	54.6b	65.0b	121.0
40GC+60ARGİ	3341.7ab	668.7ac	77.4bc	553.9ac	55.4b	65.3b	119.2
50GC+50ARGİ	3161.8ab	656.2ac	37.9c	609.3ac	44.9b	66.3b	120.8
60GC+40ARGİ	3367.4ab	685.4ac	97.6bc	552.3ac	53.5b	68.4ab	120.7
70GC+30ARGİ	3159.0ab	542.4bc	154.9b	340.9c	46.5b	71.5ab	115.9
80GC+20ARGİ	3160.4ab	642.3ac	102.1bc	445.7ac	112.4b	72.1ab	126.6
20GC+80ARGK	3403.4ab	766.2ac	50.6c	695.6ac	37.9b	73.4ab	116.1
30GC+70ARGK	2663.2ab	546.5bc	51.9c	490.6ac	22.0b	73.9ab	116.6
40GC+60ARGK	2358.3ab	497.8bc	88.3bc	401.0bc	25.8b	74.5ab	119.3
50GC+50ARGK	2227.8**b**	478.4bc	82.0bc	386.8c	27.6b	75.5ab	117.0
60GC+40ARGK	2297.9ab	488.2bc	92.3bc	382.5c	13.4b	80.9ab	116.0
70GC+30ARGK	2788.9ab	559.0bc	116.4bc	433.7ac	44.9b	81.0ab	122.5
80GC+20ARGK	2456.9ab	470.5c	71.0bc	379.6c	19.9b	84.2ab	117.6
ARGİ	3423.6ab	691.6ac		668.3ac	59.4b		124.0
ARGK	3469.4ab	832.3ab		780.5a	69.8b		123.6
GC	3306.9ab	464.0 c	255.9a		208.0a	97.0a	

*Means with the same letter in the same row and in the same column are not significantly different at 0.05.

In this study, the highest fresh hay yield was obtained from the mixture of 20GC+80ARGİ with 4066.0 kg/da, while it was in the same statistical group as many other treatments. The lowest fresh hay yield was obtained from 50GC+50ARGK mixture. The highest dry hay yield was obtained from the mixture of 20GC+80ARGİ with 911.5 kg/da. The lowest dry hay yield was obtained from a mixture of clover and 80GC+20ARGK (464.0 and 470.5 kg/da, respectively). Generally, the hay yield of the mixtures in which Ilkadım annual grass variety was included was higher. When the hay yields of the plants in the mixtures are examined separately; The highest hay yield was obtained from pure Gelemen Clover. In the mixtures, the highest yield of clover was obtained in the mixture of 70GC+30ARGİ. The highest annual grass hay yield was determined in the 20G+80İ mixture of both İlkadım and Kocayaşar cultivars. . Soya et al. (1981) reported increasing yields in PC until the beginning of flowering and decreasing yields later on. Shoaib et d. (2016) reported higher yields for intercropping systems

than for pure stands. The highest amount of plants belonging to other families was determined in the pure gelemen clover plots. In the mixtures, the highest amount of plants belonging to other families was determined in the 80GC+20ARGİ mixture, and it is statistically in the same group as the other treatments. The longest plant height in the Gelemen clover was determined in the pure Gelemen clover plots (97.0 cm), and in the mixtures of 80GC+20ARGK (84.2 cm). The longest plant height of Ilkadım annual grass variety was determined at 126.6 cm in 80GC+20ARGI mixture, and in Kocayaşar variety with 123.6 cm and 122.5 cm in pure and 80GC+20ARGK mixture.

Conclusions

It was concluded in this study that, based on hay yield and plants belong to other families between mixture ratios should be selected as either 20GC+80ARGİ or 320GC+80ARGK. But, to clear the recommendation this type study should carry out at least 2 year.

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EVALUATION OF CO2 EMISSIONS IN A NATURAL GAS LIQUEFACTION PROCESS IN WESTERN ALGERIA, AND THEIR IMPACT ON CLIMATE CHANGE

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Introduction

Climate change is a topical issue that has emerged in the international arena. Industrial activities have come under fire since global greenhouse gas (GHG) emissions have increased. The greenhouse effect, a perfectly natural phenomenon essential to our existence, has undergone rapid change due to the increase in human activities. The amplification of the latter, since the industrial age, has helped to increase the concentration of greenhouse gases (GHGs) in the atmosphere, which has caused the temperature to rise and the earth to warm up. This phenomenon could be at the origin of climate change throughout the world, and which manifests itself in various forms: heatwaves in summer which generate serious water shortages, winter without snow, climate disruption, flooding in a region with increased risk of heavy rainfall while another experiences severe droughts. Indeed, this phenomenon has become a reality.

These events have economic, environmental and social impacts, often serious and whose challenge is essential to meet. Therefore, it is necessary to put in place a strategy to mitigate GHG emissions, particularly in industrial settings, as well as sustainable management. This strategy should be oriented on the basis of efficient research and extensive knowledge on climate change and its impacts and on measurements of emissions at the level of emitting sources.
The interest of this study was to estimate the quantities of CO2 emitted by the natural gas liquefaction process and assess its toxicity to the health of the population of Arzew and see the impact on the climate.

Material and methods

Determination of the study area

The GL1 / Z natural gas liquefaction complex (liquefied gas) is located in the town of Béthioua in the industrial zone of Arzew, about 6 km south-east of the city of Arzew and 35 km north - East of Oran, wilaya of Oran, capital of the wilaya of the same name on the Gulf of Oran (Oran), located in the North-West of Algeria (SONATRACH, 1994). Located 432 km from the capital Algiers, Oran is the second largest city in Algeria and now has around 2,000,000 inhabitants.

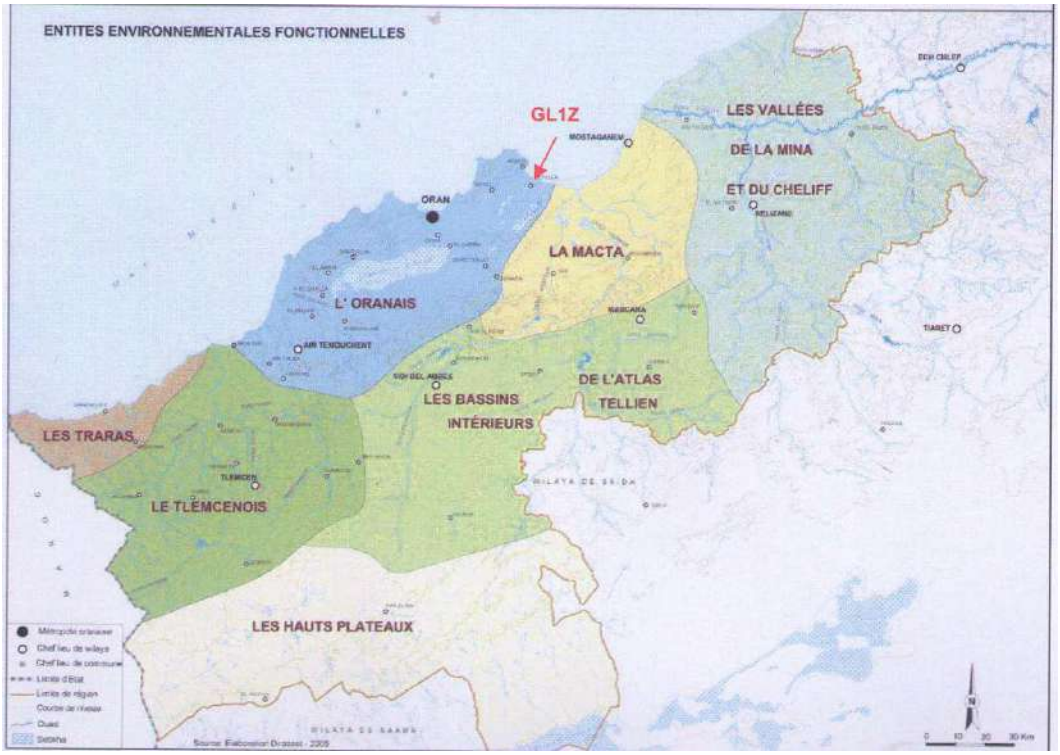

Fig. 1. Situation of GL1Z in the Oran; (SONATRACH, 1994) (GL1Z: Study area.)

Methodology

Natural gas liquefaction process

The GL1 / Z Natural Gas Liquefaction Complex is designed to transform a very large amount of natural gas from a gaseous state to a liquid state.

The GL1 / Z complex comprises three (03) essential zones (*MW. Pullman KELLOGG COMPANY; 1994*):

1st-ZONE: Utilities

Utilities constitute an important area within the GL1 / Z complex. They ensure the supply of all needs during the start-up and normal operation of the liquefaction trains.

A / Energy source: Water vapor is the energy source chosen for the complex (three high pressure 62 bar boilers and one 4.5 bar low pressure boiler)

B / Cooling source: Water is the cooling source transported by a set of six (06) high power pumps of 175,000 m3 / h.

C / Electricity production: Electricity production is provided by three (03) alternators driven by steam turbines. The turbo-alternators provide 36 MW of energy per generator plus one (01) Sonelgaz connection.

D / Desalination unit: It produces distilled water for boiler feed.

E / Compressed air production: Much of the complex's instrumentation is pneumatic. The production of compressed air is ensured by a set of four (04) compressors and an emergency air compressor.

2nd ZONE: Process

This zone is made up of six (06) trains which produce 8000 m3 / day / train of LNG (liquefied natural gas).

Each train has its own steam production, its own hydrocarbon decarbonation, drying and liquefaction section.

3rd ZONE: Terminal

This is the storage and loading area, it contains three (03) LNG tanks with a capacity of 100,000 m3, one (01) gasoline tank with a capacity of 14,500 m3.

- An LNG pumping station with a capacity of 10,000 m3 / h.
- Two (02) shipping docks with ten (10) loading arms.

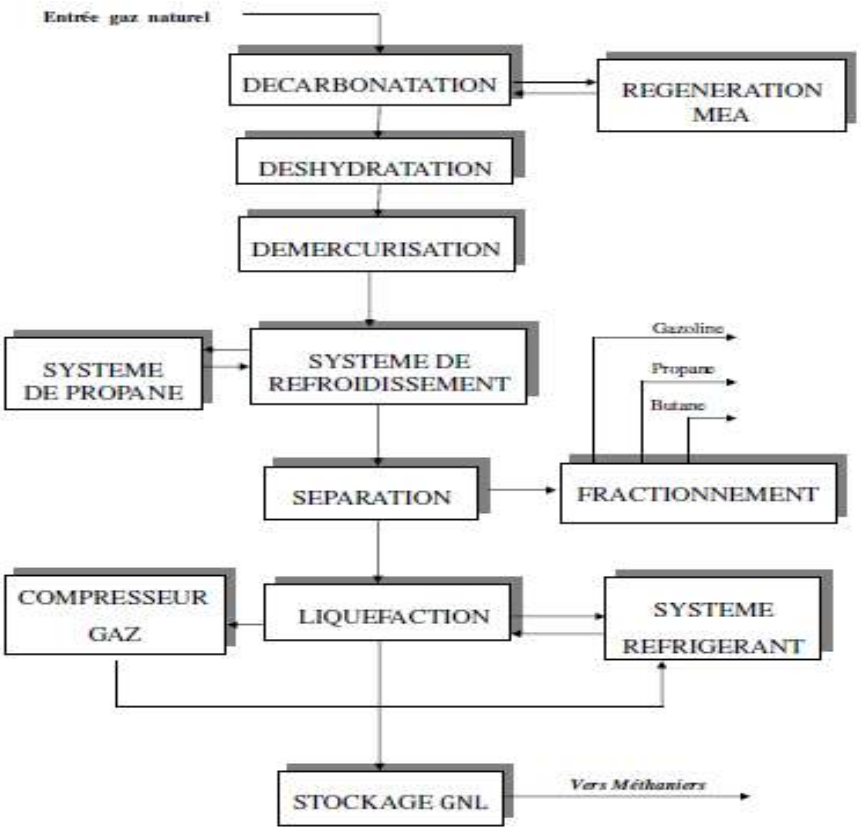

Fig. 2. Principle of natural gas liquefaction

Estimation of CO2 emissions from combustion plants

It is based on the methodology described by the French decree of March 31, 2008 relating to the verification and quantification of the emissions declared within the framework of the greenhouse gas emission allowance trading system for the period 2016. -2021.

In the absence of direct measurement of CO2 emissions from combustion plants, they are calculated according to the following formula:

CO2 emissions (tCO2) = CC x PCI * x FE x FO

(*) For natural gas, PCS can be used.

With:

CC: quantity of fuel consumed during the reporting period (t or Nm3);

PCI: lower calorific value of the fuel (TJ / t or TJ / Nm3);

PCS: higher calorific value of the fuel (MW.h / Nm3);

FE: fuel emission factor (tCO2 / TJ PCI or tCO2 / MW.h PCS for natural gas);

FO: fuel oxidation factor.

The operator can also use emission factors expressed in tCO2 / Nm3 fuel or CO2 / t fuel, the formula to apply is then as follows:

CO2 emissions = CC x EF x FO

For natural gas, it is also this formula that applies when the operator uses a quantity of fuel consumed (CC) expressed in MW.h PCS and an emission factor expressed in KgCO2 / MW.h PCS.

Decarbonation section

For the evaluation of the rate of CO2 discharged into the atmosphere in the decarbonation section in the GL1 / Z complex, the conventional titration method was used, which consists of using an electronic titrator, in order to regularly assess the CO2 rate. emitted to the atmosphere. An estimate of CO2 was made based on an approximate calculation.

Sampling from the decarbonation site requires compulsory safety clothing. For each train, a bottle of MEA (MonoEthanolAmine) rich in CO2 (column B) is taken from the regenerator inlet; and a bottle of MEA low in CO2 (column L) is taken at the outlet of the regenerator.

A 5ml aliquot from the colorless rich MEA bottle is placed in an Erlenmeyer flask and another 5ml aliquot from the colorless poor MEA bottle is placed in another Erlenmeyer flask. Two drops of red phenophthalein are added to each aliquot and titration is carried out with an H2SO4 solution until the color changes from red to transparent, then the volume of CO2 recorded on the electronic titrator is recorded.

Then to each aliquot are added 3 drops of red mixed indicator. A second titration is carried out with a solution of H2SO4 until the color changes from red to green. Then the MEA volume is raised.

The volume of CO2 released to the atmosphere is calculated as the difference between the volume of the CO2-rich MEA and the volume of the CO2-poor MEA.

Estimates of CO2 emissions by mass balance

The rate of CO2 emitted to the atmosphere during the decarbonation section is determined according to the following two formulas:

Quantity of decarbonated gas = quantity of gas entering the complex - quantity of raw gas self-consumed by the boilers.

Quantity of CO2 removed from the gas and released to the atmosphere by the decarbonation step = (Average content of incoming gas in CO2 - Objective of residual content of decarbonated gas in CO2) / 100 x Mass of decarbonated gas per month.

Estimation of CO2 emissions from flared gases

The estimate of CO2 emissions from flared gases is based on the methodology described in the French decree of March 31, 2008 relating to the verification and quantification of emissions declared under the emissions trading system of greenhouse gases for the period 2016-2021.

CO2 emissions are calculated from the quantity of gas burnt with a flare [Nm3] and the carbon content of the burnt gas (inherent CO2 included), according to the formula:

CO2 emissions = activity data x FE x FO

With:
Activity data = quantity of gas burned = quantity of gas flared;
FE = emission factor;
FO = oxidation factor.
The flared volumes are therefore calculated by mass balance:

Quantity of gas flared = the total quantity of gas self-consumed - the quantity self-consumed by the boilers.

Results and discussion

Table 1. Results of burnt gas in boilers over the period May-June 2021.

Amount of Gas consumed in the boilers		
Month	Cm3	% du GN entering
May 2021	107674943	13,28%
June 2021	100187963	12,64%

The estimate by calculation, CO2 emissions are summarized in Table 1

CO2 emissions = CC x EF x FO

CC: over the period January - June 2021, i.e. 18 months (between January 2020 and May 2021, the results will not be mentioned), the total quantity of self-consumed gas in the boilers is 2,069,955,993 m3, i.e. approximately 165,596,4794 m3 of fuel gas (80%) and 413 991 199 m3 of raw gas (20%).

The average annual PCS is 10.67 KWh / Nm3 for natural gas and 9.36 KWh / Nm3 for fuel gas.

The average monthly consumption of the boilers expressed in PCS is therefore:
- Natural gas: 413991199/18 x 10.67 / 1000 = 245405 MWh PCS.
- Fuel gas: 1655964794/18 x 9.36 / 1000 = 861102 MWh PCS.

Emission Factor: there are no specific data or measurements relating to the site's emission factors. We will consider the generic emission factors given in the decree:
- Natural gas: 185 kg CO2 / MWh PCS.
- Fuel gas: no specific data available; we will retain the same value as for the GN.
Oxidation factor: in the absence of specific data for the site, the general value will be used for gaseous fuels (0.995).

CO2 emissions = (245405 + 861102) x 185/1000 x 0.995 = 203,680 tonnes CO2 / month = 2,444,160 tonnes CO2 / year.

After calculating the CO2 emissions, we have retained that:

- ✓ The quantity of CO2 emitted by combustion plants per month is 203,680 tonnes.
- ✓ The quantity of CO2 emitted by combustion plants per year is 2,444,160 tonnes.

These amounts are found to be significant.

Rate of CO2 released to the atmosphere

Tables 2, 3, 4 and 5 give the results of the MEA titration with H2SO4 for all trains.

Table 2. MEA titration results with H2SO4 for train 100 and percentage of CO2 released to the atmosphere during the month of May.

	MEA rich on CO_2		MEA poor on CO_2		%CO_2 rehected to atmosphere
	MEA%	CO_2%	MEA%	CO_2%	
01 MAY 2021	10,38	2,84	10,38	0,74	2,1
02 MAY 2021	11,00	4,80	11,00	0,53	4,27
03 MAY 2021	12,38	5,22	12,38	1,02	4,2
04 MAY 2021	11,88	4,31	11,88	0,39	3,92
05 MAY 2021	12,00	5,15	12,00	1,30	3,85
06 MAY 2021	10,63	4,17	10,63	0,60	3,57
07 MAY 2021	12,50	5,22	12,50	1,44	3,78
08 MAY 2021	13,00	4,59	13,00	1,09	3,5
09 MAY 2021	12,00	4,45	12,00	0,11	4,34
10 MAY 2021	12,88	4,66	12,88	1,02	3,64
11 MAY 2021	13,25	4,87	13,25	1,37	3,5
12 MAY 2021	13,12	4,92	13,12	1,41	3,51
13 MAY 2021	12,88	4,52	12,88	1,02	3,5
14 MAY 2021	12,38	4,94	12,38	1,16	3,78
15 MAY 2021	12,63	4,80	12,63	1,23	3,57
16 MAY 2021	12,13	4,73	12,13	0,95	3,78
17 MAY 2021	12,63	4,41	12,63	0,41	4,00
18 MAY 2021	12,75	5,04	12,75	1,27	3,77
19 MAY 2021	12,58	5,32	12,58	1,27	4,05
20 MAY 2021	12,13	4,80	12,13	1,27	3,53
21 MAY 2021	12,75	5,15	12,75	1,37	3,78
22 MAY 2021	13,03	4,81	13,03	1,24	3,57
23 MAY 2021	12,16	4,56	12,16	1,06	3,5
24 MAY 2021	12,34	4,87	12,34	0,54	4,33
25 MAY 2021	12,78	3,96	12,78	0,89	3,07
26 MAY 2021	12,75	4,73	12,75	1,52	3,21
27 MAY 2021	12,88	4,97	12,88	1,43	3,54
28 MAY 2021	13,12	5,01	13,12	1,24	3,77
29 MAY 2021	12,65	4,56	12,65	1,34	3,22
30 MAY 2021	12,98	4,30	12,98	1,67	2,63
31 MAY 2021	12,83	4,25	12,83	1,60	2,65

Table 3. MEA titration results with H2SO4 for train 200 and percentage of CO2 released to the atmosphere during the month of May.

	MEA rich on CO_2		MEA poor on CO_2		%CO_2 rejected on atmosphere
	MEA%	CO_2%	MEA%	CO_2%	
01 MAY 2021	8,88	1,86	8,88	0,88	0,98
02 MAY 2021	10,00	3,75	10,00	1,23	2,52
03 MAY 2021	9,75	3,75	9,75	1,16	2,59
04 MAY 2021	9,88	3,05	9,88	0,74	2,31
05 MAY 2021	10,38	4,38	10,38	1,30	3,08
06 MAY 2021	9,88	2,91	9,88	0,81	2,1
07 MAY 2021	9,75	2,70	9,75	0,74	1,96
08 MAY 2021	10,50	3,40	10,50	1,86	1,54
09 MAY 2021	11,13	3,82	11,13	1,02	2,8
10 MAY 2021	10,75	3,82	10,75	1,02	2,8
11 MAY 2021	11,00	3,96	11,00	1,16	2,8
12 MAY 2021	10,75	4,06	10,75	1,18	2,88
13 MAY 2021	9,25	3,96	9,25	0,81	3,15
14 MAY 2021	9,00	3,68	9,00	1,16	2,52
15 MAY 2021	8,63	3,61	8,63	0,67	2,94
16 MAY 2021	10,00	3,82	10,00	0,67	3,15
17 MAY 2021	10,38	3,68	10,38	0,46	3,22
18 MAY 2021	10,25	4,17	10,25	0,78	3,39
19 MAY 2021	10,88	4,31	10,88	0,88	3,43
20 MAY 2021	9,5	3,47	9,5	1,23	2,24
21 MAY 2021	9,00	3,41	9,00	1,09	2,32
22 MAY 2021	8,13	3,40	8,13	1,12	2,28
23 MAY 2021	7,25	2,28	7,25	0,95	1,33
24 MAY 2021	8,38	2,91	8,38	0,74	2,17
25 MAY 2021	11,25	4,10	11,25	1,30	2,8
26 MAY 2021	9,00	3,40	9,00	0,95	2,45
27 MAY 2021	8,75	3,19	8,75	0,60	2,59
28 MAY 2021	10,00	4,03	10,00	1,76	2,27
29 MAY 2021	9,00	3,40	9,00	0,95	2,45
30 MAY 2021	8,00	3,33	8,00	1,09	2,24
31 MAY 2021	9,54	4,20	9,54	0,74	3,46

Table 4. MEA titration results with H2SO4 for train 300 and percentage of CO2 released to the atmosphere during the month of May.

	MEA rich on CO_2		MEA poor on CO_2		%CO_2 rejected on atmosphere
	MEA%	CO_2%	MEA%	CO_2%	
01 MAY 2021	13,50	5,15	13,50	2,28	2,87
02 MAY 2021	14,75	6,55	14,75	2,70	3,85
03 MAY 2021	15,00	6,76	15,00	2,77	3,99
04 MAY 2021	14,75	6,48	14,75	2,07	4,41
05 MAY 2021	13,63	3,34	13,63	2,42	0,92
06 MAY 2021	14,25	5,71	14,25	2,14	3,57
07 MAY 2021	15,00	6,20	15,00	2,28	3,92
08 MAY 2021	14,75	5,22	14,75	2,28	2,94
09 MAY 2021	15,13	5,85	15,13	2,63	3,22
10 MAY 2021	14,88	6,23	14,88	2,00	4,23
11 MAY 2021	15,00	6,64	15,00	2,56	4,08
12 MAY 2021	13,75	5,65	13,75	2,35	3,3
13 MAY 2021	16,13	6,99	16,13	2,49	4,5
14 MAY 2021	15,50	6,42	15,50	2,70	3,72
15 MAY 2021	14,13	5,36	14,13	2,14	3,22
16 MAY 2021	13,75	5,87	13,75	2,42	3,45
17 MAY 2021	14,63	6,41	14,63	2,28	4,13
18 MAY 2021	15,00	6,27	15,00	2,54	3,73
19 MAY 2021	15,25	6,47	15,25	2,28	4,19
20 MAY 2021	13,38	6,34	13,38	2,35	3,99
21 MAY 2021	13,13	5,71	13,13	1,51	4,2
22 MAY 2021	14,38	5,92	14,38	2,35	3,57
23 MAY 2021	15,13	7,04	15,13	1,93	5,11
24 MAY 2021	14,50	6,47	14,50	2,35	4,12
25 MAY 2021	15,63	6,13	15,63	2,28	3,85
26 MAY 2021	14,25	6,06	14,25	2,42	3,64
27 MAY 2021	14,00	6,13	14,00	2,07	4,06
28 MAY 2021	14,50	5,99	14,50	2,42	3,57
29 MAY 2021	14,38	5,57	14,38	2,87	2,7
30 MAY 2021	13,98	5,36	13,98	2,19	3,17
31 MAY 2021	14,25	5,99	14,25	1,78	4,21

Table 5. MEA H2SO4 titration results for train 500 and percentage of CO2 released to the atmosphere during the month of May.

	MEA rich on CO_2		MEA poor on CO_2		%CO_2 rejected on atmosphere
	MEA%	CO_2%	MEA%	CO_2%	
01 MAY 2021	13,63	5,92	13,63	2,70	3,22
02 MAY 2021	14,25	5,78	14,25	2,14	3,64
03 MAY 2021	14,25	6,55	14,25	2,56	3,99
04 MAY 2021	16,00	6,48	16,00	2,70	3,78
05 MAY 2021	16,00	2,95	16,00	1,30	1,65
06 MAY 2021	15,63	2,63	15,63	1,70	0,93
07 MAY 2021	15,00	3,12	15,00	1,86	1,26
08 MAY 2021	15,25	6,34	15,25	2,91	3,43
09 MAY 2021	15,88	6,83	15,88	3,89	2,94
10 MAY 2021	15,75	6,90	15,75	3,96	2,94
11 MAY 2021	16,25	7,04	16,25	5,29	1,75
12 MAY 2021	16,25	5,78	16,25	2,92	2,86
13 MAY 2021	16,00	6,83	16,00	3,68	3,15
14 MAY 2021	15,88	6,90	15,88	3,89	3,01
15 MAY 2021	16,63	6,83	16,63	2,84	3,99
16 MAY 2021	16,25	7,39	16,25	4,10	3,29
17 MAY 2021	16,88	7,70	16,88	4,76	2,94
18 MAY 2021	16,88	7,51	16,88	3,89	3,62
19 MAY 2021	16,25	6,41	16,25	3,05	3,36
20 MAY 2021	15,88	6,69	15,88	3,68	3,01
21 MAY 2021	15,50	6,55	15,50	3,40	3,15
22 MAY 2021	15,63	6,48	15,63	3,68	2,8
23 MAY 2021	16,00	6,48	16,00	3,33	3,15
24 MAY 2021	16,09	6,38	16,09	3,16	3,22
25 MAY 2021	15,63	7,18	15,63	3,46	3,72
26 MAY 2021	16,25	7,04	16,25	4,66	2,38
27 MAY 2021	16,50	7,04	16,50	3,61	3,43
28 MAY 2021	16,00	6,69	16,00	2,84	3,85
29 MAY 2021	16,53	6,28	16,53	3,96	2,32
30 MAY 2021	15,13	6,27	15,13	3,47	2,8
31 MAY 2021	15,25	6,34	15,25	3,76	2,58

From tables 2, 3, 4, 5 we notice the following:

- The CO2-rich MEA has been regenerated into CO2-poor MEA which still contains traces of CO2.
- The percentage of CO2 released into the atmosphere by the vent is significant in the 4 trains concerned.

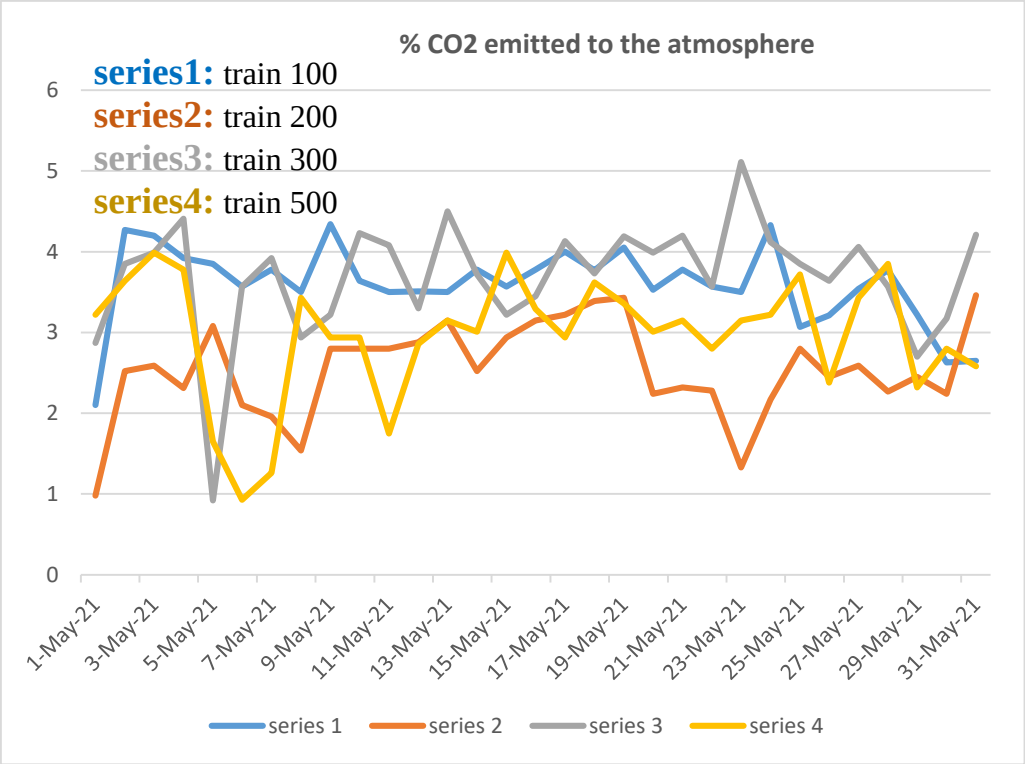

Fig 3. Variation of CO2 emitted into the atmosphere in the decarbonation section during the month of May 2021.

From the above graph, we notice that the values of CO2 released into the atmosphere vary from day to day, however if we compare the variations in the values of CO2 emitted into the atmosphere of the 4 trains (train 100, train 200, train 300, train 500) they are similar.

Results of the estimation of CO2 emissions from the decarbonation section

Quantity of decarbonated gas = quantity of gas entering the complex - quantity of raw gas self-consumed by the boilers.

Quantity of gas entering the complex from January 2020 to June 2021 = 14647777476 m3.

Total quantity of gas self-consumed in the boilers from January 2020 to June 2021: 2069955993 m3.

The boilers are overwhelmingly supplied with a mixture of fuel gas (decarbonated) and raw gas (non-decarbonated); the average composition of the mixture is 80% fuel gas and 20% raw gas. The quantity of raw gas consumed by the boilers is therefore 0.2 x 2069955993 = 413991198.6 m3.

Quantity of decarbonated gas = 1467777476 - 413991198.6 = 14233786278 m3 = 790765904 m3 / month.

Average molar mass of the incoming gas: 18.791 g / mole.

Mass of carbonated gas per month: 619,137 tonnes (on a 24 l / mole basis).

Average CO2 content of the incoming gas = 0.22% by mass.

Objective of residual CO2 decarbonated gas content: 10 ppm = 0.001% by mass.

Molar mass of CO2 = 44 g / mole.

Quantity of CO2 removed from the gas and released to the atmosphere by the decarbonation step = (0.22-0.001) / 100 x 619137 = 1356 tonnes CO2 / month, or 1883 kg CO2 / hour.

After having estimated by calculation the CO2 emissions into the atmosphere, we deduce that there is a very large quantity of carbon dioxide which is released into the atmosphere, at the level of the decarbonation sections of the GL1 / Z complex which is of 1356 tonnes of CO2 / month.

It is important and necessary to rid all natural gas of its CO2 in order to protect the installations from the corrosive risk and the risk of CO2 solidification which will lead to blockage of the pipes. Therefore, a maximum of CO2 must be evacuated for the good progress of the liquefaction of natural gas.

Table 6. CO2 flux at 100% and 110% production

	Charge 100%	Charge 110%
Light load	– Acid gas molar flow rate: 38250 mol / h – molar% of CO2: 87.32 – CO2 flux = 38250 x 0.8732 x 44/1000 = 1469 kg / h per train x 6 trains = 8817 kg / h	– Acid gas molar flow rate: 41920 mol / h – – molar% of CO2: 87.32 – – CO2 flux = 41920 x 0.8732 x 44/1000 = 1610 kg / h per train x 6 trains = 9663 kg / h
Heavy load	Acid gas molar flow rate: 22680 mol / h – molar% of CO2: 86.3 – CO2 flux = 22680 x 0.863 x 44/1000 = 861 kg / h per train x 6 trains = 5167 kg / h	– Acid gas molar flow rate: 24170 mol / h – – molar% of CO2: 86.46 – – CO2 flux = 24170 x 0.8646 x 44/1000 = 919 kg / h per train x 6 trains = 5517 kg / h

Results of the estimation of CO2 emissions

Quantity of gas flared = the total quantity of gas self-consumed - the quantity self-consumed by the boilers.

Table 7. Quantity of gas flared during the period May 2021 - June 2021.

	Gas Balance – GL1Z					
	Total self consumption		Burnt gas in boilers		Flared gas	
	Cm^3	% du GN entring	Cm^3	% du GN entring	Cm^3	% du GN entring
MAY 2021	113461000	13,99	107674943	13,28	5786057	0,71
June 2021	107893000	13,61	100187963	12,64	7705037	0,97

CO2 emissions = activity data x FE x FO

Activity data = quantity of gas burned = quantity of gas flared = 10,353,056 m3 / month on average over the period January 2020- June 2021.

EF = emission factor = tCO2 / Nm3 of flared gas. In the absence of specific data for the site, the emission factors given in the decree will be used:

– Type H gas: 2.14.10-3 tCO2 / m3.

– Type L gas: 1.82.10-3 tCO2 / m3.

The site receives gas of both types. The average composition is a little closer to a type L gas. As a first approach, in the absence of more precise data, an average emission factor of 1.98.10-3 tCO2 / m3 will be applied.

FO = oxidation factor = 0.995.

CO2 emissions **=** 10353056 x 1.98.10-3 x 0.995 = 20396 tCO2 / month.

After calculating the rate of CO2 emissions from the flaring gases, we were able to obtain the approximate value in tonnes of CO2 per month emitted by the flares, which is 20,396 tonnes.

By comparing the results of the different parts studied for the emission of CO2 to the atmosphere, we have:

- ✓ For boilers: 203,680 tonnes of CO2 per month;
- ✓ For the decarbonation section: 1,356 tonnes of CO2 / month;
- ✓ For the torch section: 20,396 tonnes of CO2 per month.

It will be clearly seen that the CO2 emissions are much higher by the boilers.

Remember that CO2 emissions are permanent and non-stop, as natural gas liquefies 24 hours a day in the GL1 / Z complex.
In addition, we conclude that the MAYn sources of atmospheric pollution generated by the activities of the GL1 / Z complex are therefore: boilers and flares.

Conclusion

This present work takes place in the perspective of evaluating the CO2 emitted by the installations of the GL1 / Z complex.

In the light of the results obtained, we concluded the following:

- ✓ The boilers are the MAY sources of CO2 emissions in the GL1 / Z complex, but in proportions that still comply with regulatory standards.
- ✓ The CO2 content released and quantified at the various stages of the decarbonation of natural gas is significant.
- ✓ I had the opportunity to manipulate the chromatograph which allowed us to separate the different constituents of the flared gases. We were able to quantify the CO2 emitted by the flares. It is recognized that flaring leads to incomplete combustion of hydrocarbons, leading to harmful compounds that can have a significant effect on the health of the population and on agriculture, particularly during periods of acid rain.

However, this estimate of CO2 emissions into the atmosphere shows that a very large quantity of carbon dioxide is released into the atmosphere, at the level of the decarbonation sections and this is inevitable and of capital interest in the process of liquefaction. In fact, rid all natural gas of its CO2 in order to protect the installations from the corrosive risk and the risk of CO2 solidification which would lead to blockage of the pipes. Therefore, a maximum of CO2 must be evacuated for the good progress of the liquefaction of natural gas.

Through the Sonatrach Group, Algeria is a founding member and stakeholder of the "Global Initiative for the Reduction of Gas Flares" (GGFR) which aims to reduce greenhouse gas emissions responsible for global warming.

Algeria has made significant investments to reduce the volume of gas flared. It is one of the top 20 countries that have reduced their gas flaring volumes since 2006.

The measures to adapt and reduce CO2 emissions, the MAYn greenhouse gas believed to be responsible for climate change, can be summarized as follows:

- Reduction of natural gas flaring.

- Geological sequestration and CO2 capture. The principle consists of capturing the CO2 at its point of emission, concentrating it and transporting it to a geological site suitable for its storage. (such as the In-Salah project).

- The capture of CO2 at its point of emission and its reuse as a reagent in other processes.

- Forestry and reforestation in an industrial environment.

Carpooling in business to eliminate CO2 emissions from cars.

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SONATRACH; National Society for Research, Production, Transport, Transformation, and Marketing of Hydrocarbons; geographical location of the GL1 / Z complex and its history; 1994;

OPTIMIZATION AND CHARACTERIZATION OF POLYPHENOL OXIDASE ENZYME OF IPOMOEA PURPUREA PLANT GROWN IN VIVO AND IN VITRO

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ABSTRACT

Ipomoea purpurea (I. purpurea) is widely used for ornamental and medicinal purposes. In medicine, the stems, seeds, roots and flowers of *I. purpurea* have been utilized in many treatments such as laxative, hallucinogen, purgative, syphilis, infertility, rheumatism, fungal infection, liver protection, acne, urinary infection, diarrhea and constipation. Although it has a wide use, studies on *I. purpurea* are quite limited. In this study, it was aimed to obtain polyphenol oxidase enzyme and compare its activities from Common-morning glory (*I. purpurea*) plants grown *in vivo* and *in vitro*. For this purpose, leaves obtained from local plants in Kocaeli region and from *in vitro* cultured plants were used to prepare crude extracts. Optimization, characterization and activities of polyphenol oxidase enzyme from obtained crude extracts were compared. Among the plant crude extracts, it was determined that the leaves of the *in vitro* plant possessed higher polyphenol oxidase activity than local plant when catechol used as substrate.

Keywords: Enzyme characterization, Optimization, *Ipomoea purpurea*, Polyphenol oxidase, Polyvinylpolypyrrolidone**.**

INTRODUCTION

One of the most important problems faced by the world today, as a result of the consumption of excessive fossil resources by the industries, the balance of nature is disturbed, leading to global warming, water, air and soil pollution. For this reason, the economy based on fossil resources leaves its place to the economy based on biological resources in order to protect nature and meet the need for exhaustible energy and material. The mostly green leaves, seeds and fruits of plants constitute an inexhaustible source of raw materials for carbohydrates, proteins, enzymes, lipids, various phenolic substances and phytochemicals (Kamm et al., 2006).

Phenolic substances compose the most important groups of natural antioxidants. These are polyphenolic components found in all parts of plants. Depending on their chemical structure, plant phenolic compounds can be separated into phenolic acids, flavonoids, tannins, stilbenes and lignans. It is known that they protect easily substances found in foods (fruits and vegetables) from oxidation (Dai and Mumper, 2010).

Polyphenol oxidases (PPO), which play a role in the oxidation of phenolic substances, constitute a very large family of enzymes. Despite the uncertainty in the classification of PPOs, they can be divided into three groups according to the literature: laccases (or *p*-diphenol: oxygen-oxidoreductase, E.C. 1.10.3.2), catechol oxidases (or *o*-diphenol: oxygen-oxidoreductase, E.C. 1.10.3.1) and tyrosinases (or monophenol-monooxygenase, E.C. 1.14.18.1) (Kocabas et al., 2011).On the other hand, peroxidases (E.C. 1.11.1.X), sometimes included in phenol oxidases, are enzymes that oxidize phenolic substances in the presence of hydrogen peroxide. Polyphenol oxidases have a wide range of uses in industry including food,

chemical, pharmacy, wine, beer and fruit juice production (removal of phenolic substances), wastewater treatment, plastics, paper industry and melanin synthesis (Gasmalla et al., 2015; Maki et al., 2006).

In this study, it was aimed to investigate the presence of polyphenol oxidase enzyme in extracts obtained from leaf parts of *Ipomoea purpurea*, known as a medicinal and ornamental plant, grown both *in vitro* and *in vivo*. At the same time, it was planned to analyze the biochemical properties of the enzyme.

As a result, it has been shown for the first time that *I. purpurea* can form cheap and renewable biomass of agricultural origin for the production of polyphenol oxidase enzyme, which has an area of use in the industrial sector.

MATERIAL AND METHOD

The seeds of Common-Morning Glory (*Ipomoea purpurea*) were purchased from Anadolu Tohum Production and Marketing Incorporated company. The chemicals used during the experiment with the highest purity grade were obtained from Sigma-Aldrich (St. Louis, MO, USA) and BioRad (California, USA).

Young leaves of *I. purpurea* were collected from its natural environment in Kocaeli (Turkey). The leaves were used in experiments within a few hours after collection or stored at -80°C until used for further experiments. To compare the effect of cultivation on PPO production, the seeds of *I. purpurea* were also grown in LS (*Linsmaier & Skoog*) medium in the Plant Tissue Culture laboratory of the Biology department (Kocaeli University). Young leaves were collected immediately and used for further PPO extraction (Figure 1).

Figure 1. Local (a) and *in vitro*-cultured (b) plants

Plant leaves were thoroughly washed with distilled water (dH_2O) before use in the experiment. In the preparation of the crude extract, 30 grams of leaves were homogenized by thumping in 200 ml 0.1 M sodium phosphate buffer (pH 7.0) solution in a mortar at +4°C. Polyvinylpolypyrrolidone (PVPP) concentration was performed as the initial optimization condition for the crude extract. PVPP was added at a final concentration of 5, 12.5, 25, 37.5, 50, 62.5, 75 mg/ml to remove the phenolic substances in the environment, and the mixture was filtered through cheesecloth and centrifuged at 10000×g for 30 minutes at +4 degrees (Kocabas et al., 2011).

The second optimization condition was to determine the effect of different pH on *I. purpurea* PPO activity, it was measured using 100 mM catechol as substrate at several pH

values ranging from 4.0 to 9.0. The buffer solutions used in the experiment were 0.1 M citrate buffer for reactions between pH 4.0-5.0, 0.1 M phosphate buffer for pH 6.0-7.0, 0.1 M Tris buffer solution for pH 8.0, and 0.1 M glycine-sodium hydroxide buffer for pH 9.0 (Gulcin et al., 2005; Kavrayan and Aydemir, 2001; Kocabas et al., 2011).

PPO activity was measured by observing quinone production at 420 nm at room temperature on a spectrophotometer. To measure enzyme activity assay, the sample cuvette was prepared by adding 500 µl substrate (pyrocatechol) prepared at a concentration of 100 mM, 1 ml sodium phosphate buffer solution (100 mM, pH 7.0) and 500 µl enzyme solution diluted in the appropriate amount (Alici and Arabaci, 2016). The control (blank) cuvette was prepared in the same way but without the enzyme (Figure 2). All experiments were performed duplicate in enzyme activity.

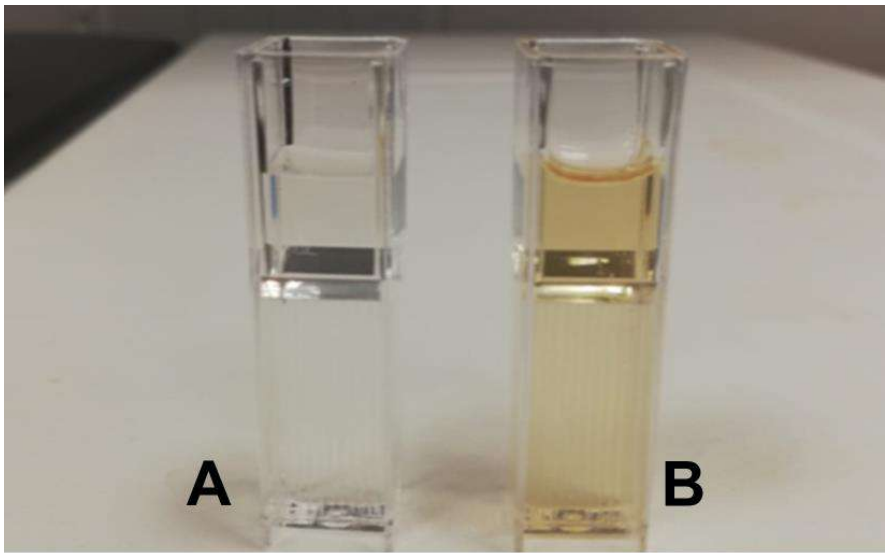

Figure 2. Enzyme activity assay. A – Blank cuvette, B – Sample cuvette.

RESULTS AND DISCUSSION

PPO enzymes have been widely used industrially in wastewater treatment, biosensor development, and the treatment of many diseases, including Parkinson's disease, vitiligo, cancer, phenylketonuria, and some *Streptococcus* infections. In addition, PPOs have been found useful for their applications in the synthesis of many pharmaceutical compounds and in the food industry to enhance the flavor of many hot and cold beverages (Gasmalla et al., 2015; Jukanti, 2017). Due to the wide range of application areas of the PPO enzymes, its isolation and purification from different sources has gained great importance in recent years. The enzyme has been isolated and purified using common chromatography techniques from different plant (Mestmäcker et al., 2018; Kumar et al., 2014), bacterial (Sivapathasekaran et al., 2009) and fungal (Singh et al., 2020) sources and has been presented for application after characterization.

In this study, *I. purpurea* was chosen as PPO source due to their broad ornamental and medicinal uses and presence of their limited study in the literature. For PPO isolation from *I. purpurea*, the first step was the optimization of extraction conditions. The leaves of *I. purpurea*, grown both naturally (local plant) and in LS medium (*in vitro*-cultured plant), were collected, and compared in terms of their PPO activities. Extraction conditions were investigated in terms of PVPP concentration and pH.

PVPP optimization

PVPP is a compound capable of preventing hydrogen bonding between phenolics and PPO enzyme (Smith and Montgomery, 1985). For this reason, PVPP was used during extraction and its concentration was optimized to improve PPO yield. PVPP was added to extraction medium in a concentration range of 5-75 mg PVPP/ml. As seen in Table 1, 25 mg PVPP/ml appeared to be a suitable concentration to remove phenolics for all samples tested. This was consistent with previous reports where PVPP was used at a broad range of 10-60 mg/ml concentrations (Kocabas et al., 2011; Pelalak et al., 2021; Rocha et al., 2001). 25 mg PVPP/ml was chosen for further extraction optimization analysis.

Table 1. PVPP optimization results

PVPP concentration (mg/ml)	PPO activity of *in vitro*-cultured plant (U/ml)	PPO activity of local plant (U/ml)
5	1937±96	870±43
12.5	2310±115	985±49
25	**3252±160**	**1000±50**
37.5	3007±150	932±46
50	1876±94	812±40
62.5	3028±151	836±42
75	1510±75	803±40

pH optimization

To optimize the pH of the environment, extraction media was prepared under conditions ranging from pH 4.0 to 9.0. Afterwards, extracts were obtained, and enzyme activity was determined under standard conditions in the spectrophotometer. According to the results given in Table 2, the highest activity was observed in the extraction medium with pH 7.0. Thus, further screening experiments were performed at pH 7.0.

It was also observed that the samples of *I. purpurea*, which were grown in two different ways, reacted differently to pH changes in the extraction medium. Plant samples obtained from tissue culture appeared to be active in a wider range of pH than local plant samples. In addition, explants exhibited higher PPO activities at pH values of 6.0-9.0. It is believed that this may provide an advantage in terms of the enzyme stability.

Table 2. pH optimization results

pH	PPO activity of *in vitro*-cultured plant (U/ml)	PPO activity of local plant (U/ml)
4	0	12±1
5	532±27	854±42
6	2201±110	690±34
7	**3328±165**	**1031±51**
8	1943±97	0
9	1250±62	0

CONCLUSIONS

As a result, crude extract samples from local plant and plant leaves grown *in vitro* in LS (*Linsmaier & Skoog*) medium were prepared under optimum conditions and compared in terms of polyphenol oxidase activities. Accordingly, it was determined that the leaves of the plant produced in vitro from the crude plant extract samples had higher polyphenol oxidase activity against the catechol substrate compared to the local plant sample.

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EVALUATION OF PHYSICO-CHEMICAL PARAMETERS OF BERRY FRUITS MARKETED IN ALBANIA

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ABSTRACT

Berry fruits are considered essential components of a healthy diet, playing an important role in human nutrition, and recently the interest on them by food scientist and consumers is growing. This study undertake to evaluate physico-chemical parameters of berry fruits blueberry (*Vaccinium spp.),* blackberry *(Rubus spp.),* raspberry *(Rubus idaeus)* and strawberry (*Fragaria ananassa*), which were marketed in Albania. Fruits were collected randomly in Tirana markets, and were evaluated for fruits dimensions, total dry matter (D.M.), total soluble solids (TSS), total titratable acidity (TTA), pH, total ash, vitamin C, and colour for L*, a* and b*. Determined parameters resulted: fruit length 10.80-45.92 mm and width 13.03-35.38, D.M. 20.27-43.4 g/100 g f.w., g/100 g f.w., TSS 9-15 ^{o}Brix, TTA 3.74-6.73 g/100 g of sample fresh weight (f.w.), pH 3.1-3.7, total ash 0.31-0.71 g/100 g f.w., L* 17.05-30.26, a* 1.58-18.93, and b* from -4.01 to 6.49, vitamin C ranged 10.6-48.8 mg/100 g f.w. From comparison between fruits blackberry resulted with the highest content of D.M., ash, pH, and with the lowest TTA, strawberry resulted with the highest vitamin C content and blueberry with the highest TSS, whereas L* value was higher in blueberry and lower in blackberry, had the highest a* and b* values in raspberry, and the lowest in blueberry. Variation were noted for TTA, TSS and vitamin C, whereas no significant differences were noted for other determined physico-chemical parameters. Future studies may be focused on investigation of total polyphenol content and antioxidant potential of these fruits.

Key words: blueberry; blackberry; raspberry; strawberry; physico-chemical parameters.

INTRODUCTION

Berries have been recognized to play an important role in human nutrition providing health-benefits (Manganaris et al., 2013).

Albania is placed in the Mediterranean Basin, offering enormous opportunities to grow many fruit crops, which have found spontaneous and wild forms of blackberries, raspberries (Rubus), strawberries (Fragaria) and vacciniums, and are part of a natural ecosystems (Kullaj et al. 2012).

They are consumed both as fresh fruit and are processed to form value-added products such as juice, puree, concentrate and frozen berries beverages, ice cream, yogurt, milkshakes, jams, jellies, spreads, syrups, wines, teas, and dairy products (Curi et al., 2016; Kadivec et al., 2013).

This study undertake to evaluate physico-chemical parameters of berry fruits blueberry (*Vaccinium spp*.), blackberry (*Rubus spp*.), raspberry (*Rubus idaeus*) and strawberry (*Fragaria ananassa*), which were marketed in Albania.

MATERIAL AND METHOD

Berry fruits selected for this study blueberry (*Vaccinium* spp.), blackberry (*Rubus* spp.), red raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*). Fruits were randomly collected in 2021 in Tirana markets, Albania, and were transported immediately to the laboratory. Prior analyzation a pre-selection was done based on fruits maturity, shape, size, and color.

Fifty berry fruits of each cultivar was individually weighed and measured for the principal dimensions. For determination of dry matter was weighed exactly 5 g of the well-homogenized sample into a pre-dried and tarred container and was placed the container in the moisture analyzer (model OHAUS) at 105 ± 2ºC and let it dry, and results were expressed as g/100 g of sample in fresh basis. Total soluble solids (TSS) was measured at 20ºC using the ABBE refractometer and results were expressed in ºBrix. Total titratable acidity (TTA) was determined by potentiometric method for which the titration with a standard alkali solution (0.1N NaOH) (in the presence of 0.3 mL phenolphthalein) was performed until its pH reached 8.1 and results were expressed in g citric acid per 100 g of sample f.w. Also, was determined the ratio of TSS/TTA. pH was measured, after the pH-meter was standardized using standard buffers with pH 4 and 7. For total ash content determination was weighted 5 gm (approximately 0.001 g) test sample into a previously dried and tarred crochet and incinerated in a muffle furnace at a temperature of 525 ± 25ºC, and results were calculated g/100 g of sample f.w. The surface color of the berry fruits was measured using a portable colorimeter (model NH310) to determine L* (lightness or darkness), a* (redness or greenness) and b* (yellowness or blueness) values. The ascorbic acid content was determined using the method of 2,6-dichlorophenol indophenol, as described by AOAC (2000).

The analyzes were performed at least in three replications, and were calculated as Mean values, ± standard deviation.

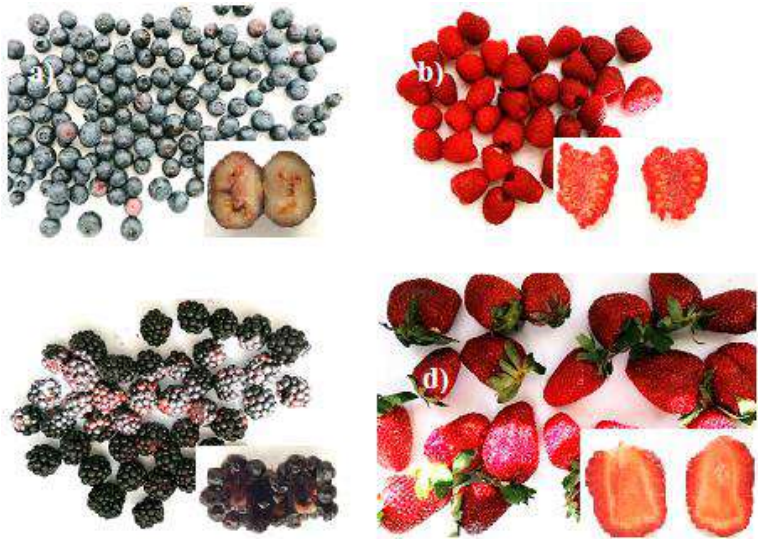

Figure 1. Berry fruits of the study: a) blueberry, b) blackberry, c) red raspberry, and d) strawberry

RESULTS AND DISCUSSION

Based on the study results, the berry fruits are small fruits with a weight per fruit 1.08-25.10 g, length 10.80-45.92 mm, width 13.03-35.38 mm and fruit shape index 0.83-1.3 (Table 1), among them blueberry is the smallest and strawberry the biggest. Such fruits are

characterized by soft flesh that lacks a peel or inner core, making them highly perishable (Bates, et al., 2001).

Table 1. Weight, dimensions and fruit shape index of berry fruits (expressed as Mean values ± SD)

Fruits	Weight per fruit	Length (mm)	Width (mm)	Fruit shape index
blueberry	1.08 ± 0.39	10.80 ± 0.999	13.03 ± 1.69	0.83 ± 1.34
blackberry	4.78 ± 0.27	19.32 ± 1.84	17.79 ± 1.30	1.09 ± 1.09
raspberry	5.27 ± 0.45	23.22 ± 1.72	20.72 ± 1.75	1.12 ± 0.982
strawberry	25.10 ± 0.89	45.92 ± 6.93	35.38 ± 3.76	1.3 ± 5.35

Fruit color is a key component of fruit quality (García-Viguera et al., 1998). Colors form the color spectrum and usually include red, reddish-purple, purple, blue-purple, blue, blue-green, green, yellow-green, yellow, and orange. Light is the difference between brightness and darkness, or the amount of white or black present in a color. The value range of L* (light) is from 0 = black to 100 = white, the range a* is negative for green to positive for red and the values b* are negative for blue and positive for yellow (Palonena and Weber 2019). The coloration is generally the first characteristic observed in fresh foods, and very often predetermines the consumer expectation in relation to the flavor and quality.

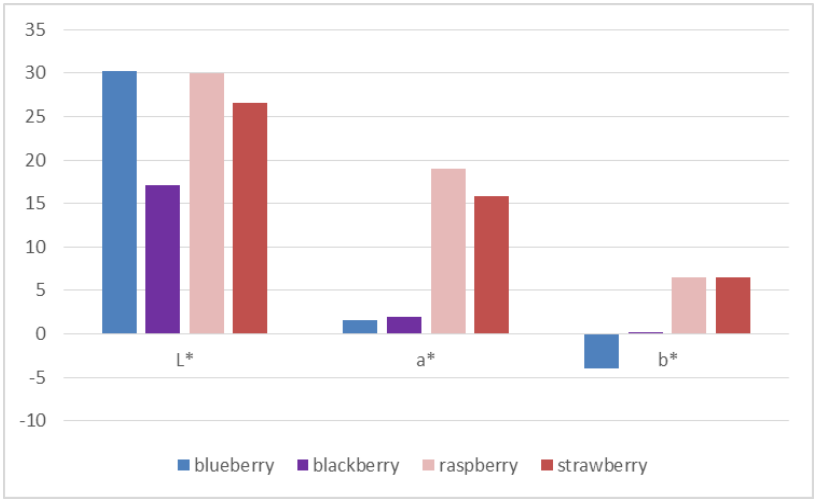

Figure 2. The color parameters of the berry fruits determined using CIE L* a*, b*

The color parameters of the samples were determined using CIE L* a*, b*, and the values resulted respectively 17.05-30.26; 1.58-18.93 and -4.01 to 6.49 (Figure 2). Among the red fruits, the blueberry had the highest L* value (30.26), and the lowest blackberry (17.05). Raspberry resulted with a higher positive value of a * (18.93) indicating that they are redder in the skin, and with a lower value resulted blueberry (1.58). Raspberry had the higher positive value b * (6.49), and with lower values resulted blueberry (-4.01).

Size, color, and shape, are considered important for external appearance of fruits, and for consumers. Color is a key parameter of post-harvest quality for red fruits and value L*, a*, b* are highly influenced by genotype, storage temperature and storage duration. In addition, physical factors, such as color and its uniformity, are parameters that directly define the quality of the fruits, because it is considered to interfere with features, such as flavor and intensity (Moreno and Deaquiz, 2016).

During the ripening process, which is a complex process, fruit undergoes continuous physicochemical changes that affect acceptability, quality, and storage time (Ayala et al., 2013) often associated with an increase in soluble solids, total sugars, total ascorbic acid, pH, and a decrease in acidity among others (Moreno and Deaquiz, 2016).

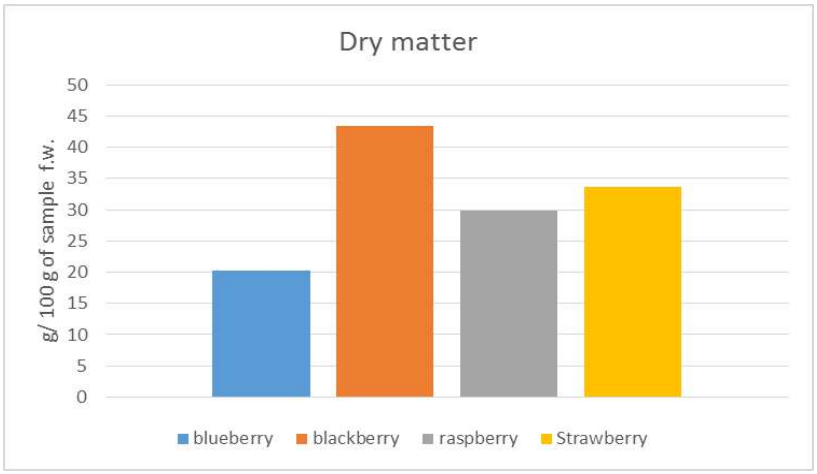

Figure 3. Dry matter of berry fruits

Dry matter of berry fruits resulted 20.27-43.4 g/100 g of sample f.w. (Figure 3), where blackberry had the higher content (43.4 g/100 g) and blueberry the lowest content (20.27 g/100 g). Dry matter for berry fruits in this study resulted higher than the study of Françoso et al. (2008), Souza et al. (2014) and similar to the study of Moraes et al. (2007).

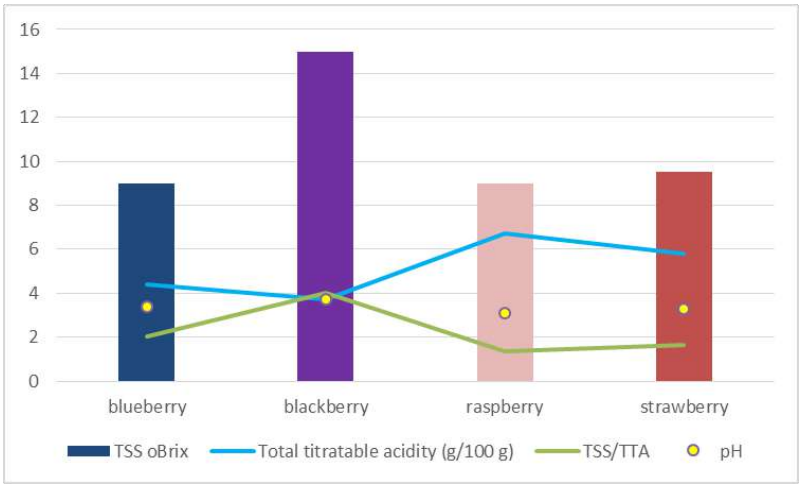

Figure 4. Total soluble solids, total tritable acidity, pH and TSS/TTA values of berry fruits

The soluble solids content is an important characteristic for products that are sold fresh, since consumers have a preference for sweeter fruits (Mahmood et al., 2012; Silva et al., 2002). The total soluble solids content of all the samples showed considerable differences as resulted 9-15^{o}Brix (Figure 4), where highest content had blackberry, no significant differences existed among blueberry, raspberry and strawberry. Total soluble solids is affected by cultivar and harvest maturity, and berries should be harvested at a near full ripe stage to retain the sweetness and appropriate flavor as the soluble solids content of berries remain unchanged post harvesting (Mitcham, 2007).

Acidity is one of the criteria that affect the classification of fruit. Total titratable acidity of all samples differed from one another and the results were higher than those reported by Moura et al. (2011) and Hirsch et al. (2012).

The measured physico-chemical properties corresponded very well, i.e., the higher the dry matter content, the higher TSS content; and the higher the pH value, the lower the TTA.

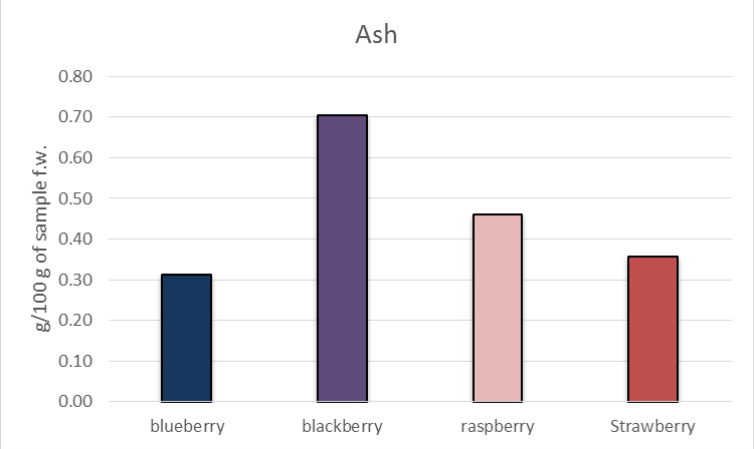

Figure 5. Total ash content of berry fruits

Regarding total ash content blueberry had the lower ash content (0.31g/100g of sample f.w.) and blackberry had the higher content (0.71 g/100 g). Results were higher than those found by Moraes et al. (2007), and close to the study of Hirsch et al. (2012) and Françoso et al. (2008).

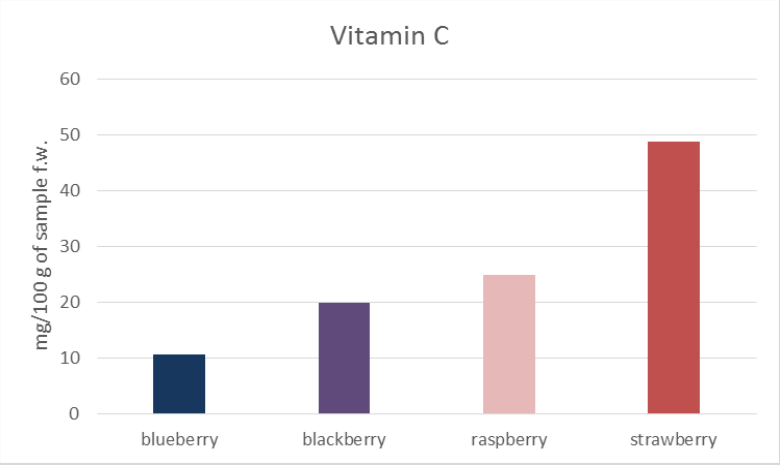

Figure 6. Vitamin C content in berry fruits

Vitamin C content resulted 10.6-48.8 mg/100 g of sample f.w., where strawberry was noted for higher content, and blueberry for the smallest, no significant differences existed among blackberry and raspberry.

Beside changes that occur during the maturation on physico-chemical parameters, depending on various factors such as light, temperature, moisture, and soil fertility among others, also their changes are important during postharvest handling of berry fruits, and to ensure the quality and marketing requirements (Gómez-Romero et al, 2010). Furthermore, the physico-chemical parameters are considered important as pre-processing parameters that can affect the quality of value-added berry products (Mitcham, 2007), and the obtained data provide significant information for berry consumers and processors for use and selection of appropriate

varieties for various applications. Based on requirements for the processing industry as suggested by Hokanson and Finn (2000), berry fruits would be excellent because of their high TSS and acid, deep red color, and relatively uniform size and shape.

CONCLUSIONS

Based on the study, physico-chemical parameters have differences among blueberry, blackberry, raspberry and strawberry in this study and when compared to other studies. Changes on physico-chemical parameters may have been affected by cultivar and maturation.

The berry fruits are small fruits with a weight per fruit 1.08-25.10 g, length 10.80-45.92 mm, width 13.03-35.38 mm and fruit shape index 0.83-1.3, among them blueberry is the smallest and strawberry the biggest. Color is a key parameter of post-harvest quality for red fruits, and the blueberry had the highest L* value (30.26), and the lowest blackberry (17.05), raspberry resulted with a higher positive value of a * (18.93) indicating that they are redder in the skin, and with a lower value resulted blueberry (1.58), and raspberry had the higher positive value b * (6.49), and with lower values resulted blueberry (-4.01).

From comparison between fruits blackberry resulted with the highest content of D.M., ash, pH, and with the lowest TTA, strawberry resulted with the highest vitamin C content and blueberry with the highest TSS. Variation were noted for TTA, TSS and vitamin C, whereas no significant differences were noted for other determined physico-chemical parameters.

The obtained data from this study on berry fruits provide significant information for postharvest handling of berry fruits, linked with their quality and marketing requirements assurance, as well as pre-processing parameters that can affect the quality of value-added berry products, and for use and selection of appropriate varieties for various applications.

Future studies may be focused on investigation of total polyphenol content and antioxidant potential of these fruits.

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DESIGN AND IMPLEMENTATION OF A SENSOR NODE WITH ITS POWER CONSUMPTION ANALYSIS

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ABSTRACT

In this study, A sensor node used in the cloud-based monitoring system designed and manufactured for collecting and recording of temperature and humidity data in destructive and nondestructive test laboratories are presented. The sensor node work as a slave structure and waits in sleep mode to save power until the requirement of a master receiver/transmitter module. When the requirement comes to the slave sensor node, the node wakes up and obtains the data from the temperature and humidity sensors and transmits them to the master receiver/transmitter module via Bluetooth. The power of a sensor nodes is supplied by a battery. The components of the sensor node including the Arduino Pro Mini module and the HM-11 Bluetooth module was configurated to operate with low power consumption because power consumption is important for self-powered structures. A detailed analysis of power consumption of the sensor node is given in the study. A special measurement method based on relation between capacitance and charge of capacitor is used to determine the battery life of the sensor node. Besides, battery life of the sensor node depending on the configuration preferences is presented.

Keywords: Bluetooth Low Energy(BLE) , Environment monitoring, Wireless sensor network,

INTRODUCTION

It is estimated that the number of Internet users in the World has reached almost 5.2 billion as of March 2021 (World internet usage and population statistics n.d.). While North America and Europe have the highest penetration rates of the population for internet access with 95% and nearly 88%, respectively, Africa and Asia have the lowest penetration rates with nearly 40% and 63%, respectively. Developments in internet access and sensor technologies have given rise to Wireless Sensor Networks (WSN) which can collect, record, analyze data and communicate with each other. Connecting WSNs to the internet, provides an environment for things to be able to connect with each other, computer to person/machine to machine interaction and communication created the last ring of the chain; "Internet of Things (IoT)". It is reported by IoT-Analytics that the market for Internet of Things has seen a somewhat unexpected acceleration in the first two quarters of 2018 and has elevated the total number of IoT devices that are in use to 7 billion devices (State of the IoT 2018: Number of IoT devices now at 7B – Market accelerating n.d.).

Tracking and monitoring systems are topics of great interest in the literature. Various monitoring systems have been developed for daily life monitoring, remote health monitoring, conserving quality of life of elder adults and monitoring environment parameters in laboratories, museums or agricultural lands. A data collection, transportation and monitoring system which collect daily vital signs at home and send them to the hospital via the internet was realized for chronic heart failure (CHF) patients in (Fanucci et al. 2013). An IoT-based smart hospital system using radio frequency identification (RFID), WSN and smart mobile was

proposed for automatic monitoring and tracking of patients, personnel and biomedical devices within hospitals and nursing institutes in (Catarinucci et al. 2015). Some elderly monitoring systems, smartphone-based activities of daily living detector without requiring user interaction (Andò et al. 2015), a WSN and cloud-based behavioral monitoring system (Mora, Matrella, and Ciampolini 2018) and integrated sensor network based tracking system (Monteriù et al. 2018) were developed to improve the life quality of disabled or elder people and decrease the restriction of their motion and supply a safer environment. Monitoring and tracking are also fundamental for smart home technologies. A low-cost home monitoring systems with flexible connection mechanisms was improved to reduce the energy consumption of a home in (Kelly, Suryadevara, and Mukhopadhyay 2013). Wi-Fi-based smart home monitoring system aimed at higher data rates and lower power consumption was discussed for active/ambient-assisted living (AAL) technologies with a large number of "plug and play" behavioral sensors in (Bassoli et al. 2017). Another smart home application presents a received signal strength indicator (RSSI) based localization and identification approach to estimate the location of the user in a home (Bianchi, Ciampolini, and De Munari 2018). Additionally, a road surface conditions monitoring system using vehicle motion sensors as accelerometers and gyroscopes to determine road surface anomalies by analyzing the common road surface types and irregularities as well as their impact on vehicle motion (El-Wakeel et al. 2018), a photovoltaic systems monitoring at panel level to detect the causes of efficiency losses (Ando et al. 2015). One of the most popular monitoring systems is environmental monitoring. Museums are another important places where measurement of environmental conditions such as temperature, humidity and air pollution are necessary. In the study (Camuffo, Grieken, and Busse 2001), a stand-alone structure for monitoring environmental conditions four museums in Europe; Correr Museum, Venice (Italy) Kunsthistorisches Museum, Vienna (Austria); Royal Museum of Fine Arts, Antwerp (Belgium) and the Sainsbury Center for Visual Arts, Norwich (UK) is presented. Environmental monitoring systems have been designed as stand-alone (Camuffo, Grieken, and Busse 2001) or IoT-based systems, especially reported recently(Blanco-Novoa et al. 2018; Folea and Mois 2015; Huang et al. 2018; Idrees, Zou, and Zheng 2018; Lee et al. 2019; Lombardo et al. 2018; Mois, Folea, and Sanislav 2017; Mois, Sanislav, and Folea 2016).

The ambient parameters as temperature and humidity are critical for food production or storage centers, data centers, test laboratories and some museums. The correctness of environment parameters is crucial to guarantee reliable and reproducible results in such laboratories. Although this fact, the consideration and attention for monitoring and validating of these functions is little important in many academic laboratories and many non-accredited test laboratories. Because the cost of the equipment and/or technical complexity of existing commercial solutions. Basic equipment such as incubation, ventilation, chilling, freezing and cooling systems have a critical importance in nearly all aspects of the traditional biological research laboratory (Gurdita, Vovko, and Ungrin 2016). It has been noted that even relatively small temperature differences meaningfully affect the growth of prokaryotic and eukaryotic cells, and furthermore, thermally sensitive surfaces can deteriorate even with temporary changes in the environment. Therefore, correct functioning of the cooling equipment is essential to ensure reliable and reproducible test results. Because of these reasons, Considering the reasons given, a low-cost and simple monitoring/tracing system was developed to monitor and record the temperature, humidity and pressure of the laboratory environment in (Gurdita, Vovko, and Ungrin 2016).The developed system consists of a Raspberry Pi single board computer with internet connection and USB-connected sensor interfaces. It consists of 8 pressure or temperature sensors, 8 digital inputs, and 4 thermocouples connection capacity. The Raspberry Pi reads temperature and humidity from sensors periodically every minute as default and stores in log files that may be accessed via a web server on the Raspberry Pi. The data obtained from the system can be accessed graphically via a web interface and the operator or

user is notified by email when parameters vary beyond the normal range. Like biological research laboratories, laboratories in hospitals similarly store and analyze a wide variety of biological reagents and samples such as urine, blood and genetic samples in order to diagnose of illnesses. These samples must be kept in a controlled situation to preserve their quality. In order to track the conditions of laboratories of pathology, radiology, nuclear medicine and radio-oncology departments of a Tertiary University Hospital in South Korea, two different types of monitoring systems were installed in (Kang et al. 2018). The first is an on-premises monitoring system and the second is a cloud IoT monitoring system. The systems do not supply only to monitor required temperature and humidity of environments, but also help to prevent accidents. The components of the entire system of the on-premises monitoring system were installed in the hospital and connected to the hospital's internal network. The ZigBee communication protocol was used to transmit the data obtained from the system to the monitoring server using via the gateway. In the second system, the data obtained from sensors was transmitted to IoT cloud server via the gateway. The cloud IoT monitoring system used the Bluetooth Low Energy (BT-LE) 4.1 communication protocol. The time interval is one minutes to collect data from temperature and humidity sensors in both systems. When the temperature or the humidity exceeds the specific tolerance limit, a warning system produces an alert and sends a message through a dedicated web page or smartphone application.

One of the most important part of the distributed sensor network monitoring systems is the sensor nodes. Sensor nodes are generally self-powered and locally-independent part of the systems. Therefore, it is very important of their power consumption performance. The Bluetooth low energy (BT-LE) protocol allows to send entirely data with low power consumption and good data transmission rate to a receiver up to a distance of at least 10 m. BT-LE is mostly well-matched with nearly all low-cost embedded PCs and all modern smartphones. This supplies merely to develop receivers and local data access at the monitored location (Lombardo et al. 2018). In this study, a wireless sensor node with BT-LE protocol is designed, manufactured, and presented for monitoring temperature and relative humidity of any environment. The developed senor nodes can be used any environment where collecting temperature and relative humidity data by Bluetooth connection. The power consumption performance of the developed sensor node is also analyzed in the study. In fact, a similar analysis was performed for a commercial sensor node used in a WSN structure created for media monitoring in (Lombardo et al. 2018). The presented sensor node used in the study is commercially available module nRF51822 SoC manufactured by *Nordic Semiconductor*. Unlike the related study, the developed sensor node in this study is manufactıred by using Arduino Pro Mini 16Mhz, an HM-11 Bluetooth 4.0 BLE module, a DHT22 temperature/humidity sensor, 3.7V 1400 mAh lipo battery and a lipo battery charging circuit. The connection preferences of the sensor node can be configurated via AT commands. The power consumption performance of the nodes are also presented according to it connection preferences. The developed sensor nodes are used in an IoT based temperature and relative humidity monitoring system in laboratories of KARTEAM (Kartepe Test ve Arastirma Merkezi-Kartepe Test and Research Center) of Uzunciftlik Nuh Çimento Vocational High School (VHS) in Kocaeli University. The data obtained from the system are published on the channel 415233 of ThingSpeak™.

The rest of the paper organized as follows. The developed monitoring system and the sensor node are presented in next two sections. The power consumption performance depending on configuration preferences is given later. The paper ends with the conclusion.

THE MONITORING SYSTEM

The monitoring system has been developed to collect temperature and relative humidity data from KARTEAM laboratories of Uzunçiftlik Nuh Çimento VHS in Koaceli University. KARTEAM laboratories was established with the aim of providing method prototyping, sample preparation, and giving destructive and nondestructive test services within ISO 17025 standard in September 2018. Some of test services given in KARTEAM are metallic materials - Charpy pendulum impact test within ISO 148-1:2016, destructive tests on welds in metallic materials - impact tests - test specimen location, notch orientation and examination within ISO 9016:2012, metallic materials - tensile testing within ISO 6892-1:2016, destructive tests on welds in metallic materials - hardness testing (microhardness testing of welded joints) within ISO 9015 2-1:2016, metallic materials - Rockwell hardness test within ISO 6508-1:2016, metallic materials - bend test within ISO 7438:2016, destructive tests on welds in metallic materials - bend tests within ISO 5173:2010, nondestructive testing of welds – visual testing of fusion-welded joints within ISO 17637:2016, nondestructive testing - penetrant testing within ISO 3452-1:2013, nondestructive testing of welds - magnetic particle testing within ISO 17638:2016, nondestructive testing of welds - ultrasonic testing - techniques, testing levels, and assessment within ISO 17640:2017 and standard test method for analysis of carbon and low-alloy steel by spark atomic emission spectrometry1 within ASTM E415-17. In accordance with, the most of these tests have to be carried out at certain laboratory conditions, 23 °C ± 5 °C and 45 %RH ± 10 %RH. Furthermore, the standard requires to record the temperature and humidity of the environment. The developed monitoring system supply to reduce the workload and enable to observe the conditions whether being at laboratories, or not. The system supplies to monitor the laboratory conditions on internet and it records the data in both an external memory and a cloud environment. The developed system has three layers including sensor nodes, receiver/transmitter module, and IoT cloud storage. Figure 1. presents the architecture of the developed system.

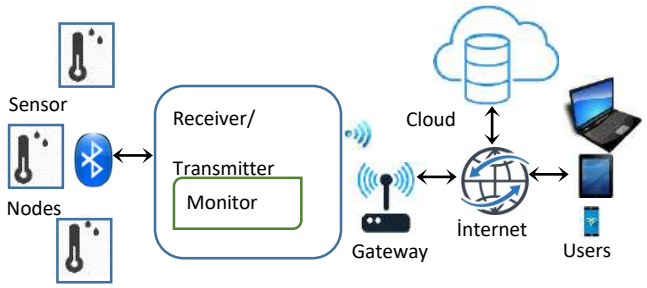

Figure 1. Architecture of developed system

The receiver/transmitter module communicates with sensor nodes via Bluetooth unique identifier data (UID). Each sensor node has a UID which is described by the firmware and cannot be altered. The receiver/transmitter module communicates with up to 250 different Bluetooth sensor nodes at the environment by searching UIDs of the nodes. In our implementation, three sensor nodes were placed in each laboratory. The UIDs was introduced to the receiver/transmitter module. The module has also a temperature and relative humidity module, HDC1080. Thus, temperature and relative humidity data from four different points are collected in each laboratory. The averages of these temperature and relative humidity values are stored and published at ThingSpeak™ cloud platform. The receiver/transmitter module and the sensor nodes are designed as master and slave, respectively. The slave sensor node waits in sleep mode to save power until the requirement of the master receiver/transmitter module. When the requirement comes to the slave sensor node, the node wakes up and obtains the data

from the temperature and humidity sensors and transmits them to the master receiver/transmitter module via Bluetooth. The receiver/transmitter module determines the data acquisition intervals from the sensor nodes. Besides, the receiver/transmitter module has a SD data storge card and an LCD monitor to display the temperature and humidity data collected from the sensor nodes. The receiver/transmitter module also sends the data to the cloud storage platform via the gateway. The relevant data can be followed by ThinkSpeak's channel 415233 and the connection link is https://thingspeak.com/channels/415233. A sample screenshot for ThinkSpeak channel 415233 is given in Figure 2.

The ThingSpeak channel 415233 was created in 2018 and more than 788000 data have been stored since created time. The most important contribution of the system is to reduce workload to measure trace, and store laboratory's conditions data, and to monitor the data from everywhere with internet connection. This system also enables the customer of KARTEAM to monitor the conditions of the laboratories. Hence, a reviewable and reliable laboratory conditions is provided for KARTEAM laboratories.

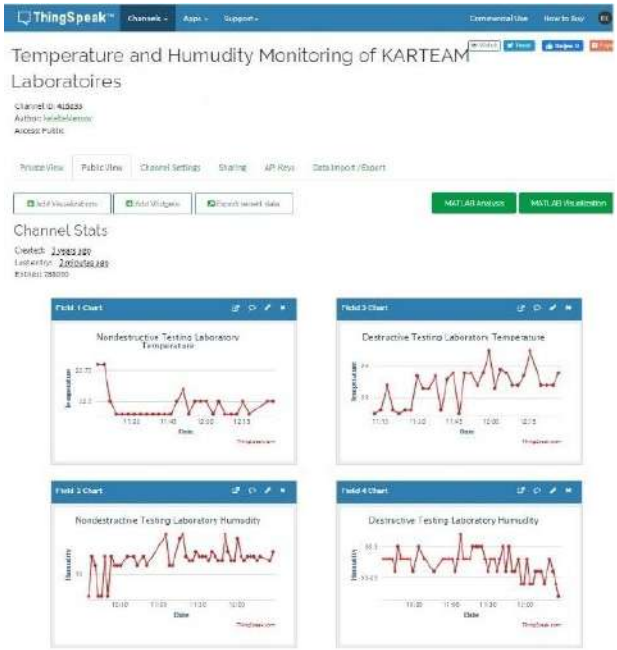

Figure 2. A sample screenshot for the channel 415233 of ThinkSpeak.

THE SENSOR NODE

The main reason for preparing the study is to investigate the developed sensor node and determine its power consumption performance. The sensor nodes are locally-independent and self-powered structures. They can be placed wherever there is a wireless connection access. In our system, BT-LE connection is preferred because of its low power consumption capability and the communication distance range is up to 100 meters. The developed sensor node includes an Arduino Pro Mini 16Mhz, an HM-11 Bluetooth 4.0 BT-LE module, a DHT22 temperature/humidity sensor, 3.7V 1400 mAh lipo battery and a lipo battery charging circuit. The developed sensor node module is placed in a box with 61 mm x 86 mm x 32 mm dimensions and its prototype is given in Figure 3.

Figure 3. A prototype of developed Sensor node

The developed sensor nodes are slave modules. A node gets the temperature and relative humidity data from DHT 22 sensor and sends them to the receiver/transmitter module via Bluetooth connection when the master receiver/transmitter module requests. Otherwise, the slave sensor node stays in sleep mode for pawer saving until the requirement of the master receiver/transmitter module. The power of a sensor nodes is supplied by a 3.7V lipo battery placed under printed circuit board. The Arduino Pro Mini module and the HM-11 Bluetooth module are established to work with low power consumption because power consumption is important for self-powered structures. The HM-11 Bluetooth 4.0 BT-LE module has 100m communication distance range in the open air and no byte limits for sending and receiving. It has programmable working features with AT commands (Bluetooth 4.0 BLE module Datasheet n.d.). The commands and the configuration preferences operated in the developed system for working features of HM-11 are given in Table 1.

Table 1. Configuration Preferences of HM-11

Command	Function of the Command	Preference
AT+ROLE	Set Master and Slaver Role	Slave
AT+ADVI	Set Advertising Interval	1285 ms
AT+MODE	Set Module Work Mode	Transmission Mode
AT+IMME	Set Module Work Type	When power on, work immediately
AT+PWRM	Set Module Sleep Type	Auto Sleep
AT+TYPE	Set Module Bond Mode	Not need PIN Code
AT+NOTI	Set Notify Con. Information	Don’t Notify

Whole modules and sensors were configurated to reduce power consumption of the sensor node because one of the most important topics is power consumption. The Arduino Pro mini has configuration features like Bluetooth module. It was also configurated to reduce power consumption. The Ardiuno module works in sleep mode to save saver. During sleeping time, the Analog to Digital Converter (ADC) module of Arduino is shut down and the Brownout Detector (BOD) module is disabled to reduce power consumption. In the developed sensor node, the Ardiuno sleeps during 8 seconds if the Bluetooth is not connected. It checks the Bluetooth connection in 8 seconds intervals by awaking. The Bluetooth module's 15th pin is the connection status pin. The pin is high level when the Bluetooth is connected to the receiver/transmitter module. It is possible to track the connection state of the Bluetooth module by following the pin. The flow chart of the sensor node is given in Figure 4.

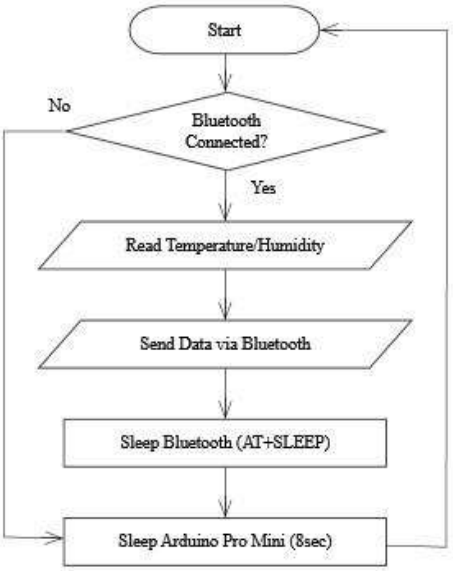

Figure 4. Flow chart for the sensor node

POWER CONSUMPTION PERFORMANCE OF SENSOR NODE

After performing above preferences, the power consumption of the sensor node decreases strikingly. As a result of the configurations, the power consumption rates are low and it is hard to determine the life time the node for a certain battery. Measuring a low current is difficult with an amperemeter. In order to determine the battery life of the node at sleep mode, the circuit given in Figure 5 can be installed.

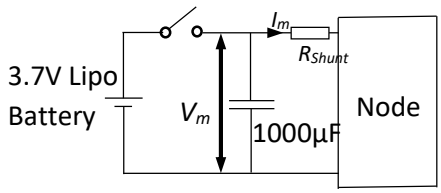

Figure 5. The circuit installed to estimate the power consumption

The measurement method in the circuit is based on relation between capacitance and charge of capacitor. The capacitance of a capacitor equals to the ratio of the electric charge (Q) on each conductor to the potential difference (V) between them. In this way, the power consumption of the node and battery life in sleep mode is obtained indirectly (Lombardo et al. 2018). When the switch is opened, the power consumption of the node can be estimated from the circuit by using equation (1).

$$V_m(t) = V_C - \int_0^t \frac{I_m(t)}{C} dt \quad \square\square\square\square\square$$

Where, V_m is the voltage of the capacitor, V_c is the initial capacitor voltage equals the battery voltage when the switch is opened, I_m is the unknown node current and C is capacitor value. The voltage of the capacitor and the shunt resistor (1Ω) at sleep mode is given in Figure 6.

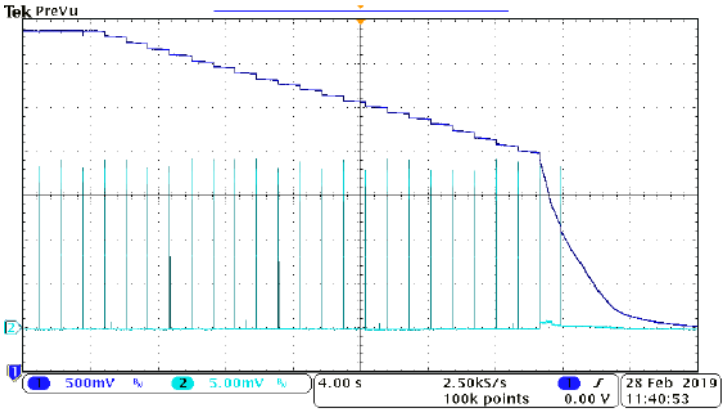

Figure 6. Blue; capacitor voltage and turquoise; voltage on shunt resistor at sleep mode

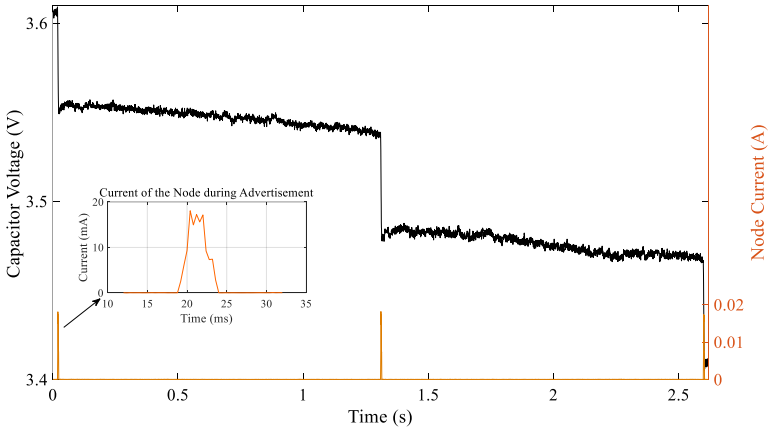

Figure 7. The capacitor voltage and the node current between three advertising

As known from Table 1, the advertising interval of the Bluetooth module is set by 1285 ms. Advertisements cause a significant drop in capacitor voltage and increase in node current during sleep mode as seen in Figure 6. In order to separately determine current consumption during advertising and sleeping between advertisements, the capacitor voltage and the node current between three advertisements are given in Figure 7. Additionally, the node current during advertising is inserted into the figure with a subplot.

The slope of the capacitor voltage between advertisements is approximately equal. If we assume the slope is fixed (-0.0126) and use the definition of the capacitance of a capacitor, time-dependent charge expression between two advertisements can be written as below.

$$Q_{m_{int}}(t) = Q_C - 0.0126t * C \square\square \quad \square\square\square$$

Where, Q_C is the charge value obtained by multiplying the capacitor voltage at the start by the capacitor value. From the measurements, the total charge amount between two advertisements (1285 ms) is about 16 µC and the average current during the time is about 12.7 µA. From voltage drops and node currents during advertising (average 4.4 ms), the charge per advertisement is about 58 µC and the average current is about 13.2 mA. For these parameters, the battery life of the node for sleep mode is theoretically more than 2.78 years. The battery life in sleep mode can be extended by increasing the advertising intervals of the Bluetooth module.

The Arduino Pro Mini in the sensor node wakes up every 8 seconds to control the Bluetooth connection. If Bluetooth is not connected to the receiver, it sleeps again for 8 seconds. Otherwise, it sends the temperature and the humidity data read from the DHT 22 to the receiver via Bluetooth and gets the Bluetooth to sleep and then enters sleep mode. The power consumption of the sensor node is given in Figure 8, when the Bluetooth and the microcontroller are sleeping, only the Bluetooth is awake and the microcontroller is asleep, both of them are awake and the system is transferring data.

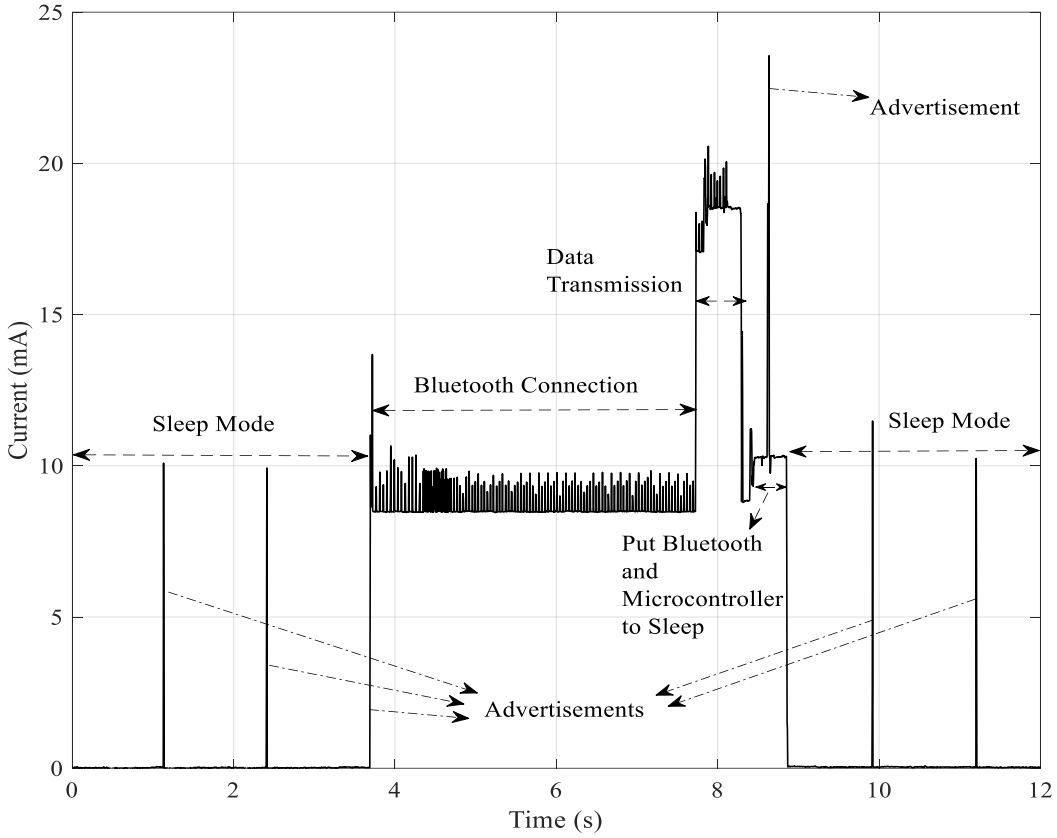

Figure 8. The power consumption of the sensor node

The charge consumed in the period between two sleep modes, including the Bluetooth connection, data transmission and entire the sleep mode again, can be written as in equation (3) (Lombardo et al. 2018),(Zhu et al. 2016);

$$Q_{Op} = \int_0^T I_{Op}(t)dt = I_{BleCon} * t_{BleCon} + I_{DataTrans} * t_{DataTrans} + I_{PutSleep} * t_{PutSleep}$$

Where, Q_{Op} is the charge during an operation cycle. I_{BleCon}, $I_{DataTrans}$, $I_{PutSleep}$ are average currents during the Bluetooth connection (t_{BleCon}), data transmission ($t_{DataTrans}$) and put the Bluetooth and the Arduino to sleep ($t_{PutSleep}$), respectively. $t_{DataTrans}$ and $t_{PutSleep}$ are fixed for each connection and equal to 0.672 s and 0.456 as seen in Figure 7. The average currents during data transmission and entering sleep mode were measured as 15.8 mA and 9.6 mA respectively. From the definition of charge which is the quantity of electricity carried in 1 second by a current of 1 A, the charge during an operation cycle except the Bluetooth connection equals to 14.98 mCoulomb (mC). Unlike $t_{DataTrans}$ and $t_{PutSleep}$, the Bluetooth connection time is entirely coincidental. It can change between 0 and 8 seconds. In order to calculate the average power consumption, the corresponding value is taken like 4 seconds. As seen in Fig. 8, the average

current during the Bluetooth connection equals to 8.6 mA. Then the average charge per connection during the Bluetooth connection equals to 34.4 mC and the average charge during an operation cycle is obtained 49.38 mC. The sensor node with an internal 1400mAh lipo battery can perform operation cycle approximately 102.231 times.

The battery life of the sensor node depends on the operation cycle frequency and advertising interval of the Bluetooth module. The Bluetooth module HM-11 has sixteen different options for advertising intervals. The battery life depending on the connection period and advertising interval for the sensor nodes is given in Figure 9.

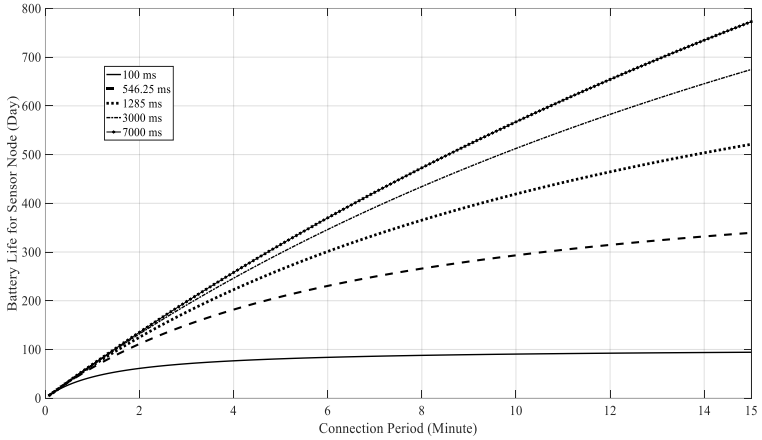

Figure 9. The battery life depending on connection period and advertising interval for the sensor node

Options of the installed system presented in the study are 1285 ms for the advertising interval and about 2.5 minutes for the connection period. The battery life of the sensor nodes for these preferences is theoretically more than 150 days.

CONCLUSION

The internet access is increasing day by day in today's world. So, tracking and monitoring systems are also topics of great interest in the literature. The increase in WSNs, distributed systems, IoT devices and internet access has supplied to rise the variety and capacity of monitoring systems. A Sensor node is a key component of these structures. Wireless access capabilities and self-powered structures reveal a flexible usage capability. The low power consumption for these structures is the most necessary point because of their self-powered nature. In this study, the sensor nodes used in the cloud-based monitoring system designed and manufactured for collecting and recording of temperature and humidity data in KARTEAM laboratories are presented.

KARTEAM laboratories has been providing method prototyping, sample preparation, and giving destructive and nondestructive test services within ISO 17025 standard since September 2018. The standards of given test services require to perform the tests in certain temperature and humidity in the laboratories and ISO 17205 requires recording the temperature and relative humidity data periodically. In traditional method, a laboratory worker records these data by manually. But the developed monitoring system supplies to carry out this work automatically and reduce the workload. Additionally, the system enables to observe the conditions of the laboratories whether being at laboratory, or not. The system supplies to monitor the laboratory conditions on internet, and it records the data in both an external memory and a cloud environment. The temperature and relative humidity data are stored and published at ThingSpeakTM cloud platform with channel 415233.

The developed system has three layers including sensor nodes, receiver/transmitter module, and IoT cloud storage. The receiver/transmitter module and the sensor nodes are designed as master and slave, respectively. One of the most important layers of the system is the sensor nodes. The slave sensor node waits in sleep mode to save power until the requirement of the master receiver/transmitter module. When the requirement comes to the slave sensor node, the node wakes up and obtains the data from the temperature and humidity sensors and transmits them to the master receiver/transmitter module via Bluetooth. The power of a sensor nodes is supplied by a battery**.** The components of the sensor node including the Arduino Pro Mini module and the HM-11 Bluetooth module was configurated to operate with low power consumption because power consumption is important for self-powered structures. A detailed analysis of power consumption of the sensor node is given in the study. A special measurement method based on relation between capacitance and charge of capacitor is used to determine the battery life of the sensor node. Besides, battery life of the sensor node depending on the configuration preferences is presented.

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IDENTIFICATION OF TRANSPORT PATHWAYS AND POTENTIAL SOURCE AREAS OF PM_{10} AND SO_2 DURING WINTER SEASON IN KIRKLARELI (TURKEY)

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ABSTRACT

In this study, the variation in daily PM_{10} and SO_2 concentrations during the winter season in Kirklareli city center were investigated. It was observed that PM_{10} concentrations had the lowest concentration values in January, SO_2 concentrations had the lowest concentration values in February, and PM_{10} and SO_2 concentrations had the highest concentrations in December. During the study period, air masses backward trajectories obtained using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) Model were run and clustering analysis was performed. Backward trajectories are clustered in five major clusters. Cluster 1 was determined to represent the highest percentage of all backward trajectories. Potential source areas of PM_{10} and SO_2 were determined by the Potential Source Contribution Function (PSCF) and Concentration Weighted Trajectory (CWT) models.

Keywords: PM_{10}, SO_2, Cluster analysis, PSCF, CWT

INTRODUCTION

Knowing the effects of wind and turbulence on the movements of particles or gas molecules released into the atmosphere is necessary to predict the transport and distribution of pollutants. The atmosphere region planetary boundary layer (approximately the lowest thousand meters), which represents the height at which the earth affects the wind structure in the atmosphere, governs the transport and distribution with about urban air pollution. Estimating wind direction and height as an effect of the variability of wind speed, and the temperature profile with surface roughness, is the main problem with the planetary boundary layer (with regard to air pollution). Predicting the distribution of pollutants is the main goal of air pollution meteorology. The distribution of pollutants depends on the physical structure of the pollutant, wind speed and direction, atmospheric stability, turbulence level, emission conditions, source configuration. In general, particles in the troposphere can be characterized by their own sources (Seinfeld, 1975). The particles can be distinguished as aerosol particles and hydrometeor particles. The hydrometeor particle contains much more water than the aerosol

particle (Jacobson, 2002). Fine and coarse particle modes, which are generally evaluated separately, are separated from the atmosphere by different mechanisms (Seinfeld and Pandis, 2006). Knowing how long the pollutants remain in the atmosphere and the removal process are of great importance (Seinfeld, 1975). It has been stated that pollutants can spread upwards depending on meteorological conditions and be transported to the uncontaminated troposphere and to the earth (Jacobson, 2002).

In many studies, air mass origins arriving the receptor region have been classified using cluster analysis. Statistical models such as PSCF and CWT were used to identify potential pollutant source areas (Yu et al. 2014; Xin et al. 2016; Li et al. 2017; Yu et al. 2020; Neykova and Hristova, 2020).

Regional sources and transportation routes were investigated for some air pollutants in Hangzhou by Yu et al. (2014). It was noted that sources affecting SO_2 concentrations in Hangzhou (mainly on the southeast coast of Zhejiang and Fujian provinces and Shanghai) show different sources for $PM_{2.5}$ and PM_{10} during heavy haze period. It was stated by Xin et al. (2016) that the set of backward trajectories clustered in four main trajectory paths. They showed that Xining is easily affected by inner trajectories in all seasons but pointed out that different trajectories have different effects on average PM_{10} concentrations. They stated that daily average PM_{10} concentrations showed a seasonal variation with lower concentrations in summer-autumn seasons and higher concentrations in winter-spring seasons. Li et al. (2017) stated in their study that the greatest potential sources for $PM_{2.5}$ and PM_{10} occur in the spring season. They stated that after the spring season, it is in the winter and autumn seasons, and then in the summer season. Potential source regions of $PM_{2.5}$ and PM_{10} were also noted to be similar. Neykova and Hristova (2020), in their study in four urban areas of Bulgaria, stated that as a result of the clustering analysis of the backward trajectories of air masses at an altitude of 1500 m above ground level, the backward trajectories were clustered in five main trajectory paths. They are specified average PM_{10} concentrations for Burgas are 33.7 μg m^{-3} in cluster 1, 30.5 μg m^{-3} in cluster 2, 35.7 μg m^{-3} in cluster 3, 33.6 μg m^{-3} in cluster 4, and 36.8 μg m^{-3} in cluster 5. They stated that the PSCF analysis results for the 3 different altitudes they examined (500 m, 1500 m, and 2000 m) followed the predominant percentage of south-southwest air masses in Pleven and Sofia, west-northwest in Plovdiv and north-northeast in Burgas. Liang et al. (2016) stated in their study that northwest airflow is an important factor in extreme pollution events, and sandstorms partially contribute to PM_{10} concentration in fast northwest transportation routes. They also stated that PM_{10} concentrations in winter and spring seasons have higher values than autumn and summer seasons.

In this study, the variation in daily PM_{10} and SO_2 concentrations in Kirklareli city center during winter season (between 01 December 2016 and 28 February 2017) were investigated. The transport routes and possible sources of PM_{10} and SO_2 were determined using trajectory clustering and PSCF and CWT methods.

MATERIAL AND METHOD

Located in the northwest of Turkey, Kirklareli is located in the Marmara Region and is a border province adjacent to Bulgaria (Figure 1). A continental climate is observed in Kirklareli, which has a 60-kilometer border with the Black Sea, in the city center and in the inner parts away from the sea (ÇDR, 2020). In this study, daily PM_{10} and SO_2 concentration data obtained from the Air Quality Monitoring Station of Republic of Turkey Ministry of Environment and Urbanization for Kirklareli city center between 01 December 2016 and 28 February 2017 were used (MEU, 2021).

Figure 1. Sampling location

The National Oceanic and Atmospheric Administration (NOAA) HYSPLIT model (version 5.0.0) was used to determine the origins and transport routes of air masses. The backward trajectories were run and clustered for 72 hours at 1-hour intervals and with arrival altitudes (1500 m above sea level). The weekly archived Global Data Assimilation System (GDAS) data with 1º resolution was used as input (Stein et al. 2015; Rolph et al. 2017). In cluster analysis used to classify data, trajectory coordinates are used as clustering variables. Air mass sources arriving the receptor site can be classified using cluster analysis (Carslaw and Ropkins 2012; Carslaw 2019; Neykova and Hristova 2020). PSCF and CWT analyzes were performed to

identify potential source areas. Cluster, PSCF and CWT maps were created with R-Studio program using OpenAir statistical analysis package. The criterion value was determined as 90 percent of all samples (Carslaw and Ropkins 2012; Carslaw 2019).

RESULTS AND DISCUSSION

The minimum, mean, and maximum values of daily PM_{10} concentrations in December are 41.05 µg m^{-3}, 89.83 µg m^{-3}, and 158.36 µg m^{-3}, respectively. In January, daily PM_{10} concentration values range from 24.05 µg m^{-3} to 136.41 µg m^{-3}. The average value of PM_{10} concentrations in this month is 72.19 µg m^{-3}. In February, the minimum value of PM_{10} concentration values is 32.41 µg m^{-3}, while the maximum value is 156.62 µg m^{-3} and the average value is 73.93 µg m^{-3}. Daily PM_{10} and SO_2 concentrations are shown in Figure 2 and Figure 3, respectively. Daily SO_2 concentrations ranged from 20.07 µg m^{-3} to 168.06 µg m^{-3} in December, with an average value of 79.04 µg m^{-3}. The minimum, average, and maximum values of daily SO_2 concentrations in January are 19.05 µg m^{-3}, 60.76 µg m^{-3}, and 154.33 µg m^{-3}, respectively. In February, the average value of daily SO_2 concentrations is 25.84 µg m^{-3}. In this month, daily SO_2 concentrations range from 5.79 µg m^{-3} to 123.71 µg m^{-3}.

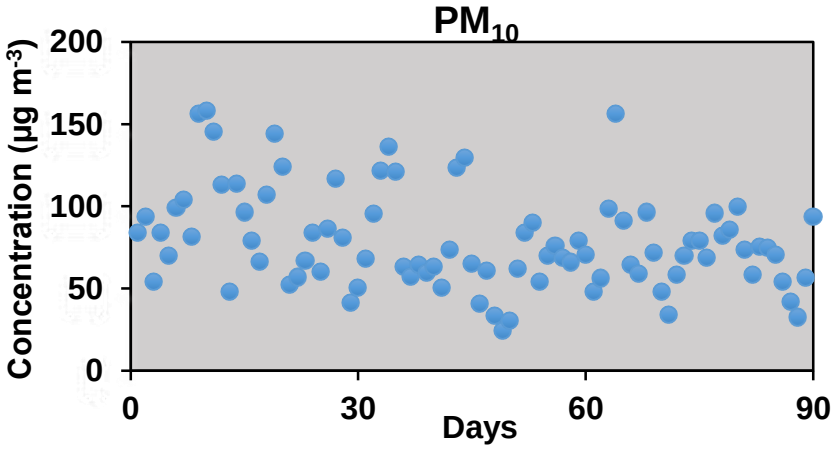

Figure 2. Daily PM_{10} concentrations

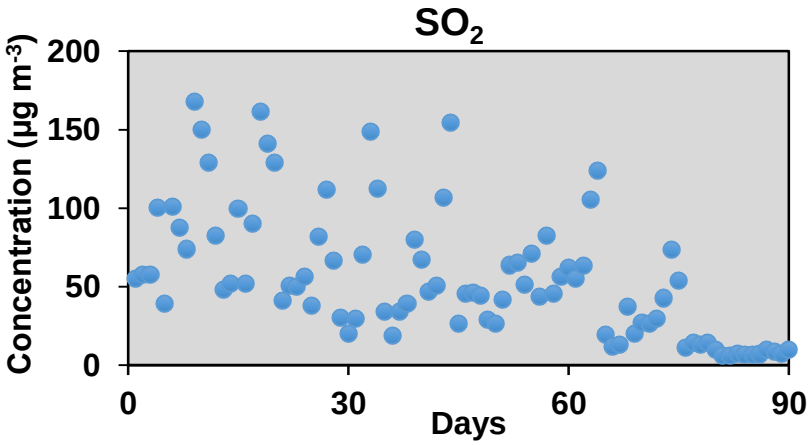

Figure 3. Daily SO_2 concentrations

It has been determined that the backward trajectories represent the air masses arriving the receptor site by clustering in 5 main clusters. Clusters of backward trajectory analysis are shown in Figure 4. According to the results of the cluster analysis, it was determined that Cluster 1 represents 84.1% of all backward trajectories. The minimum and maximum values of PM_{10} concentrations in Cluster 1 are 30.21 µg m^{-3} and 158.36 µg m^{-3}, respectively. The average PM_{10} concentration value is 81.00 µg m^{-3}. The SO_2 concentration values in this cluster ranged from 5.79 µg m^{-3} to 168.06 µg m^{-3}, while the average SO_2 concentration value was 58.36 µg m^{-3}. It is seen that the transport in Cluster 1 arrives the receptor site, starting from Poland and passing through Ukraine, Romania, and Bulgaria. Cluster 2 accounts for 5.7% of all backward trajectories. The average value of PM_{10} and SO_2 concentrations in this cluster is 85.63 µg m^{-3} and 42.55 µg m^{-3}, respectively. PM_{10} concentrations range from 41.86 µg m^{-3} to 145.34 µg m^{-3}, while SO_2 concentrations range from 10.13 µg m^{-3} to 128.85 µg m^{-3}. It is seen that the transport in Cluster 2 and Cluster 5 started from the Mediterranean. It has been determined that the transport in Cluster 2 arrives the receptor site by passing through France, Italy, Croatia, Bosnia and Herzegovina, Montenegro, Albania, Kosovo, North Macedonia, Bulgaria, and Greece. The percentage that Cluster 3, Cluster 4, and Cluster 5 represent all backward trajectories is the equal (3.4%). The average values of PM_{10} concentrations in these clusters are 67.93 µg m^{-3}, 50.39 µg m^{-3}, and 56.87 µg m^{-3}, respectively, while the average values of SO_2 concentrations are 47.74 µg m^{-3}, 38.58 µg m^{-3}, and 33.76 µg m^{-3}, respectively. It is seen that Cluster 3, which started in the North Sea, arrived the receptor site by passing over the Netherlands, Belgium, Luxembourg, Germany, Czechia, Slovakia, Hungary, Romania, and Bulgaria. It is seen that Cluster 4, which started from Germany, passed through Austria, Italy, Croatia, Bosnia and Herzegovina, Montenegro, Serbia, Bulgaria, and Greece and arrived the

receptor site. It is seen that the transport in Cluster 5 passes through Montenegro, Albania, Kosovo, Serbia, Bulgaria, and the Black Sea while arriving the receptor site.

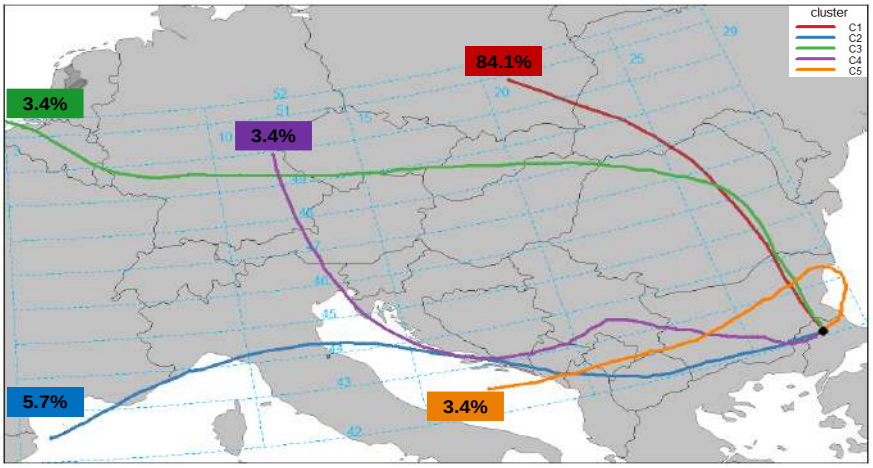

Figure 4. Clusters of backward trajectory analysis

PSCF and CWT models were used to be able to identify possible potential source areas of PM_{10} and SO_2. PSCF and CWT analysis of PM_{10} are shown in Figure 5 and PSCF and CWT analysis of SO_2 are shown in Figure 6. Xin et al. (2016) stated that when PSCF and CWT methods are compared, CWT shows a superior potential compared to PSCF and is more efficient and comprehensive for identifying potential resource areas. For PM_{10}, higher PSCF and CWT values were observed than other places, especially in Albania, Kosovo, North Macedonia, Serbia, and Bulgaria. For SO_2, higher PSCF and CWT values were observed than other places, especially in Romania, Austria, Slovenia, Serbia, Bulgaria, North Macedonia, and Albania.

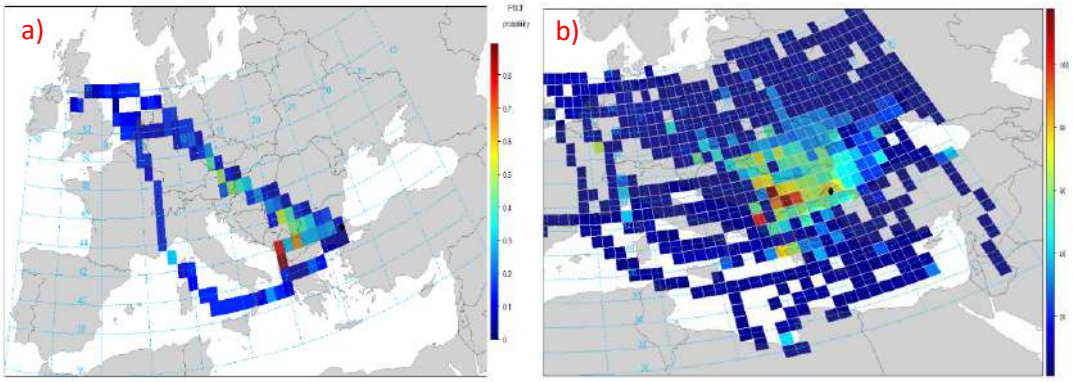

Figure 5. (a) PSCF and (b) CWT analysis of PM_{10}

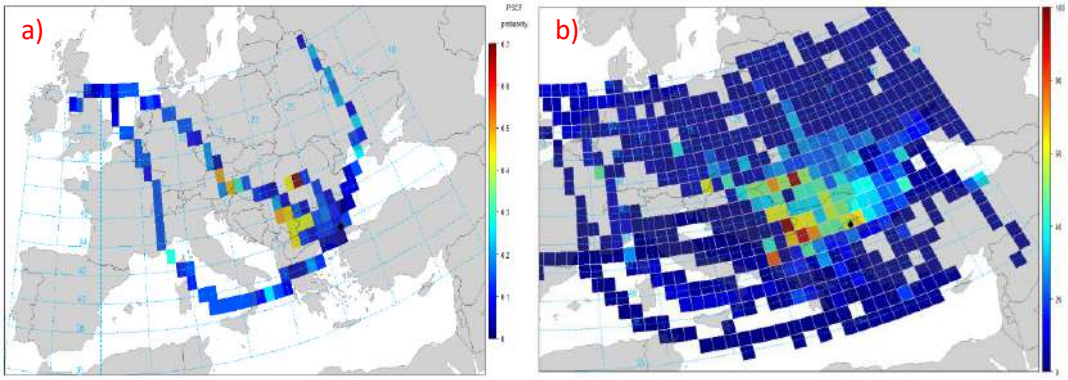

Figure 6. (a) PSCF and (b) CWT analysis of SO_2

CONCLUSIONS

During the study period, daily PM_{10} concentrations ranged from 24.05 µg m^{-3} to 158.36 µg m^{-3}. The average value of daily PM_{10} concentrations is 78.81 µg m^{-3}. The average value of daily SO_2 concentrations is 56.19 µg m^{-3}. The minimum and maximum values of daily SO_2 concentrations are 5.79 µg m^{-3} and 168.06 µg m^{-3}, respectively. It has been determined that backward trajectories represent the air masses arriving the receptor site by clustering in five main clusters. The order of magnitude of mean PM_{10} concentrations in Clusters is Cluster 2 > Cluster 1 > Cluster 3 > Cluster 5 > Cluster 4 and the order of magnitude of mean SO_2 concentrations is Cluster 1 > Cluster 3 > Cluster 2 > Cluster 4 > Cluster 5. According to the results of PSCF and CWT analyzes, it is seen that the potential source areas of PM_{10} and SO_2 are located especially in the western parts of the receptor site.

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SEASONAL EFFECT OF THE NORMALIZED DIFFERENCE VEGETATION INDEX OF CHETTABA FOREST (CONSTANTINE)

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ABSTRACT

A remote sensing approach is increasingly used by scientists in the study and monitoring of vegetation changes. The normalized difference vegetation index (NDVI) has been used extensively to do this. In this study, we have tried to characterize the NDVI vegetation index of 24 points of holm oak forest Chettaba, which is located southwest of Constantine (Algeria) from Landsat 8 satellite image data for each month of two seasons (winter and summer). The objective is to try to discriminate the seasonal variations of the year 2019-2020 on the behavior of holm oak. The comparison of the values of the Normalized Difference Vegetation Index (NDVI) in holm oak was carried out using the analysis of variance with a fixed criterion. The statistical results obtained from the NDVI of the 24 points indicate that the maximum value is recorded in winter in the month of December, while The lowest values are observed in summer in the month of June when the species studied is exposed to periods of high temperature, sunshine and lack of rain. Thus, the holm oaks undergo a thermal stress in summer, which causes the yellowing of their leaves and therefore a decrease in the normalized difference vegetation index.

Keywords: holm oak, NDVI, Landsat 8, seasonal behavior.

INTRODUCTION

Forest degradation has become a serious problem, particularly in developing countries. In 2000, the total area of degraded forests in 77 countries was estimated to be 800 million hectares, of which 500 million hectares had been converted from primary to secondary vegetation (OIBT, 2002). Among its various negative impacts, the process of forest degradation accounts for a significant share of greenhouse gas emissions. There is an urgent need to measure and analyze this process in order to design an action that can reverse it.

The application of a remote sensing data analysis method to observe forest degradation. Nowadays, satellites allow the collection of a huge volume of Earth observation data (Nativi et al., 2015). Satellite imagery can be used for remote monitoring of man-made and natural phenomena such as urban growth, changes in natural habitats, changes in agricultural land use, and the effects of climate change (Batista et al., 2014).

The change in spectral response measured at the satellite sensor is an indicator of environmental change. If we focus on the soil and vegetation themes, subtle changes in the color and mineralogy of the former and variations in the structure and spatial distribution of the latter can be indicators of environmental change and degradation (Maimouni, 2011).

The normalized difference vegetation index (NDVI), provided by satellite images, easy to calculate (Bariou et al., 1985; Caloz and Collet, 2001), widely used, as it is quite generic (Jensen, 2000), partly normalizes the effects of illumination or slopes (Bariou et al., 1985; Caloz and Collet, 2001). It is used to characterize plant health, to identify phenological changes, to estimate green biomass and yields and in many other applications. There are limitations to the use of NDVI as a measure of forest degradation, but also areas for potential improvement. Phenology plays an important role in the analysis of change processes.

The objective of this work is to monitor the evolution of the degradation process and the seasonal effect from the NDVI of forest areas where holm oak trees present by GIS (Geographic Information System) and aerial and satellite images.

MATERIAL AND METHOD

Presentation of the study area

Forest of Chettabah is located southwest of Constantine (Algeria). The estimate terrain elevation above sea level is 865 meters. The study area is located on the map topographic Constantine Scale 1/200 000 sheet N° 17 and located between the coordinates 36°19'4" north latitude and 6°28'36" East longitude. The forest of Chettabah spreads over an area of 2398 ha and 94a, and is perfectly limited and divided into six districts. Extreme altitudes of the forest is about 1104 m (maximum altitude) and 652 m (minimum altitude), corresponding to each of them respectively following map coordinates: (x = 839, y = 344), (x '= 839.9, y' = 340.3). Its bioclimatic is semi-arid to sub-humid. The average annual rainfall is estimated between 670 and 800 mm and the mean annual temperature of the region is 18°C, with an average of the warmest month above 35°C and the coldest month varies between 1.25 and 3.05°C. A large plant grouping as the forest of Chettabah can be studied in its entirety, especially when it concerns hundreds of acres to be treated in the detail.

24 stations of 30m by 30m of holm oak in the Chettaba forest were randomly selected to explain their phenological character using satellite images (Figure 1).

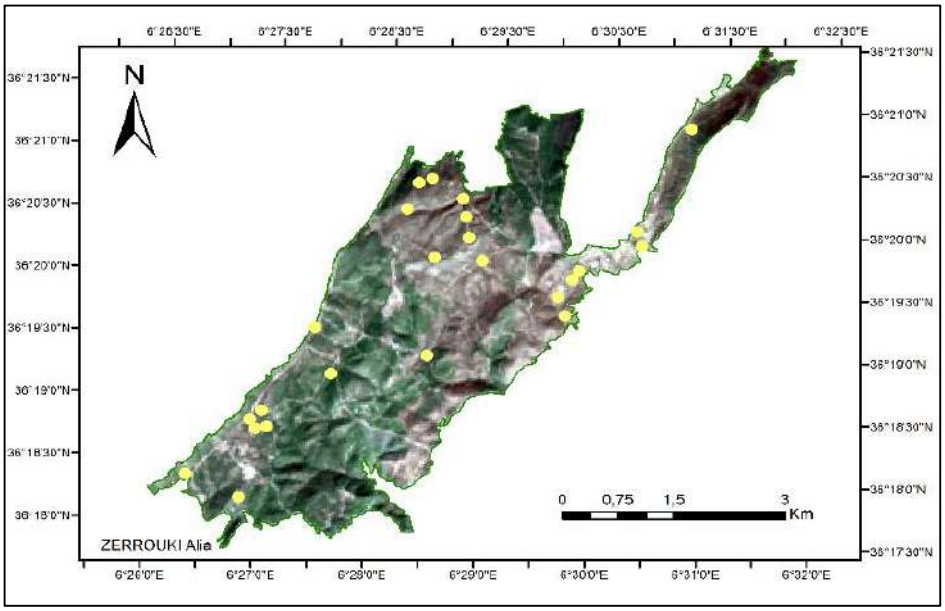

Figure 1. Location of the study stations in the Chettaba forest.

The monitoring of forest cover is essential to better understand the influences of climate change, we analyzed Landsat 8 satellite image composites of two different seasons winter (December 24, 2019, January 4, 2020, February 10, 2020) and summer (June 10, July 12, August 13, 2020) to extract the seasonal NDVI data. For the processing, analysis and representation of the geographic information, we used ArcGIS version10.5 software developed by ESRI (Environmental Systems Research Institute). The calculation of the natural vegetation index (NDVI) is based on two spectral bands, red R and infrared IR (Vermote, 2016) using the following formula: NDVI = (PIR - R)/ (PIR + R)

The obtained results are analyzed by the analysis of variance to know the seasonal variation of the natural vegetation index (NDVI).

RESULTS AND DISCUSSION

Based on the study results, the classical sunflower hybrids exhibited higher The Normalized Difference Vegetation Index (NDVI), provides estimated values of the "green intensity" of forests, resulting from the analysis of satellite data. The approach is based on the premise that NDVI is an indicator of plant health, in the sense that a degradation of the vegetation of an ecosystem, or a decrease in green intensity, would result in a decrease in the NDVI value. One of the major applications of remote sensing is the monitoring of processes occurring on the planet. Images can be used to analyze short-term processes, for example to observe the growth cycle of certain crops in order to evaluate the yield of a given crop.
Images can also be used to study medium and long term processes. Analyses of forest degradation and land use change are major examples of the application of this approach. It is indeed possible to compare images from different years. These images must be taken at the same time of year, so as to minimize the expression of variables such as light quality, observation geometry and, in the case of plant ecosystems, differences in the behavior of a community over the year (Singh, 1986; Chuvieco, 1998).

Both of these approaches are phenological. Phenology is the study of the sequence of events in the life cycle of plants and animals, especially in relation to changes in season and climate.

In our case, we tried to map the seasonal NDVI of the year (2019-2020), to spatially determine the production of forest biomass between seasons (winter and summer). From Figure 2, we notice a clear seasonal variation in photosynthetic activity, the forest vegetation in our case shows a higher vegetative activity during the winter season. On the other hand, the summer season marks the lowest values of the NDVI.

Changes in light reflectance during growth are evident and occur over short periods of time. In the case of forest ecosystems, natural processes, and approaches to observing them, are prolonged. The behavior of an individual tree extends over a long period of time (5 to 25 years), and the same rule applies to forest plantations described as "pure ecosystems" (i.e., consisting of even-aged stands).

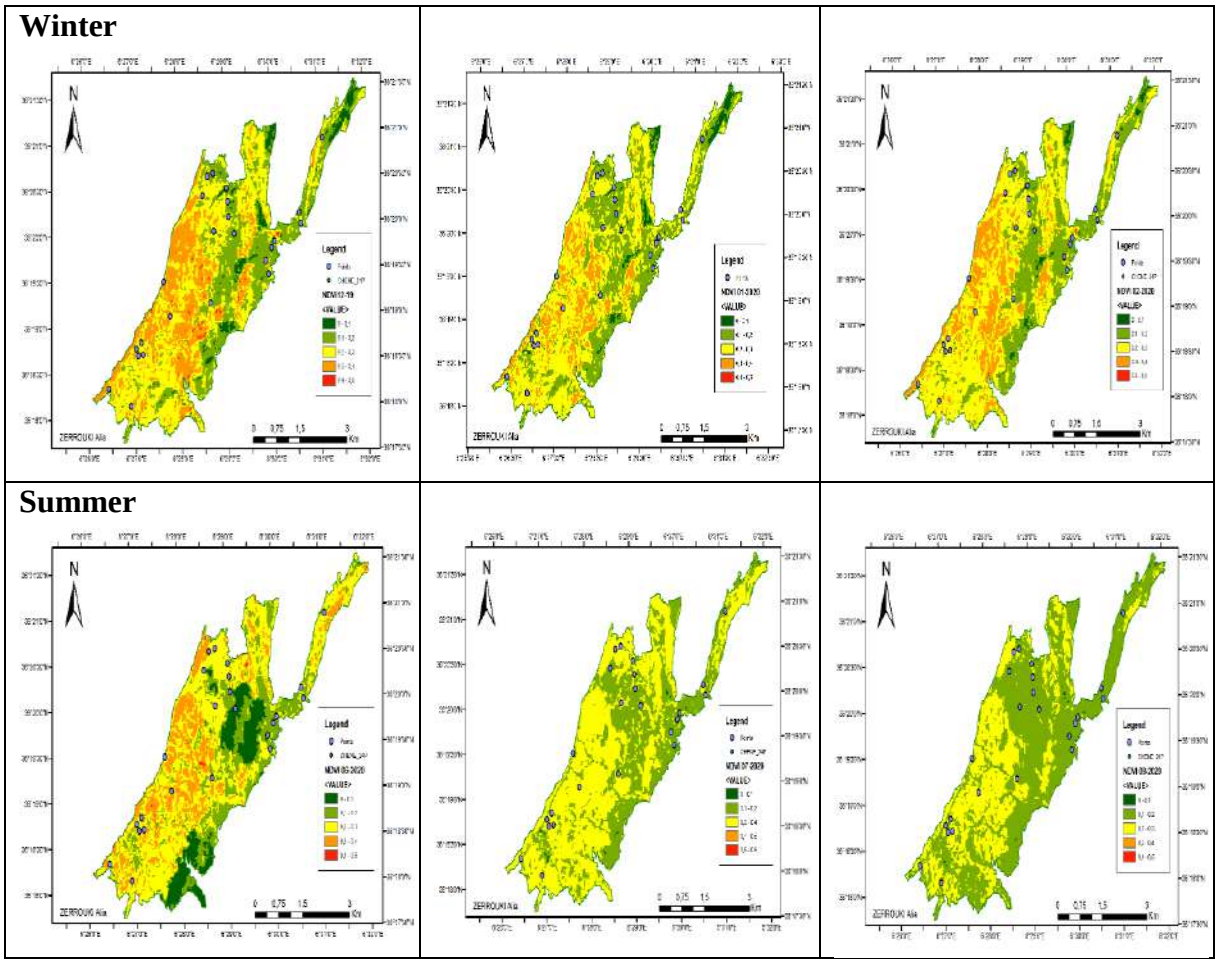

Figure 2. Seasonal NDVI calculated for the year (2019-2020); A: winter, B: summer.

The results of the descriptive statistical analysis of the variance of the holm oak NDVI indicate that the maximum values of NDVI are observed in December for winter and in June for summer while the minimum values are observed in January and August respectively (Table 1).

Table 1. Detailed NDVI ratio of the 6 least

NDVI	Observations	Observations without missing data	Minimum	Maximum	Mean	Variance
24/12/2020	24	24	0.115	0.387	0.243	0.006
09/01/2020	24	24	0.143	0.358	0.234	0.004
10/02/2020	24	24	0.122	0.380	0.240	0.005
10/06/2020	24	24	0.073	0.385	0.254	0.007
12/07/2020	24	24	0.157	0.341	0.227	0.003
13/08/2020	24	24	0.144	0.269	0.193	0.002

Table 2. Analysis of variance of NDVI of the 6 least

Source of variation	All months groups	Summer groups	Winter groups
Sum of squares	0.0531	0.0009	0.045
Degree of freedom	5	2	2
Mean of squares	0.0106	0.0004	0.0225
F	2.3761	0.0973	5.6178
Probability	0.0419	0.9073	0.0054
Critical value for F	2.2782	3.1296	3.1296

Concerning the month effect, the analysis of variance showed a significant effect on the variation of NDVI values for the studied holm oak trees. On the other hand, the seasonal effect showed a non-significant effect for winter and highly significant for summer (Figure 3). The summer season recorded a decrease in the percentage of natural vegetation index, because the summer season experienced a shortage of water and high temperatures that affect the holm oak which leads to the yellowing of its leaves (a negative correlation can be explained by the strong negative covariance existing between temperatures and precipitation).

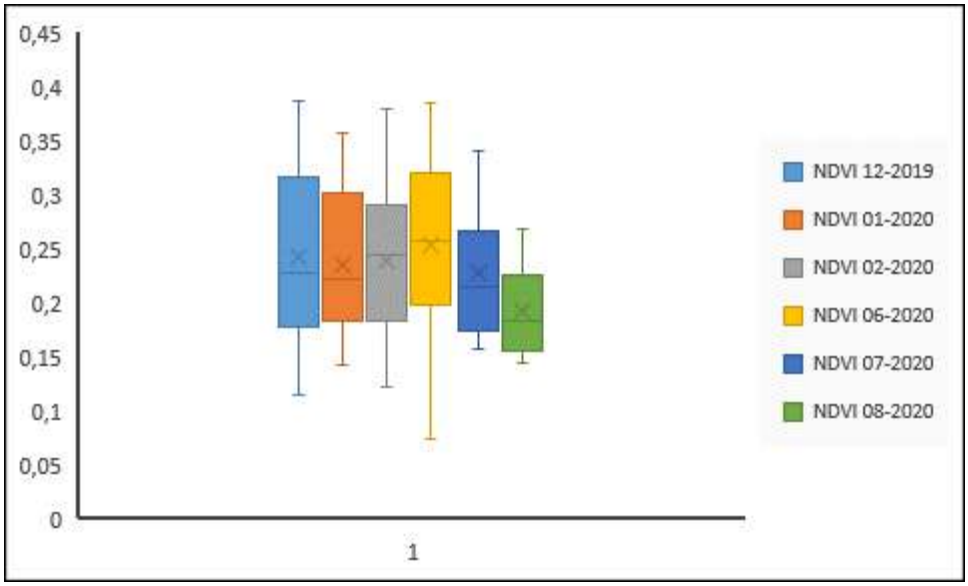

Figure 3. Boxplot of NDVI values by month.

CONCLUSION

The results obtained give an idea on the intensity and the seasonal effect of the vegetative activity of the Chettaba forest. They show a decrease of this activity from winter to summer, this is due to its exposure to thermal stress in summer. Thus, any increase in temperature compared to the average leads to a significant decrease in photosynthetic activity of the leaves of the trees, which is reflected in a significant drop in the values of the NDVI. This negative correlation can be explained by the strong negative covariance between temperature and rainfall. At the end of this study, we can say that it is possible to derive relevant information on the forest vegetation of the Chettaba forest from the use of satellite images and the various techniques of geographic information system (GIS).

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EFFECTS OF PENCANOZOLE TREATMENT ON CAPSICUM ANNUUM

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ABSTRACT

In agricultural areas, unconscious use of pesticides might cause harmful effects on growth and development of non-target crop plants and decreases yield and quality of the plants. Penconazole is a systemic fungucide that is used commonly in agricultural areas. Besides, it is known that use of penconazole at low concentration decreases negative effects of environmental stresses. In this study, it was examined that changes induced by penconazole application at different concentrations in pepper plants exposed to 0 and 200 mM NaCl treatment. For this purpose, different concentrations of penconazole treatment (15 mg/l, 25 mg/l, 50 mg/l ve 100mg/l) on pepper seedlings was conducted and leaves of the plants were harvested 10 days after the treatment. Total chlorophyll, carotenoids, malondialdehyde, ascorbate peroxidase, glutathione S-transferase and glutathione reductase analyses were performed in harvested leaves. It was determined that penconazole affected antioxidant defence responses, depending on the concentrations applied, of peppers that are both treated and not-treated with salt stress.

Keywords: Penconazole, Oxidative Stress, Pepper

INTRODUCTION

Growth of plants is optimum in conditions that are ideal for them. However, some conditions can adversely affect plant growth. These factors that negatively affect growth and development are called "stress". Plants are exposed to various stress factors such as drought, salinity, temperature and pesticides in their natural environment, and these stresses cause changes in some physiological and biochemical functions of plants (Kalefetoğlu and Ekmekçi, 2005, Çulha ve Çakırlar 2011).

Salt stress is one of the important stress factors affecting plant growth. The negative effects of salinity vary depending on the type of salt, the level of stress and the species of plants. Some plants are sensitive to salinity, while others tolerate stress through physiological, biochemical and molecular defense responses (Çulha ve Çakırlar 2011).

Another stress factor for plants is pesticide application in agricultural areas. Pesticides are chemicals used to combat diseases and pests. Penconazole (1-(2,4- dichloro-propylphen ethyl)-1H-1,2,4-triazole) (Pen) is a systemic fungicide and is used to combat powdery mildew in various plants (Bedil, 2015). Besides, it is known that use of penconazole at low concentrations decreases negative effects of environmental stresses (Shaki ve ark 2018, Hassanpour ve ark. 2013).

The purposes of this study can be summarized as follows: (1) to determine phytotoxic changes caused by Pen on non-target plant pepper, (2) to evaluate multiple stress interaction by comparing phytotoxic changes as a result of Pen application alone and simultaneous application of Pen and salinity, (3) to examine the effect of Pen application at low concentrations on salt stress.

MATERIALS AND METHODS

Plant material and treatments

In this study, "Mert F1" seed was used as pepper seed. Seeds are planted in pots containing turf and perlit after they are being kept in distilled water for 6 hours. Plants were grown under conditions having an average temperature of 28 ˚C and an average humidity of 60-65% in climate room. Seedlings (of approximately 6 weeks) were divided into 4 different groups (control, fungicide, salt stress, multiple stress). Stress applications were made as shown in Table 1. In 10th day after stress treatments, leaves of all plant were harvested and were stored at -80˚C freezer for analysis.

Treatments	**Groups**
Distilled water	CONTROL
15mg/L Pen	FUNGICIDE STRESS
25 mg/L Pen	
50 mg/L Pen	
100 mg/L Pen	
200 mM NaCl	SALT STRESS
15mg/L Pen+200 mM NaCl	MULTIPLE STRESS
25 mg/L Pen+200 mM NaCl	
50 mg/L Pen+200 mM NaCl	
100 mg/L Pen+200 mM NaCl	

Table 1. Experimental protocol of stress treatments

Physiological and Biochemical Analyses

Physiological and biochemical analyses were performed according to the methods developed by different researchers. Total chlorophyll and carotenoids contents were determined following the methods of De Kok and Graham (1980). The MDA content was analyzed according to Heath and Packer (1968). Proline analysis was made according to the method of Bates et al. (1973). The APX activity was determined according to Nakano and Asada (1981). GST activity was determined according to Habig et al. (1974). GR activity was analyzed according to Carlberg and Mannervik (1985).

RESULTS AND DISCUSSION

Total Chlorophyll and Carotenoids Contents

Changes in chlorophyll content in plants under stress are considered as an important stress biomarker. In this study, the highest total chlorophyll content was found as 14.64 μg^{-1} g at group treated with 15 mg/L Pen, the lowest total chlorophyll content was found as 4.9 μg^{-1} g at groups treated with 100 mg/L Pen+NaCl (Fig. 1).

The carotenoid content increased at all concentrations of Pen (except for 100 mg/L Pen) compared to the control, while NaCl application decreased the carotenoid content. The lowest carotenoid content was found as 0.98 μg^{-1} g at groups treated with 100 mg/L Pen+NaCl (Fig. 2).

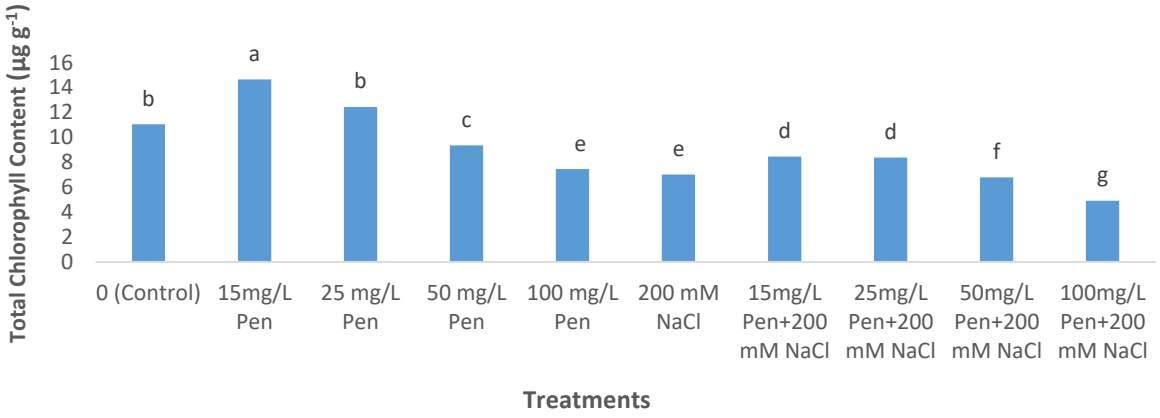

Fig. 1. Changes in total chlorophyll content in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

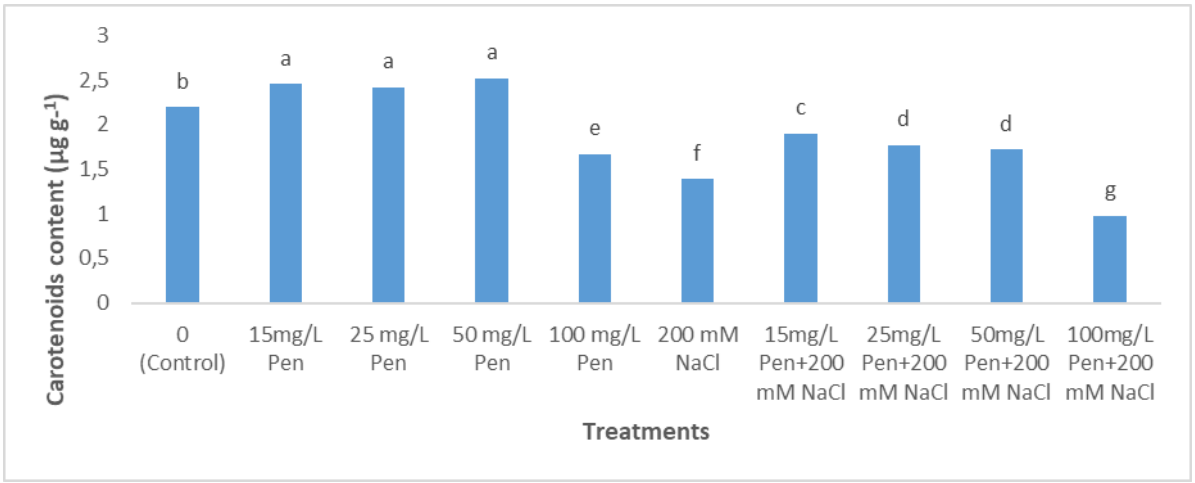

Fig. 2. Changes in carotenoids content in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

Proline Contents

Proline acts especially as osmoregulator in plants exposed to drought and salinity and also it has an important role on the adaptation of plants to various environmental stress factors (Delauney and Verma, 1993). In this study, proline content increased in all stress groups compared to control. The highest proline content was found as 0,019 µg mg^{-1} FW at group treated with 15 mg/L Pen (Fig. 3).

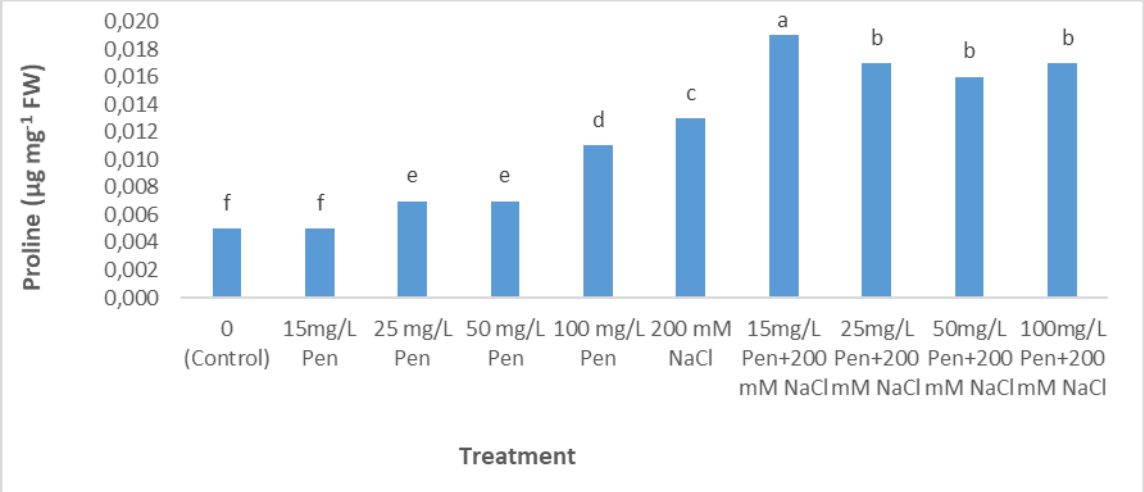

Fig. 4. Changes in proline content in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

Malondialdehyde (MDA) Content

MDA is the end product of lipid peroxidation and is also an important stress biomarker (Gill and Tuteja, 2010). In this study, MDA content increased at all stress groups (except for 15 mg/L Pen) compared to the control and the highest MDA content was found as 7.89 g^{-1} FW at groups treated with 100 mg/L Pen+NaCl (Fig. 5).

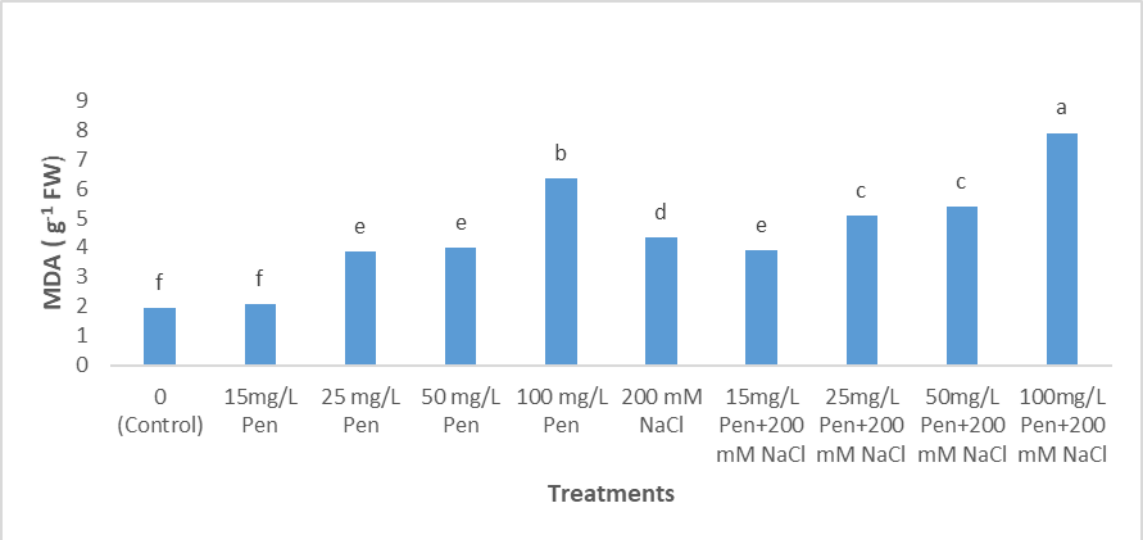

Fig. 5. Changes in MDA content in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

Antioxidant Enzyme Activity

Antioxidant enzymes in plants regulate the level of reactive oxygen species (ROS) and enable plants to survive under stress conditions (Mittler 2002; Çulha ve Çakırlar 2011; Büyük ve ark. 2012). It was determined that in both treated and non-treated salt stress plants, penconazole affected antioxidant enzyme activities. GST and GR acivities increased at all stress groups compared to the control. The highest GST and GR acivities were found at groups treated with 100 mg/L Pen+NaCl as 6.31 and 13.29 µmol min^{-1} mg^{-1} protein, respectively (Fig. 6 and 7).

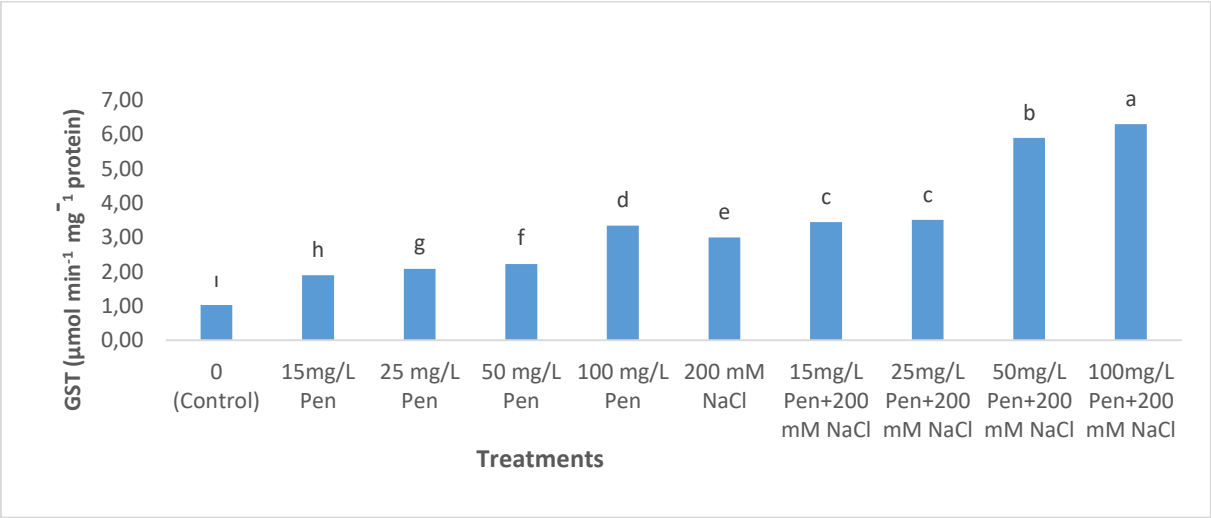

Fig. 6. Changes in GST activity in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

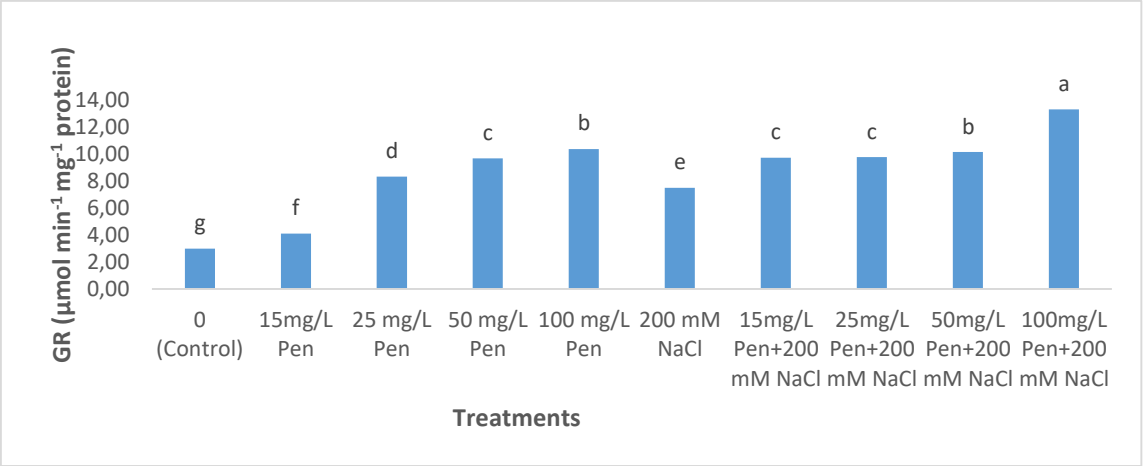

Fig. 7. Changes in GR activity in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

APX activity generally increased at all stress groups (except for 50 mg/L Pen+200 mM NaCl and 100 mg/L Pen+200 mM NaCl) compared to the control. At groups treated with 50 mg/L Pen+200 mM NaCl and 100 mg/L Pen+200 mM NaCl, APX activity decreased compared to control (Fig. 8). The decrease in APX activity in the last two treatment groups may be associated with increased ROS at these concentrations. Excessive accumulation of ROS in tissues may inhibit APX activity.

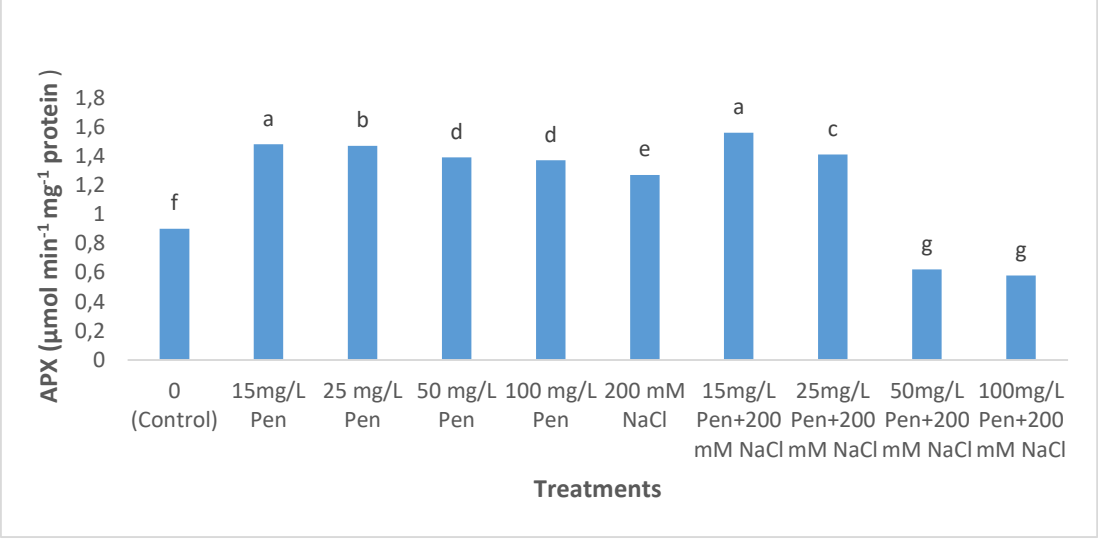

Fig. 8. Changes in APX activity in pepper leaves exposed to stresses. The different lower-case letters are significantly different from each other ($p<0.05$) among different stress groups according to Duncan's test.

As a result; both separate and simultaneous applications of Pen and NaCl induced antioxidant defence responses in the pepper plant. In particular, multiple stress applications induced these responses in more significant level. Low-dose Pen application (15 mg/L) decreased the MDA content and increased the pigment and proline content in plants under salt-stress.

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EVALUATION OF ECOSYSTEM SERVICES OF A PERI-URBAN FOREST IN THE CITY OF ALGIERS

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ABSTRACT

Forests provide a significant number of ecosystem services, related to economic, ecological, and social domains as well. The present work aims at evaluating the ecosystem services of the peri-urban forest of Bainem, which belongs to the coastal sub-sector.
Located at 15 km west of the downtown, the Bainem forest represents the largest green space in the Algiers city .However, throughout its history; it has suffered various anthropic and climatic aggressions resulting in continuous changes of its vegetation cover and land use, which in turn have repercussions on the quality of the services provided by these various components.
The objective of this study is to determine the ecosystem services provided by this forest and to map their spatial distribution to enhance better values and to develop them.
For that purpose, we conducted phytoecological surveys taking into consideration the ecological diversity of this area. This study was completed by surveys with a questionnaire to scientists and nature managers as a working tool.
The results show that this forest contains more than 150 species represented by 50 families including 84 species of known medicinal interest, 29 aromatic and medicinal species, 94 melliferous species, and 29 edible species including 4 mushrooms.
In terms of regulation, and lying on relief, this forest protects the whole city located at bottom, against natural hazards such as floods, landslides, erosion, and runoff that can cause material and human damage.
In addition, it contributes to climate regulation by reducing greenhouse gas emissions, by absorbing carbon dioxide. With an area of 504 ha composed by different forest ecosystems, this forest can absorb more than 2.2 million kg of total carbon.
The full expansion and transformation of Algiers into a large metropolis has an interest in conserving and enhancing of the peri-urban forest of Bainem.

Keywords: Ecosystem services, peri-urban forest, aromatic and medicinal plants, carbon sequestration, Baïnem forest.

INTRODUCTION

Ecosystem services have become, over the last twenty years, a concept recurrently mobilized in public policies for biodiversity preservation (Maillefert &Petit, 2017). Forests provide a large number of ecosystem services, linked to economic, ecological, and social domains (Carnol &Verheyen, 2010). They correspond to the benefits derived by humans from biological processes (CGDD, 2010). The concept of ecosystem services highlights all the material and immaterial benefits that ecosystems provide to humans (MEA, 2005). It has become the essential model for the link between ecosystem functioning and human well-being (Fisher & al. 2009).

The diversity of ecosystem services provided by forests reveals the importance of the conservation of forest ecosystems about the goods and services they provide to humans, whether in terms of provisioning services, regulating services, or cultural services which, according to Daily & al. (1997), are for the most part difficult to substitute.

Scientists, managers, and policymakers are increasingly recognizing the diverse contributions of ecosystems to human well-being, prompting them to seek and develop the tools and knowledge needed to manage these systems and identify and quantify ecosystem services and evaluate their benefits (Binder & al. 2017).

The goal of ecosystem service assessment is to provide detailed information that will contribute to environmental management decisions by identifying high-priority ecosystem services and assessing their environmental, sociocultural, and economic dynamics and importance (Preston & Raudsepp-Hearne, 2017).

Biodiversity is at the heart of thinking about ecosystem services. Indeed, it is likely to provide regulatory, support, and cultural services, and the maintenance of biodiversity through forest management is, therefore, an essential service provided by forests (Dreye &Landmann, 2012).

Located in the Mediterranean region, Algerian forests are home to a wide range of biological diversity including trees, plants, insects, fungi, animals that represent an essential element of the climatic, physical, and socio-economic balance of rural areas in particular and the country in general.

Algeria covers an area of 2 381 741 km ², 10% of the total area represents forest formations that are located in the northern regions of the country because of climatic and soil conditions favorable to their development.

Peri-urban forests are "natural" spaces within or on the bangs of an urbanized area, they are protected and removed for many from economic activities: they are assigned primarily a social recreational, landscape, and ecological role (Monot, 2017).

Located 15 km west of the center of the city of Algiers, the peri-urban forest of Bainem represents the largest green space in Algiers. However, throughout its history, it has suffered various anthropic and climatic aggressions which are translated by continuous changes of its vegetal cover and occupation of ground, and which repercussion on the quality of the services provided by these various components. This forest presents a great variety of landscapes and remarkable ecosystems, it is endowed with an exceptional landscape and patrimonial value, which deserves particular attention.

The problem that arises is related to the lack of studies on the ecosystem services of the components of this forest and its environment and by the importance that the populations that live in the surroundings give to it.

Aware of the primordial role played by urban and peri-urban forests on the environmental, social, and landscape levels, and aware of the multiple pressures to which these forests are subjected, land covetousness, not always organized frequentation and pollution, to name only the main ones (Laaribya, 2006).

The present work aims to evaluate the ecosystem services of the peri-urban forest of Bainem which belongs to the coastal sub-sector and to map their spatial distribution to better valorize and develop them.

MATERIAL AND METHODS

Our study was conducted in the peri-urban forest of Bainem which has a public status is subject to the forestry regime and managed by the Directorate General of Forests, under the Ministry of Agriculture. This forest is subject to massive frequentation generating an excessive anthropization. It extends over an area of 504 ha, with a topography rather uneven in the massif of Bouzaréah (fig.1).

15 Kilometers west of Algiers, in a coastal position less than 1 kilometer from the Mediterranean Sea. The bioclimate is subhumid with warm winter. The highest point of the forest reaches 320 m. With the geographical coordinates 2°56'59" to 2°59'08" of East longitude and 36°47'52" to 36°48'30" of North latitude.

The terrain is diverse with the presence of metamorphic rocks (schists, mica schists, gneiss) (ISL-BRGM, 2006).

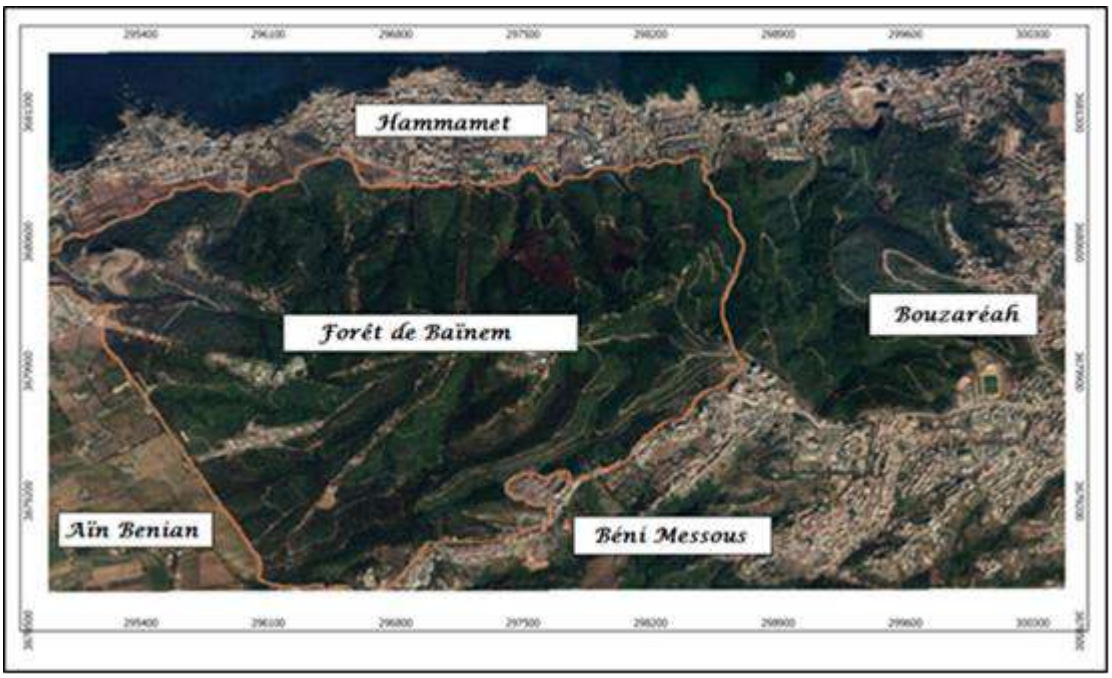

Figure 1. Geographic situation of the study area (Google Earth)

A preliminary field visit was conducted during April 2021. Then, several field trips were made to try to identify as closely as possible the richness of plant biodiversity. Thus, the methodological approach adopted to achieve the map of ecosystem services involved 55 phytoecological surveys that were conducted in the field throughout the peri-urban forest of Bainem taking into account the ecological diversity of this area.

In the case of our study, we opted for subjective sampling which consists in choosing a sample of the zones appearing particularly homogeneous and representative of the whole (Gounot, 1969).

The surface of each phytoecological survey was 400 m2. Each species was given abundance-dominance coefficients, based on the codification of (Braun-Blanquet, 1932).

The common species being recognized in the field, those that we could not identify were carefully collected, photographed, and brought back to the laboratory for identification. Thus 151 species were identified using the flora of Quézel and Santa (1962-1963). The nomenclature was updated using the synonymic index of the flora of North Africa (Dobignard & Chatelain,, 2010-2013).

The second method of our study was to conduct a field survey as a working tool questionnaire previously prepared and established according to our problem. The administration of the questionnaire was done instantly, i.e. in a face-to-face situation between the investigator and the respondent.

Our surveys had to target the actors involved in the subject of our study at all levels. To do this, we developed a list of stakeholders involved in ecosystem services assessment. The sample is composed of 100 actors with four types of actors: foresters (managers), INRF researchers, kiosk owners, and visitors.

After photo-interpretation of a satellite image and thanks to the Qgis 3.4 Madeira software, we obtained isophenous zones, which informed on the phytoecological plan allowed us to identify and characterize all the facies and thus to obtain a map of the land occupation.

The correlation between the survey and the results of the land occupation allowed us to highlight the different ecosystem services of the Bainem forest.

RESULTS AND DISCUSSION

1. Map of the occupation of the forest of Bainem

The isophenous zones informed on the ecological plan allowed us to elaborate the map of the occupation of the lands, in this last one we were able to bring out the various physiognomic types to know, matorral, forest, scrub, and plantations, the remainder of the forest is occupied by an arboretum, naked ground and rocky outcrops (fig.2).

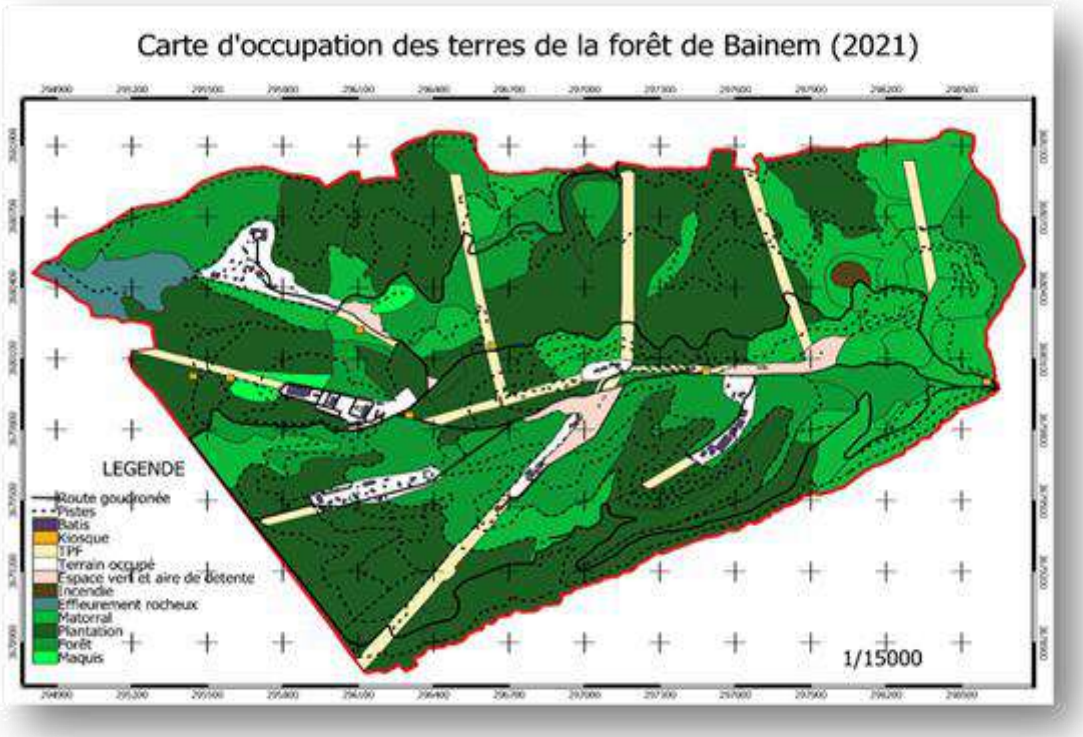

Figure 2. Map of land use of the forest of Bainem

1.1 Physiognomic types

Almost half of the forest is occupied by plantations (Aleppo pine, Eucalyptus, Acacia) or 49%, while natural forests occupy only 17%. The state of degradation of forest formations is observed by the area of matorrals that occupies almost double the forest formations or 29% (Fig. 3).
The floristic inventory allowed us to identify 151 species distributed in 50 botanical families.
Because of the high public use of wooded areas, we cannot forget that the recreational service allows different categories of the population to enjoy the landscape qualities of the forest and to practice activities beneficial to health and well-being.

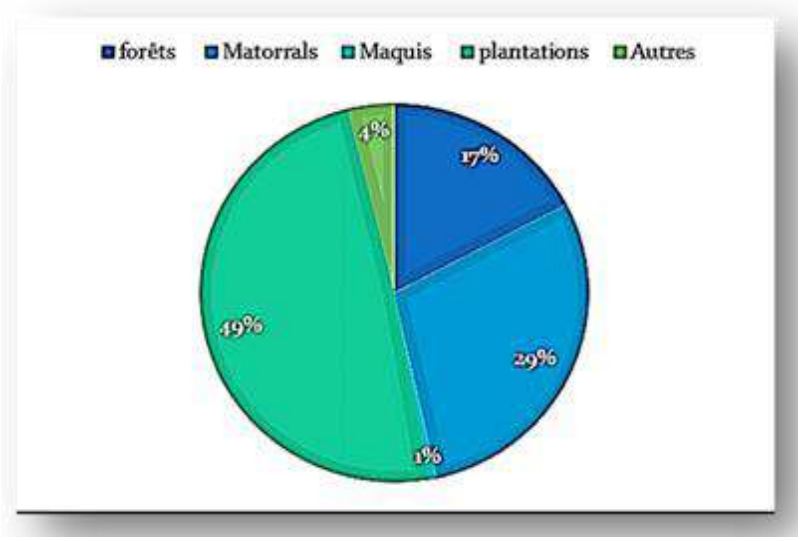

Figure 3. Area of land use in the Bainem Forest

2. Results of the surveys

A total of 100 people in this peri-urban forest participated in the survey (Fig. 4), 78% represented by visitors, 11% by researchers from the National Institute of Forest Research (INRF), 7% by foresters followed by owners of kiosks of the forest or 4%.

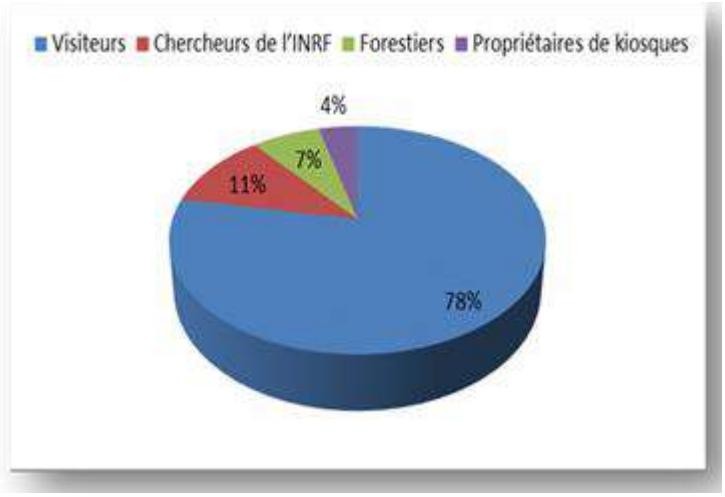

Figure 4. Representation of the percentages of the respondents according to their status

2.1. Choice of the forest of Bainem

According to the results obtained, we note that the primary purpose of visitors is in favor of children with a rate of 32.69% (Fig. 5) followed by the value of 19.23% which represents the relaxation because it brings them significant well-being and 9.61% to be in contact with nature.

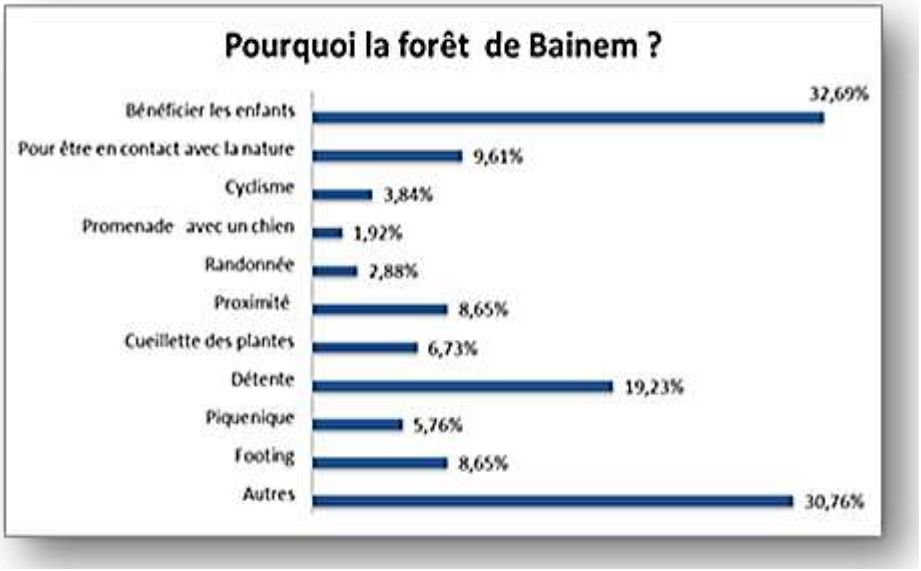

Figure 5. Main reasons for which the respondents come to the forest of Bainem

2.2. The rate of frequentation

It emerges from the analysis of the obtained result that 54.05% of the visitors come to this forest on Friday (fig.6), 27.02% visit this forest on Saturday, 12.16% on Tuesday, and 6.75% of the answers for the rest of the days of the week.

Figure 6. The days of visits to the forest of Bainem

The frequentation of forests is often a function of the season. The peri-urban forest of Bainem has become one of the privileged sites for different activities such as walking or mountain biking. This frequentation has become excessive especially in spring insofar as almost all visitors frequent the forest during this season with a significant rate of attendance of 51.35% (Fig. 7), and more particularly the weekends with a rate of 39.18% (Fig. 6), then come the fall with a percentage of 16.21%, winter with a percentage of 13.51% and finally the summer with a percentage of 8.10%.

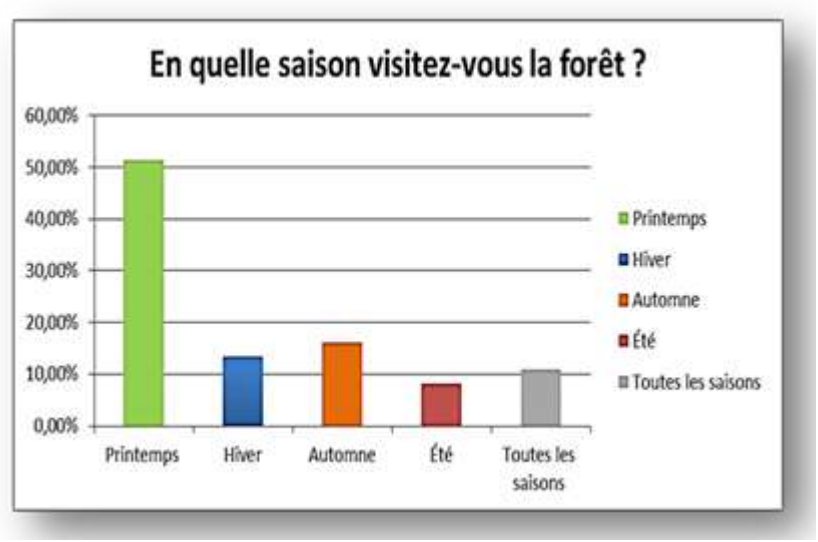

Figure7. The seasons of the visit of the forest of Bainem

This frequentation contributes directly or indirectly to the degradation of the forest of Bainem by the compaction of the grounds by the vehicles to reach the forest, the trampling, motorcycles, cars circulating and parked everywhere even under the trees and on tracks, etc. This frequentation then generates harmful consequences for the forest stands.

3. Ecosystem services

After analyzing and interpreting the results of our survey and maps, we were able to conclude all the services of this forest ecosystem.

The results of this study show that the peri-urban forest of Bainem offers a diversity of ecosystem services:

3.1 Cultural ecosystem services

In recent years, the managers of the peri-urban forest of Bainem have improved the possibilities of recreation and nature education, which was inaccessible at the time by developing pedestrian and educational trails. Given its location, close to urban centers, it has a higher recreational value than other more remote forests. It is a study site where many scientists can research the fauna and flora.

- **Recreation and relaxation**

This peri-urban forest offers visitors a space for relaxation and sports exercises (jogging) and recreational activities for children and adults (hiking, cycling) contributing to their mental and physical well-being.

Group of cyclists in the forest of Bainem
Pictures L. MEHRI 2021

The total area of recreational areas is 8,456 ha (fig.8), a small area compared to the total area of the forest of Bainem for that the managers of the forest must release other areas distributed throughout the forest.

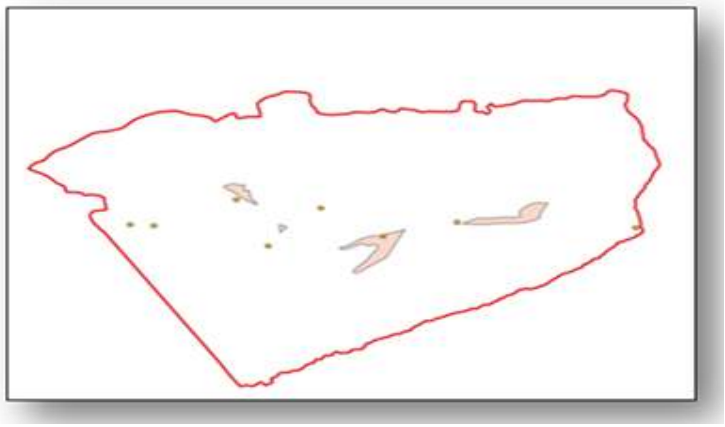

Figure 8. Map of the location of the most frequented areas and kiosks in the forest of Bainem

- **Education**

This forest represents a site of great importance for research and scientific studies.

Researchers of the INRF and students of Algiers 1 University in the Bainem forest
Pictures S.ABLA 2018

- **Category of beneficiaries of the cultural ecosystem services of this forest:**

- Professionals: in this category, the first beneficiaries are the researchers because this forest contributes to the valorization of their scientific knowledge, in second place, the owners of kiosks and the cleaning agents by offering them jobs.
- The visitors: they are beneficiaries who come to this forest during their free time to relax or to practice various recreational activities.
- The people who live near the forest enjoy the recreational benefits of the forest and the unpolluted climate.

3.2. Ecosystem services for provisioning

- **Medicinal and edible species**

Among the various benefits provided by the peri-urban forest of Bainem, we have non-wood products (NTFP) which are products used in the manufacture of medicines. We were able to identify:

- 84 medicinal species ;
- 29 aromatic and medicinal species.

The medicinal plant species found in this forest constitute natural remedies that can be used as curative and preventive treatments.

The majority of respondents collect myrtle (*Myrtus communis*), lavender (*Lavandula stoechas*), and nettle (*Urtica urens*).

Only 20% of the respondents collect mushrooms: the blood lactary (*Lactarius deliciosus*), button mushrooms (*Agaricus bisporus*), lepiotes (*Macrolepiota procera*), and boletus (*Boletus*) (fig. 9).

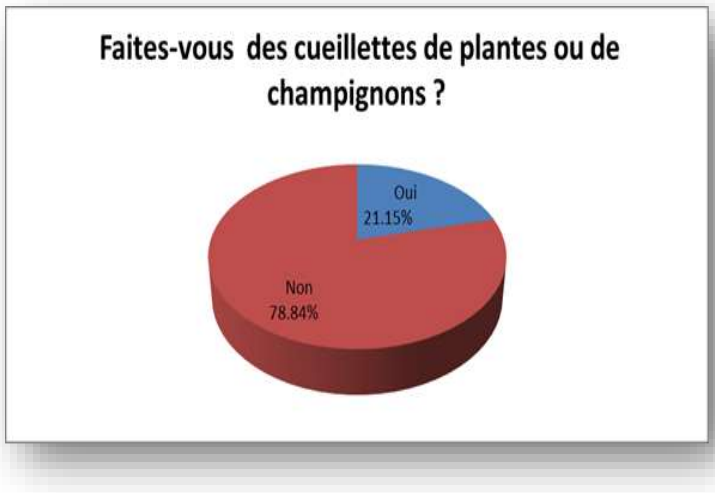

Figure 9. Collection of plants

3.3. Regulating ecosystem services

- **Carbon sequestration**

Plant species in the Bainem forest contribute to climate protection by absorbing CO2 from the atmosphere through photosynthesis and wood formation and by storing carbon in the soil over the long term.

The highest values are attributed to conifers which are represented in majority by the Aleppo Pine store 36.6 kg/m3 followed by hardwoods (Eucalyptus, Acacia and Oaks) which store 23kg/m3, the average values are those of the scrubland which store 7.2kg/m3, finally the lowest values are those of mixed formations which store 4.5kg/m3 (fig.10).

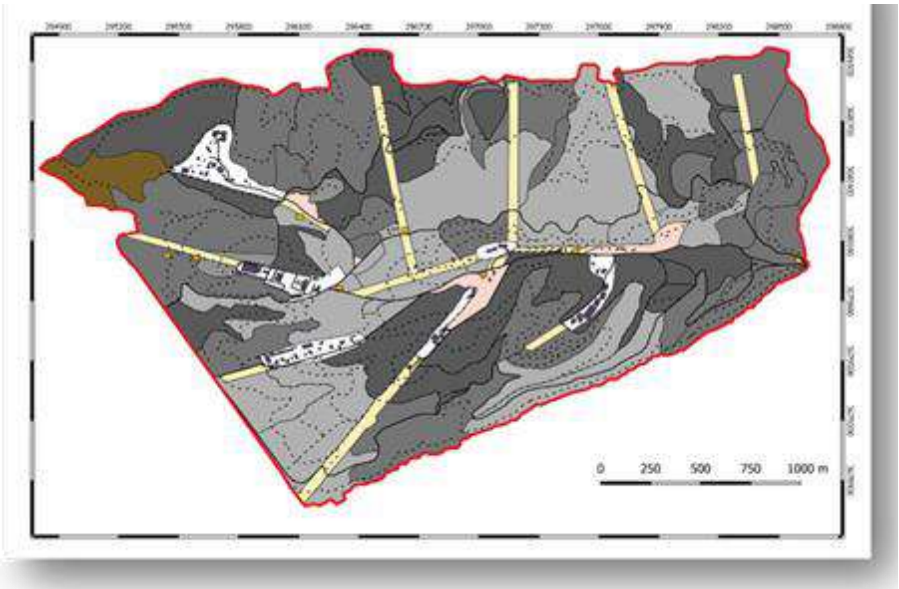

Figure10. Map of carbon sequestration in the forest of Bainem

- **Erosion control**

Vegetation cover, particularly the above-ground and below-ground structures of plants, is important for retaining soil and stabilizing slopes (CSBQ, 2017). Our study area consists mainly of Eucalyptus plantations that have a protective effect against erosion.

- **Pollination**

Pollination is necessary for the reproduction of most plants. Insect pollination has a stabilizing effect and increases the yield of food crops.

In our study area, we could find 94 melliferous species that attract numerous pollinating species that contribute to this regulation service.

3.4. Supporting ecosystem services

- **Soil formation**

All forests have a role in soil formation and thus determine their typology. Softwood stands and plantations such as Pinus halepensis plantations contribute to the formation of acidic soils through the decomposition of their needles, which release organic acids that can contribute to the acidification of horizons. Unlike coniferous trees, hardwoods have tissues rich in cellulose, the decomposition of their leaves contributes to the formation of good quality and fertile soils.

CONCLUSION

The results of this study show that the peri-urban forest of Bainem offers a diversity of ecosystem services. This study also allowed us to identify 151 species with 29 species of aromatic and medicinal plants, 84 medicinal species and 94 melliferous species, and 4 species of mushrooms and 29 edible species.

In addition to its protective role, it contributes to climate regulation by reducing greenhouse gas emissions, by absorbing carbon dioxide. With an area of 504 ha composed of different ecosystems, this forest can absorb 2 249 862, 6 kg of total carbon.

There are few sectors and organizations in Algeria that integrate the theme of goods and services provided by natural ecosystems and the economy of ecosystems and biodiversity in general. The goods and services provided by natural ecosystems are important for many sectors, and to preserve and enhance them, it is necessary to establish strong partnerships with other sectors

such as Agriculture, Forestry, Fisheries, Tourism, Energy Industry, Water, Land Management and the Ministry of Culture.
The peri-urban forest of Bainem plays an important and primordial role both environmentally, socially, and landscape, for the well-being of city dwellers and the enjoyment of landscapes
Aware of the importance of the urban and peri-urban forest in the life of the population, of the multiple services it offers to city dwellers, and of the problem of its degradation, the public authorities must give a particular interest to this space.
The work done can serve as a basis for further work on the ecosystem services of the peri-urban forest of Bainem a forest heritage to be preserved by all means for future generations.
The city of Algiers in full expansion and transformation into a large metropolis has an interest in preserving and enhancing the peri-urban forest of Bainem.

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DETECTION OF ROAD POLLUTION BY USING OF A BIOACCUMULATIVE PLANT FOR THE EVALUATION OF HEAVY METAL CONCENTRATIONS IN WESTERN ALGERIA

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Introduction

Several researchers have shown that sensitive plants react as true bio-indicators of pollution. This "bio-monitoring" technique uses plant organisms that can store pollutants in their tissues or on their surfaces through fixation and transfer mechanisms. The measurement of pollutant levels in these sensitive plants makes it possible to detect the degradation of air quality before it severely affects the biotope or man. Plants considered sensitive are used as bio-indicators to monitor the stress generated by air pollution (Rupa and Venkatachalam 2018).The evaluation of heavy metal emissions from motor vehicle traffic is the objective of this work. It provides a better understanding of these emissions, whose deposition is harmful to the environment and humans through a plant species used as a bio-accumulator of airborne heavy metal contamination from road traffic. Heavy metal emissions from road traffic are mainly in the form of fine particles that are then collected by leaf surfaces (Sulaiman and Hamzah 2018).

Materials and Methods

The study area includes the town of Sidi –Bel- Abbes and a service station on the national road linking Sidi-Bel-Abbes to Telagh. It is located in Western Algeria characterized by a Mediterranean climate of the semi-arid type with an average annual rainfall of 350 mm with a rainfall regime of the HPAE type. The study area is characterized by very dense and elevated road traffic all year round, inducing particles, dust and organic complexes from vehicles.
Plant sampling: Needle samples were collected from Aleppo pine trees and samples of litter under the trees at both sites. Each site has been divided into 3 different zones to assess the degree of heavy metal contamination from road traffic. For the chemical analysis, 200 grams of needles and 200 grams of litter were collected for each zone. The 12 samples collected were kept in airtight bags and labeled for analysis.
Extraction method: All media and equipment used for the analyses were washed with distilled water, then soaked in HNO3, rinsed with distilled water and dried in an oven. The needles were separated from their supports and dried in a filtered air oven for 4 days at a temperature of 40°C without any treatment and then ground to a powder with an agate mortar. The analytical protocols used are based on ICI (Inter-Institute of Analytical Techniques) methods and dry mineralization was used for Cd, Cu, Fe, Ni, Pb, Cr and Zn. The solution of the metallic elements is carried out on a 1 g ground sample, then the procedure applied: calcination at 420°C for 4 hours in an oven, then recovery of the ashes with 5 ml of HNO3 65%, let evaporate for 3 minutes, then add 10 ml of HNO3 50%. Filtration is then carried out for 24 hours on filter paper (10µm). Six solutions are prepared for each site (3 solutions for the Pine needles "Zone1, 2, 3"and 3 solutions for the litter needles "Zone1, 2, 3"in total 12 solutions are prepared. A blank control solution of 65% HNO3 and distilled water was prepared in the same way as the 12 solutions mentioned above.

Elements analyzed: The metals sought in the samples "Cd, Cr, Cu, Fe, Ni, Pb and Zn" are subjected to analysis by "flame atomic absorption spectroscopy": FAAS (Rayleigh WFX-130BAAS). An air/acetylene flame was used for the excitation of the metal atoms and specific lamps for each metal were used for the detection of each element. The limit of quantification of pollutants in plants is, according to themethodused: 0.01, 0.05, 0.13, 0.045, 0.03, 0.1 and 0.007 mg/kg for Cd,Cr,Cu,Fe, Ni, Pb and Zn.

Results and discussion

The classification of heavy metals according to their concentrations in descending order at the two study sites: for the Urban site Pb> Fe> Zn> Cr> Cu> Ni> Cd, and for the Rural site Pb> Fe> Zn> Cu> Cr> Ni> Cd. Cr enriched road dust from our urban site has higher total concentrations than our rural site. Apeagyei et al (2011) observed that urban road dust was significantly enriched in Cr compared to rural road dust. Athanasopoulou and Kollaros (2016) observed that total heavy metal concentrations in road dust came mainly from highways. With vehicles traveling at 80 km/h, heavy metal levels in road dust were much higher than those emitted by vehicles traveling at 50 km/h (Duong and Lee 2011). The major sources of Ni were determined to be diesel emissions, brake abrasion and vehicle corrosion. Cu was mainly derived from brake abrasion and combustion exhaust. Zinc concentration was influenced by vehicle emissions and tire wear (Duong and Lee 2011).Copper in street dust may be a result of wear and tear on automobile engines, while tire wear and lubricating oils are possible sources of zinc and cadmium. Exposure to halogen and pollutant emissions from motor vehicle traffic is detrimental to human health and associated with an increased risk of respiratory disease (Hirshon et al 2008);

Table 1. Summary of heavy metal concentration results "mg/kg" at the two study sites.

Metals	Sidi Bel Abbes Bosquet		Telagh gas station		Deviations	
	"Urban site		" Rural site "			
	Needle	Litter	Needle	Litter	Needle	Litter
Fe	293,77	343,10	312,60	393,17	+ 18.83	+ 50.07
Cu	104,60	213,60	193,27	307,30	+ 88.67	+ 93.70
Ni	44,70	162,27	52,13	177,63	+ 7.43	+ 15.36
Pb	325,27	378,67	391,70	486,00	+ 66.43	+ 107.33
Zn	202,50	312,07	233,87	356,73	+ 31.37	+ 44.66
Cd	22,90	127,47	37,67	105,57	+ 14.77	- 21.90
Cr	161,87	233,67	165,77	284,00	+ 3.90	+ 50.33
Total	1 155,60	1 770,83	1 387,01	2 110,40	+ 231.40	+ 339.57

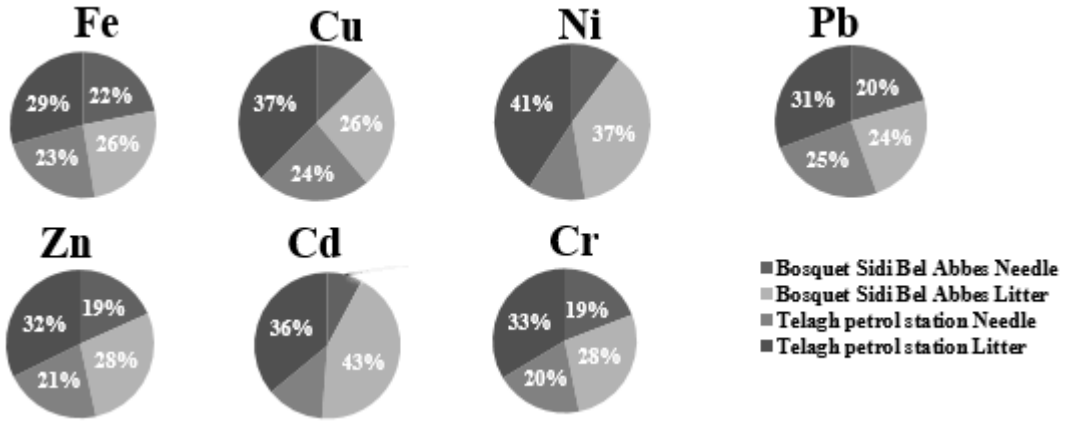

Figure 1: Percentage representation of each metal in Aleppo pine needles and its litter at the two study sites.

Conclusion

A total of 1155.60 mg/kg and 1387.01 mg/kg are recorded for needles for the urban and rural sites respectively. Concentrations in litter are 1770.83 mg/kg and 2110.40 mg/kg for the urban and rural sites respectively. Road traffic is an important source of heavy metals for the environment. And Aleppo pine is a very good accumulator of heavy metals. The litter accumulates more heavy metals compared to pine needles. The results also show that the areas exposed to road traffic (Zone 1) were found to be more polluted than the other sampling areas (Zones 2 and 3) for both study sites. The rural site is more polluted than the urban site due to the presence of a service station containing the lubricants and has very frequent heavy truck traffic throughout the year.

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INVESTIGATION OF BIOLOGICAL ACTIVITY AND SOLAR PROTECTION FACTOR OF Ziziphus jujuba FRUIT AND BRANCH EXTRACTS

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ABSTRACT

Nowadays, it is of great importance to investigate the use of natural additives in the food, pharmaceutical and cosmetics sectors. *Ziziphus jujuba* (Hünnap), an herb widely used in Chinese folk medicine, is very popular due to its high nutritional value. In our study, *Z. jujuba* water fruit and branch extracts were prepared using sonicator device (SN) and hot water bath (HWB). Antibacterial and antifungal activities were investigated on food-borne test microorganisms (*Escherichia coli* O157:H7 and *Listeria monocytogenes* ATCC 7644), clinical test microorganisms (*Pseudomonas aeruginosa* ATCC 27853, *Candida glabrata* RSKK 04019 and *Candida albicans* ATCC 10231) and fish originated pathogens (*Aeromonas hydrophila*, *Vibrio anguillarum* M1, *Vibrio anguillarum* A4, *Lactococcus garvieae*, *Yersinia ruckeri*). The antimicrobial activity of the *Z. jujuba* water fruit and branch extracts was investigated by disc diffusion and micro-dilution assays. The disc diffusion assay results indicated that among the food and clinical test microorganisms, the highest antimicrobial activity was determined against *C. albicans* ATCC 10231 with 16.89 mm inhibition zone diameter in SN water fruit extract. Among fish pathogens, HWB water fruit extract showed the highest antibacterial activity against *V. anguillarum* A4 with 16.96 mm inhibition zone diameter. The lowest inhibition zone diameter of all the tested extracts was determined against *A. hydrophila* with 6.46 mm for the SN water branch extract. Minimal inhibition (MIC) and minimal bactericidal or fungicidal (MBC or MFC) concentrations of HWB and SN water fruit and branch extracts on all tested microorganisms were determined between 25 and 100 µg/µl. The solar protection factor (SPF) of *Z. jujuba* HWB and SN water fruit and branch extracts was determined by in-vitro assay. The SPF values of the *Z. jujuba* extracts were varied from 0.17 to 1.20. The highest SPF value was obtained from SN water branch extract (1.20). The results we obtained in our study suggest that *Z. jujuba* HWB-SN water fruit and branch extracts can be recommended as natural additives in various industrial areas such as food, pharmaceutical and cosmetics.

Keywords: Hünnap, Food pathogen, Clinical pathogen, Fish pathogen, Antimicrobial activity, Solar protection

INTRODUCTION

The rapidly growing world population leads to increase in the amount of food needed. In recent years, diseases caused by food-borne pathogens have become an important health problem worldwide and cause important economic problems (Hemalata et al., 2016; Zhao et al., 2014; Akbar and Kumar, 2011; Bedasa et al., 2018).The main reason for the occurrence of food-borne diseases and spoilage of food; bacteria, viruses, fungi (Hemalata et al., 2016; Zhao et al., 2014). *L. monocytogenes* is an important food-borne pathogen that can survive even at high salt concentrations and low pH (Pal and Awel, 2014; Dhama et al., 2013). Due to the capability of this pathogen to survive and grow in a wide variety of hard environmental conditions makes it

a large worry for ready-to-eat (RTE) produce (Gkerekou et al., 2021). In the last report published by the European Food Safety Authority (EFSA), they reported that the case of listeriosis in humans in 2018 was 2549 (EFSA and ECDC, 2019). *E. coli* O157: H7 is also an important food-borne pathogen that can cause a high incidence of disease. It was reported as the main cause of an estimated 74,000 cases and 61 deaths in the United States of America (USA) (Bedasa et al., 2018). *P. aeruginosa* is a Gram-negative human pathogen and is considered one of the main pathogen associated with nosocomial infections (Zhao et al., 2020). *Candida* species are fungi that can be commonly colonized on various mucosal surfaces such as the oral cavity, intestine, and vagina. More than 90% of Candida-related infections are caused by *C. albicans, C. glabrata, C. krusei, C. parapsilosis* and *C. tropicalis* (Singh et al., 2020).

Fish and fishery products meet a large part of people's food needs. The most important reason encountered in aquaculture is the high rate of product loss due to diseases caused by pathogenic microorganisms. It has been reported to cause high fish mortality and serious economic damage due to the infection it causes (Olivares-Fuster et al., 2008; Mian et al., 2009; Soto et al., 2015). *Y. ruckeri* is a facultative anaerobic pathogen that is the main cause of yersiniosis, a serious septicemic disease (Tobback et al., 2007: Acuña et al., 2020). *V. anguillarum* and *A. hydrophila* are pathogenic microorganisms that cause disease in fish. They are responsible for significant economic losses worldwide. There are many antibiotics used to prevent diseases that have occurred in fish farms recently. Due to the widespread use of antibiotics, resistant bacterial strains began to develop (Mirand Zemelman, 2020; Seyfried et al., 2010). The possibility of transferring antimicrobial resistance genes from aquatic animals to humans (Cabello, 2006; Romero et al., 2012) has increased the search for alternative natural antimicrobial sources. Plants constitute the most important group of alternative natural antimicrobial sources due to the phenolic compounds they contain. Therefore, plant materials constitute the most important group of alternative natural antimicrobial sources. *Z. jujuba* is a widely used herb in Chinese folk medicine since the past. Known as its homeland China, *Z. jujuba* is widely growth in Russia, the Middle East, India, Southern Europe, Anatolia and North Africa (Mengjun, 2003; Reichl, 1991). *Z. jujuba*, which is very popular today due to its high nutritional value, has been used as a food ingredient, food additive and sweetener for many years in addition to its medicinal use. In Chinese folk medicine, it has been shown to implement numerous health-enhancing effects, such as protecting the gastrointestinal system, anti-inflammatory, antioxidant, antimicrobial, and apoptotic effects in breast cancer cells (Plastina et al., 2010; Yu et al., 2012). *Z. jujuba* has a very rich content in terms of carbohydrates, proteins, fats, minerals, vitamins, phenolic compounds and flavonoids (Liu et al., 2007). Flavonoids are a class of natural products found in fruits, vegetables, and beverages. They are synthesized by plants have many important effects, such as protection against pathogens and ultraviolet B (UV-B) radiation (de Cooman et al., 1998). Ultraviolet radiation (UV) accelerates skin aging by causing changes in collagen and elastic fibers, and causes skin cancer by causing DNA damage (de Cooman et al., 1998). Ultraviolet radiation consists of three parts: ultraviolet A (UV-A), UV-B and ultraviolet C (UV-C). Most of UV-C (200-290 nm) is absorbed by the atmosphere (Allen and Bain, 1994). Radiation effective in the development of skin cancer are UV-A (320-400 nm) and UV-B (290-300 nm) (Sarkar, 2004). As a result of the epidemiological studies of the World Health Organization (WHO), it has been shown that the main etiological cause of skin cancer is exposure to UV radiation during childhood and puberty period (WHO, 2001). In this study, the antimicrobial activity of water extracts from *Z. jujuba* fruits and branch was investigated on various originated test microorganisms. The solar protection factor (SPF) of the *Z. jujuba* fruits

and branch extracts were also examined to determine potential use as natural additive in cosmetics industry.

MATERIAL AND METHOD

The *Z. jujuba* fruit and branches were washed and then dried. After grounding with a Waring blender, the powdered fruit and branch samples were extracted with water by using hot water bath and sonicator devices. In the sonication method, the ground fruit and branch materials were extracted with water on ice for a total of 30 minutes at 10 minute intervals. In the hot water bath, the fruit and branch materials were extracted with water at 100°C for 36 h. The solvents of the extracted samples were evaporated and then the dried extract was obtained. The water fruit and branch extracts were dissolved in dimethylsulfoxide (DMSO) and then sterilized with 0.45 µm filter.

Determination of Antimicrobial Activity

The antimicrobial activity of *Z. jujuba* water fruit and branch extracts was determined by disc diffusion method. Nutrient Broth (NB)/Agar (for *P. aeruginosa* ATCC 27853, *A. hydrophila* and *E. coli* O157: H7), Yeast Extract Peptone Dextrose (YPD)/Agar (for *C. glabrata* RSKK 04019 and *C. albicans* ATCC 10231), Tryptic Soy Broth (TSB)/Agar (for *L. monocytogenes* ATCC 7644, *L. garvieae* and *Y. ruckeri*), Tryptic Soy Broth/NaCl (for *V. anguillarum* A4 and M1 strains) were used as growth medium. The test microorganisms were inoculated onto solid media after their concentrations were adjusted to 0.5 McFarland. Sterile discs were placed on the Petri dishes and then 20 µl (5000 µl/disc) of *Z. jujuba* fruit and branch extracts were then dropped onto the discs. Petri dishes were incubated for 24 h at appropriate temperatures. At the end of the incubation period, the zones around the discs were measured and recorded. All experiments were done in triplicate.

Determination of minimal inhibition (MIC) and minimal bactericidal or fungicidal (MBC or MFC) concentrations

MIC and MBC or MFC values of the extracts were determined against the test microorganisms by micro-dilution method. The test microorganisms adjusted to 0.5 McFarland were added to each tube containing extract and medium and then vortexed. The tubes containing the mixture were incubated at appropriate temperatures for 24 h. After incubation, the concentration of the extract in the broth medium with no growth was recorded as MIC values. Then, the samples from the tubes were inoculated on the solid media by using spot dropping method and the plates were incubated for 24 h at appropriate temperatures. At the end of the incubation, the extract concentrations that prevent the growth of bacteria on solid media were evaluated as MBC or MFC values.

Determination In-Vitro Solar Protection Factor (SPF) of Extracts

The SPF of *Z. jujuba* juice fruit and branch extracts was determined in-vitro. The extracts were prepared at a concentration of 2 µg/µl in ethanol (96%) in triplicate. The homogeneous mixture obtained was measured in a spectrophotometer (Beckman Coulter) at 5 nm intervals in the wavelength range of 290 nm-320 nm. The Mansur equation is used to calculate the sun protection factor (Mansur et al., 1986).

Solar protection factor (SPF) = **CF x** $\sum_{290}^{320} \boldsymbol{EE}(\boldsymbol{\lambda})$x $\boldsymbol{I}(\boldsymbol{\lambda})$x $\boldsymbol{Abs}$ $(\boldsymbol{\lambda})$(1)

CF = Correction factor (= 10); **EE(λ)** = Erythematogenic effect radiation wavelength (λ);

I (λ) = Solarlight intensity at wavelength (λ); **abs (λ)** = Absorbance of the extracts at wavelength (λ).

RESULTS AND DISCUSSION

Z. jujuba have been very popular recently due to their high nutritional content. The antimicrobial and antifungal activities of *Z. jujuba* SN-HWB water fruit and branch extracts were determined by using various methods. The results of the disc diffusion assay for HWB extracts showed that the highest inhibition zone diameter (14.18±1.06 mm) was determined against *L. monocytogenes* ATCC 7644 in water fruit extract among the food-borne test microorganisms. In clinical test microorganisms, the highest inhibition zone diameter in water fruit extract was detected on *C. albicans* ATCC 10231 with 16.12±1.45 mm.

Table 1. Antimicrobial activity of HWB water fruit and branch extracts

Test Microorganisms	Inhibition zone diameter (mm)		MIC (µg/µl)		MBC or MFC (µg/µl)	
	HWB fruit	**HWB branch**	**HWB fruit**	**HWB branch**	**HWB fruit**	**HWB branch**
***E. coli* O157:H7**	12.92± 0.16	13.71± 0.36	100	100	100	100
***L. monocytogenes* ATCC 7644**	14.18± 1.06	7.77± 0.70	50	50	50	50
***P. aeruginosa* ATTC 27853**	15.31± 0.90	7.93± 1.08	50	100	50	100
***C. albicans* ATCC 10231**	16.12± 1.45	10.08± 0.96	100	100	100	100
***C. glabrata* RSKK 04019**	12.24± 1.19	10.61± 0.99	100	100	100	100
***V. anguillarum* M1**	13.21 ±0.66	13.15± 0.18	50	50	50	50
***V. anguillarum* A4**	16.96± 1.64	11.7± 0.37	50	50	50	50
Y. ruckeri	15.36± 0.59	10.01± 0.37	50	50	50	50
A. hydrophila	10.16± 1.56	11.95± 0.75	50	100	50	100
L. garvieae	14.33± 0.94	15.52± 1.54	50	50	50	50

Among fish pathogens, the water fruit extract exhibited the highest antibacterial activity on *V. anguillarum* A4 with 16.96±1.64 mm inhibition zone diameter. For SN extracts, the water branch extract showed the highest inhibition zone diameter (13.19±0.45) against *E. coli* O157:H7 among the food-borne test microorganisms. For clinical test microorganisms, SN water fruit extract exhibited the highest inhibition zone diameter of 16.89±1.32 on *C. albicans* ATCC 10231. The inhibition zone diameter of SN water fruit extract was 16.12±1.01 mm,

showing the highest inhibition zone diameter on *L. garvieae* among fish pathogens. MIC and MBC or MFC values of *Z. jujuba* water fruit and branch extracts on all tested microorganisms were determined between 25-100 µg/µl (Table 1-2). *Z. jujuba* fruits have been the subject of many studies due to their high nutritional content. However, according to our literature research, no study was found related to the antimicrobial activity of its branches. The results we obtained in our study it was determined that some of *Z. jujuba* water branch extracts showed antimicrobial activity close to water fruit extracts.

Table 2.Antimicrobial activity of SN water fruit and branch extracts

Test Microorganisms	**Inhibition zone diameter (mm)**		**MIC (µg/µl)**		**MBC or MFC (µg/µl)**	
	SN fruit	**SN branch**	**SN fruit**	**SN branch**	**SN fruit**	**SN branch**
***E. coli* O157:H7**	12.92± 0.16	13.19± 0.45	100	100	100	100
***L. monocytogenes* ATCC 7644**	11.45± 0.72	6.56± 0.54	50	100	50	100
***P. aeruginosa* ATTC 27853**	13.53± 0.26	7.68± 1.48	50	100	50	100
***C. albicans* ATCC 10231**	16.89± 1.32	10.4± 1.19	100	100	100	100
***C. glabrata* RSKK 04019**	12.60± 0.16	11.38± 1.33	100	100	100	100
***V. anguillarum* M1**	14.05± 0.52	6.83± 0.45	50	50	50	50
***V. anguillarum* A4**	15.96± 0.81	10.01± 0.47	50	50	50	50
Y. ruckeri	14.54± 0.69	13.95± 0.36	50	50	50	50
A. hydrophila	14.66± 1.75	6.46± 0.85	25	100	25	100
L. garvieae	16.12± 1.01	15.64± 1.64	50	50	50	50

In the study conducted by Asely El et al. (2020), they investigated the activity of *Ziziyphus mauritiana* leaf powders added to fish feeds in order to minimize the mortality rate caused by *A. hydrophila* infections in *Oreochromis niloticus* and to repair the organs damaged as a result of the infection. The results of their study indicated that the deaths caused by *A. hydrophila* infections decreased and the damage caused by infection was minimized on *O. niloticus* fed with *Z. mauritiana* leaf powders. As a result of the literature review, there are very few studies investigating the antimicrobial activity of *Z. jujuba* on fish pathogens. In a study investigating the antimicrobial activity of *Z. jujuba* water extract on *E. coli*, they reported that *Z. jujuba* water extract had no antimicrobial activity on *E. coli* (Beg et al., 2016).

Excessive exposure to solarlight is one of the most important causes of skin cancer. Solar-screens are widely used in the cosmetic industry to protect from solarlight. Nowadays, the research for natural solar protection factors has been increasing. The SPF of *Z. jujuba* HWB-SN fruit and branch extracts was tested. The results obtained were calculated according to the

Mansur equation and the results are given in Table 3. The SPF value of *Z. jujuba* HWB water fruit and branch extracts was found to be 0.17 and 0.32. SN water fruit and branch extracts presented good SPF value of 0.20 and 1.20. In our study, the SPF of SN water branch extracts showed higher SPF value than fruit extracts.

Table 3. SPF values of *Z. jujuba* water fruit and branch extracts

Extracts	**SPF**
HWB fruit	0.17
HWB branch	0.32
SN fruit	0.20
SN branch	1.20

As a result of the researches, it has been reported that many plants have solar protection factors (Cefali et al., 2018). The high flavonoid presence they contain gives plants the ability to absorb UV radiation between 200 and 400 nm, making them suitable for use as natural solar-screen agents (Silveira et al., 2009; Ahmad et al., 2006). As a result of our literature review, any study was found on the solar protection factor of *Z. jujuba* extracts.

CONCLUSIONS

Today, synthetic additives are frequently used in common areas such as medicine, food and cosmetics. However, since the health damages of synthetically produced substances are known by consumers, the search for natural substances in these industries has become frequent. In the study, *Z. jujuba* HWB-SN water fruit and branch extracts were found to have antibacterial or antifungal activity on tested food, clinical and fish pathogens. There are a lot of materials in the cosmetics sector to protect from the solar. *Z. jujuba*HWB-SN water fruit and branch extracts were found to have solar protection factor. As a result of the literature review, there are not many studies on the antimicrobial activity of *Z. jujuba* on fish pathogens. *Z. jujuba* HWB-SN water fruit and branch extracts have been determined to have potential as natural additives in cosmetics, food and pharmaceutical industries.

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INVESTIGATION OF USAGE POTENTIAL OF KUMQUAT FRUIT AND LEAF METHANOL EXTRACTS FOR AQUACULTURE AND COSMETICS INDUSTRIES

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ABSTRACT

The adverse health effects of chemical additives used in many industrial sectors are widely known. Consumers have been recently looking for alternative natural additives due to the side effects of existing chemical additives. Various plants are the most important part of natural source. In the present study, the potential use of kumquat (*Fortunella margarita*) fruit and leaf methanol extracts in the fishery and cosmetic industries was investigated. The antimicrobial activity of the kumquat extracts on fish bacterial pathogens was tested to determine the potential usage of kumquat fruit and leaves as a natural antimicrobial additive in the feed industry for fish. The antimicrobial activity of the kumquat extracts against *Vibrio anguillarum* M1, *Vibrio anguillarum* A4 and *Vibrio alginolyticus* was investigated by disc diffusion and micro-dilution assays. Kumquat fruit and leaf methanol extracts had antimicrobial activity on the three microorganisms tested. The disc diffusion assay results indicated that the kumquat fruit extract showed the highest antibacterial activity on *V. alginolyticus* with 21.16 mm inhibition zone diameter. For the leaf methanol extract, the highest inhibition zone diameter was determined on *V. anguillarum* M1 (24.80 mm). Minimal inhibition (MIC) and minimal bactericidal (MBC) concentrations of kumquat fruit and leaf methanol extracts were determined between 25 and 100 µg/µl. Prolonged exposure to ultraviolet radiation (UV) from the solar can cause serious health problems. Plants have also been used in the cosmetic industry for centuries. The solar protection factor (SPF) of kumquat fruit and leaf methanol extracts was also determined. The kumquat leaf methanol extract presented a good SPF value with 26.09. SPF of the fruit methanol extract was recorded as 7.35. Therefore, the kumquat fruit and leaf methanol extracts can be used as natural additive for fisheries and cosmetic industries.

Keywords: Antimicrobial, Fish pathogen, Sun protection factor, *Fortunella margarita*

INTRODUCTION

The world population has been rapidly increasing. It is of great importance to meet the food needs of the growing population. Fish farming, provides a large part of the world's food capacity, is a rapidly growing animal food sector with a growth rate of 10.4% year-on-year to meet the increasing human population (FAO, 2013). The rapid development of large-scale aquaculture systems in response to increasing food demand has resulted in a significant increase in the number of infectious diseases (Zhao et al., 2020). Protection of fish against infectious diseases is a large challenge in aquaculture worldwide, and damages due to infectious diseases limit profitability (Jia et al., 2000). In modern great fish farms, a wide diversity of disinfectants and antibiotics are given choice as agents used against pathogens (Turker and Birinci, 2015). Excessive use of antibiotics in the therapy of diseases reasoned by pathogenic microorganisms causes an increase in the level of antimicrobial compounds in the environment, accumulation of antibiotic drug residues in fish tissues (Rigos and Troisi, 2005; Samanidou and Evaggelopoulou, 2007; Romero et al., 2012;), alteration of the normal microbiota in farmed

fish and may cause resistance of pathogens (Navarrete et al., 2008; Romero et al., 2014). *V. angillarium* and *V. alginolyticus* are important fish pathogens that cause pathogenic diseases in fish farms. *V. alginolyticus* is an important pathogen threatening fish farming worldwide by causing fish mortality (Chen et al., 2000). *V. angillarium* species is the main cause of fatal vibriosis disease in fish. Due to the possible side effects of chemical antibiotic drugs in the treatment of diseases in fish farming, the research for alternative natural antimicrobial agents has been increasing in recent days. Plants have been used in the treatment of many diseases for many years. Kumquat (*F. margarita*) belongs to the *Rutacea* family. The fruits and leaves of *Fortunella* species are widely used in Chinese folk medicine (Sadek et al., 2009). It has been used in alternative medicine for many years in the treatment of many diseases such as fever, gallstones, digestive disorders, and asthma (Scordino et al., 2011; Nalbantbaşı and Gölcü, 2009). Kumquat, which is a good source of antioxidants due to the flanovoid compounds it contains, is also a natural alternative for food preservation with its antimicrobial properties (Sadek et al., 2009; Fitsiou et al., 2016). Antioxidant activities of plants also play an important role in UV protection (Kittiwannachot et al., 2008). Depending on the quantity and create of UV radiation, long-term exposure can cause damage such as photoaging, skin cancer (Ichihashi et al., 2003). Solar ultraviolet (UV) radiation is disunited into three territories: UVC (290–200) nm, UV-B (320–290) nm, and UV-A (400–320) nm (Roy, 1998). UV-A radiation reaches the stronger layers of the epidermis and dermis and prevents early aging of the skin. UV-C radiation is filtered by the atmosphere before it reaches the earth. UV-B radiation is not quite filtered out by the ozone layer and is accountable for the harms due to sunburn (Dutra et al., 2004). Human skin the UV radiation range that damages in the UV-B range, this range is used as a base in SPF studies. Sunscreens are widely used to prevent the absorption of harmful UV radiation from the sun. However, recent research reports have revealed that most synthetic sunscreen applications have undesirable effects on the skin in the short or long term. Therefore, there is a worldwide need for effective and safe UV filters, especially of natural origin (Ahmady et al., 2020). Plants have UV absorption and antimicrobial activity due to the phenolic compounds they contain. In presented study, antimicrobial activity on fish pathogens and solar protection factor of kumquat fruit and leaf extracts were investigated to determine usage potential in aquaculture and cosmetics industries.

MATERIAL AND METHOD

Supply of Fruit and Leaf Samples

Kumquat leaves and fruits were obtained from Alata Horticultural Research Institute (Turkey).

Preparation of Extracts

The fruit and leaf materials were washed. After drying, they were ground with Waring blendır. Soxhlet system was used to prepare kumquat fruit and leaf methanol extracts. The solvents were evaporated using a rotary evaporator and the dry extract was obtained. Kumquat fruit-leaf extracts were dissolved in methanol and sterilized with a 0.45µm filter.

Determination of Antimicrobial Activity

Disc diffusion method

Disc diffusion method was used to determine the antimicrobial activity of methanol extracts of kumquat fruit and leaf. The fish originated test microorganisms of *V. anguillarum* (M1 and A4 strains) and *V. alginolyticus* were cultured in TSB/agar medium containing 2% NaCl and incubated at 25°C. The test microorganisms were inoculated onto agar medium after their concentrations were adjusted to 0.5 McFarland. Sterile discs were placed on the inoculated

Petri dishes and then kumquat fruit and leaf extracts (20 µl) were dropped onto the discs. Petri dishes were incubated at 25°C for 24 hours. At the end of the incubation period, the zones around the discs were measured with calipers and recorded. All experiments were done in duplicate.

Determination of minimal inhibition (MIC) and bactericidal (MBC) concentrations

MIC or MBC values of the extracts were determined against fish test microorganisms by using micro-dilution method. Test microorganisms adjusted to 0.5 McFarland were added to each tube containing extract and medium and vortexed. Then, the mixture was incubated at 25°C for 24 hours. After incubation, the concentration of the extract in the broth medium with no growth was recorded as MIC values. Then, the samples from the tubes were inoculated on the solid media by using spot dropping method and the plates were incubated for 24 h at appropriate temperatures. At the end of the incubation period, the concentrations of the extract that inhibited the growth of bacteria on the solid medium were evaluated as MBC values.

Determination In-Vitro Solar Protection Factor (SPF) of Extracts

The solar protection factor of kumquat fruit and leaf methanol extracts were determined in-vitro. The extracts were prepared in triplicate at a concentration of 2 mg/ml in ethanol (96%). The homogeneous mix was measured in 3 repetitions in a spectrophotometer (Beckman Coulter) at 5 nm intervals in the wavelength range of 290 nm-320 nm. The Mansur equation is used to calculate the sun protection factor (Mansur et al., 1986).

Solar protection factor (SPF) = **CF x** $\sum_{290}^{320} \boldsymbol{EE}(\boldsymbol{\lambda})$x $\boldsymbol{I}(\boldsymbol{\lambda})$x $\boldsymbol{Abs}$ $(\boldsymbol{\lambda})$(1)

CF = Correction factor (= 10); **EE(λ)** = Erythemetogenic effect radiation wavelength (λ);

I (λ) = intensity of solarlight at wavelength (λ); **abs (λ)** = Absorbance (λ) of the extracts at wavelength.

RESULTS AND DISCUSSION

The antimicrobial activity assay results of kumquat fruit and leaf methanol extracts against fish pathogens are given in Table 1. The disc diffusion assay results indicated that the kumquat fruit extract showed the highest antibacterial activity on *V. alginolyticus* with 21.16 mm inhibition zone diameter. The highest inhibition zone diameter in leaf methanol extract was determined as 24.80 mm against *V. anguillarum* M1. MIC and MBC values of the extracts were determined between 25 and 100 µg/µl against the three microorganisms tested.

Table 1. Antimicrobial activities of kumquat fruit and leaf ethanol extracts

Test microorganisms	Inhibition zone diameter (mm)		MIC (µg/µl)		MBC (µg/µl)	
	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf
***V. anguillarum* A4**	18.69±0.21	24.41±0.07	50	25	100	25
***V. anguillarum* M1**	20.20±1.75	24.80±0.80	50	25	100	25
V. alginolyticus	21.16±1.10	22.69±0.96	50	25	100	25

Antimicrobial susbstances evaluated as bacteriostatic if the MBC/MIC ratio is >4 and bactericidal if the MBC/MIC ratio is ≤4 (Krishnan et al., 2010; Hazen, 1998). The kumquat fruit methanol and leaf extracts in the study can be classified as bactericidal agents against all tested fish microorganisms (Table 2).

Table 2. MBC/MIC ratio of kumquat fruit and methanol extracts

Extracts	**MBC/MIC**		
	***V. anguillarum* A4**	***V. anguillarum* M1**	***V. alginolyticus***
Fruit	2	2	2
Leaf	1	1	1

The aquaculture industry meets a large part of the food need today. The treatment of diseases occurring in fish farms is of great importance. In a study conducted by Turker and Birinci (2015), the antimicrobial activity of some Turkish plants against fish pathogens was determined. The results of their study, the highest inhibition zone diameter against *V. anguillarum* was determined as 25.5 mm for *Anemone nemorosa*, 19.6 mm for *Fragaria vesca*, 17.9 mm for *Alchemilla mollis* and 19.1 mm for *Sideritis taurica* in ethanol and water extracts. In the current study, kumquat fruit and leaf extracts showed higher antimicrobial activity on fish pathogens. According to our knowledge, there is no study on the fish pathogens of kumquat in the literature. The results obtained in our study show that kumquat fruit and leaf extracts can be used in a new industrial area as natural feed additive.

The solar protection factor values of kumquat fruit and leaf methanol extracts are presented in Table 3. The results indicated that the kumquat leaf methanol extract showed higher SPF value with 26.09 than the fruit methanol extract (7.35). SPF value for sunscreen above 2 is considered as having good sunscreen activity (Alexander and Andrew, 2004). According to the table value reported by Imam et al. (2015), the percentage of UV inhibited by kumquat fruit methanol extract is approximately 80%. The percentage of UV inhibited in kumquat leaf methanol extract is greater than about 96%. The obtained results show that the solar protection activity of kumquat fruit methanol extracts are especially quite high.

Table 3. SPF values of kumquat extracts

Extracts	SPF
Fruit methanol	7.35
Leaf methanol	26.09

Prolonged exposure to UV radiation can reason many critical problems such as skin cancer and oxidative stress. Natural sources have recently been recognized as potential solar screens due to their antioxidant activity (Bonina et al., 1996) and their absorption in the UV region (Liu et al., 1996). In a study, investigating the in vitro solar protection factor of vegetable oils used in the cosmetics industry, the SPF value was determined as 3.97 for orange oil and 2.8 for lemon oil (Kaur and Sara., 2010). In another study, Khelker et al. (2017) investigated the SPF of *Curcuma longa L.* and *Citrus sinensis L*. The result of their study indicated the SPF as 0.330 for *Curcuma longa L*. and 3.086 for *Citrus sinensis L*. SPF values of kumquat fruit and leaf methanol extracts used in our study were found to be higher than these studies. As a result of our literature research, there is no study on the solar protection factor of kumquat fruit and

leaf. The results obtained in the present study offer a new perspective on the use of kumquat fruit and leaf extracts.

CONCLUSION

Antimicrobial activity against fish originated bacterial microorganisms and SPF value of kumquat fruit and leaf methanol extracts were investigated. The results obtained in our study showed that kumquat fruit and leaf methanol extracts had antimicrobial activity on the three microorganisms tested. It has been determined that kumquat fruit and leaf methanol extracts have the potential to be used as natural feed additives in the treatment or prevention of bacterial fish diseases caused by pathogenic microorganisms. Also, the kumquat fruit and leaf methanol extracts having good solar protection factor can be used as natural additive in solar screen formulations in the cosmetic industry.

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SOIL CONTAMINATION AND HEALTH RISK ASSESSMENT OF HEAVY METAL IN ESKISEHIR-SEYITGAZI DISTRICT

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ABSTRACT

Soil is the basic component of the biosphere and is exposed to many contaminants, including heavy metals. Heavy metals accumulate mostly in soil in nature and heavy metal pollution is becoming a serious concern. This study aimed to determine the pollution level and possible sources of heavy metals in the agricultural areas in Seyitgazi district of Eskişehir, where worldwide significant a boron mine is located, and to evaluate its effects on human health. 94 soil samples were taken from Seyitgazi district of Eskişehir Province in 2014 and Cr, Fe, Ni, Cu, Zn, Cd, and Pb analyzed. Enrichment Factor (Ef), Geoaccumalation Index (Igeo), Contamination Factor (Cf) and Ecological Risk Factor (Er) were used to determine heavy metal pollution, and the effects on human health were determined by the hazard quotient (HQ) and hazard index (HI). In this study, due to background values are not available, the continental upper crust values were used as background values for calculation of pollution indices. The pollution indices (Ef, Igeo, Cf and Er) used showed that the study area was moderately polluted and signicantly enrichment by Cd. According to Pearson correlation analysis and principal component analysis, the source of Cd in the study area is anthropogenic. However, there is no non-carcinogenic health risks have been identified for children (HI: 0.56) and adults (HI: 0.002).

Keywords: Pollution Index, Pearson Correlation Analysis, Principal Contanent Analysis, Multivariate Statistical Analysis.

INTRODUCTION

In comparison to other elements of the environment, soil is widely regarded as the most vulnerable to heavy metal accumulation (Mazurek et al. 2017) Because of its toxic effects on living organisms, heavy metal pollution has become a global environmental concern that must be addressed. Heavy metals, regardless of their source, are frequently associated with soil pollution (Zhang et al. 2017). The ability of soils to accumulate heavy metals is related to soil type, physical and chemical properties, and heavy metal nature. Heavy metal accumulation in soil varies according to the ability of heavy metals to bind organic matter or the degree of decomposition of the soil's main substance (Kabata-Pandias, 2011). The organic matter, which is where the majority of heavy metals accumulate, is concentrated in the top layer of the soil. Natural processes (lithogenic and pedogenic), as well as direct and indirect human activities, can all contribute to heavy metal accumulation (Varol et al. 2020). The primary sources of anthropogenic inputs are agricultural activities (pesticide and fertilizer applications), industrial activities, domestic waste, and traffic emissions (Jia et al. 2018; Kumar et al. 2019). Long-term heavy metal accumulation in agricultural lands can result in ecological damage and environmental issues (Cao et al. 2009; Zhang et al. 2017).

Heavy metal environmental risk estimation is frequently used in determining the degree of soil pollution. Furthermore, these estimates serve as a foundation for developing pollution prevention strategies. In the assessment of environmental risks caused by heavy metals, the

enrichment factor, geoaccumulation index, and contamination factor are frequently used. Furthermore, Hakanson's (1980) ecological risk index and ecological risk factors are widely used (Jia et al. 2018; Ni et al. 2018; Varol et al. 2020). Both index methods have been shown to be effective in determining the impact of heavy metals in soil (Varol et al. 2020). Heavy metals in the soil can enter the food chain via plants and endanger human health. For these reasons, the accumulation of heavy metals in agricultural soils should be investigated alongside the reasons (Sungur 2016; Kaitantzian et al. 2013). This study has two main objectives: a) to determine the total six heavy metal (Cr, Ni, Cu, Zn, Cd, and Pb) concentrations in the agricultural soil and to evaluate their levels using pollution indexes, b) to deternine the non-carcinogenic human health risks for the farmers living in Seyitgazi district of Eskişehir Province.

MATERIAL AND METHODS

This research was carried out in Turkey's Seyitgazi district of Eskisehir providence (Figure 1). Seyitgazi geographically consists of hilly and undulating plains. It is surrounded by the Turkmen mountains in the west. With its geographical formation, important mineral deposits have been formed. The prominent sector in the district is agriculture and animal husbandry. Wheat, barley, beet, chickpea and sunflower are produced as field crops (Bebka 2021).

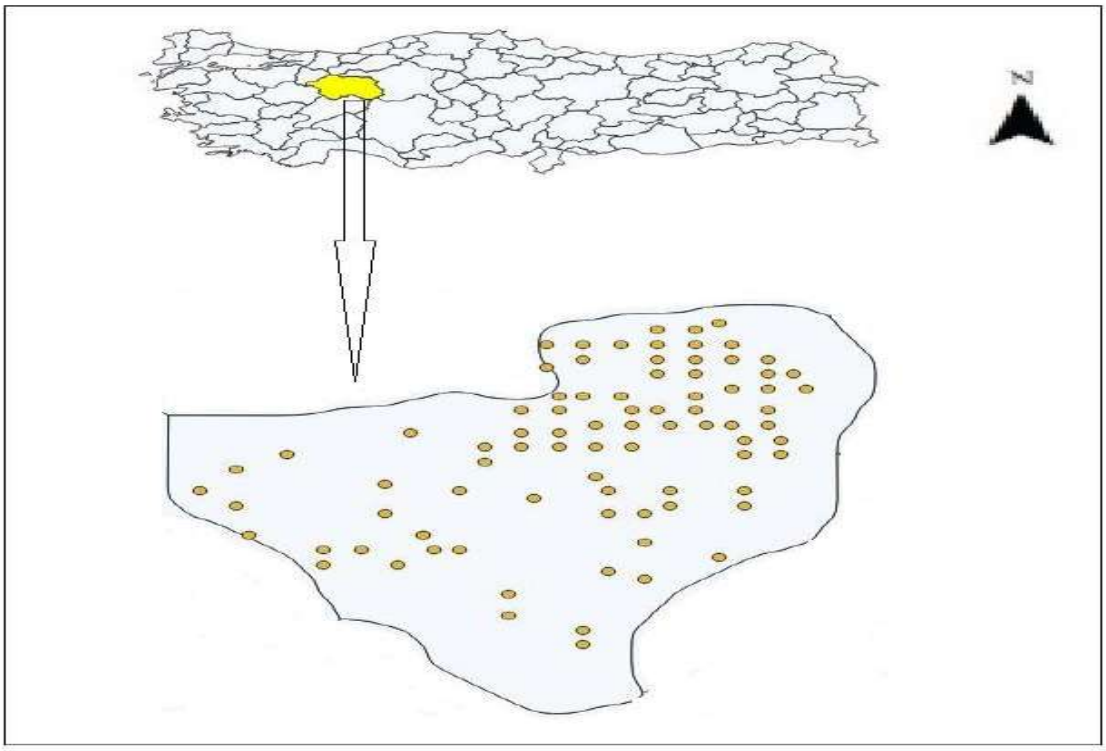

Figure 1. Soil sampling point of working area.

Soil samples were collected from agricultural lands in Eskisehir's Seyitgazi district. In 2014, 94 soil samples (0–30 cm) were collected on a grid basis from the working area.Soil samples were collected from four subsamples using a stainless steel shovel at each point and transported to the laboratory in plastic bags after being mixed. Soil samples were air-dried and sieved at 2 and 0.5 mm in preparation for analysis. Soil samples were analyzed for Pb, Cr, Ni, Fe, Co, Mn, Zn, and Cu. Soil samples were digested in 10 ml HCI and HNO3 (3:1) using a microwave digestion system for this purpose.The solutions were then diluted to 50 mL with

ultrapure water. Heavy metal concentrations were determined using Inductively Coupled Plasma – Optical Emission Spectrometry (PerkinElmer Optima 8000, USA).

Pollution indices were used to assess environmental risks. Enrichment factor (Ef), Geoaccumulation index (Igeo), Contamination factor (Cf), and Potential Ecological Risk Factor were used to calculate the degree of heavy metal pollution in soils (Er). Heavy metal contamination in soil is frequently determined using pollution indices (Ihedioha et al., 2017; Mazurek et al., 2017; Ullah et al., 2017). The Ef, Igeo, and Cf indices are based on a comparison of heavy metal levels in soils to background levels. Due to a lack of background heavy metals, the continental upper crust values reported by Rudnick and Gao (2013) were used as background values in this study. The toxic values determined by Hakanson (1980) for each heavy metal were used in the calculation of Er indices. The Ef, Igeo, Cf, and Er indices were calculated using the formulas in Eqs. 1, 2, 3, and 4, respectively (Baltas et al., 2020; Varol et al., 2020).

$$Ef = \frac{\text{Concentrations of heavy metals /Concentrations of Fe}}{\text{Background of heavy metals / Background of Fe}} \quad (1)$$

$$Igeo = \log_2 \frac{Concentrations\ of\ heavy\ metals}{(\ 1.5\ x\ Background\ of\ heavy\ metals)} \quad (2)$$

$$Cf = \frac{\text{Concentration of heavy metals}}{\text{Background of heavy metals}} \quad (3)$$

$$Er = \text{Toxicological respons factors} \times Cf \quad (4)$$

The evaluation criteria of the pollution indices are presented in Table 1 (Hakanson, 1980; Mazurek et al., 2017; Varol et al., 2020; Tokatli et al., 2021).

Table 1. Evaluation criteria of pollution indices.

Pollution Indices	Evaluation	Pollution Indices	Evaluation
Ef		Cf	
Ef <2	Minimal enrichment	Cf < 1	Low contamination
2< Ef <5	Moderate enrichment	1 < Cf <3	Moderately contamination
5< Ef < 20	Significant enrichment	3 < Cf < 6	considerably contamination
20< Ef <40	High enrichment	Cf > 6	Very high contamination
Igeo		Er	
0< Igeo	Unpolluted	Er < 40	Low ecological risk
0< Igeo <1	unpolluted to moderately polluted	40 < Er < 80	Moderate ecological risk
1< Igeo <2	moderately polluted	80 < Er < 160	Considerable ecological risk
2 < Igeo <3	moderately to highly polluted	160 < Er < 320	High ecological risk
3 < Igeo <4	highly polluted	Er > 320	Very high ecological risk

Heavy metals in the soil enter humans in three ways a) through ingestion, b) through inhalation, and c) through dermal absorption of soil particles. The chronic daily intake (CDI)

(mg^{-1} kg^{-1} $d^{-1)}$ term is used to express people's exposure to heavy metals and is calculated using the following formulas.

$$CDI_{Ing} = ((C_i \times R_{Ing} \times FC \times EF \times ED) / (BW \times AT)) \quad (5)$$

$$CDI_{Inh} = ((C_i \times R_{Inh} \times EF \times ED) / (PEF \times BW \times AT)) \quad (6)$$

$$CDI_{Der} = ((C_i \times SA \times AF \times ABS \times EF \times ED \times 10^{-6}) / (BW \times AT)) \quad (7)$$

$$CDI_{Total} = CDI_{Ing} + CDI_{Inh} + CDI_{Der} \quad (8)$$

In these formulas are; Ci is the heavy metal concentration, RIng is the ingestion rate, FC is the conversion factor, EF is the exposure frequency, ED is the exposure duration, BW is the average body weight, AT is the averaging time, Rinh is the inhalation rate, PEF is the particle emission factor, SA is the skin surface area, AF is the soil adherence factor, and ABS is the dermal absorption factor. (Chabukdhara et al. 2013; Eziz et al. 2018; Ihedioha et al. 2017, USEPA 2002). Non-carcinogenic risks for peoples were determined by Hazard Quent (HQ). The hazard quotient was calculated following formula;

$$HQ=(CDI / RfD) \quad (9)$$

By calculated HQ values of each heavy metal, the total exposure to which people were exposed was calculated and expressed as the hazard index (HI) (USEPA 1989). HI was calculated following formula;

$$HI=HQ_{ing}+HQ_{in}+HQ_{der} \quad (10)$$

To determine the relationships between heavy metals, Pearson correlation analysis was used. Principal component analysis was used to identify potential heavy metal sources (PCA). The statistics programs SPSS and R was used for all of the analysis.

RESULTS AND DISCUSSION

The study area is contaminated by Cd, according to pollution indices (Figure 2). With a value of 6.96, Cd had the highest Ef value, indicating that the study area is significant enrichment**.** Cd was followed by Ni (3.50), then Pb (2.36). The study area was moderately enriched by Ni and Pb but not polluted by Zn, Cu, or Cr (Ef < 2). Similarly, the study area was unpolluted to moderately polluted by Cd, according to Igeo (0.89). The Igeo values for Zn, Cu, Cr, Ni, and Pb do not indicate any soil pollution (0 > Igeo). Cd, like Igeo, exhibited the highest Cf value (2.87). This was followed by Ni (1.97), Cr (1.20), Pb (1.09), Zn (0.64), and Cu (0.0.62). Cf values show that the soils in the study area are moderately contaminated by Cd, Pb, Cr and Ni. Hakanson's (1980) ecological risk factor is frequently used to assess the ecological risk posed by heavy metals (Baltas et al., 2020; Mazurek et al., 2017; Tokatli et al., 2021; Varol et al., 2020). The soils in the research area have considerable ecological risk in terms of Cd (86.30), according to the Hakanson classification. The Zn Cu, Cr, and Pb Er values do not indicate any environmental risk in the study area.

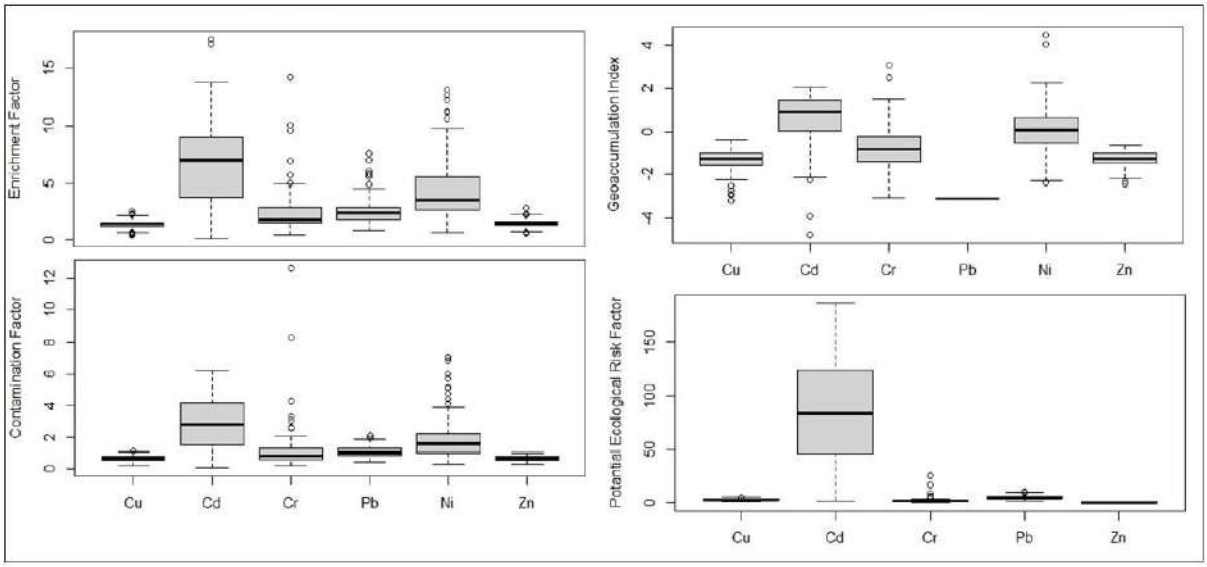

Figure 2**.** Ef, Igeo, Cf and Er values of heavy metals in Seyitgazi District

Pearson correlation analysis was used to determine the relationships between heavy metals found in the research area's territory (Figure 3). The strong correlation between heavy metals suggests that they came from similar sources or are interdependent (Baltas et al., 2020; Tholkappian et al., 2018; Wang et al., 2020; Yi et al., 2011). Correlation analysis revealed a strong positive relationship between Cr, Ni, and Cu. The highest correlation (r = 0.79) was discovered between Cr and Ni, while the lowest correlation was discovered between Ni and Pb (r = - 0.48).

Figure 3. Pearson correlation coefficient between heavy metals in study area (p <0.05)

Using a PCA analysis with varimax rotation, three main components with Eigenvalues greater than one were identified (Figure 4). The first component, which is loaded with Cr and Ni, accounts for 43.06 percent of the total variance. Furthermore, there is a positive relationship between Cr and Ni (r = 0.79, p=0.01). PC1 is better explained by the natural accumulation of heavy metals in soils as a result of the bedrock's chemical process. (Baltas et al., 2020; Chandrasekaran et al., 2015). The second component, which is loaded with Zn and Cu, accounts

for 25.00 percent of the total variance. There is a positive relationship between Zn and Cu (r = 0.54, p=0.01) and between Cu, Cr and Ni (r > 0.50, p=0.01). The third component is loaded with Cd and accounts for 13.48 percent of the total variance. Anthropogenic pollution is the primary cause of Cd soils. The main causes of Cd accumulation in soils are the use of phosphorus-containing fertilizers, pesticides, and manure applications in agricultural areas (Marrugo-Negrete et al., 2017; Ungureanu et al., 2017).

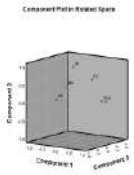

Figure 4. Principal component matrix of heavy metals

Health risk of resident children and adults were assessment through Chronic daily intake (CDI), hazard quotient (HQ) and hazard index (HI) (Table 2). The distribution of CDI_{total} values for children and adults were determined as Cr> Ni > Cu >Pb> Zn > Cd. CDI_{total} values for children ranged from 1.17E-03 – 3,69E-06 mg^{-1} kg^{-1} d^{-1} and adults ranged from 1.35E-04 – 3.97E-07 mg^{-1} kg^{-1} d^{-1}. CDI_{total} values showed that the order of heavy metal exposure in soil was CDI_{ing} > CDI_{der} > CDI_{inh}. The distribution of HQ_{total} values for children were determined as Cr > Ni >Pb > Zn > Cu> Cd. Similarly, HQ_{total} values for adults were determined as Cr > Pb > Ni > Zn > Cu> Cd. HQ_{total} values were found to be in the range of 4.19E-01 – 5.69E-03 mg^{-1} kg^{-1} d^{-1} and 4.59E-02 – 6.90E-04 mg^{-1} kg^{-1} d^{-1} for children and adults, respectively. Despite the fact that the pollution indices indicate that the study area is at risk of Cd pollution, no non-carcinogenic health risk has been identified for adults and children living in the area (HI < 1).

Table 2. Non–carcinogenic risks to chilren and adults.

Heavy	CDI_{ing}		CDI_{inh}		CDI_{der}		CDI_{total}	
Metals	Child	Adults	Child	Adults	Child	Adults	Child	Adults
Cu	2,23E-04	2,39E-05	6,28E-09	3,52E-09	6,16E-07	1,79E-07	2,24E-04	2,41E-05
Cd	3,68E-06	3,94E-07	1,03E-10	5,80E-11	1,02E-08	2,95E-09	3,69E-06	3,97E-07
Cr	1,25E-03	1,35E-04	3,98E-08	1,97E-08	3,46E-06	1,01E-06	1,25E-03	1,35E-04
Pb	2,36E-04	2,53E-05	6,64E-09	3,72E-09	6,53E-07	1,90E-07	2,36E-04	2,55E-05
Ni	1,16E-03	1,64E-04	3,27E-08	2,42E-08	3,22E-06	1,23E-06	1,17E-04	1,65E-04
Zn	5,52E-04	5,92E-05	1,56E-08	8,71E-09	1,53E-06	4,44E-07	5,55E -04	5,97E-05
Heavy	HQ ing		HQ inh		HQ der		HQ total	
Metals	Child	Adults	Child	Adults	Child	Adults	Child	Adults
Cu	5,58E-03	5,98E-04	1,57E-07	8,79E-08	5,14E-05	1,49E-05	5,63E-03	6,13E-04
Cd	3,68E-03	3,94E-04	1,03E-07	5,80E-08	1,02E-03	2,95E-04	4,69E-03	6,90E-04
Cr	4,17E-01	4,48E-02	1,37E-03	6,80E-04	1,15E-03	3,38E-04	4,19E-01	4,59E-02
Pb	6,74E-02	7,23E-03	1,89E-06	1,06E-06	1,23E-03	3,58E-04	6,87E-02	7,59E-03
Ni	5,82E-02	8,20E-03	1,59E-06	1,17E-06	5,96E-04	2,28E-04	5,88E-02	8,43E-03
Zn	1,84E-03	1,97E-04	5,18E-08	2,90E-08	2,55E-05	7,40E-06	1,87E-03	2,05E-04
HI	Children				Adults			
	0,56				0,002			

CONCLUSION

Heavy metal pollution in agricultural areas in the mining region, its possible sources, and the health risks to people living in the region were assessed in this study. The study's pollution indices revealed that agricultural lands were contaminated with Cd. The source of Cd in soils is anthropogenic, according to principal component analysis and Pearson correlation analysis. Despite the fact that pollution indices revealed that the soils in the study area were contaminated with Cd, no non-carcinogenic health risks were found in adults or children. Although there is no risk to human health in this study, the region should be monitored on a regular basis and risks reduced due to the risk of soil pollution.

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THE INVESTIGATION OF THE IMPACT OF DIFFERENT PLANT GROWTH REGULATORS ON MICROPROPAGATION OF ARONIA (*ARONIA MELANOCARPA.* L)

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ABSTRACT

The low rate of germination of *Aronia melanocarpa* species, whose value added has increased significantly in recent years, and the production of plants obtained by seed, which is a generative method, show expansion. Therefore, the usage of micro-propagation techniques has more importance for the production of Aronia species. This thesis has investigated the mass production of Aronia plant which will contribute to fruit diversity of our country with tissue culture methods which allow rapid, virus and disease-free proliferation. For that purpose, ' Viking " and " Nero nodal segments of Aronia are used as explants, and different doses of sodium hypochlorite (5%, 10%, 20%) in surface sterilization, different plant growth regulators, chemicals that will increase the success of shoot development, tillering and rooting and various combinations of regulators and chemicals are used to identify the best practices for mass production. The best result in surface sterilization is obtained from 10% sodium hypochlorite treated 10 minutes. DKW (Driver and Kuniyuki) containing Gamborg B5 Vitamins as basic plant media, MS (Murashige and Skoog), plant growth regulators (0,1mg/L from IBA and 0,1mg/L of GA3) and different doses of cytokinin source (BAP and Kinetin), 0.5, 1 ve 2 mg/L, are used. The best shoot growth and the maximum tillering are obtained from the combination of 2mg/L BAP+0.5 Kinetin + 0. 1mg/L IBA+0.1mg/L GA_3. ½ MS primary nutrient containing Gamborg B5 Vitamins, different doses of IBA (1, 2 ve 3 mg/L) and NAA' (1, 2 ve 3 mg/L) are used for rooting stage. The best result is obtained from the ½ MS plant media combined of 2 mg/l IBA +0,5 mg/l NAA.

Keywords: Aronia, micropropogation, PGRS, tillering, rooting

INTRODUCTION

Aronia (*Aronia melanocarpa. L)* also known as black chokeberry and whose fruits have been consumed since the middle of the last century, is a member of the Rosaceae family. Elliot, one of the species of aronia, is also considered as an ornamental plant especially in Asian and European countries due to its red leaves in autumn (Hirvi and Honkanen, 1985; Şutan et al. 2017). The 3 most well-known species of aronia are respectively A. *arbutifolia* (L.) Persian (red fruit), A. *prunifolia* (Marsh.) Rehd. (Purple fruit) and A. *melanocarpa* (Michx.) Ell. (Black fruit).

Aronia plant is defined as shrubs with "multiple branches and shed their leaves in winter" and it is known that its homeland is North America and Eastern Europe (Hirvi and Honkanen, 1985). Originating in Eastern Europe and Russia. It was used as an ornamental plant before World War II.

Aronia is also among the landscape plants due to its white flowers in spring and black or red fruit in summer and autumn. It has great potential as an ornamental plant because its leaf color turns red in autumn. Aronia is a durable plant species with high tolerance to environmental conditions. Aronia fruit, which has the ability to destroy cancerous cells with its strong

antioxidant feature, has also been called a super fruit due to its contribution to human health. It contains an antioxidant polyphenolic natural mixture that fights against free radicals that damage cells created by stress, environmental pollution and activities of daily living in our body. In addition, Aronia has become popular in Europe due to its nutritional content and benefits (Seidemann, 1993).

Many researchers have stated that Aronia fruit is rich in fiber, protein, minerals, vitamins and organic acids, which have protective and therapeutic properties against diseases, as well as high phenolic compounds and carotenoids (Waver et al., 2006; Koponen et al., 2007; Chrubasik et al. et al., 2010; Snebergova et al., 2014).

Recently, Aronia has attracted attention not only because of its biological and nutritional value, but also because of its high income. An Aronia tree gives about 20-25 kg of fruit. In addition, Aronia plants are a suitable plant species to be harvested by machine. In addition to these, Aronia is a plant species that needs very little nutrients during the growth period and has self-fertility characteristics. The most important problem in aquaculture is the ingestion of fruits by birds. For these reasons, it is of great importance to harvest the fruits on time. On the other hand, the production cost is lower compared to other berry fruit groups.

Aronia can reproduce easily by seed, but this method is not recommended due to some heterozygotes and delayed fruiting of plants (Litwinczuk, 2002). By using in vitro methods, the propagation of the plant can be performed faster and a disease-free plant is obtained in a short time (Brand and Cullina, 1992). The first studies on this plant species in our country started with the production of saplings at Atatürk Horticultural Central Research Institute in 2012 and a plantation was established in the trial area. As a private sector, the first studies on reproduction in Aronia were made by Beta Fidancılık. Some Aronia cultivars were planted in breeding plots and it was determined that some cultivars met the need for chilling at an altitude of 200-300 m in the Çukurova gateway region of Turkey.

Borsai and Clapa (2017) compared essential nutrient media to establish a suitable protocol for the in vitro growth of Aronia *Aronia melanocarpa (Michx*.) Elliott. They tried different media and plant growth regulators to determine the nutrient medium in which tillering is maximum with the in vitro propagation method of plants. DKW, MS, WPM media were compared using 0.5 mg/l BAP. The best results were obtained from the medium containing 0.5 mg/l BAP using DKW basic nutrient medium (Driver and Kuniyuki., 1984). The investigators also emphasized that they achieved the lowest multiplication coefficient with the use of 2 mg/L Zeatin, or 5 mg/L 2-IP.

The aim is to investigate mass production possibilities with the tissue culture method that allows the rapid, virus and disease-free reproduction of the Aronia plant, which has a very high added value, and to contribute to Turkey's fruit diversity.

MATERIAL AND METHOD

In the research, 'Nero' and 'Viking' Aronia varieties, which are in the Aronia *Melanocarpa* (Michx) Elliot species, were used. Media trials related to tissue culture were carried out in the plant tissue culture laboratory of Beta Private Company, where research and mass production were carried out. The plants obtained by tissue culture techniques were transferred to the acclimatization greenhouses in the same company.

Content and preparation of nutrient media

Murashige and Skoog (MS) medium which is widely preferred plant tissue culture growing medium and was also used as nutrient medium. In addition, Plant regeneration heavily depends on the basal media factor. Recent studies showed the potential role of Driver and

Kuniyaki Walnut (DKW) medium as an alternative to popular MS medium. So, DKW (Juglans) media was used in the experiment, and these two media were compared (Table 1).

The MS nutrient medium was enriched by plant growth regulators belonged to three different groups.MS basic nutrient medium containing thiamine, nicotinic acid, pyridoxine HCl and myo-inositol vitamins and 4 mg/l 2-isopentenyladenine (2-IP) cytokine in the starting medium, 30 g/l sucrose, 7.0 g/l agar and pH 5.7 has been prepared. The prepared media were boiled in a heater with a stirrer up to 100°C and then distributed in glass test tubes, approximately 10 ml each, and sterilized in an autoclave at 121°C for 20 minutes.

The nutrient media in glass tubes were planted with one explant in each tube (100 tubes). After planting, the tubes were placed in the plant growth chamber where controlled conditions were provided. The temperature of the growth chamber was 24±1°C and the light conditions were set as 16/8 (light/dark) hour photoperiod.

Table 1. MS and DKW basic medium content.

MS (Murashige ve Skoog, 1962)	
Micro Elements	**mg/l**
KI	0.83
H_3BO_3	6.20
$MnSO_4 7H_2O$	22.30
$ZnSO_4$	8.60
$NaMoO_4$	0.25
CuSO4	0.025
$CoCl_2$	0.025
Macro Elements	**mg/l**
$CaCl_2$	332.02
KH_2PO_4	170.00
$MgSO_4 7H_2O$	370.00
NH_4NO_3	1650.00
KNO_3	1900
Vitamins	**mg/l**
Glycine	2.00
Myo-Inositol	100.00
Nicotinic Acid	0.50
Pyridoxine HCl	0.50
Thiamine Hydrochloride	0.10

DKW (Driver and Kuniyaki Walnut) Juglans	
Micro Elements	mg/l
$CuSO_4 5H_2O$	0.25
FeNaEDTA	44.63
H_3BO_3	4.80
$MnSO_4 H_2O$	33.80
$NaMoO_4 2H_2O$	0.39
$ZnSO_4 7H_2O$	17.00
Macro Elements	**mg/l**
$CaCl_2$	112.5
$Ca(NO_3)_2 2H_2O$	1664.64
KH_2PO_4	265.00
K_2SO_4	1559.00
$MgSO_4$	361.49
NH_4NO_3	1416.00

Determination of the effect of different cytokinin sources on proliferation

In the experiment, 0.1mg/L of IBA and 0.1mg/L of GA_3 were kept constant in DKW basic nutrient medium containing Gamborg B5 Vitamins, and the effects of 1, 2 and 3mg/L doses of different cytokine sources such as BAP and Kinetin on proliferation were compared with MS basic medium (Table 2).

Plants waiting for 3-4 weeks in sibling media were transferred to root media. $^{1/2}$MS medium containing IBA and NAA auxin combinations was prepared at 30g/l sucrose, 7g/l agar and pH 5.7. Plants waiting in the growing chamber at 25 degrees and 16/8 light period were allowed to take root.

Table 2. DKW and MS medium with PGR (Plant Growth Regulators) combinations used in the shoot propagation stage of the study

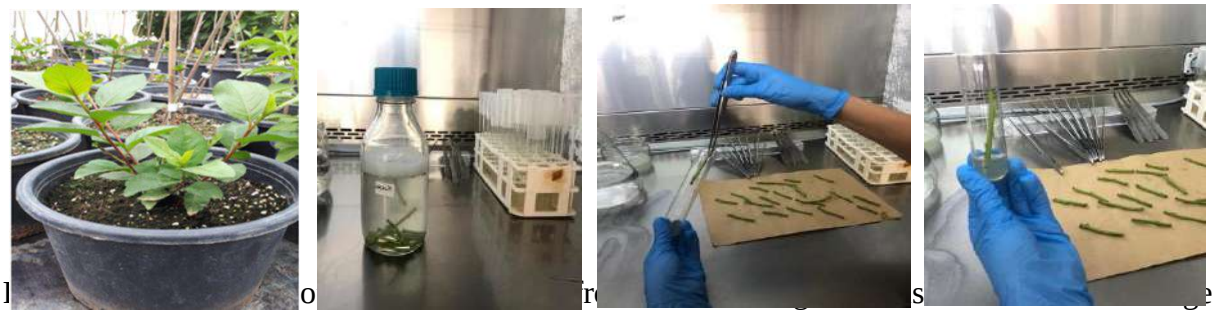
DKW and MS Medium	**Amount of plant growth regulators (mg/l)**
Control	0 BAP + 0 KİN + 0 IBA + 0 GA_3
1	0.5 BAP + 0.1 IBA + 0.1 GA_3
2	1 BAP + 0.1 IBA + 0.1 GA_3
3	2 BAP + 0.1 IBA + 0.1 GA_3
4	0.5 KİN + 0.1 IBA + 0.1 GA_3
5	1 KİN + 0.1 IBA + 0.1 GA_3
6	2 KİN + 0.1 IBA + 0.1 GA_3
7	0 BAP + 0 KİN + 0.1 IBA + 0.1 GA_3
8	0 .5 BAP + 0.5 KİN + 0.1 IBA + 0.1 GA_3
9	1 BAP + 1 KİN + 0.1 IBA + 0.1 GA_3
10	2 BAP + 2 KİN + 0.1 IBA + 0.1 GA_3

Surface Sterilization

Nodal segments taken from 1-2 month old shoots of Viking and Nero Aronia cultivars in the summer of 2018 were used as starting material. The green leaves were cleaned and the explants with axillary buds were cut 2-3 cm in length and subjected to surface sterilization processes. Explants were kept in running tap water for 60 minutes. Afterwards, it was cleaned with antibacterial soap and rinsed with tap water 3 times. Rinsed shoots were kept in 70% alcohol for 5 seconds. Then, it was treated with 5%, 10% and 20% sodium hypochlorite for 10 minutes. Afterwards, rinsing was done 3 times in sterile distilled water and the disinfection success (%) of sodium hypochlorite applications was determined. After all these procedures, the explants were cut to 0.5-1 cm each using scalpel and forceps and prepared for planting in the culture medium (Fig 1)

Statistical Analysis

The experiment was carried out with three replications. Means separation was determined by LSD test. Statistical analyses were carried out using JMP 5.0.1 version.



l o r s

Table 4. Different Auxin and doses used in the rooting stage of the study

½ MS medium	**Amount of auxin components and combinations (mg/l)**
Control	0 IBA+0 NAA+0 GA3
MS1	1 IBA+0 NAA+0.1GA3
MS2	2 IBA+0 NAA+0.1 GA3
MS3	3 IBA+0 NAA+0.1 GA3
MS4	0 IBA+0.5 NAA+0.1 GA3
MS5	0 IBA+1 NAA+0.1 GA3
MS6	0 IBA+2 NAA+0.1 GA3
MS7	0 IBA+3 NAA+0.1 GA3
MS8	2 IBA+0.5 NAA+0.1 GA3

RESULTS AND DISCUSSION

In the study, 1-2 month old shoots of Aronia plant were treated with different NaOCl (sodium hypochlorite) solutions (5%, 10% and 20%). It was observed that all of the explants placed in the environment in the 5% NaOCl dose application died due to contamination (contamination) caused by fungal and especially bacterial disease factors. In the 20% NaOCl dose application, it was observed that all nodal explants burned and dried. The highest success in surface sterilization applications was obtained from 10% NaOCl application. In 10% NaOCl dose application, it was observed that 90% of the nodal explants placed in the medium remained viable and 10% of them had bacterial and fungal contamination.

BAP and Kinetin were used as the source of cytokinin, and DKW and MS were used as the nutrient source. In the study where IBA as an auxin source and GA_3 as a plant growth regulator were used at a fixed dose, the best number of siblings and shoot length were observed in 2 mg/l BAP+0.5 mg/l Kinetin +0.1 IBA+0.1 GA_3 and DKW basic nutrient media. In the researches, it was observed that the tillering coefficient of Viking variety (3.27) was detected higher than and Nero (3.07) variety. While the best tillering ratio in Viking variety was obtained in combination of 2 mg/l BAP+0.5 mg/l Kinetin, in Nero variety the best tillering ratio was obtained in combination of 3 mg/l BAP+1 mg/l Kinetin (Table 5)

Table 5. The mean number of tillers (tiller/ explant) of aronia cultivars

Varieties	**Tillering Coefficient**
Viking	3.27a
Nero	3.07b
$Lsd_{\%5}$	0.09

In the experiment, IBA (0, 1, 2 and 3mg/L) and NAA (0, 0.5, 1, 2 and 3mg/L) were added to ½ MS basic nutrient medium containing Gamborg B5 vitamins in 8 different media consisting of different doses and combinations. In terms of length, the best rooting medium was determined. Sucrose was used as 2% in the media. Trials were made in triplicate and 50 explants or 50 plants were used in each application.

As a result of the research, the best shoot length was obtained in the combination of 2 mg/l BAP+0.5 mg/l Kinetin in Nero (3.80a.) variety. In Viking (3.80a) variety, the best shoot length was obtained in combination of 3 mg/l BAP. As a result of another study, researchers The highest shoot number were obtained at MS basal medium containing a combination of growth regulators at different concentrations (2.0 mg l-1 BA +0.01 mg l-1 IAA+0.1 mg l-1 GA_3) (Pırlak et al, 2018).

Plants waiting for 3-4 weeks in sibling media will be transferred to root media. The rooting medium was prepared with MS medium containing combinations of IBA and NAA auxins, 30 g/l sucrose, 7 g/l agar and pH 5.7. Plants waiting in the growth room at 25 degrees, 16/8 light period were allowed to take root. The best results in aronia rooting were achieved in the medium containing 2 IBA + 0.5 NAA auxin combinations. 80% success was observed in the plants that we adapted from the medium where the best results were observed to the external environment. Researchers (Pırlak et al. 2018) stated that 1-2mg/l doses of IBA in Ms medium are effective in rooting. Our findings were found to be accordance with the studies.

CONCLUSIONS

Based on the study, It has been determined that in vitro mass production of aronia plant can be successfully carried out with appropriate sterilization methods and the use of plant growth regulators in appropriate combinations. The tillering rates of Nero and Viking cultivars in tissue culture are the same, but in shoot length measurement and analysis studies, it was determined that the shoot length of Viking cultivar was higher. The best results in Viking variety were obtained in the medium containing DKW+2 mg/l BAP+0.5 mg/l Kinetin +0.1 mg/l IBA+0.1 mg/l GA_3. The best results in Nero variety were obtained in the medium containing DKW+3 mg/l BAP+1 mg/l Kinetin +0.1 mg/l IBA+0.1 mg/l GA_3. According to the measurement and analysis results obtained, it was determined that the best variety in terms of success in production was Viking variety.

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STRUCTURAL CHARACTERIZATION OF THE MACHROUHA FOREST (EAST-ALGERIA)

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ABSTRACT

The objective of our study is to collect data concerning the current ecological state of the Machrouha forest (East-Algerian) and the woody resources in order to establish a forest reference state. The methodology adopted allows the knowledge of quantitative data relating to these resources from the dendrometric parameters collected on 4 plots of cork oak (*Quercus suber*). The various analyzes carried out indicate an average level of viability for this species. This level of viability is related to the quality of the stands which are moderately stable; the mortality and regeneration rates are low. The relative density of the stand is too high in plot P1 with 256 trees / ha and on the other hand, plot P3 has a very low abundance with 22 trees / ha. The basal areas of cork oak are significantly different at the plot level ; plot P4 has the highest average, which is 50.40 m^2 / ha. The average slenderness ratio is 21.95 in the 4 plots. The variation of H/D is irregular; this finding suggests that the slenderness ratio is a function of the average diameter and therefore the age of the stand. Indeed, this work constitutes a database for the forest but it represents only one of the facets to be taken into consideration in order to protect and restore it.

KEYWORDS: Machrouha, dendrometric parameters, cork oak, viability, slenderness coefficient

INTRODUCTION

Forests play a crucial role in sustaining life on the planet (Myers, 1996). They play a role in regulating global and regional climate systems (Gedney and Valdes, 1999). Forest products are important resources for all nations because of their significant role in the economies of countries.

Mediterranean forests represent a fragile natural environment that is deeply disturbed by human activity. Barbero (1990), points out that these forest ecosystems are characterized by two types of criteria: their spatial heterogeneity and their vulnerability due to their irregular exploitation by man.

The genus Quercus is one of the most species-rich forest genera. It includes several hundred woody species from temperate and Mediterranean zones, America, Europe and Asia, among which are some species of great economic importance. The oaks alone constitute, practically, various types of landscape highly characteristic of the Mediterranean world. In fact, the deciduous oaks are found in humid bioclimatic environments, particularly in the supra-

Mediterranean stage. On the other hand, the sclerophyllous oaks preferentially characterized the Mediterranean vegetation stage with a subhumid bioclimatic environment (Quezel, 1975).

In Algeria, the oaks represent a forest capital where they cover nearly 40% of the Algerian forest. Cork oak forests, like other forests, are particularly important because they constitute an essential element of the physical, climatic and especially socio-economic balance of the populations of rural areas and of the country in general (Alatou, 1994).

Few research works have been carried out so far on oaks in Algeria, however, oak forests are the only forests in the country capable of producing hardwoods suitable for several uses (fine carpentry, furniture, railway sleepers and uses of high quality mechanical resistance, ...). Also, they are of great interest from the ecological, biological, aesthetic, socio-economic, landscape and hunting point of view.

This study focuses on the municipality of Machrouha, its area is 22623 ha, the forest area is 16448 ha and the afforestation rate 73%. It is the most wooded commune of the wilaya of Souk-Ahras. It is considered a tourist region, it represents a place of rest and treatment of respiratory diseases in view of its healthy and clean climate.

The objective of this study is to collect data on the current ecological status of the forest of Machrouha (East Algeria) and woody resources in order to establish a reference state of forest.

MATERIAL AND METHODS

Study site

The Ouled Bechih forest is located north of Souk Ahras (Algeria). The study area is located between the coordinates 36°21'26" north latitude and 7°50'08" east longitude (Figure 1). It covers an area of 6582 ha, mainly composed of zeen oak and cork oak (Bouhraoua and Villement, 2005). This forest represents more than 50% of the underground forest of Souk Ahras. This region is characterized by a subhumid climate. The average annual temperature is 16°C and the average annual rainfall is 625 mm, with an atmospheric humidity of 68%. The altitude of the Ouled Bechih forest varies from 790 m to 1050 m, with slopes of over 15% (Boudy, 1955). The hydrographic network is very important. Several wadis and streams cross this forest massif: Oued Hemimine, Oued El Ouarida and Oued Medjerda.

Methods

Choice of the study plots

4 plots of cork oak are randomly selected with a rectangular shape and an equivalent area of 900m2 (30m x 30m), within each plot all individuals are inventoried (Rached-Kanouni et al, 2019). The parameters taken into consideration are:

- The vernacular names of the species encountered in the different study plots;

- The diameter of trees is measured on bark at chest height (1.30m) above the ground. The measurement can be made with a forestry compass.

- The total height (Ht) defined by the length of the straight line joining the foot of the tree at ground level to the end of the terminal bud of the stem.

- The geographical coordinates (longitude and latitude) of each plot were taken using a GPS (Global Positioning System).

Data processing and analysis

All measurements and observations collected on the sampled trees during field trips (vegetation survey, GPS coordinates, etc.…) are copied on the Excel spreadsheet to facilitate their processing.

Viability analysis

Stability

The stability of a stand is given by the value of the slenderness coefficient (EC), which is the ratio of height to diameter (Robisoa et al., 2008).

$$\boldsymbol{CE = H/D}$$

When: CE < 100 represents a regular and stable stand with a complete and dense cover.
CE > 100 it means that the regular stand is unstable.

Abundance (A)

Abundance is an important parameter for describing a stand. It provides an estimate of the stand density (number of stems per hectare: N/Ha) of a forest type (Rakotomalala, 2008).

$$\boldsymbol{A = n/s}$$

n: number of trees inventoried in the plot;
s: plot area (in ha); s = 0.09 ha.

Dominance (G)

The dominance evaluates the basal area of a stand. It is the total area of stem sections at 1.30 m height for a given forest area. The dominance reflects the degree of filling of the forest (Razanatsimba, 2005). Dominance is given by the formula:

$$\mathbf{G = \Sigma g}\boldsymbol{i} \mathbf{= \pi /4 \Sigma di^2\ (m^2/ha)}$$

di: diameter at 1.30m from the ground of each stem (in m).

Regeneration

Natural regeneration refers to the ability of an ecosystem (generally forest) to spontaneously recover, after removal of all or part of the forest cover, either by clear cutting, partial cutting or creation of gaps or clearings. This is one of the ways of renewing the forest, which can also be done by clear cutting followed by replanting. This last solution, simple and proven, also allows a cultural rotation, to choose a species and/or an origin better adapted to the site (improved variety), even according to the desired landscape. In addition, natural regeneration can lead to a regular or irregular forest. The regeneration rate (Tr) indicates the ratio between the number of regenerated individuals (n) and the number of seed individuals (N) (Robisoa, 2008).

Mortality

Mortality represents the natural death of forest species and should vary by diameter class. Indeed, it is higher in young stems than in old stems. The mortality rate (Tm) is the ratio between the number of dead trees (windfall and standing death) and the total number of trees in the plot per unit area (Robisoa, 2008).

RESULTS AND DISCUSSION

The horizontal structure of a species gathers the distribution of stems and basal area by diameter class. Since density (abundance), basal area (dominance) and stand development are strongly linked, the study of one cannot be done without the introduction of another (Rajoelison et al., 2008). This study will be done by taking into account at least two of the factors studied. This analysis consists in studying the spatial structure of the stands from the point of view of abundance and dominance. The results obtained in our study are illustrated in Table 1.

Stand density is high in plot P1 (256 individuals/ha), while plot P3 has the lowest abundance with 22 individuals/ha.

The basal area of cork oak is significantly different in 4 plots; plots P3 and P4 have the highest values, which are 0.30 m2/ha and 0.36 m3/ha respectively. The youngest of all is plot 2 with an average diameter at breast height of 32.09 cm; this stand is in the young forest stage. The average diameters are observed in plots 3 and 4 with values respectively is 61.94 and 58.16 cm, the trees have reached the stage of mature forest with diameters of exploitation (Andriamahazo, 2003). The trees in plots P1 and P2 are characterized by low height values, with 9.03 and 7.41 m respectively, compared to P3 where the height reaches its maximum (Table 1).

Table 1. Dendrometric characteristic of cork oak.

Plot	D (cm)	H (m)	H/D	A (Na/ha)	G (m^2/ha)
P1	40,28	9,03	22,5	256	0,14
P2	32,09	7,41	23,01	178	0,08
P3	61,94	16,28	13,64	22	0,3
P4	58,16	11,58	22,65	144	0,36

For all plots, with a mean diameter between 7.41 and 16.28 cm, show low slenderness coefficients between 13.64 and 23.01%. Plots 2, 1 and 4, which have respectively 7.41.25 cm, 9.03 cm and 11.58 cm of average diameter, the variation of H/D is irregular (Fig. 3). This finding allows us to assume that the slenderness coefficient is a function of the average diameter and therefore of the age of the stand.

From Figure. 1, the slenderness coefficients vary between 13.64 and 22.65 in the 4 plots, and thus below 100 means that this stand is stable with a complete and dense canopy. This finding allows us to assume that the slenderness coefficient is a function of the average diameter and therefore of the age of the stand. The figure below shows the slenderness coefficient as a function of the average diameter of the stand; as well as the polynomial trend line of the function.

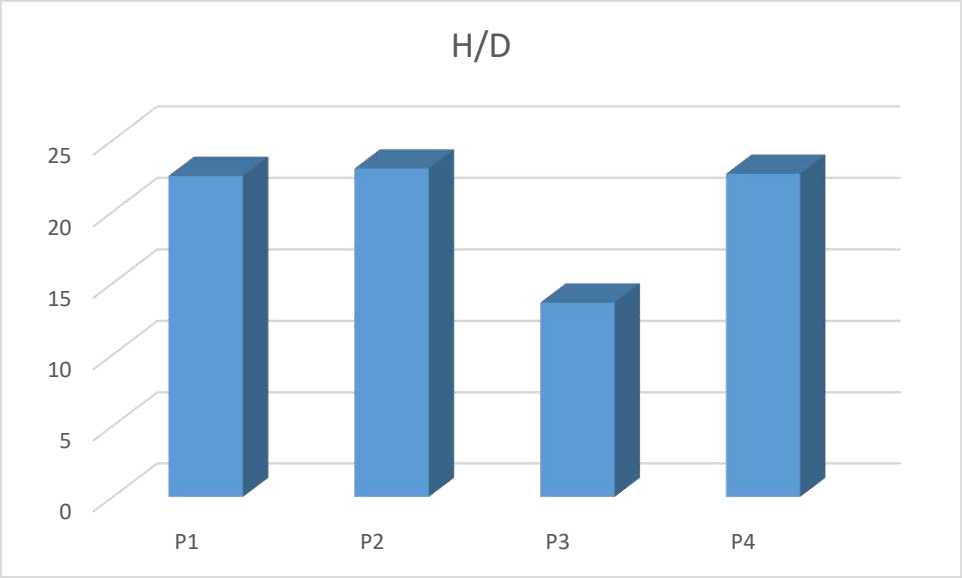

Figure 1. Elongation coefficient of 4 plots

From Figure 2, the slenderness coefficient is a negative function of the mean diameter. The coefficient of determination $r^2 = 0.6221$ of the trend curve means that 37% of the observed values are not explained by the trend curve. This 10% must correspond to the almost constant portion of the curve at the 7 cm to 9 cm interval and two plots mentioned above.

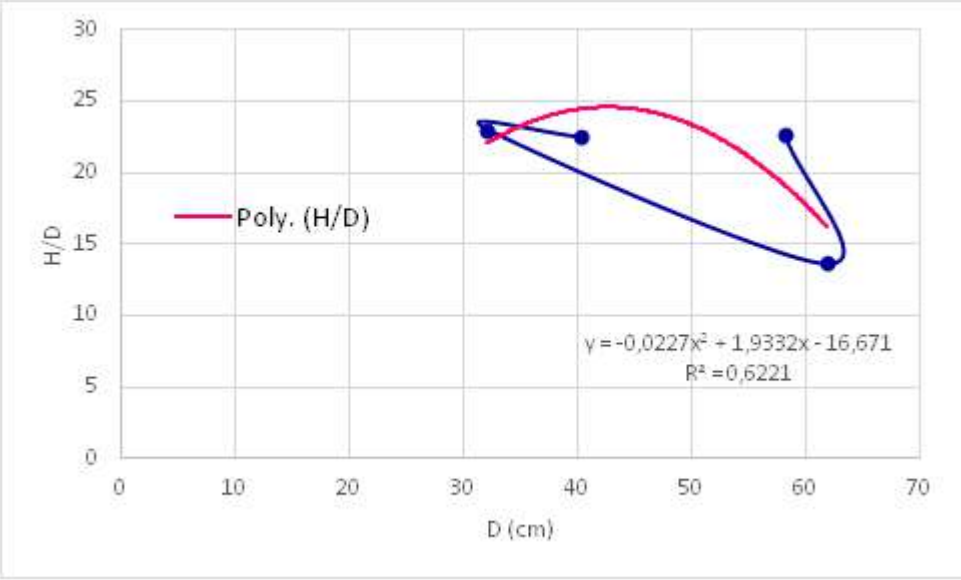

Figure 2. Slenderness coefficient as a function of the diameter of the cork oak stand

The natural regeneration is presented by the young wood of less than 5 cm of diameter at man's height, thus of not yet measurable basal surface. It corresponds precisely to the state of thicket. As in the previous studies, the study of the natural regeneration of cork oak will be done in increasing order of average stand diameter. The cork oak stand is characterized by a low rate of regeneration and mortality.

CONCLUSION

The monitoring of a forest allows to detect changes over time. Any living environment is in perpetual change. The study of the dendrometric characteristics of cork oak following the study plots showed that this stand is stable and regular with a complete and dense cover, characterized by trees of young and adult stage with a low rate of regeneration and mortality.

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COMPARATIVE ANTIMICROBIAL ACTIVITY OF BACILLUS SPP. ISOLATED FROM WASTEWATER TREATMENT PLANT AND SOIL SAMPLES

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ABSTRACT

The aim of the present study was to isolate *Bacillus* spp. from different geographical locations, capable of producing compounds with antimicrobial activity. Soil and water samples were collected from different areas under different climatic conditions. A total of 42 microorganisms were isolated from soil and wastewater samples, but five microorganisms from soil and six from wastewater samples were selected as the most active after the preliminary tests. The antimicrobial activity of the strains was determined against a great number of Gram-positive and Gram-negative bacteria, as well on fungi, using the agar well method. All of them were tested against sensitivity to the antimicrobial action of *Bacillus subtillis* ATCC 6633 for the very first time. Two strains, one from the soil sample and one from the wastewater sample exhibited very broad activities against both Gram-positive and Gram-negative microorganisms. The isolates from both samples showed inhibitory effect against *Escherichia coli* ATCC 8739 and *Bacillus subtillis* ATCC 6633. The isolates from the wastewater samples showed a larger zone of inhibition against the Gram positive test microorganisms, compared with the isolates from the soil samples. All six isolates from the wastewater samples showed antifungal activity, while only two out of five isolates from the soil samples had inhibitory effect on the tested fungi. The results showed that wastewater and soil are a good source for isolation of microorganisms that produce secondary metabolites with antimicrobial activity.

Keywords: *Bacillus* spp., antibacterial activity, antifungal activity, soil, wastewater

INTRODUCTION

The widespread use of pesticides has contaminated the environment, and the emergence of various resistant strains has raised public awareness and prompted a desire for a reduction in the usage of artificial chemical compounds. Biological control using natural microorganisms offers a possible alternative to industrial pesticides in this context. *Bacillus* strains have a major advantage in terms of survival in many ecosystems due to their ability to produce numerous antibacterial compounds and their sporulation capacity (Dimkić et. al., 2017). Antibiotic synthesis by some particular bacteria must be detected in order to determine its ability to operate as a biocontrol agent. In compared to the traditional way of selection, screening candidate strains for antibiotic production followed by direct detection of their antibiotic profiles provides a quick approach (de Souza and Raaijmakers, 2003). The synthesis of antimicrobial compounds has been hypothesized as one of the main methods by which they defend the host against harmful microorganisms, based on the idea of bacterial interference. These substances may influence bacterial metabolism or toxin generation in addition to reducing the number of viable cells (Dunne and Shanahan 2002). Vescovo et al., 1993; Bernet-Camard et al., 1997; Boris et al., 2001; Eijsink et al., 2002 are among the most recent scientific papers and reviews on the antibacterial activity and its mediators in lactic acid probiotic bacteria. The study of those engaged in the genus *Bacillus*, on the other hand, is far less understood. In medicine and industry, *Bacillus* capability to generate a wide range of biologically active metabolites, particularly those with antibacterial and/or antifungal activity, has been extensively explored. Furthermore, when used as biological control agents, the capacity of these bacteria to create antimicrobial compounds is critical for their ability to control plant diseases (Raaijmakers, 2002). As previously stated, a bacterial strain's antagonistic activity is determined by their ability to create a variety of chemicals, including molecules with extremely specific spectrums and modes of action, such as bacteriolytic enzymes, bacteriocins, and antibiotics. *Bacillus* produces all of these substances.

Antimicrobial activity was discovered many years ago, as a result of some bacteria being able to produce secondary metabolites. The antimicrobial activity can be antibacterial, antifungal or antiviral. Among the various groups of organisms that can produce metabolites, the genus *Bacillus* also can be found. *Bacillus* species are Gram positive, rod shape, catalase-positive bacteria, endospore-forming, aerobic or facultative anaerobic (Gordon et al., 1973) found in nature especially in soil and wastewater treatment plants. Among the genus, *Bacillus* spp. is the organism of interest. Besides spore forming, *Bacillus* spp. has the ability of broad spectrum antimicrobial activity (Sansinenea and Ortiz 2011). Bioactive compounds from *Bacillus* spp. were reported to inhibit several bacteria, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtillis*, *Salmonella typhi* (Xie et al. 2009). Broad antifungal activity of *Bacillus* spp. was reported by Rossall (1994).

In the therapeutic context, the mechanism of resistance highlights a severe difficulty in the treatment of pathogenic bacteria. Serious bacterial and fungal infections are becoming more well recognized as leading causes of morbidity and mortality, particularly among the elderly (Gullo, 2009; Peleg and Hooper, 2010). Modern medicine is focusing on natural items for

innovative antibiotics and antimicrobials to combat rising antimicrobial resistance. *Bacillus subtilis* strains, the model system for gram-positive organisms, can manufacture over two dozen antibiotics with various structures and activities, depending on the ecological niche or induced systematic resistance (Abriouel et. al., 2011). *Bacillus* isolates are well known for producing a diverse range of antimicrobial chemicals, including lipopeptides such as iturin, surfactin, fengycins, bacteriocins, and bacteriocin-like inhibitory substances (BLIS) (Stein, 2005). Various *Bacillus* strains produce a wide range of antibacterial substances that can be used to combat multidrug resistance. Only a small part of the antibacterial compounds that this genus is capable of producing has been identified (Ramachandran et al., 2014).

Therefore, in this study, we'll look at what we know about *Bacillus* spp. antimicrobial activity, particularly in strains that have been isolated from soil and wastewater samples collected from different areas under different climatic conditions. We shall look at how these Bacilli bacteria produce antimicrobial compounds.

MATERIALS AND METHODS

Sample collection

Different soil samples were taken from different three regions in North Macedonia, under different climatic conditions. The wastewater samples were collected from a wastewater treatment plant in Kumanovo, North Macedonia. The collected soil and wastewater samples were kept for screening and isolation of various microorganisms with antimicrobial properties.

Isolation of microorganisms

Each 10 g of the samples were suspended in 9 ml sterile distilled water and shaken vigorously for 2 minutes. The soil samples were heated at 70°C for 30 minutes in a water bath. Then the liquid was serially diluted in sterile distilled water, and the dilution from 10^{-1} to 10^{-7} was plated on nutrient agar medium. Plates were incubated at 37°C for 24 hours.

Test microorganisms

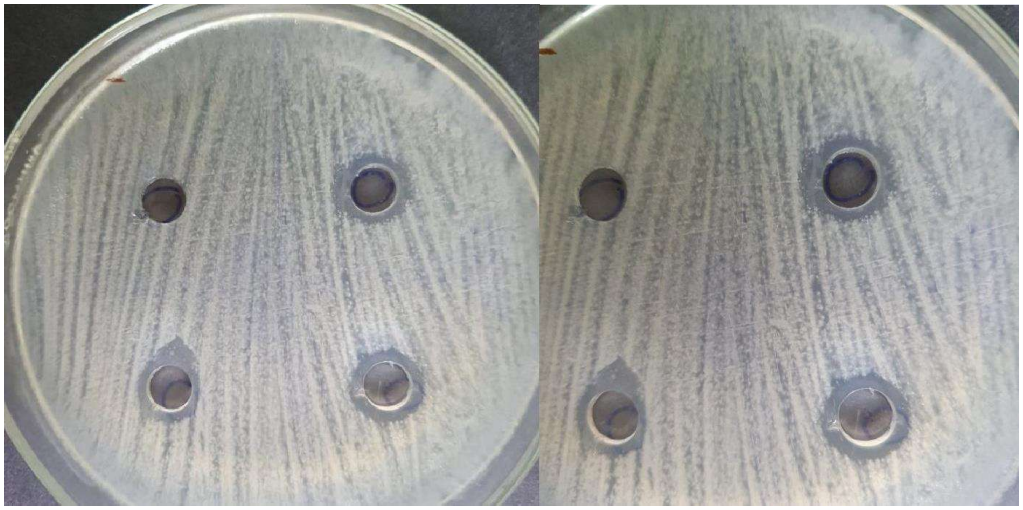
The test bacteria (*Salmonella enterica* ATCC 10708, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Listeria monocytogenes* ATCC 13393, *Bacillus subtillis* ATCC 6633, *Staphylococcus aureus* ATCC 6538) and fungi (*Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404) used in this study were obtained from the Culture Collection of the Microbiology Laboratory at the Department of Microbiology and Microbial Biotechnology at the Faculty of Natural Sciences and Mathematics in Skopje, North Macedonia. The bacteria were incubated at 37°C and all were activated by incubation for a period of 24 hours in a nutrient broth. The fungi were incubated at 25°C and were activated by incubation for a period of 72 hours in a Sabourad dextrose broth.

Inhibitory effect by the agar-well diffusion method

The determination of the inhibitory effect of isolates on test bacteria was carried out according to the agar-well diffusion method. All bacteria were cultured on nutrient broth medium and incubated at 37 °C for 24 hours. Nutrient agar medium (15 ml) was poured into each sterile Petri dish. Suspensions (100 μL) of target strain cultured for 24 hours were spread on the plates, and wells of 6 mm diameter were punched in the agar with a sterile steel borer. The *Bacillus* cultures were incubated in nutrient broth at 37 °C for 24 hours. Each sample (80 μL) were filled into the wells of agar plates directly. The inoculated plates were incubated for 24 hours at 37 °C, and the diameter of the inhibition zone was measured with ruler as mm. The measurements were done basically from the edge at the zone to the edge of the wall (Reinheimer et al., 1990). Besides, the antimicrobial activity of bacteria was compared with antibiotics. Standard antibiotic discs used for control were Gentamycin (80 mg/2ml) for the gram-negative bacteria, Penicillin for the gram-positive bacteria and Cyclohexmide for the fungi.

RESULTS AND DISCUSSION

The bacterial isolates obtained from the wastewater and soil samples collected from different regions in North Macedonia were isolated and screened using Muller-Hinton medium. Morphological studies indicated that the isolates are Gram-positive, sporulating, rod-shaped bacteria. Screening of the antimicrobial activity of 11 isolates was carried out against 9 test microorganisms. The majority of strains (81%) inhibited *Escherichia coli* ATCC 8739 growth. Eight isolates (72%) inhibited the growth of *Bacillus subtillis* ATCC 6633 and three isolates (27%) inhibited the growth of *Staphylococcus aureus* ATCC 6538. *Salmonella enterica* ATCC 10708 was inhibited by only one isolate, while none of the isolates showed inhibitory activity against *Listeria monocytogenes* ATCC 13393 and *Pseudomonas aeruginosa* ATCC 9027 (Table 1). Antifungal activity against *Candida albicans* ATCC 10231 was detected for six isolates (54%), against *Aspergillus niger* ATCC 16404 was detected for eight isolates (72%) (Table 2). Only three out of five isolates from the soil sample showed inhibitory activity against *Escherichia coli* ATCC 8739, while all isolates from the wastewater samples demonstrated inhibitory activity against *Escherichia coli* ATCC 8739 (Figure 1). Compared to the inhibition of growth against the gram-positive test microorganisms, all isolates from the soil samples showed inhibitory activity against *Bacillus subtillis* ATCC 6633, while only three isolates from the wastewater samples showed the same activity (Figure 2). Four out of six isolates from the wastewater samples inhibited the growth of *Staphylococcus aureus* ATCC 6538 (Figure 3). As for the antimicrobial activity of the *Bacillus* strains on test fungi, all six isolates from the wastewater samples showed inhibitory activity, while only two isolates from the soil samples inhibited the growth of the test fungi (Figure 4). The sensitivity levels of some isolates are more in comparison to some antibiotics. Diameters of inhibition zone (mm) exhibited against test bacteria of standard antibiotics are shown in Table 3.



A. B.

Figure 1. Agar well diffusion method for determining A) the antibacterial activity of *Bacillus* spp. from the soil samples against *Escherichia coli* ATCC 8739. B) the antibacterial activity of *Bacillus* spp. from the wastewater samples against *Escherichia coli* ATCC 8739.

A. B.

Figure 2. Agar well diffusion method for determining A) the antibacterial activity of *Bacillus* spp. from the soil samples against *Bacillus subtillis* ATCC 6633. B) the antibacterial activity of *Bacillus* spp. from the wastewater samples against *Bacillus subtillis* ATCC 6633.

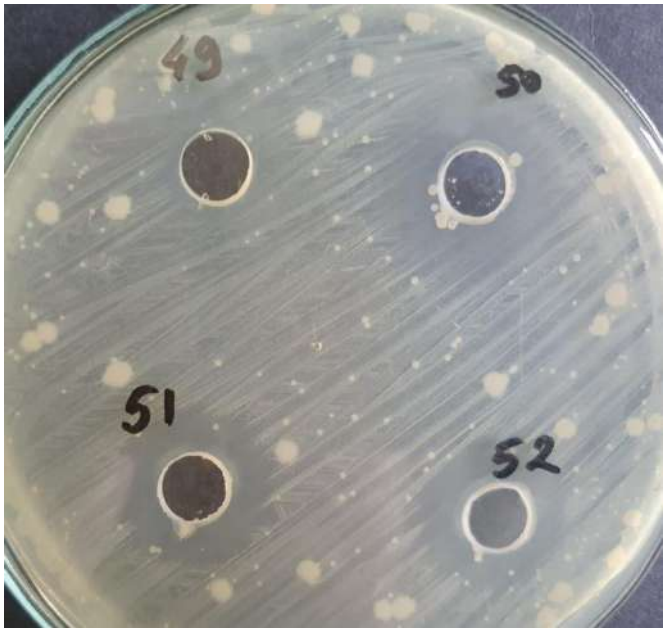
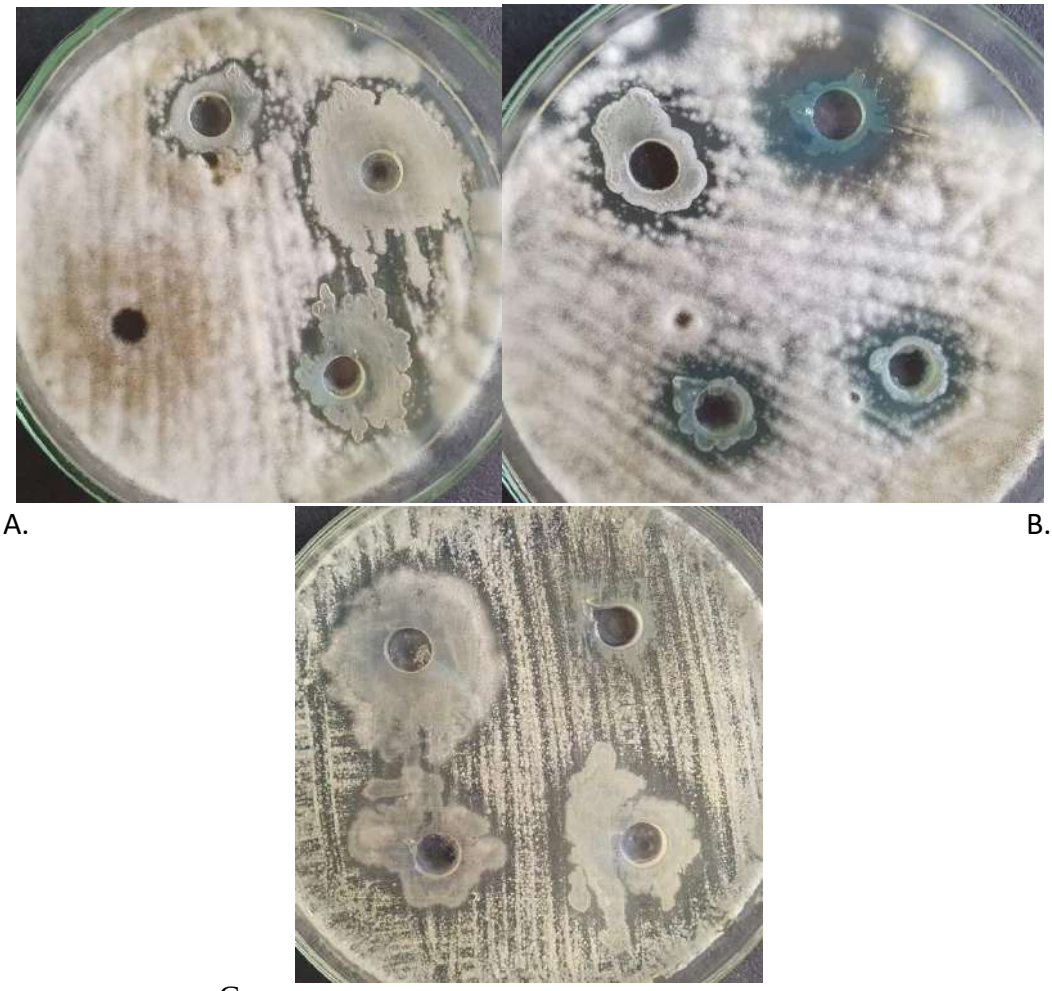


Figure 3. Agar well diffusion method for determining the antibacterial activity of *Bacillus* spp. from the soil and wastewater samples against Staphylococcus aureus ATCC 6538.

Table 1. Antimicrobial activity of *Bacillus* strains on test bacteria.

Inhibition zone (diameter, mm) against tested bacteria						
Bacillus Strains	*Salmonella enterica* ATCC 10708	*Escherichia coli* ATCC 8739	*Pseudomonas aeruginosa* ATCC 9027	*Listeria monocytogenes* ATCC 13393	*Bacillus subtillis* ATCC 6633	*Staphylococcus aureus* ATCC 6538
Soil 1	/	/	/	/	8 mm	/
Soil 2	/	4 mm	/	/	4 mm	/
Soil 3	/	/	/	/	8 mm	/
Soil 4	/	2 mm	/	/	4 mm	/
Soil 5	/	4 mm	/	/	10 mm	/
Wastewater 1	/	6 mm	/	/	/	10 mm
Wastewater 2	/	4 mm	/	/	4 mm	10 mm
Wastewater 3	/	2 mm	/	/	2 mm	8 mm

Wastewater 4	/	4 mm	/	/	/	/
Wastewater 5	/	2 mm	/	/	/	/
Wastewater 6	6 mm	8 mm	/	/	4 mm	12 mm



A. B. C.

Figure 4. Agar well diffusion method for determining A) the antifungal activity of *Bacillus* spp. from the soil samples against *Aspergillus niger* ATCC 16404. B) the antifungal activity of *Bacillus* spp. from the wastewater samples against *Aspergillus niger* ATCC 16404. C) the antifungal activity of *Bacillus* spp. from the soil and wastewater samples against *Candida albicans* ATCC 10231.

Table 2. Antimicrobial activity of *Bacillus* strains on test fungi.

Inhibition zone (diameter, mm) against tested fungi		
Bacillus Strains	*Candida albicans* ATCC 10231	*Aspergillus niger* ATCC 16404
Soil 1	/	/
Soil 2	2 mm	12 mm
Soil 3	/	/
Soil 4	4 mm	12 mm
Soil 5	/	/
Wastewater 1	/	6 mm
Wastewater 2	6 mm	4 mm
Wastewater 3	6 mm	4 mm
Wastewater 4	10 mm	4 mm
Wastewater 5	8 mm	2 mm
Wastewater 6	20 mm	2 mm

Table 3. Diameters of inhibition zone (mm) exhibited against test bacteria and fungi of standard antibiotics.

Test bacteria	Gentamycin	Penicillin	Cyclohexmide
Salmonella enterica ATCC 10708	14 mm	/	/
Escherichia col, ATCC 8739	20 mm	/	/
Pseudomonas aeruginosa ATCC 9027	20 mm	/	/
Listeria monocytogenes ATCC 13393	/	20 mm	/
Bacillus subtillis ATCC 6633	/	16 mm	/
Staphylococcus aureus ATCC 6538	/	20 mm	/
Candida albicans ATCC 10231	/	/	10 mm
Aspergillus niger ATCC 16404	/	/	10 mm

Antimicrobial chemical synthesis is frequent among these bacterial strains, according to this study. Antimicrobial activity was found in all of the isolates against one or more indicator bacteria and fungi. Gram-positive bacteria were mostly affected by the inhibitory impact. *Bacillus subtillis* was inhibited by the majority of the strains. The antimicrobial spectra, which are essentially limited to gram-positive bacteria, could indicate that the antimicrobial substances produced are linked to a gram-positive bacterium trait. It is generally known that the activity of Gram-positive bacteria's bacteriocins is limited to other gram-positive bacteria (Riley et. al., 2002). Despite the fact that these bacteria have yet to be classified to the species level, morphological and biochemical traits indicate that they belong to the genus *Bacillus*. *Bacillus* produces a wide range of antimicrobial substances, many of which are classified as peptides, lipopeptides, or phenolic derivatives (Nakano and Zuber, 1990). *Bacillus* spp. isolated from soil (Bizani and Brandelli, 2002; Oscariz et al., 1999) and plant tissues have been linked to bacteriocin-like compounds (Bechard et. al., 1998; Yoshida et. al., 2001).

Microorganisms are responsible for the development of some of the most important medications ever devised. They provide life-saving antibiotics and fungicides for bacterial and fungal illnesses. Despite past secondary metabolite research's tremendous success, the number of terrestrial antibiotics appears to be approaching a saturation curve, with an apparent limit in the near future. The growing number of duplications and the urgent need for novel leading structures in pharmacology has compelled researchers to look for metabolites in previously unknown environments. Microorganisms belonging to the genus *Bacillus* piqued our interest because they have shown interesting antibacterial potential. The current discovery emphasizes the significance of additional research in order to gain novel antibacterial agents from the genus *Bacillus* in North Macedonia's unexplored habitat. These are a diverse and mostly unscreened ecology, as well as the least researched area for the isolation of antibiotic-producing Bacilli.

Microbial strains found in this belt could give us with uncommon and unique industrial enzymes, antibiotics, or metabolites that could be more effective than the current regime in curing diseases. The findings of this study show that these microorganisms have a lot of promise

for producing antimicrobial substances that may be used in a variety of applications, and that this potential needs to be further studied.

CONCLUSIONS

We may conclude that the soil and wastewater samples are rich in *Bacillus* spp., which are antimicrobial agents with a broad scope, but the isolates from the wastewater samples showed bigger inhibitory activity against the test bacteria and fungi, compared with the isolates from the soil samples. More research is needed to establish the active metabolites of these isolates and to identify the strain at the molecular level.

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DIAGNOSTIC OF ALEPPO PINE IN EL HAMIMET FOREST (NORTHEASTERN ALGERIA)

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ABSTRACT

The aim of this work is to make a diagnosis of the current state of Aleppo pine plots of El Hamimet forest (Algeria). The importance of environmental conditions (climate and altitude) is considered. The methodology adopted is based on tree measurements; Dieback and dendrometric parameters (height, diameter, circumference and basal area) are analyzed in four stations. There are significant differences between stations on survival and the dendrometric parameters. With an average diameter between 15.31 and 29.88 cm, these stands are in the state of young forest. The total density of Aleppo pine trees with a diameter at man's height greater than 5 cm varies from 78 to 489 trees per hectare. The youngest of all is the one in plot 1 with an average DBH of 15.31 cm. The highest density and basal area are obtained in station 4 (489 trees/ha) and station 2 (33.01 m^3) respectively. The total decline affected 1 tree and 10 others are affected by a partial decline. The decline rate is low and indicates that the environmental conditions of this forest are more or less favorable for Aleppo pine. The Aleppo pine is chosen as having the best performances for future reforestation programmes.

Keywords: Tree diagnosis, Aleppo pine, Decline, Reforestation.

INTRODUCTION

Mediterranean forests cover about 81 million hectares (9.4% of the world's forest area) and consist of a mosaic of deciduous and coniferous forest species (Mugnossa et al., 2000). These forests provide ecological, social, economic and aesthetic services necessary for human life and well-being (Luyssaert et al., 2010; FAO, 2010).

The Algerian forest covers a total area estimated at 4.7 million ha or an afforestation rate of 11% for the North of Algeria including the steppe area (DGF, 2008). It is distinguished by its ecological value, its richness in forest species and occupying the most variable bioclimates. The most important species in economic terms are the cork oak with 21% of the forest area and the Aleppo pine, dominant by its stands distributed in the form of large clumps throughout northern Algeria and populates even the most hostile areas of the steppe to the margins of the Sahara. The Aleppo pine covers 35% of the wooded areas of Northern Algeria, i.e. about 800 000 ha (Bentouati et al., 2005).

The main objective of monitoring the health status is to assess the current health status of the forest of El Hamimet. This is due to the importance of its surface estimated at 1460 ha and the altitude which varies between 800 m and 1039 m like the mountainous reliefs which characterize the chain of Aures.

The forest of El Hamimet is formed by a natural set characterized by a plant cover, consisting mainly of reforestation with a main species the Aleppo pine (*Pinus halpensis*) accompanied by: *Cypressus sempervirens*, *Accasia cyanophilla* (Internal report of forests, 2005).

MATERIAL AND METHOD

Presentation of the Study Area (The Forest Of El Hamimet)

The area of El Hamimet is located in the wilaya of Oum El Boughi, between 35° 58' 26" N and 7° 11' 11.7" E. Mount El Hamimet is located in the north of the wilaya of Oum El-Boughi, extends over the territories of the communes of Ksar Sbihi, Ain Diss, Ain Babouche, Oum El Boughi, Berriche and Zorg. It extends over an area estimated at 78 000 ha. It is limited (Figure 1):

- North by the ridge line of the chain of Chebket- East- Sellaoua.
- In the South, it is bounded by the plains of Ain Babouche.
- In the West by the ridge line of Djebel Es Sensa.

The reforestation area El-Hamimet extends over an area estimated at 1460ha.

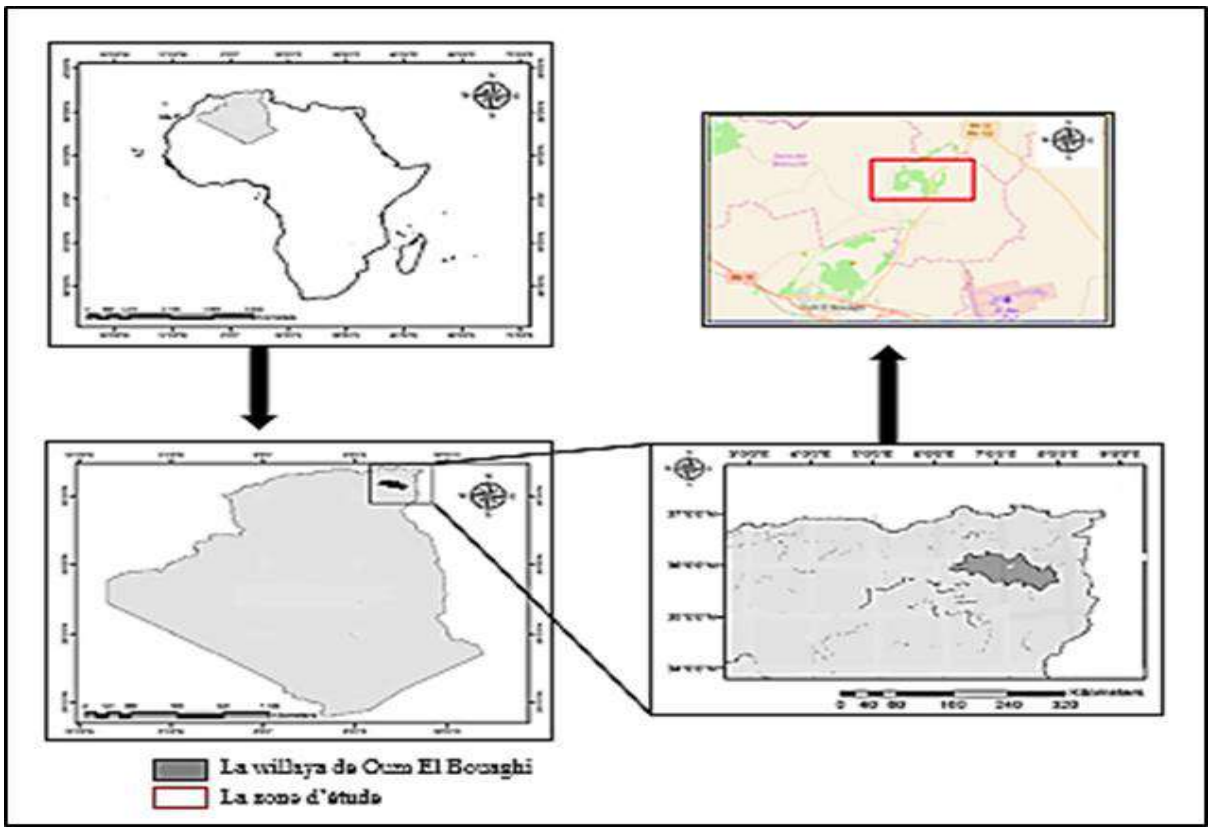

Figure 1. Geographical location of the study area (the forest of el Hamimet).

Characteristics of the plots are in table.

Table 1. Characteristics of the study plots.

Plots	Vegetation	Exposition	Altitude (m)	Geographical coordinates
P1	Aleppo pine	NW	875	X: 35°57'55.470" Y: 7°12'12.209"
P2	Aleppo pine	NW	873	X: 35°57'53.994" Y: 7°12'06.719"
P3	Aleppo pine	NW	905	X: 35°57'54.798" Y: 7°11'57.168"
P4	Aleppo pine	NE	855	X: 35°58'07.535" Y: 7°12'08.586"

Dendrometric data collection inventory and description of the stands are a prerequisite for any successful forest management and sylvicultural planning (Rajaonarisoa et al., 2002).We are interested in achieving this inventory to highlight the structure, stand density and the difference of perspective development for each station. The structure of the stand is defined as the manner in which these are arranged dendrometric variables (Roger Edmond et al., 2007). The tree inventory was conducted in each plot. Dendrometric measurements are:

- The diameter (D) at 1.30 m is estimated using calipers.
- The circumference (C) at 1.30 m is estimated with a tape measure.
- The total tree height (H) measured with the "Smartphone application". The Swedish unit is, recommended in this type of study requiring maximum precision; the permissible error is negligible with direct readings that require no calculation. The measurement accuracy depends on the quality of the inclinometer of Apps and stability with which the device is held.
- The basal area of a stand is the area of all cross sections of trunks, 1.30 m tall, and the trees on one hectare of forest.
- Abundance gives the number of stems of a species (*Pinus halepensis*) in the stand. It is expressed as N per hectare (N/ha) (Rakotomalala et al., 2008).
- The dominance evaluates the basal area G. The stand depends on both the density and the elongation of the trees (Razanatsimba, 2005).

The purpose of studying tree spread is to determine the factors that influence its value and its relationship to stand stability. Generally, the slenderness coefficient 100 corresponds to the stability threshold of a stand, but for a species sensitive to disturbances this threshold goes down to 80. It is formulated by: $G = \Sigma g = \Sigma (\Pi d^2/ 4)$ and is expressed in $m^2/$ ha.

The Mortality represents the natural death of forest species and should vary by diameter class. Indeed, it is higher in young stems than in old stems. The mortality rate (Tm) is the ratio of the number of dead trees (windfall and standing dead) to the total number of trees in the plot per unit area (Robisoa, 2008).

RESULTS AND DISCUSSION

The horizontal structure of a species combines the distribution of stems and the distribution of basal area by diameter class (Table 2). Since density, basal area and stand development are strongly linked, the study of one cannot be done without the introduction of another.

This study will be done by taking into account at least two of these factors. Table 2 summarizes the main stand characteristics of *Pinus halepensis* in each plot, concerning trees with measurable basal area.

With an average diameter between 15.31 and 29.88 cm, these stands are in the young forest condition. The total density of Aleppo pine trees with a diameter at man's height greater than 5 cm varies from 78 to 489 feet per hectare. The youngest of all is the one in plot 1 with

an average diameter at breast height of 15.31 cm. This stand contains a large number of young trees (489 trees/ha).

The basal area of Aleppo pine is significantly different in the 4 plots; plots P2 and P4 have the highest values, which are 33.01 m2/ha and 37.68 m2/ha respectively.

Table 2. Quantitative characteristics of the stands of Aleppo pine.

Plots	Species	D (cm)	H (m)	H/D	g (m^2)	gh (m^2)	g-gh (cm^2)	N/ha	G (m^2/ha)
P1	Aleppo pine	15.31	29.23	39.23	0.02	0.01	0.70	489.00	7.80
P2	Aleppo pine	29.88	26.47	26.47	0.10	0.01	2.97	344.00	33.01
P3	Aleppo pine	24.54	25.89	25.89	0.06	0.01	0.80	156.00	8.87
P4	Aleppo pine	29.84	30.6	30.60	0.10	0.01	3.39	278.00	37.68

From figure 2 the slenderness coefficients vary between 25.89 and 39.23 in the 4 plots, and thus lower than 100 it means that this stand is stable and regular with a complete and dense cover (Erlbeck et al., 2002).

The slenderness coefficient gives an idea about the ecological stability of the tree stratum (Rajoelison et al., 2008). Considering the H/D ratio, this factor is of the order of 30.54. Therefore, the trees are under too much competition and should not be able to withstand the wind well. They have a height that is far too high in relation to their diameter. This growth rate can be explained by their strong competitive power (Massenet et al., 2011). This observation allows us to assume that the slenderness coefficient is a function of the average diameter and therefore of the age of the stand (Figure 2). Therefore, the slenderness coefficient is less than 100, which means that these stands are stable and regular.

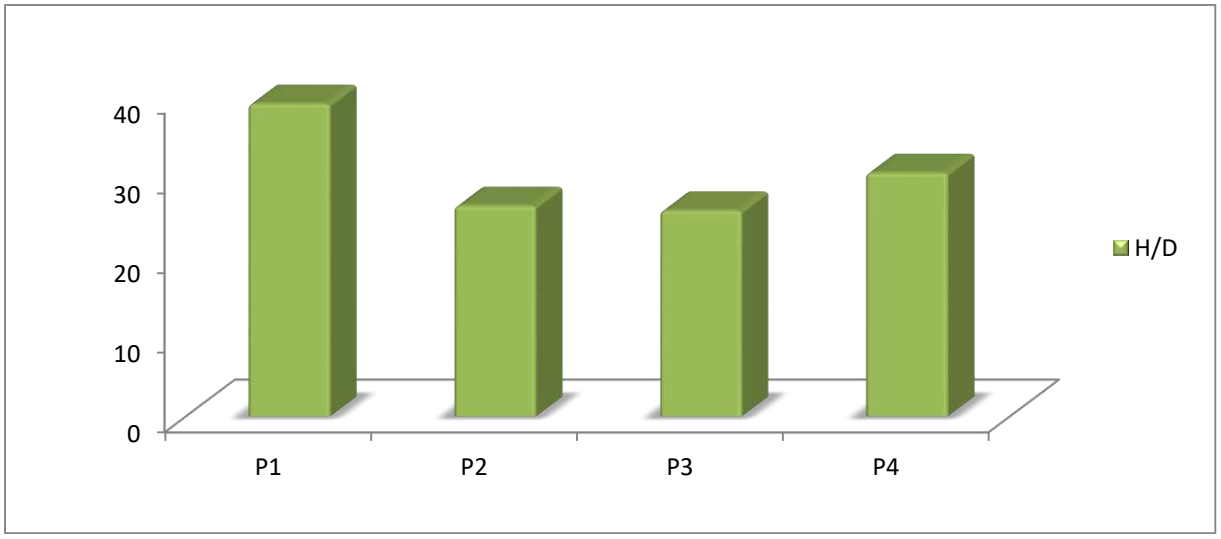

Figure 2. Slenderness coefficient of 4 plots.

Figure 3 shows the slenderness coefficient as a function of stand diameter, as well as the polynomial trend line of the function. The coefficient of determination R^2 =0.9232 of the trend line.

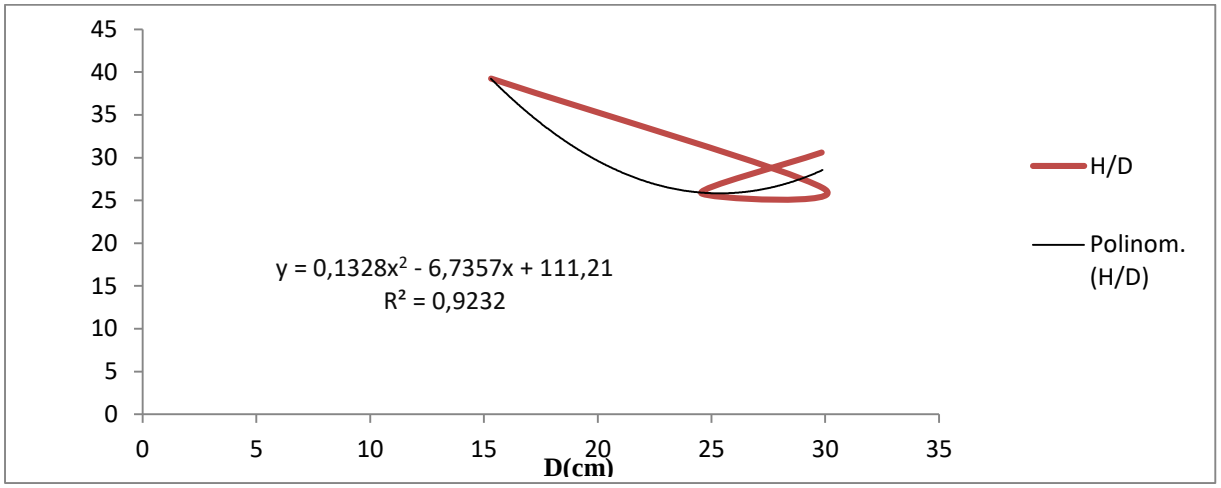

Figure 3. Slenderness coefficient as a function of the diameter of the stand of Aleppo pine.

CONCLUSION

The Dendrometric characteristics suggests that, in general, the forest of El Hamimet as an ecosystem is viable even if pressures. Therefore, if the degree of pressure increases and reduces the forest area, the sustainability of the forest will be threatened. The use of Aleppo pine in reforestation for timber production will not only be a way to value the various qualities of its wood, but also a way to diversify the products on the timber market and a way to protect the bare soil of the highlands. Due to its interesting physical-mechanical properties, the wood of Aleppo pine has a multitude of possible uses that classify it in the category of quality woods. This species is particularly successful in the study site, with rapid growth and successful natural regeneration.

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VERTICAL LINKAGE IN TIMBER PRODUCTION: A CASE STUDY IN TUYEN QUANG PROVINCE, VIETNAM

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Abstract

Vertical linkage between woodworking companies and local forest plantation communities is the best development direction for wood processing industry. Timber production has been significantly contributed to the poverty reduction and rural livelihoods at Tuyen Quang Province, Vietnam in recent years. By using descriptive analysis to explore the role of vertical linkage in timber production to the rural development in Tuyen Quang, the results have shown that vertical linkage both helped wood enterprises to ensure input resources for producing in a long-term and provides growers with better market information concerning timber demand, reduce risks and gain higher income from forest. This linkage model should be replicated in the forest plantation community because it not only brings benefits to the parties but also improves the local ecological environment protection.

Keywords: *Forestry, Rural Development, Timber Processing Company, Timber Grower, Vertical Linkage, Tuyen Quang, Timber Production.*

INTRODUCTION

Vertical linkages is the trend of some timber processors because it typically benefits wood firms by providing potential to increase resource security for long-term through diversity of resources supply; efficiency and profits by controlling all the stages of timber production; reducing financial risks of landholding and timber growing, labour and management costs (Race el at., 2009). The forest communities get the attraction to the partnerships by expectation on chances to obtain the reliable cash flow, in terms of increasing income and spread the market risks through ensure sales; silvicultural physical support and advice with skills in tree crop management and establishment (Yokota el at., 2014).

As a mountainous province in the North of Vietnam, Tuyen Quang has 446.691 hectares of forestland that takes around 76% of the total natural land area. With good weather conditions and fertile soil, Tuyen Quang has great potential for forestry economic development. Therefore, planting trees to meet the demand of raw materials for the wood processing industry at Tuyen Quang and other provinces has been realized by local people for a long time. By the end of 2020, the exploitation of timber in Tuyen Quang has reach 880.958 m3, accounting for 7,3% of the country's timber production (General Statistic Office of Vietnam, 2020). Initially, it has met the need of timber for wood processing in the area. The effectives of forest plantations, harvesting, wood processing and forestry products have significantly contribute to job creation and alleviation of poverty, improve livelihoods for people in rural area and protect the ecological environment.

One of the primary reasons for the interesting results in woodworking industry above is thanks to effective implement of linkage between wood companies and local forest communities. This is a good models that brings many benefits to the parties. It helps timber growers solve the problem of output and the wood processors can stabilize the source of input materials. This

research aimed to (1) identify theories on vertical linkage in timber production; (2) examine the current status of vertical linkage in timber production in Tuyen Quang; (3) access the role of vertical linkage in timber production to rural development in Tuyen Quang. A literature review of vertical linkage in timber production was conducted as a first step for this study. Descriptive statistics and qualitative analysis method were used to explore the role of forestry company-community partnership to the rural development. Data was collected from June 2019 to June 2020 from related publication, forestry reports and farmer interviews. Participatory Rural Appraisal (PRA) was used to the respondents using open questionnaires for data collection.

Literature Review

The role of vertical linkage in timber production

Under the Vietnam's forest policy, since 2014 timber was not allowed to exploit from natural forest, replacing this all materials would be used from planted forest and import. Thus, grower schemes play an important role not only for significant contribution to the rural community's income but also being one of the main supply chains of materials for wood industry (Phuc, 2017).

The connection between wood processing enterprises and local grower communities are based on the trust and voluntary agreements (The World Bank, 2009). Accordingly, the growers provide forest land and trees management while the companies provide initial finance, management and market opportunities (Boulay el at, 2013).

Permadi et al. (2018) in the study on relationship between forestry enterprises and farmers in Indonesia concluded that the factors that lead growers to participate in the link with the company are some commitments mentioned in the contract from the company such as improving the local roads, raising the household's income, securing in timber consumption when harvest.

Research on "The current status and solutions to develop linkage models following timber plantation value chain" by Son (2017), the author has shown that the benefits from participating in the commercial relationship with wood processing companies for farmers is gaining about 15% to 20% higher income from forest than non-participating households.

Generally, the results of studies agreed that vertical linkage in timber production and marketing between wood company and forest based community is necessary. It is because the link will be able to solve the basic problems in the wood processing industry such as: helping company to avoid spreading investment; highly improving the quality and output of timber for growers; balancing supply and demand in the timber market. Especially, the members participating in the linkage will have the same voice and responsibility for the final product. Therefore, it is easy to trace the origin of wood and the parties easily share information. It not only contribute to improving the competitiveness of the wood processing companies but also increase market opportunities for them in meeting large order requirements.

Important factors for an effective vertical linkage

Firstly, the arrangement must be legally valid and fully bargained by parties. It is necessary for a commercial timber contract to follow the law because this would increase and emphasize the responsibility of partnerships in the implementation agreements or just in case, if there is any disagreement happening between partners, third party will play the role to arbitrate. Furthermore, if the issues and interests in each issues are identified clearly by both sides, it will lead partners to have common expectations in their connection and to find effectively the best opportunities for prospective collaboration. The value of joint ventures will be limited when either the grower or company is restricted in their ability to negotiate linkage arrangements (Alexander, 2020)

Secondly, the parties must have mutual respect on each partner's legitimate aims and trust one another when create a relationship. It is right to consider that the core values for the

successful linkage is the trust and fair sharing system among participants. Because the company and grower take part in joint venture voluntarily to find from each other mutual benefit so that belief is one of the necessary important requirements to maintain and develop the reality integration for long-term. Without trust and respect, uncertainties will run high and finally negate positive elements of both sides. Small problems may become large problems easily.

Thirdly, benefit and risks must be shared equitably base on the contribution of each sides. For every economic linkages especially in forestry area, only after the benefit can be increased and the risks in production, market, social and environmental terms can be reduced, it would be secure for a sustainable development.

Research And Discussions

Typical model of vertical linkage in timber production in Tuyen Quang

In Tuyen Quang Province, mostly trees growing for timber are Eucalyptus and Acacia. By the end of 2020, there are 9 large wood processing enterprises and about 382 small forestry business companies in the province, of which approximately 95% belong to private businesses and 5% are from government. These are the main sources of wood consumption in the area (Tuyen Quang Department of Statistic, 2020). The estimate of total forest land covered in Tuyen Quang till the year of 2020 is 448,681 hectares, in which the planted forest that belong to timber growers is 251.954 (ha) and natural forest is 196.727 (ha) (Tuyen Quang Department of Agriculture & Rural Development, 2020).

The widespread typology of vertical linkage in forestry production in Tuyen Quang is out-grower scheme conducting by 2 main parties: timber processing company and forest growers. This link has been established and on the development in the province for more than 15 years. Out grower schemes has involved some 3.200 smallholder tree growers on about 60.000 hectares of planted forest land (Tuyen Quang Forest Protection Department, 2020). This scheme has been operated effectively by An Hoa Paper Company, the biggest wood processing institution in Tuyen Quang, and local farmers. Following this model, the timber processor will provide landholder with physical inputs such as seedlings, silvicultural training for forest establishment and maintenance, amount of loan following the purchasing agreements in the contract. Meanwhile the farmer have forest plantation lands and labours. They grow and maintain trees on their land under the controlling of the company over wood production. When the trees are reached to standard quantity and quality of harvesting, the company will buy timber with current market prices. On the other hand, this model is based on the belief that the resources of the linkage parties will be maximized. Specifically, the processing company has the potential of investment capital, technique and technology, management capacity and output coverage for products; Households have the sources of forest plantation land and labour. The out-grower integration is attractive growers by some advance payments for their works and guaranteed market when harvesting. The company find the benefit through saving investment in developing their own forest assets and avoiding the potential of expensive liabilities.

The role of vertical linkage in forestry production to rural development in Tuyen Quang

The area of forestry plantation communities are mostly located in remote mountainous where people living in are still poor and their income base totally on agroforestry production activities. Moreover, forest growers are almost ethnic minorities with low educational level. Thus, forest are closely connected to social issues and play an important role in improving the livelihood of poor rural communities. Trees are considered as an important form of savings and informal collateral for low-income households. Since An Hoa Paper Company has established in the area and the out grower scheme has been conducted, it has helped to improve benefits for the households and ensure a steady supply of timber for the companies (Table 1 & Table 2).

Table 1. Total volume of Timber consumption of An Hoa Paper Company

Year	Demand (m3)	Supply (m3)	Ratio (%)
2016	445.000	332.168	74,6
2017	430.000	347.429	80,8
2018	550.000	472.577	85,9
2019	600.000	530.666	88,4
2020	650.000	555.003	95,4

Source: An Hoa Paper company, 2020

Table 2. Comparing benefits from the linkage to the participated and non-participated households

Benefits	Participated (%)	Non-Participated (%)
Good seedlings	100,0	53,3
Improve knowledge	92,5	46,7
Stable consumption	96,3	38,3
Good price	81,3	53,3
Higher income	87,5	56,7
Better market information	63,8	41,7
Reduce risks	81,3	38,3

Source: Calculated from Survey Data, 2020

First, the collaboration will help growers save investment resources in the process of forestation. At the beginning of planting, farmers have to pay some costs such as: seedlings, fertilizer, forest establishment and maintenance, expenses for exploitation and transportation of wood to the log yard of the company. Due to the specific characteristics of timber raw materials for woodworking industry so growers have to wait for at least 6 -7 years later to get money back after harvesting. Hence, in some difficult economic areas, people with low income need the initially necessary support from the company. Through the linkage, company will provide growers free seedlings, technical assistance and ensure to buy timber when harvest with good prices. By this way, farmers can save money right the beginning of forest growing process and get higher income when selling timber. According to An Hoa paper company statistic, from 2017 to 2020, implementing the forest development policy and stabilizing wood raw material areas, the company invested more than 7,3 billions of acacia seedlings to about 1.500 households in 4 districts: Chiem Hoa, Ham Yen, Son Duong, Yen Son. Farmers participating in this program said that owing to seedlings come from good resources and are planted properly following technical process, their plantations are growing very well. Moreover, the company also provide technical advice in soil preparation, fertilizing, digging holes for trees planting. As a result, the family saved a lot of costs in forest plantation under the program. This findings was consistent with the study of Phuc (2017) and Son (2017) on assessing the benefits from the linkage between forestry company and community partnership in afforestation.

Second, out grower scheme provide growers with market information concerning timber demand and price and access to market agents. Because of the lack of knowledge market specifications and often substandard quality of their products, farmers have less advantage in

negotiating with brokers and mobile saw millers who come to villages in search of trees to fell. They also have little knowledge of how to assess the value of their trees and how and where to market them, especially when the open market is volatile. Therefore, joining in the relationship with the company, farmers may have a guaranteed price (probably higher) than get the market price. Moreover, outgrowing arrangements can be useful to communities as stepping stones to greater and more profitable market involvement. In Tuyen Quang recent years, many small-scale farmers are attracted to out grower schemes as a source of the financial, technical and labour inputs that make it possible to bring under-utilised land into more profitable production.

Third, for many farmers, tree planting is an economically driven activity, providing a source of income. Companies' investments in local human capital can be a useful route to development for rural communities. Through short training courses in timber planting and tending technical transfer or extension services, farmers have chances to improve silvicultural skills, plantation management, capacity in scientifically planning trees and skills required to maximize productivity. These are very especially necessary to ethnic group because they are the parts that normally grow trees following traditional way and sell them whenever they want. Hence, farmers will know how to maintain and develop their forest. Accordingly, they can increase their income because the bigger size of wood it is, the higher price they can get.

Fourth, out grower deals help to improve the local ecological environment by increasing the new forest area annually. In 2020, Tuyen Quang planted more than 12.063 hectares of new forest, increase the ratio of forest cover up to 65% of the total province area (Tuyen Quang Department of Statistic, 2020). It has contributed significantly to the improvement of underutilized agricultural land with poor access and low productivity, protect soil and water for local.

Finally, the link provide opportunities for community members, whether a tree grower or not, to work in the plantations and give them valuable practical experience on cultivation practices. According to the statistic of the Department of Agriculture in Tuyen Quang, by the end of 2020, more than 70% of the province's labourers are agricultural and forestry workers, of which, forestry accounts for about 13%. Moreover, community members could receive assistance from the company for social funds and road infrastructure under outgrowing program.

With above result findings, this linkage model should be encouraged to apply in other wood companies in the province. The farmers should participate in the link with enterprises to get better benefit from planting forest work.

Conclusions

In recent years, forestry production have contributed more than one-third of Tuyen Quang's GDP. The linkage makes many benefits such as using efficient resources, increasing the specialization, expanding market access and have contributed substantially to household income. Three important impacts leading to effective forest company-community relationship are legally valid and fully bargained in the arrangement; mutual respect and trust relationship; sharing equitably benefit and risks. The results showed that vertical linkage between forest company and local growers play an important role to the rural development such as: (1) saving investment resources in the process of forestation; (2) providing growers with market information concerning timber demand and price and access to market agents; (3) providing a source of income; (4) improving the local ecological environment protection and (5) providing opportunities for employment and social security.

The limitations of this study are not to mention the difficulties and factors affecting the implementation of links between woodworking companies and households. Accordingly, some recommendations for improving linkage in the future have not been propose. These are the idea for the next studies.

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EFFECTS OF dam AND seqA GENE MUTATIONS ON BIOFILM PHENOTYPES, CELLULASE ENZYME PRODUCTION AND MOTILITY IN SALMONELLA TYPHIMURIUM

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ABSTRACT

Biofilms are microorganism communities consisting of single or multiple species, attached to a surface within a matrix structure, distinctly differentiated from planktonic forms in gene expression and physiological aspects. The fact that the members of these structures show a structure and task differentiation similar to multicellular organisms, increase in their virulence characteristics and contain high levels of resistance to adverse environmental conditions cause serious problems in food and health. In this study, biofilm formation, cellulose production is one of the main components affecting biofilm morphotypes. Accordingly, cellulase production, motility properties were investigated in mutants created by deletion of the *dam* and *seqA* genes homologous site recombination in *S.* Typhimurium strain. As a result of the studies, it was determined that biofilm production capacity, cellulose and cellulase enzyme production, and motility decreased significantly in both mutants. These data indicate that both genes are new genes effective on biofilm production in *S.* Typhimurium. In the light of these findings obtained from the study, the use of inhibitors of deoxy adenine methylase (Dam) and DNA sequestration A (SeqA) proteins encoded by these genes has emerged in the fight against *Salmonella* biofilms.

Key Words: *S.* Typhimurium, *dam, seqA*, cellulose, cellulase, motility

INTRODUCTION

Biofilm is defined as the multi-layered structure formed in a network of macromolecules (extracellular polymeric structure, EPS) comprising many repetitive subunits produced by the cells in question and secreted out of the cell in this process (Davey and O'Toole, 2000). Environmental conditions that encourage bacterial cells to form a biofilm are usually stressed factors that make it difficult for bacteria to persist or reproduce in this environment. These can be factors such as the ratio of hydrogen ion [H^+] and hydroxide ion [OH^-] concentrations, insufficiency of carbon and nitrogen sources in the environment, temperature changes that deviate significantly from the optimum growth temperature, oxygen level changes in the niches where bacteria reside, or ionic forces (Kostakioti et al., 2013).

In order to survive against such challenging conditions that bacteria usually encounter in their environment; It is known that they can produce very different phenotypic, genetic, or epigenetic responses. It was determined that a significant part of bacterial genomes differed in terms of expression, especially during the transition of bacteria from planktonic life form to biofilm form. The differentiation of the genes responsible for the formation of biofilm forms constitutes the primary step in the emergence of the species-specific phenotype of the biofilm or the modification of the existing biofilm phenotype (Sabbagh et al., 2010). This genetic flexibility of bacteria to adapt to changing environmental conditions

is of great importance in understanding the way they perceive their environment and the biology of the responses they create in parallel with this perception. While motile cells form a stable biofilm by attaching to a surface and producing EPS, it is generally accepted that the genes responsible for movement are inactive, and the genes necessary for matrix production are activated (Corcoran et al., 2014).

It has been determined that epigenetic mechanisms regulate many physiological and structural characters in bacteria. Nowadays, the evidence that *Salmonella* biofilms are under strict epigenetic regulation has started to accumulate, dramatically increasing the interest in this field (Dubnou and Losick, 2006; Casadesus and Low, 2013). One of the most important enzymes involved in epigenetic regulation in bacteria is DNA methylases. It has been determined that the main methyltransferase enzyme of the Gammaproteobacteria taxon, including *S.* enterica, is the deoxyadenosine methyltransferase (Dam) enzyme. Dam methylation is carried out at position 6 of the adenine base in the 5'GATC3' recognition sequences. The Dam methyltransferase enzyme uses the S-adenosine methionine cofactor (SAM) as a methyl group donor, similar to other methyltransferases. The functions of DNA methylation can be counted as regulation of gene expression in bacteria, intra-genome or inter-genome mobility of mobile DNA elements, the timing of DNA replication, distribution of replicated chromosomal or plasmid DNA copies to newly formed cells (Balbontin et al., 2006; Chatti et al., 2008). On the other hand, SeqA protein works in coordination with Dam methyltransferase and acts as a replication origin sequestering the protein to regulate DNA replication time. Besides this essential function; it has been determined that the protein in question participates in the regulation of different genes in *Salmonella* and that in *seqA* gene mutants, adhesion to host cells and especially host cell invasion is significantly reduced (Chatti et al., 2008; Jakomin et al., 2008; Uğur et al., 2018).

This study aimed to detail the effects of these genes on *Salmonella* biofilms by examining the changes in biofilm-related phenotypic characteristics in mutant strains in terms of the *dam* and *seqA* genes.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

The wild-type *S.* Typhimurium 14028 used in this study and its mutant strains in *dam* and *seqA* gene were obtained from the culture collection created in Ankara University Faculty of Science, Department of Biology, Prokaryote Genetics Laboratory. Strains were grown in Luria Bertani Broth (LB) broth at 37 °C at 200 rpm shaking speed for 18 h. Since mutant strains contain chloramphenicol gene cassette, chloramphenicol antibiotic (20 µg/mL) was added to their growth media. Bacteria samples used in the biofilm experiment were used in a salt-free LB Broth (LB-NaCl) medium, and bacteria were grown in static conditions at 20 °C in this medium. During the study, all cultures were stored at -20 °C, and -80 °C in Luria-Bertani (LB) broth with 15% glycerol added to sterile microfuge tubes.

Determination of Biofilm Production of Wild-type and Mutant Strains on Polystyrene Surfaces

To determine the amount of biofilm produced by *S.* Typhimurium strains (wild type, Δ*dam*, and Δs*eqA*) on the polystyrene surface, the method proposed by Woodward et al. (2000) was used. Biofilm measurements were carried out at 20 ℃ at the end of 24, 48, and 72 hours incubation periods.

Detection of Cellulose Presence in Biofilm Structure

The strains were grown on NaCl-free LB agar media containing calcofluor, a cellulose indicator (fluorescent brightener 28, Sigma-Aldrich, China, 200 µg/L). These media were incubated at 20 °C for

8 days and evaluated and photographed under 366 nm UV light according to whether they produced cellulose, an extracellular matrix component (Kodak Gel Logic 200 Imaging System). The study was carried out in 2 parallel and 2 repetitions. Samples showing fluorescent properties under 366 nm UV light were evaluated as positive in cellulose production, while those that did not show were evaluated as negative in cellulose production (Vestby et al., 2009).

Determination of Cellulase Activity

Agar well diffusion test was used to evaluate cellulase activity. Mutant and wild-type cultures were incubated in LB broth containing 5% carboxymethyl cellulose and free of NaCl for 18 hours under shaking conditions. After incubation, culture suspensions were prepared with OD600=5 in PBS. 100 µL of the prepared culture suspension was transferred to the wells by opening wells with a diameter of 5 mm in NaCl-free LB solid media containing 5% carboxymethyl cellulose. After 48 hours of incubation at 20 °C, the PBS solution containing 0.1% Congo Red was spread on the agar surface and incubated for 30 minutes. At the end of the incubation, the agar plates were washed 3 times with PBS for 15 minutes. Degradation zone diameters formed around the wells were evaluated as an indicator of cellulase activity (Ahmad et al., 2016).

Evaluation of Bacterial 'Swimming' and 'Swarming' Motility

'Swarming' movement is a multicellular surface movement that occurs with the rotation of the flagella. The 'swimming' movement is the individual movement in the liquid medium supported by the rotation of the flagella (Henrichsen, 1972; Mattick, 2002). In the study to evaluate the 'swimming' movement, *Salmonella* strains were first incubated at 37 °C overnight in LB agar medium. At the end of the incubation, a single colony was taken from the agar surface and inoculated on a 0.3% LB agar surface, and incubated in two stages for 5 hours at 28 °C and 4 hours at 37 °C, respectively. Strains were similarly incubated overnight at 37 °C on LB agar medium to detect 'swarming' activity. At the end of the incubation period, a single colony was taken from the agar surface and inoculated into a 0.5% LB solid medium containing 0.5% glucose. Agar plates were evaluated by incubation at 28 °C for 5 hours and 37 °C for 4 hours. The movement was determined by measuring the radius from the inoculation zone to the edge of the swimming and swarming zone (Ahmad et al., 2016).

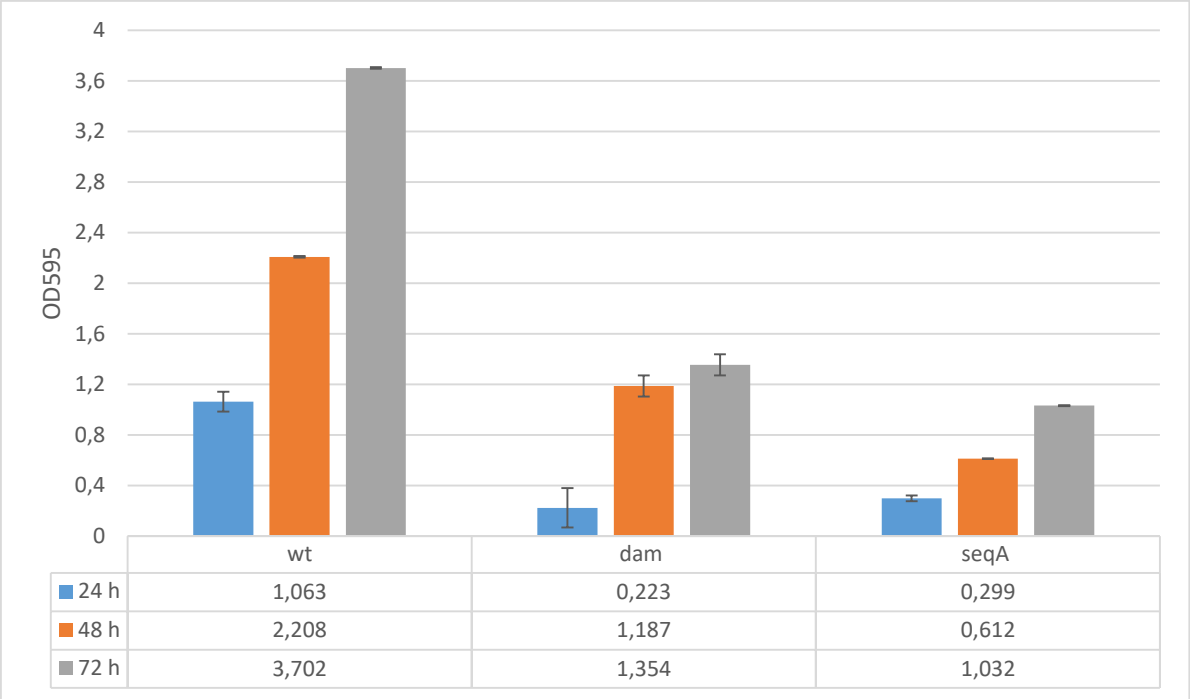
Statistical Analysis

Comparisons between means of tests performed in the study are in Minitab® statistical software (version 18.1). Post hoc Tukey test was used for more than two means, Student's t-test and two-way analysis of variance (ANOVA) were used for two means.

RESULTS

Biofilm Production in the Natural Strain and Its *dam* and *seqA* Mutants

In the biofilm formation experiments carried out on polystyrene surfaces, it was determined that the wild-type strain reached the optimum biofilm production level in 72 hours. It was determined that biofilm production decreased at statistically significant levels ($p<0.05$) in the *dam* and *seqA* mutants of this strain at all incubation periods used in the study (Figure 1).



	wt	dam	seqA
24 h	1,063	0,223	0,299
48 h	2,208	1,187	0,612
72 h	3,702	1,354	1,032

Figure 1. Biofilm production levels in wild-type strains and mutants

Comparison of Bacterial 'Swimming' and 'Swarming' Movements in Wild-Type and Mutant Strains

The experiment, carried out to determine the 'Swarming' and 'Swimming' motility due to the flagella, was evaluated by measuring the radius of the movement from the inoculation zone on the agar plates to the edge of the swimming and swarming zone. The swimming movement observed in *S.* Typhimurium 14028 wild-type strain was abolished in the Δ*dam* mutant strain, while it was reduced by 85% in the Δ*seqA* mutant strain. The swarming movement was also reduced by approximately 50% in both mutants (Figure 2 and Figure 3).

Figure 2. The "swimming" movements of the *S.* Typhimurium 14028 wild-type strain and its *dam* and *seqA* gene mutants

Figure 3. The "swarming" movements of the *S.* Typhimurium 14028 wild-type strain and its *dam* and *seqA* gene mutants

Evaluation of cellulase activity

The zone resulting from the cellulase activity, which was observed with a diameter of 4 cm in the *S.* Typhimurium 14028 wild-type strain, was detected as 2.4 cm in the *seqA* gene mutant and 3 cm in the *dam* gene mutant (Figure 4).

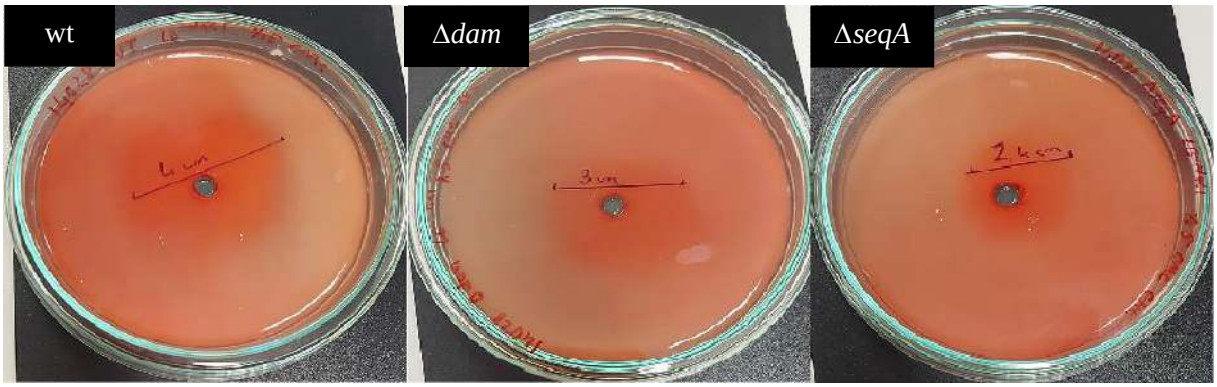


Figure 4. Cellulase activity zones *of S.* Typhimurium 14028 wild-type strain, and its *dam* and *seqA* mutants

Cellulose Production in Biofilm Matrix

In order to determine the cellulose production of *Salmonella* strains, a calcofluor binding experiment was carried out. In the Petri plates examined under 366 nm UV light, *S.* Typhimurium wild-type strain gave strong fluorescence due to their cellulose production, while dramatic decreases were determined in this radiation in *seqA* and *dam* mutant strains compared to the wild-type strain (Figure 5).

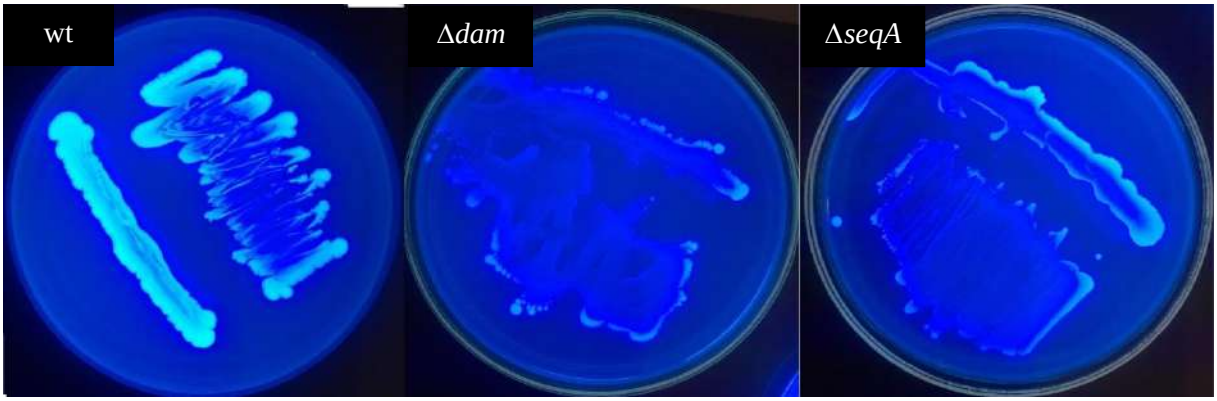


Figure 5. Cellulose production levels in *S.* Typhimurium 14028 wild-type strain and its *dam* and *seqA* mutants

DISCUSSION AND CONCLUSION

As a result of previous studies, it was determined that the deletion of the *dam* and *seqA* genes in *Salmonella* caused a decrease in biofilm production and a change in biofilm morphotype (Uğur et al., 2018). Our findings have proven that these effects occur by reducing cellulose production in the biofilm matrix and cellulase activity in the cells. It is also known that in the absence or reduction of cellulose, the biofilm matrix becomes more vulnerable to environmental effects (Pietro et al., 2006; Hamed et al., 2019). In this study, the molecular mechanism of these effects are detailed.

In addition, the identification with morphological tests that both individual (swimming) and community movements (swarming) are significantly inhibited in the *dam* and *seqA* mutants contributes to the explanation of the formation and stability of biofilm. Because the movement of flagella, which are the basic elements of chemotaxis, is critical in planktonic forms of *Salmonella*. This feature is also essential in the adhesion process, which is the first stage of biofilm formation (Aloui et al., 2010). On the other hand, swarming movement, which is responsible for the movement of the biofilm structure and provided by the flagella bundles, is also considered a critical character for the stability of mature biofilm structures (Biao et al., 2020).

When all these findings are evaluated together, it is possible to say that an effective method can be developed to prevent the formation of *Salmonella* biofilms by using *dam* and *seqA* gene inhibitors.

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IDENTIFICATION OF STINK BUGS INSECTS (PENTATOMIDAE) IN ECOSYSTEMS OF CENTRAL MOUNTAIN RANGE IN ALBANIA

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ABSTRACT

This study aims to present a identification to the stink bugs insect *Pentatomidae* family (Hemiptera), in the different ecosystems in central mountain range. The collection of biological material is performed during the period 2018- 2019 in hilly habitats. The study analyzed 121 individuals, which represented 19 genus and 27 species. The collected biological material revealed the genera *Carpocoris*, *Holcostethus* and *Stagonomus* are more represented, with 3 species and a frequency of 11.11%. Habitats of Iba station were represented by more species compared to other stations, 14 species and frequency of 51.85%. While with less represented species was Ndroqi station with 9 species and a frequency of 33.33%.

Based on the "*Jaccard index*", Dajti with Farka and Iba with Ndroqi stations, present higher coefficient of similarity compared to other stations, 27.77%, which mean a similarity between these habitats.

Zoogeographic Mediterranean regions represent most of the species, with 9 species and frequency 33.33%.

Keywords: Stink Bugs, *Pentatomidae,* Ecosystem, Zoogeographic regions, *Carpocoris*

Introduction

Stink Bugs (*Pentatomidae* Family Leach, 1815) are insects with antennas that are constituted by 5 segments. This family includes individuals of middle to big dimensions and are predominantly small. Their body is of oval shape and covered by a solid mantel. Their scutellum is big and of triangle shape as 'mantel' (Servadei 1967). They present green, yellow and metallic bright colours. Tarsus is constituted by 2 or 3 segments (Tremblay 1981). They are classified as phytophagy species. Their negative impact in agriculture is mainly encountered in crops, rice, fruit trees, etc. They stand grouped and sack the liquid of the hosted tree and present considerable resistance toward pesticides (Gennaro 1977; Miller 1971; Pollini 2002). Also, in this group are included predators that are feed with other insects (Silvestri 1939; Servadei el al. 1972).

Our study considers species of this family in the ecosystems with geography of lower to hilly-mountainous altitudes of the Tirana Region, attempting to present a general panorama of this family in this habitat. Conclusions are drawn up through the analysis.

Materials and Methods

Collection of the biological samples was conducted for the period 2018-2019, in different habitats of the sampling stations of Dajti, Iba, Vora, Farka and Ndroqi. The collection of individuals was achieved through random procedures during the warm part of year May-September, for each station, during the day time 09^{00}-15^{00}.

Instruments used for collection were mainly entomological nets of 80 cm diameter. Shaving of the insects was conducted in diagonal equal surfaces of 100 m^2 (10m x 10m), passing 5 times across each rectangle diagonals (Colas 1969). In our field expedites were used also air nets.

After the field collection, individuals were placed in plastic bottles, and were labelled, by giving information on place and date, respectively. Regarding to the tinny samples they were placed in plastic flacons of 150-200cc. The biological material, in scientific laboratory, was kept in bottles with Ethanol solution (95%), acetic acid, distilled water in ratio 80:5:20 (v/v/v) and some ether drops added consequently (Colas 1969; Chapman 1985).
Scientific determination of the biological material was conducted through investigation with stereomicroscope Trinocular Stero Microscope (*with still camera model 50240003 n/s C88794*) in the MSN lab employing. The individuals were determined by using the determination keys for each family, collections and previous scientific publications (Aukema et al. 1999; Dolling 1991; Halimi 2015; Schuh 1995; Ribes 2008; Tremblay 1990).
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 *et al*. 2018).

Results and Discussions

Determination of species that belong to the *Pentatomidae* family (Anex 1) includes species encountered in the lower altitude ecosystems and hilly-mountainous ecosystems of Tirana. For every species we have given information on the number on each station: Dajti, Iba, Vora, Farka and Ndroqi, accompanied by information related to the zoogeographical region.
From investigation of the biological samples, in our study, were encountered 121 individuals, of *Pentatomida* family, which represented 19 genera and 27 species (Table 1). In that aspect was determined also the frequency per every species according to equation:

$$F = \frac{n}{N} \times 100$$

Where: n- number of species for each family; N- number of species in total encountered

Table 1: Number of species according to genera

Nr	Scientific name	Species number	Species frequency (%)
1	*Aelia*	2	7,41
2	*Bagrada*	1	3,70
3	*Carpocoris*	3	11,11
4	*Codophila*	1	3,70
5	*Dolycoris*	1	3,70
6	*Eurydema*	1	3,70
7	*Graphosoma*	2	7,41
8	*Holcostethus*	3	11,11
9	*Mustha*	1	3,70
10	*Neottiglossa*	1	3,70
11	*Nezara*	1	3,70
12	*Palomena*	1	3,70
13	*Picromerus*	1	3,70
14	*Piezodorus*	1	3,70
15	*Stagonomus*	3	11,11
16	*Staria*	1	3,70
17	*Thalagmus*	1	3,70
18	*Ventocoris*	1	3,70
19	*Zicrona*	1	3,70
	Total	**27**	**100**

Analysis and interpretation of the data results in our study, indicates that according to species' diversity, *Carpocoris*, *Holcostethus* and *Stagonomus* genera are represented with more species, three respectively, and frequency 11.11%, *Aelia* and *Graphosoma* genera are represented by two species and frequency 7.41%, while other genera were represented by only one species and frequency 3.70%.

According to the species variety, more represented resulted Iba station, respectively with 14 species, or frequency 51.85%, followed by Dajti station with 12 species or frequency 44.44%, Farka station with 11 species and frequency 40.74%, and last resulted Ndroqi station with 9 species or 33.33% frequency (Table 2, Figure 1).

Table 2: Number of species according to station

Station	Number of species	Species frequency
Dajt	12	44.44
Iba	14	51.85
Vora	10	37.04
Farka	11	40.74
Ndroqi	9	33.33

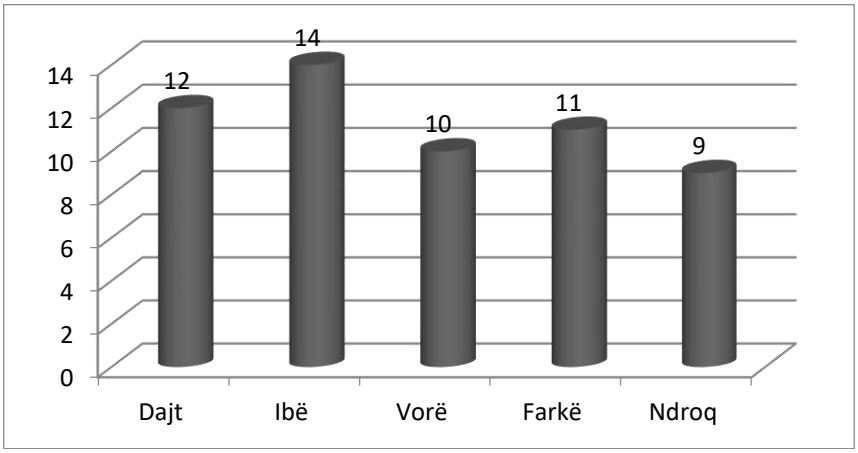

Figure 1: Distribution of species according to the station in study (number of species)

Calculation of the coefficient of similarity Jaccard gave an indication on the species similarity among the stations (Jaccard, 1901). In the table are presented the numbers of common species (C), according to the stations, and the coefficient of similarity (C_J) for each station (Table 3).

Stations present according to their geographical distribution a diversification of the ecological conditions and ecosystems. Hence is studied the correlation between the species to highlight the influence of the conditions in the species distribution, by taking in consideration the species itself, own their ecological valence.

Table 4: Number of common species and similarity coefficient according to each station

	Iba	**Vora**	**Farka**	**Ndroqi**
Dajti	C = 4 C_J = 18.18%	C = 3 C_J =15.78%	C = 5 C_J = 27.77%	C = 4 C_J =23.52 %
Iba		C = 3 C_J =14.28%	C = 5 C_J = 25%	C = 5 C_J =27.77 %
Vora			C = 2 C_J =10.52 %	C = 1 C_J =5.55 %
Farka				C = 1 C_J = 5.26%

From analysis, we concluded that the higher values of the coefficient of species' similarity stands among Dajti and Farka stations, as well as Iba with Ndroqi, by 27.77% and 5 common species, followed from similarity among stations Iba and Farka by 25% and with 5 species, Dajti and Ndroqi by 23.52% and 4 common species. The lowest value stands among Dajti and Iba, by 18.18% and 4 species, Dajti and Vora by 15.78% and 3 common species, Iba and Vora with coefficient 14.28% with 3 common species, Vora and Farka with 10.52% and 2 species, between Vora and Ndroqi as well as Farka and Ndroqi station by 5.26% and 1 common species. Analysis of similarity of species structure , give indication on the affinity regarding to species structure for these stations, as well as impact of ecological factors in general, and particularly the anthropogenic factor impact.

From the study of the zoogeographical groups (Table 4, Figures 2), the nucleus of the *Pentatomidae* family is the Mediterranean Zoogeographic group with 9 species and frequency 33.33%, and consecutively Paleartik group with 7 species or 25.93%, Holarctic with 3 species or 11.11%, and in very small number of species Euro-Siberian and Euro-Mediterranean by 2 species or 7.41%, and Euro-Africans, Central European-Asian, Cosmopolitan and Balcanic by 1 species or 3.70%.

Table 3: Number of species according to the zoogeographical regions

Zoogeographical region	Number of species	Species' frequency
Holarctic	3	11.11
Paleartik	7	25.93
Euro – Siberian	2	7.41
Euro- Africans	1	3.70
Euro – Mediterranean	2	7.41
Mediterranean	9	33.33
Central European-Asiatic	1	3.70
Cosmopolitan	1	3.70
Balcanic	1	3.70
TOTAL	**27**	100,00

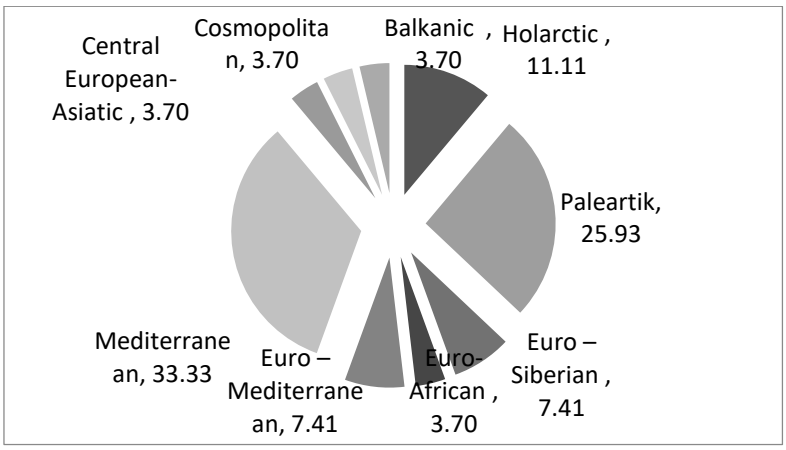

Figure 2: Distribution of species frequency according to zoogeographical regions

Aelia acuminate photo: E.Halimi

Codophila varia Photo: E.Halimi

Conclusions

This study presents a systematic and ecological analysis for 121 exemplars of *Pentatomidae* family, in ecosystems of Tirana region. These exemplars represented by 19 genera and 27 species. Results give indication that *Carpocoris, Holcostethus an*d *Stagonomus* genera are presented with the maximum values of diversity, by 3 species and frequency 11.11%.
Iba station dominates regarding to the species diversity, by 14 species or 51.85%, followed by Ndroqi station with 9 species or by 33.33%.
Maximum value of coefficient of species' similarity stands between Dajti and Farka stations, as well as Iba and Ndroqi stations, by 27.77%, the minimum value of coefficient stands among Vora and Ndroqi, as well Farka and Ndroqi stations by 5.26%.
More represented with species is Mediterranean zoogeographic region by 9 species or 33.33%, Euro-African region, Central European-Asiatic, Cosmopolitan and Balcanic by only one species or 3.70%.

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Anex : List of species for *Pentatomidae* Family

No.	Scientific name	No exemplar	Dajt	Iba	Vora	Farka	Ndroqi
1	Genus Aelia						
1	*Aelia acuminata* Linnaeus, 1758	6	+				+
2	*Aelia rostrata* Boheman, 1852	5		+		+	
2	Genus Bagrada						
3	*Bagrada abeillei Puton, 1881-*	4	+		+		
3	Genus Carpocoris						
4	*Carpocoris fuscispinus Boheman, 1853*	5		+	+		
5	*Carpocoris purpureipennis* De Geer, 1773	4		+	+		
6	*Carpocoris melanocerus Mulsant & Rey, 1852*	6		+			+
4	Genus Codophila						
7	*Codophila varia* Fabricius, 1787	4	+			+	
5	Genus Dolycoris						
8	*Dolycoris baccarum* Linnaeus, 1758	16	+	+		+	
6	Genus Euryderma						
9	*Eurydema ornate* Linnaeus, 1758	3		+			+
7	Genus Graphosoma						
10	*Graphosoma lineatum* Linnaeus, 1758	10	+				+
11	*Graphosoma semipunctatum* Fabricius, 1775	3		+		+	
8	Genus Holcostethus						
12	*Holcostethus fissiceps Horvàth, 1906*	3			+	+	
13	*Holcostethus sphacelatus* Fabricius, 1794	3	+				+
14	*Holcostethus vernalis Wolff, 1804*	1			+		
9	Genus Mustha						
15	*Mustha spinosula* Lefebvre, 1831	3	+	+			
10	Genus Neottiglossa						
16	*Neottiglossa bifida Costa A, 1847*	3			+	+	
11	Genus Nezara						
17	*Nezara viridula Linnaeus, 1758*	4		+		+	+
12	Genus Palomena						
18	*Palomena prasina* Linnaeus, 1761	2			+		
13	Genus Picromerus						
19	*Picromerus conformis Herrich – Schâffer, 1894*	4	+		+	+	
14	Genus Piezodorus						
20	*Piezodorus lituratus* Fabricius, 1794	2		+	+		
15	Genus Stagonomus						
21	*Stagonomus amoenus* Brullé, 1832	2	+			+	
22	*Stagonomus bipunctatus Linnaeus, 1758*	4	+		+		+
23	*Stagonomus pusillus* Herrich – Schâffer, 1830	4		+			+
16	Genus Staria						
24	*Staria lunata* Hahnn, 1835	5	+	+		+	
17	Genus Thalagmus						
25	*Thalagmus flavolineatus* Fabricius, 1798	3		+			+
18	Genus Ventocoris						
26	*Ventocoris trigonus Krynicki, 1871*	2	+	+			
19	Genus Zicrona						
27	*Zicrona caerulea* Linnaeus, 1758	3				+	

THE EFFECT OF ADDITION ARTICHOKE BRACT POWDER ON THE PHYSICAL AND RHEOLOGICAL PROPERTIES OF WHEAT FLOUR

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ABSTRACT

Evaluation of nutritionally and functionally valuable by-products is important in terms of both food enrichment and economic gains. The stem and bract parts make up about approximately 60% of the total weight of the artichoke plant (*Cynara scolymus* L.). These parts are discarded and not used in the food industry. In this study, artichoke bracts were dried and ground as powder and added to the wheat flour at various rates (0 - 2.5 - 5 - 7.5 - 10%) to improve and enrich some nutritional and rheological properties of wheat flour.

In the study, the addition of artichoke bract powder to wheat flour increased the ash content of the flour mixtures and decreased the Zeleny sedimentation and falling number values. It was observed that the addition of artichoke bract powder significantly decreased the L* (lightness) color value compared to wheat flour and increased the b* (yellowness) value significantly ($p<0.01$). The addition of artichoke bract powder to wheat flour increased the water absorption capacity and dough development time of doughs. While the addition of artichoke bract powder significantly ($p<0.01$) increased the stability time of the dough compared to wheat flour; this value decreased as the amount of added artichoke bract powder increased. While the addition of artichoke bract powder increases the resistance of the dough; it significantly ($p<0.01$) decreased its extensibility and elasticity. As a result, it was determined that the energy values of the doughs also decreased.

At the end of the study, it was determined that the addition of artichoke bract powder affected and improved various physical and rheological properties of wheat flour. The addition of artichoke bract powder up to 5 % was found acceptable in terms of the rheological properties of the dough.

Keywords: Artichoke bract powder, Food by-products, Fortification, Rheology

INTRODUCTION

The fruit and vegetable processing industry produce large amounts of wastes and residues (like leaves, stems, pomaces, etc.) annually. Some of these residues often referred to as food by-products, are rich in vitamins, minerals, and secondary metabolites, including dietary fibers, polyphenols, flavonoids, and terpenoids (Farag et. al., 2013; Pandino et. al., 2011). The amount of by-products produced in the developing food industry is increasing day by day. In some plants such as artichoke, the amount of by-product can reach approximately 60 % of its total weight. Artichoke (*Cynara scolymus* L.), which is a plant from the Asteraceae family, is an ancient perennial plant species native to the Mediterranean Basin (Ceccarelli et. al., 2011). The edible parts of the plant are the inner bracts and the capitulum, commonly known as heart. Artichokes, which can be also sold with fresh stems and bracts, are generally available in the

markets as peeled and canned. The artichoke canning industry generates large amounts of by-products, consisting mainly of the stems and bracts (Pandino et. al., 2011).

Globe artichoke is surrounded by the bracts, which are leaves that have been modified in some way. Bracts quietly and dutifully protect the flower from bugs, bumps, desiccation, and hungry animals. On the other hand, bracts are a natural source of phenolic acids, especially with cynarin, *p*-coumaric acid, ferulic acid, chlorogenic acid (Claus et al., 2015); some flavonoid derivatives, such as luteolin and apigenin (Moglia et. al., 2008); anthocyanins and terpenoids (i.e., sesquiterpene hydrocarbons and non-terpene derivatives) (Dabbou et. al. 2016). Polyphenols are related to a decrease in risk of chronic diseases, such as diabetes, cancer, cardiovascular disease; and so they are associated with an increase in the quality of life (Holst and Williamson, 2008). Artichoke bracts also contain large amounts of dietary fibers such as inulin (22–47 g/100 g) and pectin (19–26 g/100 g) (Domingo et. al., 2019). Inulin is a linear polydisperse carbohydrate and a soluble dietary fiber. Inulin is being digested in the alimentary canal and converted by colon bacteria into short-chain fatty acids, enhancing the gastrointestinal and immune systems (Lopez-Molina et al., 2005; Morris & Morris, 2012). Also, inulin has been added as a fat substitute in sausages formulations (Alaei et. al., 2018; Leroy et. al., 2010) and has been applied in low-fat dairy products (Faustino et al., 2019). Pectin, which can be derived from globe artichoke bracts, leaves, and stems (20 g/100 g), is used as a gelling agent in the food industry (Sabater et. al., 2018).

Consequently, by-products that are obtained during the processing of artichoke cause environmental pollution and economic losses. Evaluation of nutritionally and functionally valuable these by-products is important in terms of both food enrichment and economic gains. In this study, artichoke bracts were added to wheat flour, aimed to evaluate artichoke bracts and to improve and enrich some nutritional and rheological properties of wheat flour.

MATERIAL AND METHOD

2.1. Materials

The analyses were carried out on artichoke (*Cynara scolymus* L.) bracts which were obtained from the plant peeled for canning. All fresh plants were grown in open-field in Urla, Izmir, Turkey by local farmers. Collected bracts were dried at 50°C for 2 days by oven (Nüve FN-500, Ankara, Turkey), and then ground in a laboratory grinder through 350 µ sieve. Wheat flour was obtained from a local mill in Konya, Turkey. It contained 14.0 % of moisture (AACC 44-12), 27.1 % of wet gluten (AACC 38-12) and 96 % gluten index indicate that the flour was characterized by high gluten quality (AACC, 1990). In the preparation of the flour mixtures, five levels of additional doses were applied. Artichoke bracts powder was added in the amounts of 0 - 2.5 - 5 - 7.5 – 10 % (w/w) with relation to wheat flour.

2.2. Methods

2.2.1. Color properties

Color of raw materials and flour mixtures was evaluated by measuring the L (100 = white; 0 = black), a (+, red; -, green) and b (+, yellow; -, blue) values using a Hunter Lab Color QUEST II Minolta CR-300 (Minolta Camera, Co., Ltd., Osaka, Japan) with illuminate D63 as reference. Values are the mean of five determinations (Francis, 1998).

2.2.2. Zeleny sedimentation and falling number analysis

The Zeleny sedimentation test was performed according to the ICC standard method (116/1). Hagberg falling number determination was carried out using the ICC-approved method (107/1) (ICC, 1994).

2.2.3. Rheological properties

The farinograph test was applied according to the AACC standardized method (54–21.02). It was conducted on a Farinograph-E machine by Brabender (Germany) (AACC, 2011b). Extensography was carried out by applying the AACC method (54–10.01), using an Extensograph-E machine by Brabender (Germany) (AACC, 2011a).

2.2.4. Moisture and ash content

The moisture (method 44–12) and ash (method 08–03) contents of bract powder and wheat flour were determined using standard methods (AACC, 1990).

2.2.5. Statistical analysis

JMP (version 5.0, Izmir) software was used to perform the statistical analyses. Differences in samples due to addition of artichoke bract powder was tested for statistical significance at p = 0.05 level. Multiple range tests were used to differentiate between the mean values. Standard deviations were calculated using the same software.

RESULTS AND DISCUSSION

One of the key criteria for customers is the color of flour mixtures. Customers generally prefer white or nearly white flour. Color properties of flour mixtures were shown in Table 1. L*, a* and b* values of artichoke bract powder were found 65.8; 1.03 and 22.69, respectively; while the wheat flour values were 93.79; -0.10; and 9.78, respectively (data not shown). When the color results of flour mixtures were examined, despite the low addition rates, it was observed that the artichoke bract powder significantly ($p<0.05$) effected the L* (lightness) and b* (yellowness) values of the flour mixtures. As the addition amount of artichoke bract powder increased, the value of lightness decreased; yellowness values were increased. Artichoke bract powder addition did not affect the redness values and gave statistically similar redness values. In terms of Hue angle values, there was no statistical differences ($p>0.05$) between flour mixtures. Hue angle values varied from 90.50 to 90.84. According to the results, the highest SI value was found in the samples with 7.5 and 10% artichoke bract powder added samples, while the lowest value was obtaimed with control sample. It was found that the increasing artichoke bract powder addition in flour mixture increased the SI value.

Table 1. Color properties of artichoke bract powder added flour mixtures[1]

Artichoke bract powder level (%)	L*	a*	b*	Hue	SI
0	93.79^{a}	-0.10^{a}	9.25^{d}	90.57^{a}	9.78^{c}
2.5	87.53^{b}	-0.10^{a}	9.78^{cd}	90.84^{a}	9.26^{d}
5	84.94^{c}	-0.11^{a}	10.25^{bc}	90.73^{a}	10.25^{b}
7.5	82.58^{d}	-0.12^{a}	10.91^{ab}	90.60^{a}	10.91^{a}
10	81.12^{e}	-0.12^{a}	11.16^{a}	90.50^{a}	11.16^{a}

[1]Values followed by different superscript letters (series "a-d") within each column are significantly different at $p < 0.05$.

The results of Zeleny sedimentation test and Hagberg falling number were shown at Table 2. Zeleny sedimentation test describes the degree of sedimentation of flour suspended in a lactic acid solution during a standard time interval. This value is taken as a measure of the gluten and

baking quality. The gluten fraction of the flour swells in the lactic acid solution. The swelled gluten affects the rate of sedimentation of flour suspension. Both higher gluten content and a better gluten quality give rise to slower sedimentation and higher Zeleny test values. In short, the sedimentation value of flour depends on the amount of wheat protein, especially gluten (Shewry and Tatham, 2000). Due to artichoke bract flour does not contain any gluten, the sedimentation values of the flours decreased as the amount of artichoke bract powder addition value increased in flour mixtures. The highest sedimentation value was found in control (36 ml) and the lowest (24 ml) value was in 10 % bract powder added flour sample.

Table 2. Zeleny sedimentation and falling number values of artichoke bract powder added flour mixtures[1]

Artichoke bract powder level (%)	Sedimentation (ml)	Falling number (sec)
0	36[a]	707[a]
2.5	31[b]	596[b]
5	28[c]	512[c]
7.5	26[d]	468[cd]
10	24[e]	417[d]

[1]Values followed by different superscript letters (series "a-d") within each column are significantly different at $p < 0.05$.

The Hagberg falling number is a term that is used to indicate the alpha-amylase enzyme activity in flour. This enzyme will attack the molecules of starch, breaking them down into sugars. Falling number is used to describe the number of seconds it takes for a plunger to fall through wheat flour/water suspension. If the plunger falls quickly, it means that the starch has been converted to sugar. However, if the plunger falls slowly, the mixture is thick with starch (Cauvain, 2017). Artichoke bract powder contains about 78 % carbohydrates. Most of the carbohydrate content of artichoke bract powder consists of dietary fiber (84 %) (Umana et. al., 2021). Control flour gave the highest falling number value (707 sec) and it was observed that the falling number value decreased numerically as the amount of added artichoke bract powder increased. This was thought to be due to the lower starch content of artichoke bract powder than wheat flour. But there statistically similar values between 5 – 7.5 – 10 % bract flour added mixtures ($p>0.05$).

Rheology gives a quantitative measure for the amount of stress in the dough, which is closely related to the quality of the molecular gluten network (Bloksma and Bushuk, 1988). To define the physical properties of dough, rheological measurements are used. Rheological analyzes give information about the performance of the dough during kneading, proofing and baking and also related to product functionality (Fan et al., 1994; Shah et al., 1999; Dobraszczyk, 2003). Farinograph and extensograph are the most common instruments used for characterizing dough rheology (Song and Zheng, 2007). Farinograph is mostly used for evaluation of dough strength and dough stability whereas extensograph is for determination of dough energy and dough extensibility. They also give information about the water absorption capacity of the dough. Farinograph has been used to characterize the behavior of doughs during processing and measure the mixing time/torque and apparent viscosity. Extensograph is used for measuring the flour quality and stretching behavior of dough. Extensional properties, which determine the course of dough expansion during proofing and baking, have a direct effect on loaf volume and quality of texture of bread crumb (Antoni and Dariusz, 2013). The results of farinograph and extensograph analyzes were shown at Table 3 and 4, respectively.

A prerequisite for making dough is water, which plasticizes the dough. The control of water content of critical importance in mixing. It determines the ability of the dough to be extended and to resist extension. The water absorption capacity of flour often defines its quality and its tendency to form viscoelastic dough. The hydration of flour is severe in the food industry because it affects its functional properties and the quality of cooking products (Berton et al.,

2002). According to farinograph results in Table 3, while the addition of artichoke bract powder significantly ($p<0.01$) increased the water absorption capacity of the dough compared to control sample (Table 3). But no significant difference was observed between 7.5 and 10 % artichoke bract powder added samples ($p>0.05$). Results were indicated that artichoke bract powder characterized by greater ability of increasing the water absorption of the mixtures compared to wheat flour. Fibre-rich preparations are known for their ability to absorb considerable amounts of water. That ability is mainly determined by the presence, in fiber structure, of a large number of hydroxyl groups which enter into interactions with water via hydrogen bonds (Rosell et. al., 2001).

Table 3. Farinograph properties of artichoke bract powder added flour mixtures[1]

Artichoke bract powder level (%)	Water Absorption Capacity (%)	Development Time (min)	Dough Stability (min)	Softening Degree (BU)
0	66.6^{d}	1.5^{d}	3.5^{d}	66^{c}
2.5	68.7^{c}	1.4^{d}	12.0^{a}	75^{c}
5	71.5^{b}	3.4^{c}	11.5^{ab}	84^{bc}
7.5	73.7^{a}	6.8^{b}	9.1^{bc}	112^{ab}
10	75.2^{a}	8.2^{a}	7.6^{c}	133^{a}

[1]Values followed by different superscript letters (series "a-d") within each column are significantly different at $p < 0.05$.

Development time in farinograph graphics provides the time between the time zero and its maximum peak of the curve. It is used when the flour mixing requirements change, to make adjustments during mixing in commercial processes. Stronger flours with higher protein content have a longer development time than weaker flours (Posner and Hibbs, 2011). The interaction between fiber and gluten prevents protein hydration, and thus increases dough development time (Kohajdová et. al., 2012).When the development time of the flour mixtures compared, the highest development time (8.2 min) was found in 10 % artichoke bract powder added sample. It was observed that the development time was shortened as the amount of added artichoke bract powder increased and there was no statistical difference between the control (1.5 min) and 2.5% artichoke bract powder added sample (1.4 min). The increased dough development time has been considered due to the water hydration and gluten network development. In another study, oat flour and carob fiber were added to wheat flour and, an increase was observed in the development time and water absorption capacities of doughs compared to the control, similarly (Miś et. al., 2012).

Dough stability is defined as the time difference between the point where the top of the curve first intercepts the 500 BU line and the point where the top of the curve leaves the 500 BU line (Sim et al., 2011). It is a measurement of how well flour resists overmixing. Stronger flours are usually more stable than weaker ones from the same wheat class. While the addition of artichoke bract powder significantly ($p<0.01$) increased the stability time of the dough compared to wheat flour; this value decreased as the amount of added artichoke bract powder increased. Similarly, the stability times of wheat flours with insoluble date fiber added samples were higher than the control (Ahmed et. al., 2013). The dough development time and stability values decreased with reduced protein content, but the value of mixing tolerance index increased (Fu et al., 2008).

Degree of softening is the difference in height between the center of the graph at maximum resistance to mixing and the center of the graph at a point 12 minutes later (Sahi, 2012). The degree of softening is considered to be associated with the reduction of gluten content and destruction of gluten network (Šporin et al., 2018). While the softening degree of control flour was 66 BU, 10 % artichoke bract powder added sample was 133 BU. The addition of artichoke bract powder significantly ($p<0.01$) increased the degree of softening of doughs compared to the wheat flour (Table 3).

Rheology of wheat flour enriched with food-by products which contain high dietary fiber, has been investigated. Hussein et. al. (2013) added carrot pomace powder to wheat flour and found that water absorption, dough development time and dough stability increased as the increase of carrot pomace powder. Wheat flour was partially substituted with wholegrain oat flour for making traditional bread (Salehifar and Shahedi, 2007), and according to results water absorption, dough development time and dough softening increased as the increase of wholegrain oat flour; however, dough resistance and extensibility decreased. Whole grain rye flour, wholegrain barley flour, and oat flakes meal used to replace refined wheat flour for bread making respectively, and the results showed that the wholegrain flour incorporation increased dough water absorption and development time, while decreased the resistance to extensibility and stretching energy compared to control bread with 100% refined wheat flour (Koletta et al., 2014) Oat flour and carrot pomace powder are a gluten-free additives, thus the addition of these enhance the water absorption and dilutes the wheat gluten, which further results in the increased dough development time and decreased dough resistance and extensibility

The results of extensograph analyzes of the samples were shown in Table 4. With increase in the addition level of artichoke bract powder in flour mixtures, the extensibility of dough gradually decreased (Table 4), from 148 to 115 mm. With increase in water absorption, there was a decrease in the extensograph extensibility of dough enriched with artichoke bract powder, and a decrease in its resistance and energy. While the addition of artichoke bract powder increased, the resistance of the dough significantly ($p<0.01$) decreased. As a result, it was determined that the energy values of the doughs also decreased. The fibre-rich additions were the dominant factor modifying the extension behaviour of the doughs.

Table 4. Extensograph properties of artichoke bract powder added flour mixture[1]

Artichoke bract powder level (%)	Extensibility (mm)	Extension Resistance (BU)	Energy (cm^2)
0	148^{a}	504^{a}	90^{a}
2.5	139^{a}	454^{b}	76^{b}
5	134^{ab}	414^{c}	73^{bc}
7.5	123^{bc}	407^{cd}	68^{bc}
10	115^{c}	397^{d}	63^{c}

[1]Values followed by different superscript letters (series "a-d") within each column are significantly different at $p < 0.05$.

According to results (data not shown), since the ash content of the artichoke bract powder (5.92 % dry basis) was considerably higher than that of wheat flour (0.68 %). Flour mixtures gave similar moisture values and moisture content of flour samples varied from 9.72 to 10.11%. There was no significant differences between all samples of the moisture content ($p>0.05$). The same results for ash content of artichoke bract powder also were reported by Ruiz-Cano et. al., (2014) (4.7 – 6.3 %), Umana et. al (2021 (5.40 %). There was no significant differences between all samples of the ash content ($p>0.05$), but the ash content of the flour mixtures increased as the addition level of artichoke bract powder increased, descriptively.

Table 5. Moisture and ash content of artichoke bract powder added flour mixtures[1]

Artichoke bract powder level (%)	Moisture (%)	Ash (%)
0	9.72^{a}	0.68^{a}
2.5	10.01^{a}	0.80^{a}
5	10.03^{a}	0.94^{a}
7.5	10.05^{a}	1.09^{a}
10	10.11^{a}	1.21^{a}

[1]Values followed by different superscript letters (series "a-d") within each column are significantly different at $p < 0.05$.

CONCLUSIONS

It is very important to evaluate the by-products of the food industry, which has become an environmental problem today, by using them in different fields. As a result of rheological studies, it was determined that artichoke bract powder can be added to wheat flour up to 5 %. Thus, artichoke leaves, which are very rich in nutritional content, are evaluated and offered to the consumer. Also, it is thought that the nutritional content of wheat flour will be enriched and the ratio of dietary fiber will increase.

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THE ESTABLISHMENT AND POPULATION CHARACTERISTICS OF THE INVASIVE BLUE CRAB CALLINECTES SAPIDUS IN THE LAGOON OF NARTA, ALBANIA

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ABSTRACT

The aim of this study is to evaluate the establishment of blue crab population in Narta Lagoon, to monitor its distribution, to assess its abundance and to analyze its population structure and biometric characteristics. Comparative analyses were done between data collected in 2012 and those collected during 2014 - 2015. Blue crab individuals were collected from gillnets and fyke nets of local fishermen. Abundance, carapace width and height, as well as weight of each collected individual have been evaluated. Questionnaires were also distributed to local fishermen, in order to gather additional information on the presence, state and impact of the blue crab to other populations of Narta Lagoon. Based on the collected data and analysis in this study, the population of the blue crab *Callinectes sapidus* can be considered as established in the Narta Lagoon.

Keywords: *marine invasive species, biometric characteristics, Adriatic Sea.*

INTRODUCTON

The blue crab *Callinectes sapidus* Rathbun 1896 is an invasive species in the Mediterranean Sea (Streftaris and Zenetos 2006). The first scientific record for the presence of this species in Albanian coast was considered in 2006 from Patoku Lagoon (after Beqiraj & Kashta, 2010). Narta Lagoon, situated on the south-west of Albanian coast, at the Adriatic Sea, has a surface of 41.8 km^2 and is the second largest lagoon of Albania. It is situated within the boundaries of the Vjosa - Narta Protected Landscape and has been recognized as an Important Bird Area (IBA) and an Important Plant Area (IPA) at international scale. Based on personal communications with local fishermen of Narta Lagoon, the blue crab appeared in the Narta area in 2003. The first scientific observations and analysis of this species in Narta Lagoon have been done in 2012 and followed during 2014 – 2015, which are being presented here in this paper.

MATERIAL AND METHODS

The lagoon of Narta is situated in the south-west of Albanian coast, at the Adriatic sea, in north of the bay of Vlora, on the eastern shore of the strait of Otranto and boundaries of the Vjosa-Narta. It has a surface area of 41.8 km^2 with a maximal depth of 1.5 m. Since the presence of the blue crab has been recorded in the Narta lagoon (according to Agolli et al., 2012), the periods of observations and collections of the blue crab in the lagoon was based on the literature on *Callinectes sapidus* in the Mediterranean, according to Cabal et al. ., (2006), Florio et al., (2008), Galil et al., (2006), Gennaio et al., (2006); Onofri et al., (2008), Kirincic & Stevcic, (2008), Tuncer & Bilgin (2008). According to these references, the blue crab enters the lagoon in the period March - April and leaves the lagoon in the period October - November.

Figure 1. Map of Albania, showing the position of Narta Lagoon

Figure 2. Map of Narta Lagoon

The blue crabs were collected as by-catch from gillnets and fyke nets of local fishermen. In central part of the lagoon, the collection was done from gillnets of 24 mm mesh size and a linear length of 300 m, while in the other parts of the lagoon, in front of the communication channel with the sea, the collection was done from fyke nets of 8 mm mesh size and a linear length of 10-15 m. Also, a small number of individuals were collected directly on the ``dajlan`` (a compact reed fence, that is used to close the communication of the lagoon with the sea).

It has been evaluated the report between males and females (sex ratio) (M:F) and biometric measurements have been carried out. Carapace width and height have been measured, in order to evaluate crabs' age after Hines et. al (1990) and their maturity after Cadman and Weinstein (1985). All collected crabs have also been weighted and Spearman correlation has been evaluated to assess correlation between weight, width and height.

Besides direct observations and sampling in the study area, questionnaires have also been distributed to local fishermen with the purpose of gathering information about the presence of the blue crab in Narta Lagoon, assessment of its state, its possible impact on other populations in the lagoon, as well as its overall socio-economic impact to local fishermen community in Narta area.

Figure 3. View from western part of Narta Lagoon

Figure 4. Weighting crabs for parametric correlation analysis

Data preprocessing and analysis were performed in IBM SPSS Statistics version 26.0 (IBM Corp. Released, 2019). The data is given as mean ± standard deviation unless otherwise stated. The exploration of the data is done through frequency tables and descriptive statistics. The outliers in the data are assessed by inspection of boxplots. The data for all continuous variables for each level of the factors were checked for normal distribution by Shapiro-Wilk's test ($p>0.05$). The homogeneity of variances is tested by Levene's test for equality of variances ($p>0.05$). A Mann-Whitney U test is run to determine if there were differences between two not normally distributed groups. Kruskall Wallis test with pairwise comparisons is run to determine if there were differences between more than two not normally distributed groups. The Spearman correlation is used to see if there was a correlation between parameters not normally distributed. The results are considered significant for $p<0.05$.

RESULTS AND DISCUSSION

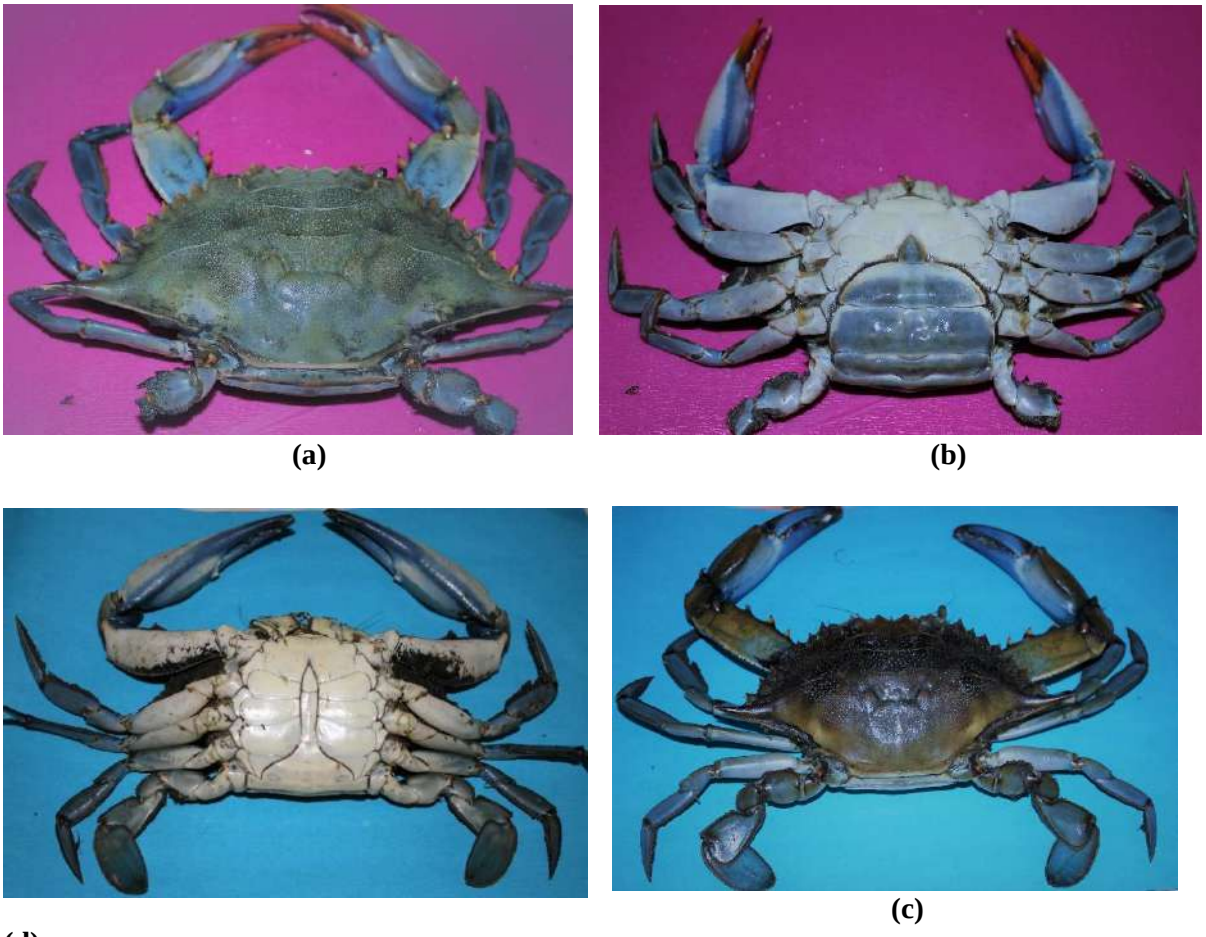
During the two sampling periods in Narta Lagoon, 193 individuals of the blue crab *Callinectes sapidus* have been collected, of which 96 individuals in 2012 and 97 individuals in 2014 - 2015. The highest presence of the blue crab in this lagoon was recorded during the period June – October each year. The highest presence of ovigerous females was recorded during June – August.

Most of the individuals found in this lagoon could be considered as matured, if referring to classification after Cadman and Weinstein (1985), which stated that maturity is reached at carapace width of 120-170 mm. 177 individuals out of the total of 193 individuals belonged to “matured” category.

Table 1. Number of males and females of the blue crab collected in Narta Lagoon

2012	**Sex**	**Total**
	Female	60
	Male	36
Total		**96**
2014 - 2015		
	Female	59
	Male	38
Total		**97**

As it is shown in the Table 1, the report between females and males (sex ratio) is almost 2:1. 119 individuals out of the total of 193 caught individuals in Narta Lagoon were females and 74 individuals were males.



(a) **(b)** **(c)** **(d)**

Figure 5. Blue crab *Callinectes sapidus* from Narta Lagoon: a) female dorsal view; b) female ventral view; c) male dorsal view; d) male ventral view.

Table 2. Descriptive statistics by year and sex

Year	Sex		N	Minimum	Maximum	Mean	Std. Deviation
2012	Male	Height	36	35	83	66.72	9.993
		Width	36	110	182	152.33	18.321
		Weight	36	83	425	265.44	86.667
	Female	Height	60	43	89	61.46	7.475
		Width	60	89	177	146.44	18.993
		Weight	60	65	395	204.29	62.420
2014	Male	Height	8	54	73	67.75	7.402
		Width	8	99	176	153.13	29.993
		Weight	8	106	341	244.75	85.304
	Female	Height	15	51	81	67.80	7.163
		Width	15	104	184	158.73	21.717
		Weight	15	98	364	237.73	66.002
2015	Male	Height	30	50	94	72.47	9.354
		Width	30	104	192	169.23	16.224
		Weight	30	108	459	319.73	79.409
	Female	Height	44	40	81	65.55	8.007
		Width	44	62	184	154.23	23.152
		Weight	44	39	385	246.05	72.145

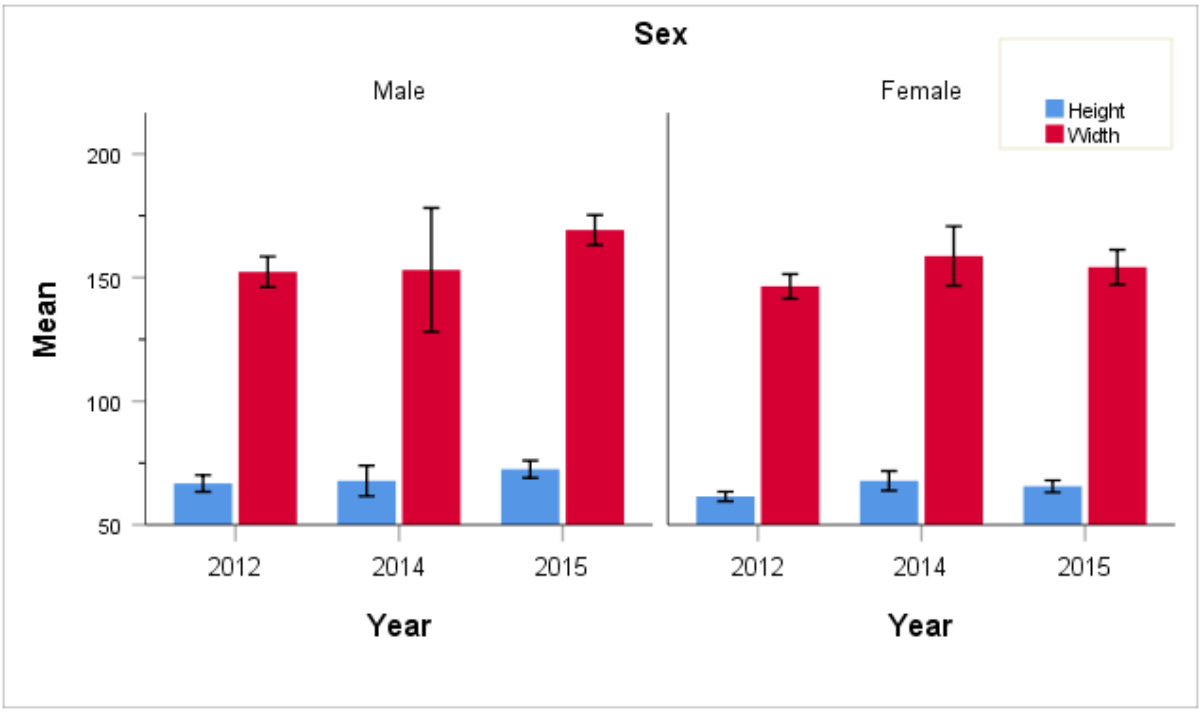


Figure 6. Mean of biometric parameters for males and females across years

The data for height, width and weight for all subgroups were not normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$). A Kruskall Wallis test was run to determine if there

were differences in height, width and weight between the three years in study. Distributions of the data were similar for all years, as assessed by visual inspection.

For males:

Median of height for all years were not statistically different, X^2 (2) = 2.78, p = 0.24. Multiple comparisons were not performed, because the overall test does not show significant differences across samples.

Median of width for all years were statistically different, X^2 (2) = 17.18, p <0.0001. Multiple comparisons showed significant differences only between years 2012 and 2015 (p<0.0001), where width shows and increase from 2012 to 2015.

Median of weight for all years were statistically different, X^2 (2) = 7.73, p =0.02. Multiple comparisons showed significant differences only between years 2012 and 2015 (p=0.04), where weight shows and increase from 2012 to 2015.

For females:

Median of height for all years were statistically different, X^2 (2) = 15.22, p <0.0001. Multiple comparisons showed significant differences between year 2012 and 2014 (p=0.003), and 2015 (p=0.007), where height shows and increase from 2012 to 2015.

Median of width for all years were statistically different, X^2 (2) = 12.07, p =0.002. Multiple comparisons showed significant differences between year 2012 and 2014 (p=0.017), and 2015 (p=0.014), width shows and increase from 2012 to 2015.

Median of weight for all years were statistically different, X^2 (2) = 9.72, p =0.008. Multiple comparisons showed significant differences only between years 2012 and 2015 (p=0.008), where weight shows and increase from 2012 to 2015.

According to the Table 3 and Figure 7, the evaluation of Spearman correlation shows a strong correlation (p<0.05) between weight, width and height for the blue crab individuals collected in each year of this study.

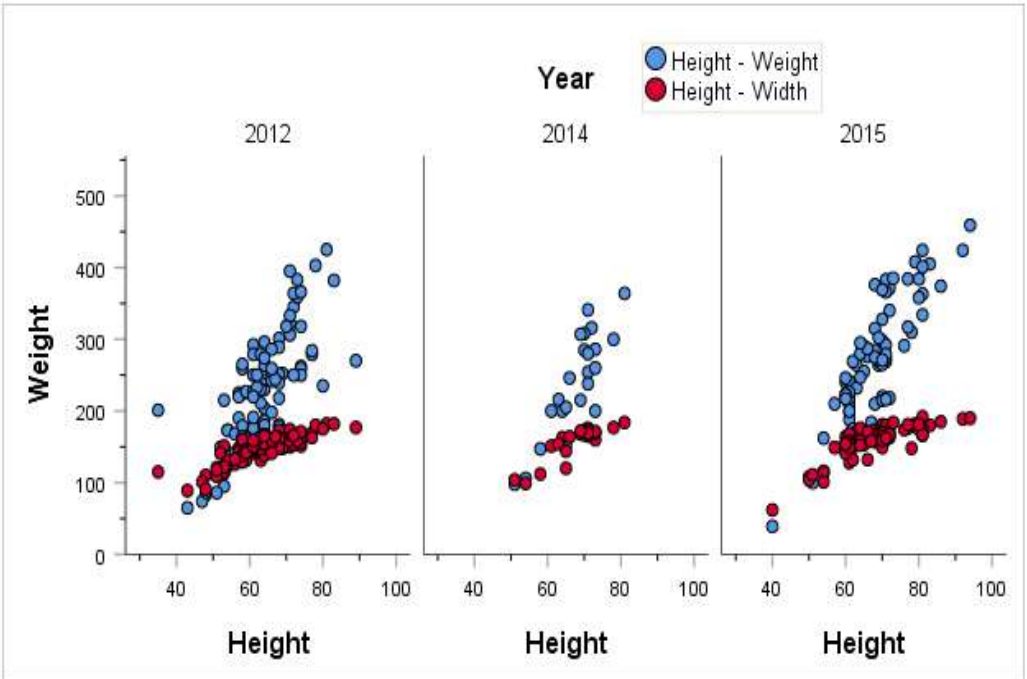


Figure 7. Correlation between parameters for each year.

Table 3. Correlations Matrix by year

Year				Height	Width	Weight
2012	Spearman's rho	Height	Correlation Coefficient		.824**	.757**
			Sig. (2-tailed)		.000	.000
			N	96	96	96
		Width	Correlation Coefficient	.824**		.764**
			Sig. (2-tailed)	.000		.000
			N	96	96	96
		Weight	Correlation Coefficient	.757**	.764**	
			Sig. (2-tailed)	.000	.000	
			N	96	96	96
2014	Spearman's rho	Height	Correlation Coefficient		.802**	.738**
			Sig. (2-tailed)		.000	.000
			N	23	23	23
		Width	Correlation Coefficient	.802**		.898**
			Sig. (2-tailed)	.000		.000
			N	23	23	23
		Weight	Correlation Coefficient	.738**	.898**	
			Sig. (2-tailed)	.000	.000	
			N	23	23	23
2015	Spearman's rho	Height	Correlation Coefficient		.731**	.801**
			Sig. (2-tailed)		.000	.000
			N	74	74	74
		Width	Correlation Coefficient	.731**		.834**
			Sig. (2-tailed)	.000		.000
			N	74	74	74
		Weight	Correlation Coefficient	.801**	.834**	
			Sig. (2-tailed)	.000	.000	
			N	74	74	74

*. Correlation is significant at the 0.01 level (2-tailed).

Most of the individuals found in this lagoon could be considered as matured, if referring to classification after Cadman and Weinstein (1985), which stated that maturity is reached at carapace width of 120-170 mm. 177 individuals out of the total of 193 individuals belonged to “matured” category. Based on the classification system of Harding system (2003), the blue crabs individuals were classified as juveniles (CW < 120 mm) and adults (CW > 120 mm) (see Table 4)

Table 4. Classification of individuals into juveniles and adults according to the Harding system (2003).

	Sex	Juveniles (CW<120 mm)	Adults (CW>120 mm)	Total
2012	Female	5	55	**60**
	Male	3	33	**36**
	Total	**8**	**88**	**96**
2014	Female	1	14	**15**
	Male	2	6	**8**
	Total	**3**	**20**	**23**
2015	Female	4	40	**44**
	Male	1	29	**30**
	Total	**5**	**69**	**74**

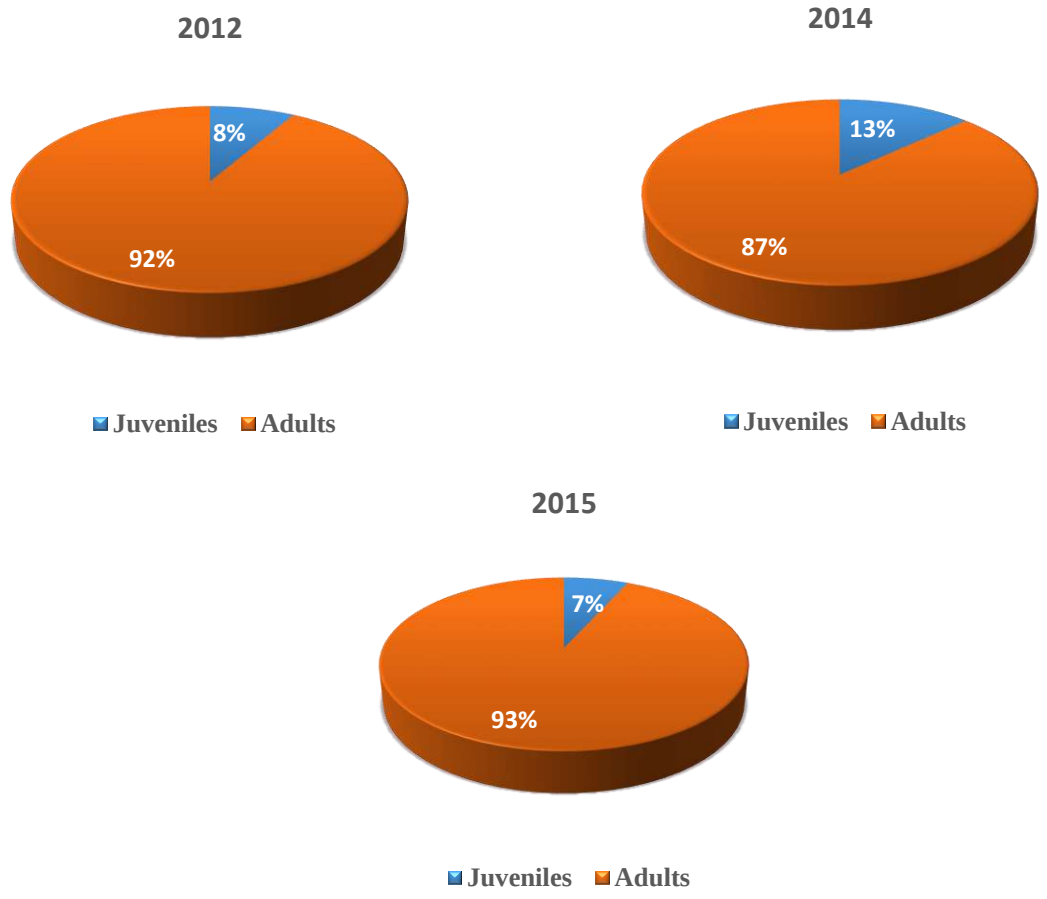


Figure 8. Ratio (in %) between juvenile and adult individuals of the blue crab in Narta Lagoon collected in 2012,2014,2015

As it is shown in the Table 4 and in the Figure 8 here above, we can evaluate a different ratio of juveniles and adults over the three years. From the analyzed data it is shown that 8 juvenile

individuals (8%) and 88 adult individuals (92%) were collected in 2012, 3 juvenile individuals (13%) and 20 adult individuals (87%) in 2014 and 5 juvenile individuals (7%) and 69 adult individuals (93%) were collected in 2015.
This high and continuous presence during the two sampling periods, as well as the finding of juvenile individuals and ovigerous females, are indicators of the stability of the blue crab in the Narta Lagoon.

The collected individuals were classified based on carapace width, in small individuals (CW <80mm), medium individuals (CW 80 - 120 mm), and large individuals (CW> 120m) according to Cadman & Weinstein (1985).

Table 5. Classification of individuals by size (based on the Cadman & Weinstein, 1985).

2012	**Sex**	**Small individuals** (CW<80m)	**Average individuals** (CW 80-120mm)	**Large individuals** (CW>120mm)	**Total**
	Male	0	3	33	36
	Female	0	5	55	60
	Total	**0**	**8**	**88**	**96**
2014					
	Male	0	2	6	8
	Female	0	2	13	15
	Total	**0**	**4**	**19**	**23**
2015					
	Male	0	1	29	30
	Female	1	3	40	44
	Total	**1**	**4**	**69**	**74**

From the analyzed data, as it is shown in the Table 5 and Figure 9, large individuals predominated significantly each year.

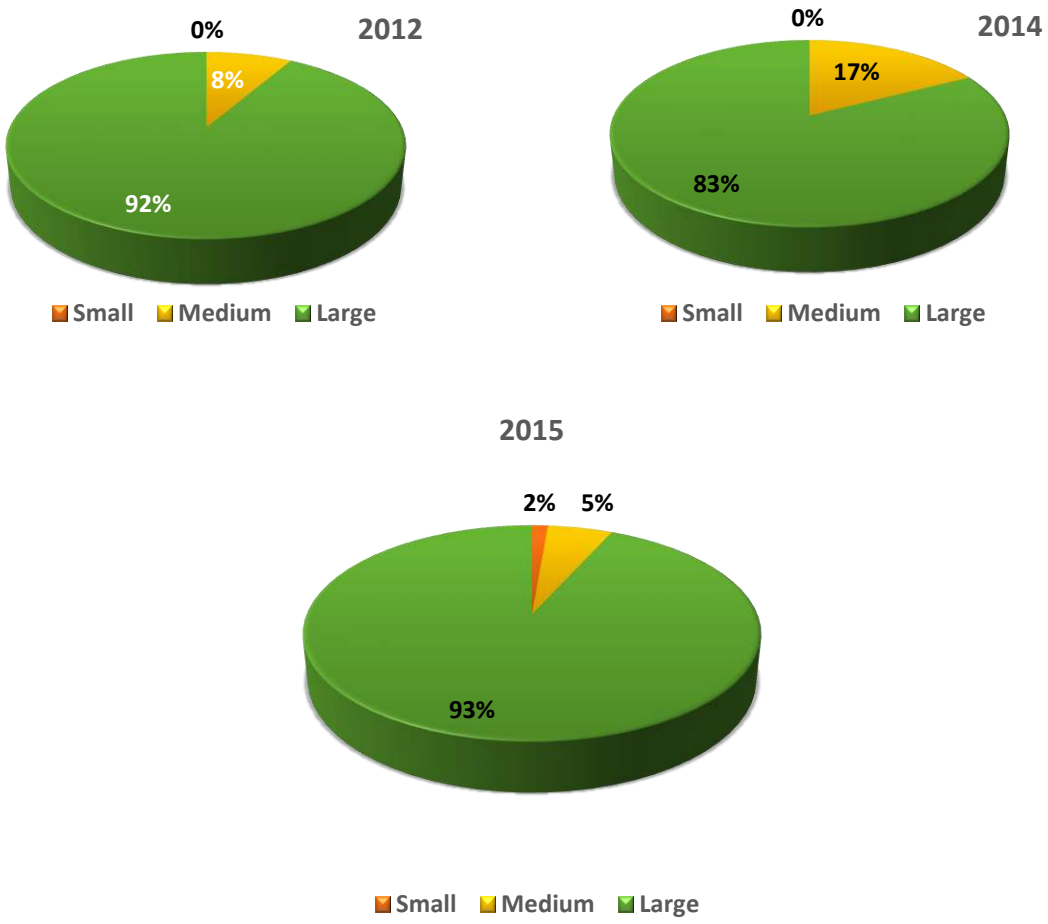


Figure 9. The percentage of individuals according to the size, small, medium and large for each year, 2012,2014,2015.

The blue crab individuals were classified based on carapace width in 1 year old individuals (CW <100 mm), individuals between 1 and 2 years old (CW 100-170 mm), and individuals over 2 years (CW> 170 mm) , according to Hines et al., 1990.
As shown in the Figure 10, here above, there is a significant increase of individuals of over 2 years from 9% to 30% and 31% and a decrease of individuals of 1 – 2 years from 89% to 66% and 68%, when comparing respectively between 2012, 2014 and 215.

Referring to questionnaires that were distributed to local fishermen in Narta area, the blue crab has already impacted populations of native species in the Narta lagoon, especially other crabs and fish. Its presence is already becoming a concern for socio-economic impact to local fishermen community.

Table 6. Classification of individuals by age (based on the Hines et al.1990).

	Sex	**Up to 1 year** (CW < 100 mm)	**Between 1 and 2 year** (CW 100 - 170 mm)	**Over 2 year** (CW > 170 mm)	**Total**
2012	Female	2	54	4	60
	Male	0	32	4	36
	Total	**2**	**86**	**8**	**96**
2014	Female	**0**	**8**	**4**	**12**
	Male	**1**	**7**	**3**	**11**
	Total	**1**	**15**	**7**	**23**
2015	Female	1	37	9	47
	Male	0	13	14	27
	Total	**1**	**50**	**23**	**74**

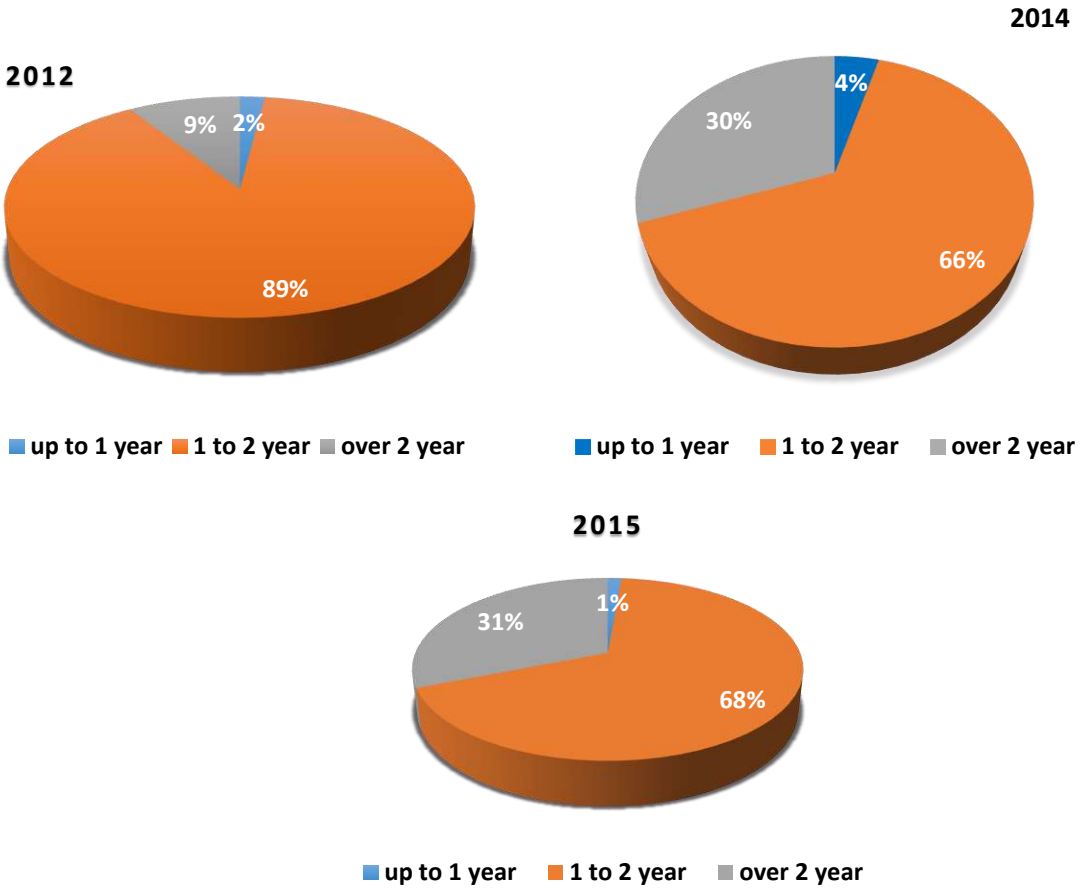


Figure 10. Ratio (in %) between individuals up to 1 year, 1-2 years and over 2 years of the blue crab in Narta Lagoon collected in 2012,2014,2015.

CONCLUSIONS

The blue crab *Callinectes sapidus* can be considered as a common species in the Narta Lagoon already with relatively high density for the most part of the year and stabilized in this lagoon. The highest presence of the blue crab in this lagoon was recorded during the period June – October, with the highest presence of ovigerous females during June – August. The correlation between three parameters, height, weight and width, is quite strong. Taking into account the high presence and abundance of the blue crab in Narta Lagoon, continuous presence of juveniles and ovigerous females, as well as increasing presence of individuals aged over 2 years, the population of the blue crab can be considered as established in the Narta Lagoon.

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MECHANICAL PROPERTIES OF BORON REINFORCED TEXTILE COMPOSITE

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ABSTRACT

Thanks to the developing technology, composite materials and their usage areas have made a great progress. In this study, usage areas of composite materials are tried to be associated with the materials used. Composite materials which are evaluated advanced technology materials have been being used in so many different fields. They have such features as high strength, high elasticity module, low density, high fatigue strength, thermal conductance, electrical conductivity, aesthetic appearance, lightness and corrosion resistance. While there are too many searches related to composite materials, it is an undeniable fact that the numbers of searches related to boron reinforced textile composite materials are highly limited. Therefore, in this study, general information about composites and composite textiles is given. Also; The mechanical properties of boron reinforced composites and jute composite prepregs were compared.

Key Words: Composite, Jute reinforced textiles composite materials, Composites, Boron reinforced textile composite materials, Mechanical properties.

INTRODUCTION

Metals, ceramics, elastomers, polymers and glasses used as engineering materials have a special place for composites. For this purpose, new composite materials are produced by combining two or more materials, namely matrix and reinforcement with superior properties.

Composite materials; they are materials with superior properties formed by the combination of two or more materials (metallic, organic, inorganic, etc.), one of which is matrix and the other is reinforcement.

Worldwide; There are more than 50000 materials used in the design and production of new products for different application areas. These materials range from ordinary materials used for centuries (copper, iron, etc.) to advanced materials developed in recent years (composites, ceramics and high-performance steels, etc.). Depending on the many different material options, today's designers and manufacturers have the privilege of choosing the right material and choosing the right production method for application (Mistik, 2009).

In this study, Vickers hardness and Charpy impact resistance behavior of jute prepreg composites and boron reinforced steel composites were investigated.

COMPOSITE MATERIALS

Due to the rapid developments in technology and industry and increasing energy costs in recent years; The need for materials with superior properties is increasing day by day. With this requirement, scientists carry out various studies for the production of new and superior materials. In line with these studies, composite materials with superior properties are produced. These superior properties are very attractive due to their high elastic modulus, high strength and low thermal expansion (Ray, 1989), (Orhan, 2007).

Composite materials consist of at least two different structural components and the transition zone between these components. The main component is called the matrix and the reinforcing component is called the reinforcement element.

In general, the main characteristics expected from the reinforcement element are:
- High part and strength,
- Low density,
- Chemical compatibility with the matrix,
- Ease of production,
- Maintaining its resistance at high temperatures,
- Being economical.

In the use of the composite to be produced in a structural application, a reinforcement element with low density, high modulus and strength is required. The most commonly used reinforcing elements in metal matrix composite materials are Al2O3, SiC, boron, TiC and carbon (Şahin, 2006; Mazumdar, 2002).

Boron reinforcement is the most expensive reinforcing element used in metal matrices. It is followed by SiC, carbon and Al2O3, respectively.

DEVELOPMENT AND CLASSIFICATION OF COMPOSITE MATERIALS

The history of composite materials actually dates back to ancient times. It has taken many years for composite materials to become usable today with technological developments from their conversion form. The use of composite materials from past to present dates back to the use of adobe.

The use of glass fibers is not unique to the present. It is known that the production of fine glass fibers was known in Egypt around 1600 BC and they were used for different purposes. However; The use of glass fibers in today's industry dates back to 1877. Today, the most common use is polyester composites equipped with glass fibers (https://www.re-coma.com/ctp/kompozit-history/).

Composite materials have been used for a long time to solve technological problems. These materials began to attract the attention of the industry with the development of polymeric-based composites in the 1960s. After that automotive parts, sporting goods, aircraft parts,

consumer goods and their production has started by being designed to be used in the maritime field (Mıstık, 2009).

Composite materials can be classified in two ways according to matrix material and reinforcement type.

According to the matrix material; metal matrix composite materials, polymer matrix composite materials, ceramic matrix composite materials, Nano composites.

According to the reinforcement material; particle-reinforced composite materials, fiber-reinforced composite materials, layered composite materials and filler-reinforced composite materials.

GENERAL PROPERTIES OF COMPOSITE MATERIALS

Composite materials, which are increasingly used in industry, are widely used in the defense industry, maritime transport, automotive, space, energy and aviation sectors; The general properties, especially the mechanical properties, can be listed as follows:

- High strength
- High modulus of elasticity
- Low density
- Good fracture toughness
- Good abrasion resistance
- Good fatigue strength
- Thermal conductivity
- Electrical conductivity
- Aesthetic appearance

its main features can be listed.

ADVANTAGES OF COMPOSITE MATERIALS

Composite materials are in demand due to their general properties, their general manufacturability, high strength and low densities. Therefore, these materials have many advantages over ordinary and convertible materials. These;

- A single composite material can be used for parts where it is necessary to use many metallic materials.

- Composite structures are materials with high hardness and have very low density compared to metals such as steel and aluminum.

- Composite materials have a very high strength to density ratio. This ratio is around 3-5 times that of steel and aluminum alloys.

- The fatigue strength of composite structures is very high.

- Composite materials have high corrosion resistance. Iron and aluminum corrode if there is water and air in the environment and require the application of special coatings or alloys. However, since the outer part of composite materials is made of plastic material, they have high resistance to corrosion and chemicals.

- Composite materials have more design possibilities and have greater dimensional stability.

- Mesh type and mesh-like structures can be produced as composite. This results in reduced processing time and cost.

- Complex parts that cannot be produced using metal can be produced as composite material without welding or riveting.

- Composite materials have high impact strength, for example glass and kevlar composites have higher impact strength than steel and aluminum.

- Sound and vibration properties of composite materials are better than metals.

- Composite materials used in engines; It is not sensitive to chemicals such as oil, hydraulic fluids, solvent, paint and petroleum.

- Due to the low pressure and temperature requirements, the production of composite materials is lower than the processing of metals.

- Due to such properties of composite materials, maintenance costs are considerably lower than metallic materials (Mistik, 2009), (Mazumdar, 2002), (Gay, 2003).

DISADVANTAGES OF COMPOSITE MATERIALS

Composite materials have some disadvantages as well as advantages. These disadvantages appear primarily in the form of high quality. These disadvantages;

- The cost of composite materials is approximately 5-20 times higher than steel and aluminum on a weight basis. The costs of some materials are as follows. Fiberglass $2.00 - $16.00/kg; carbon fiber $16 - $80/kg; boron fiber $2300/kg, epoxy $3/kg; glass/epoxy prepreg $24/kg and carbon/epoxy prepreg $24 - $1200/kg. Steel is around $0.40 - $2.00/kg and aluminum is around $1.2 - $2.00/kg.

- In the past, composite materials were produced in the production of larger parts and in small quantities. However, today, with the automation of resin transfer molding and filament winding methods, faster and desired size production is realized.

- The heat resistance of composite parts depends on the heat resistance of the matrix material. Polymer-based matrix material is generally used in composite materials. The thermal resistance of the matrix material also determines the temperature resistance of the composite structure. The polymer matrix also determines the composite's resistance to solvents, chemicals and environmental conditions.

- The moisture absorption of the composite structure affects the properties and dimensional stability of the material (Mistik, 2009), (Mazumdar, 2002).

Composite structures are usually heterogeneous and non-isotropic orthotropic or anisotropic structures. At one point of an orthotropic structure, there are different material properties in the direction of three basic axes. However, the structure is perpendicular to each other and symmetrical with respect to the three basic planes. In an anisotropic structure, there are different properties in all directions at one point. There are no planes in which these features are symmetrical. Features vary depending on direction. Composite materials are heterogeneous and non-isotropic structures. For this reason, there are two different branches of study, called

micromechanics and macro mechanics, in their design and analysis (Mistik, 2009), (Armatlı Kayrak, 1999).

PREPREGES

Prepreg is the abbreviation of the term "pre-impregnated" and the term used for resin-impregnated composite fabrics.

Prepreg is a fibrous polymer reinforcement pre-impregnated with resin. Prepregs are processed under a certain pressure and temperature. The impregnated resin hardens, resulting in a lightweight and very durable composite material with high thermal and chemical resistance. As can be seen in figure 1 below, they have the appearance of a woven fabric structure (http://www.turkchem.net/prepreg.html, 14/11/2018).

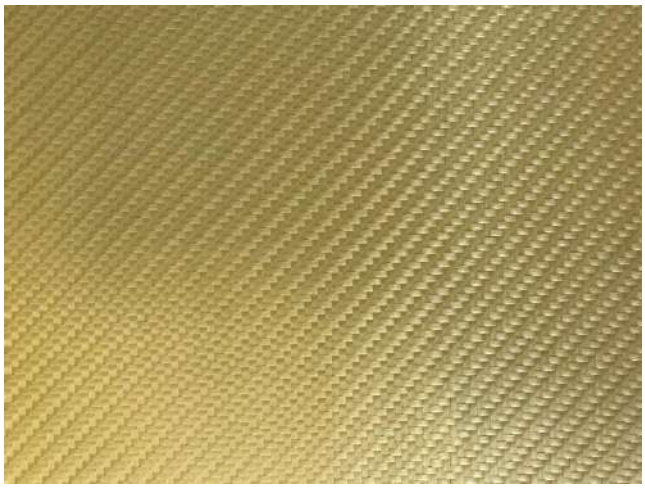

Figure 1. Prepreg material

Woven or unidirectional glass, carbon, aramid and boron impregnated with resin on the fabric and formed with semi-cure the composite product. For curing prepregs, ready for series resin without additional labor required to contain the resin and hardener mixture thus can be used (Spmkompozit, 2018).

BORON AND BORON PREPREGES

It is known that boron minerals have been used throughout human history (for example: it has been determined that different boron salts were used in mummification processes in Ancient Egypt, taking into account their long-term preservation and anti-corrosion properties).

Today, when we look at the contemporary applications brought by scientific and technological developments around the world, it is seen that boron products are used in a wide range from textile fibers to glass, from nuclear applications to new and advanced magnets and even fertilizers. In this respect, practices and activities that started as a raw material input from

boron mineral have become a "techno-economic" element that is closely and directly related to many sectors and technology fields.

Considering the richness of boron deposits of our country, it is clearly seen that it should be considered as an extremely important, vital and strategic resource. When boron deposits, boron mineral production, production methods of boron compounds, areas of use, new technologies, advanced technology applications that will mark the future, and market situations in the world and in Turkey, the following conclusions can be drawn:

• Boron deposits are concentrated in a few regions in the world and the most important deposits are in Turkey.

• Boron compounds are also classified according to their physical and chemical properties, production methods, amount of consumption, technology and usage areas.(Mistik, 2009).

As can be seen in figure 2 below, they have the appearance of a boron prepreg woven tape fabric structure.

Figure 2. Boron Prepreg

Figure 3 below shows the production process diagram of boron fiber by CVD method.

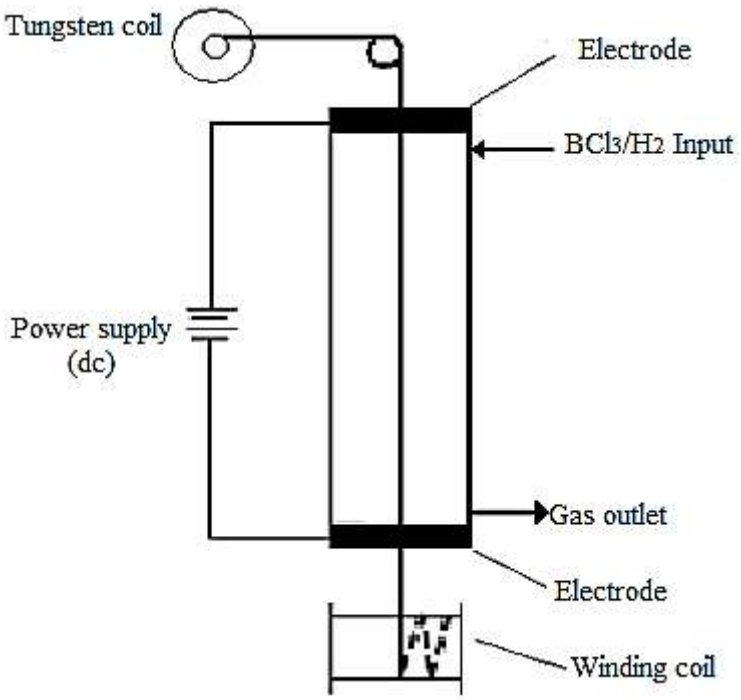

Figure 3. Boron fiber production scheme by CVD method (Reinhart, 1993).

In figure 4 below, the production process diagram for converting boron fiber into boron prepreg strip shape is given.

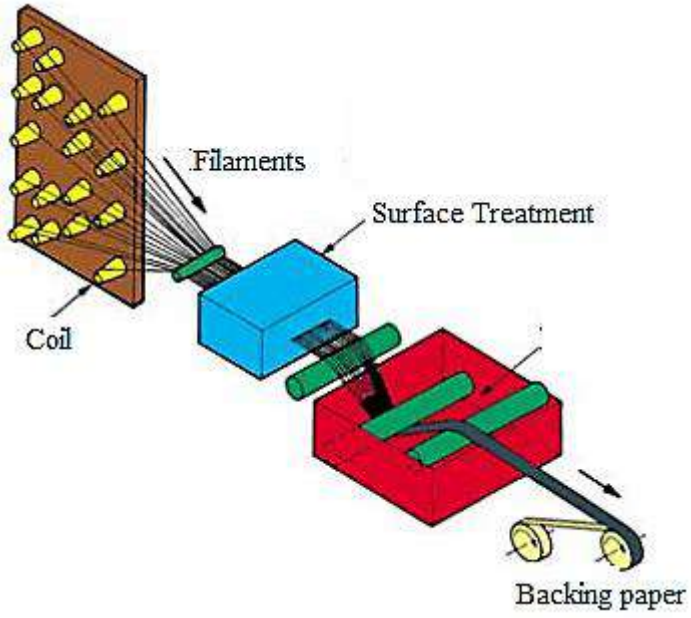

Figure 4. Boron fiber / Epoxy prepreg strip

USAGE AREAS OF BORON REINFORCED PREPREG COMPOSITES

As seen in the table below, when we look at the world's boron reserves, our country's boron reserves are more than the sum of all other boron reserves in the world.

Table 1. World total boron reserves (1000t B2O3)(Altun, 2005).

Country	**Total Reserve**	**%**
Turkey	563.000	64
USA	80.000	9
Russia	100.000	11
Chinese	36.000	4
Chile	41.000	4
Bolivia	15.000	2
Peru	22.000	3
Argentina	9.000	1
Kazakhistan	15.000	2
Total	885.000	100

Considering the reserves in the world; Boron minerals and compounds are used in the production of many different materials and products in a wide variety of industries. The main usage areas of boron and its products are;

a) Glass Industry;
- Boron silicate glasses
- Insulation fiberglass
- Textile fiberglass
- Alumina boron silicate glasses
- Optical fibers
- Glass ceramics

b) Enamel and glaze
c) Fire resistant materials
- Cellulosic insulation materials
- Plastics
- Textile

d) Soap and detergent
e) Pulp (as bleach)
f) Fertilizers and pesticides
g) Metallurgy
h) Magnetic materials
i) Nuclear materials
j) It appears in the form of other applications (Mistik, 2009).

OTHER TEXTILE SUPPLEMENTS AND PROPERTIES USED IN THE PRODUCTION OF COMPOSITE MATERIAL

1- Jute Prepreg

Properties of jute fibers and prepreg materials used for the study; The brand registration of the jute fabric was accepted by the patent institute with the brand number 2009 16576 and it was woven with plain weave. The weight of the woven jute fabric is 10 ounces, approximately 284gr/m^2. The length of the jute fibers is 18-25 cm. In its structure; It contains 60-64% cellulose, 20% lignin, and 5% pectin.

2- Carbon Fibers

Carbon fibers have lower density and higher specific strength than other classical engineering materials. Fibers containing at least 92% carbon by weight are called carbon fiber.

The density of carbon fibers varies between 1.6-2.2 g/cm3 depending on the raw material used and the processing temperature. The raw material density used in carbon fiber production varies between 1.14-1.19 g/cm3. The increase in the obtained fiber modulus increases with the increase of the graphitization temperature.

Composites made of carbon fibers are 5 times more durable and 1/5 in weight than 1020 steel constructions. Likewise, while 6061 is 7 times more durable in aluminum constructions, it is 2 times harder and 1.5 times lighter. The fatigue behavior of carbon fibers is better than all known metals. The electrical conductivity of tar-based carbon fibers is 3 times higher than that of copper (Yaman, et al. 2007).

Properties of carbon prepreg materials used for the study; Tensile strength; (min) WARP/WELF Ksi = 552 Mpa = 3800.

The force at which the fiber breaks is measured according to the width of the field. Voltage modules; (min) WARP/WELF Msi = 34.8 Gpa = 240 Elastic stiffness measurement.

3- Aramid Fibers

Aromatic polyamides have become an important material used in commercial applications with the introduction of Nomex®, a meta-aramid fiber, in the early 1960s.

The emergence of the possibility of producing high-modulus and high-strength fibers from liquid crystal solution in 1965 led to the development and commercialization of Kevlar® fiber, which is more durable and high-modulus than Nomex®, by DuPont in 1971 (Hearle, 2001), (Mistik, 2009).

There are 4 types of Kevlar fiber, These are;

1- Kevlar is generally used as a rubber reinforcement material in vehicle tires.
2- Kevlar 29 is generally used in ropes, cables, coated fabrics and ballistic protective fabrics,
3- Kevlar 49 is generally used in reinforcing composite materials used for aerospace, marine, automotive and sports purposes,

4- Kevlar 149 is more crystalline than Kevlar 49 and its modulus is 35% higher (Ulcay, 1989), (Mistik, 2009).

4- Glass Fibers

In 1935, Owens-Illinois Glass Co., Newark/Ohio, produced glass fibers that were thin enough to be spun and woven. It was first used in the aviation industry as reinforced composite materials in 1942.

Glass fiber is the most widely used reinforcing material in polymer composites. Glass fiber types used as composite reinforcement material are E, S and S-2 glass fibers. Other types of glass fibers are used in non-composite applications.

The most important properties of glass fiber are low cost, high tensile strength, high chemical resistance and very good insulating properties.

The negative properties of glass fiber are low tensile modulus and low fatigue strength. Glass fibers are isotropic due to the three-dimensional network structure of glass. The friction of the glass fibers against each other can cause damage on the fiber, this damage creates fine cracks on the fiber. To prevent this, glass fibers are coated. This process also creates a chemical bond between the fiber and the matrix (Mistik, 2009).

Table 2. Mechanical properties of glass fibers;

FEATURES	GLASS TYPES						
	E-Glass	A-Glass	M-Glass	S-Glass	C-Glass	D-Glass	R-Glass
Specific Gravity [gr/cm³]	2,54	2,45	2,89	2,49	2,45	2,16	2,58
Tensile Strength [MPa]	3600	3100	3500	4500	3400	2450	4400
Elongation at Break [%]	4,8	-		5,4	4,8		-
Tensile E-Module [GPa]	76	72	111,3	86	70	53	85
Refractive index	1,548	1,512	1,635	1,523		1,47	-
Coefficient of Thermal Expansion [cm/cmK°]	$1,6\times10^{-6}$	-		$1,7\times10^{-6}$	$2,2\times10^{-6}$	$3,1\times10^{-6}$	-
Fiber Diameter [mm]	$3\text{-}20\times10^{-3}$	-		$3,13\times10^{-3}$	-		-
Softening Point [°C]	850	700		-	690	770	990
Dielectric Constant [1MHz]	6,33	-		5,34	-	5,8	-

5- Polypropylene Fibers

Polypropylene is obtained from the polymerization reaction of propylene with a catalyst at 100 °C under a pressure of 20-30 atm. Polypropylene is the lightest known polymer material

with a density of 0.905 g/cm3. It is obtained as monofilament or multifilament by melt drawing method.

Polypropylene is not affected by moisture and chemical reagents. The moisture absorption rate is 0.05%. It is less resistant to heat, light and oxidizing substances than polyethylene. One of the most important features of polypropylene fiber is its high friction resistance (Baser, 1992).

COMPARISON OF MECHANICAL PROPERTIES OF HYBRID COMPOSITES MANUFACTURED

Charpy Impact test results applied to symmetrical, asymmetrical and random array composites on Jute Reinforced prepreg materials;

Table 3. Charpy Impact Resistance of Jute Prepreg Composites

Charpy Impact Resistance	
Sample Code	Impact Resistance Force (Nm),(J/m)
N01 S0	74,556
N08 AS0	76,518
N15 RO	76,518

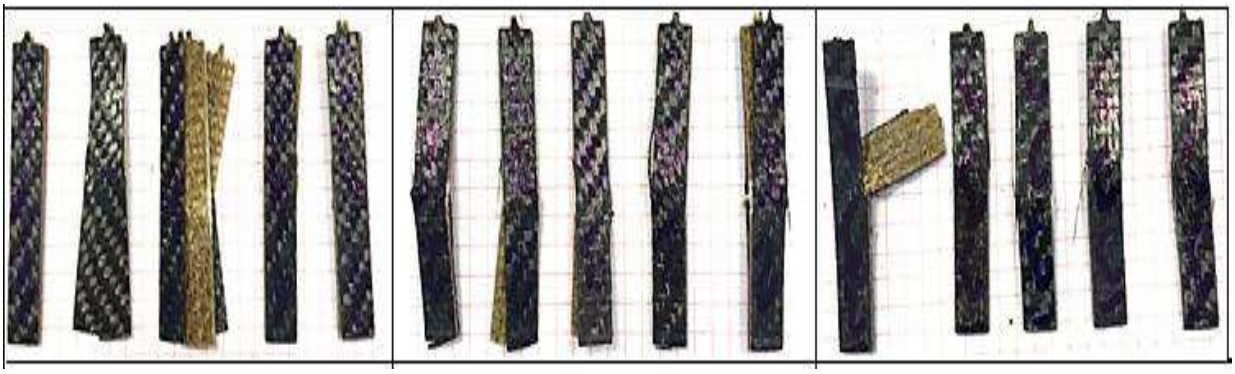

Figure 5. Post-test appearances of the Charpy Impact-tested samples of Jute Prepreg Composites

Comparison of Vickers hardness test results of Jute Prepreg Composites with boron added steel composites.

Table 4. Vickers hardness test results of high-P steels(Hong, vd., 2014).

Heat treatment	Steel	Vickers hardness(VHN)
Isothermal treatment at 550OC	2P-6	239± 31
	2P1B-6	240± 25
	2P2B-6	257± 29
	5P-6	233± 26
	5P1B-6	260± 19
	5P2B-6	277± 16

When the results of the Vickers hardness test samples were compared; Jute Reinforced prepreg materials max. value 51.24 AVE, min. the value comes out to 45.48 AVE. Boron added steel composites are 5 times harder than Jute Reinforced prepreg materials.

Comparison of Charpy Impact test results of Jute Prepreg Composites with boron added steel composites.

Table 5. Charpy Impact test results of high-P steels(Hong, vd., 2014).

Heat treatment	Steel	Upper shelf energy (J)
Isothermal treatment at 550OC	2P-6	42 ± 1.4
	2P1B-6	67 ± 4
	2P2B-6	43 ± 2.7
	5P-6	37 ± 5.9
	5P1B-6	67 ± 2.3
	5P2B-6	37 ± 1

The max. When the values are compared, better results are obtained than composite prepregs with a difference of 9,518 J.

CONCLUSIONS

Today, it is known that the development and production of new composite materials with the technological infrastructure and developing technologies are produced in the form of products with very high added values. The least known aspects of composite products; how they are produced, with which technique and method they are produced, and how and how their raw materials are used. Transferring these technologies from the producer country to another country under technology transfer, licensed production and similar names is usually very difficult or can be achieved with very high costs. Therefore, it is of strategic importance to have technological competence and production capability in the field of composite materials.

The developments in the field of composite materials reveal that these materials can be used in almost every desired area with the development of these materials. The subject of composite materials is an interdisciplinary field that encompasses various other specialties such as physical metallurgy, solid-state physics, textile science, chemistry, mechanics, ballistics,

surface science and materials analysis. The subject of composite materials will continue to be important for many years, as the selection of materials required for composite applications, their production methods, material properties and their behavior against the tests to be applied must be evaluated.

Tests on the obtained boron reinforced composite prepreg materials; mainly unit mass determination, tensile test, impact strength test, fatigue tests. These tests are; It demonstrates the strength, modulus of elasticity, density, fracture toughness, abrasion resistance, fatigue strength, aesthetic appearance and other mechanical properties of the produced boron reinforced composite prepreg materials.

As a result of this study; It is desired to investigate the effect of the production parameters of boron reinforced textile composite prepreg materials on the mechanical properties of the materials.

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BIOCHEMICAL CHARACTERIZATION AND ANTIOXIDANT ACTIVITY OF LEAVES AND ROOTS EXTRACTS FROM DATE PALM CULTIVARS (*PHOENIX DACTYLIFERA* L.) ENDEMIC TO THE LAGHOUAT REGION

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ABSTRACT

The date palm is the main phytogenetic resource of the Algerian Sahara. Considered as a tree of providence, its various parts have been used for centuries for various purposes, including traditional medicine. While the fruits have been the subject of many studies, the leaves and roots remain unexploited. In this study, we performed a biochemical characterization of the leaves (l) and roots (r) of two endemic cultivars from the Laghouat region: Taddala (TAD) and Tizzaouet (TIZ). A reflux apparatus performed the extraction of the total phenolic content. We used the colorimetric method of Singelton and Rossi for the determination of the total phenol content (TPC) and aluminum trichloride for the determination of the total flavonoid content (TFC). In addition, we tested the antioxidant activity of the extracts with the DPPH (2,2-diphenyl-1-picrylhydrazyl) test. The results showed very high levels of TPC in date palm leaves and roots with a predominance in leaves. It reached up to 825,63 mg (GAE)/100 g dry weight in TAD leaves. TFC were also quite high, ranging from 11.24 to 70.41 mg (EQ) / 100 g dry weight and were significantly correlated with TPC. The extracts showed significant antiradical efficacy compared to vitamin C. The results suggest that all parts of this plant are a good source of natural antioxidants and can be used as functional food ingredients.

Keywords: *Phoenix dactylifera;* date palm leaves and roots; total phenolic content; total flavonoid content; antioxidant activity; DPPH.

INTRODUCTION

The date palm (*Phoenix dactylifera L.*) is one of the fruit species whose culture has existed since the earliest antiquity (Munier, 1973). The date palm is a monocotyledon of the family of the Palmae, one of the genera of which are the *Coryphoideae* (Barreveld, 1993). From an ecological point of view, the date palm plays a major role in the fight against desertification and maintaining ecological balance and biological diversity. Date palm is an important crop in all of the southern regions in Algeria, approximately 18 million date palms are cultivated on an area of 169,380 ha; out of these, ten million trees are producing an annual yield of 500,000 mt of dates (Bouguedoura, 2015). More than 940 cultivars have been currently identified (Hannachi et al., 1998). However, this culture has continued to deteriorate (ICRA, 2003). The phoenicultural heritage of the Laghouat region (located in North of Sahara) has experienced

significant degradation in recent years. It is urgent to fight against the genetic erosion of this resource.

The importance of the date in human nutrition comes from its rich composition in carbohydrate (sugars such as fructose, glucose, and sucrose; dietary fiber), salts and minerals (calcium, magnesium, phosphorus, potassium, iron, zinc, copper, manganese, and selenium), vitamins (A, A1, B, B1, B2, B3, B5, B6), fatty acids, amino acids and protein (Elleuch et al., 2008 ; Livingston et al., 2002). It comes also, from its richness in antioxidant (Mansouri et al., 2005; Ghiaba et al., 2012; Hamini et al., 2015). Antioxidants such as phenolic compounds may reduce the risks of several diseases and improve general human health (Iqbal et al., 2007). Most of antioxidant capacity of fruit or vegetable may derive from compounds like flavonoids, isoflavones, flavones, anthocyanins, catechins and isocatechins (Wang et al., 1996; Dasgupta, 2007). Many of these phytochemicals may help to protect cells against the oxidative damage caused by free radicals (Wada et al., 2002; Kumaran et al., 2007). Phenolic compounds are characterized by the presence of one or more rings aromatics comprising one or more hydroxyl groups. They are classified according to the number of carbons per molecule and according to the basic skeletal structure (Harborne, 1989). In spite of extensive studies made on the bioactivities of date fruit, the antioxidant properties of leaves and roots of *P. dactylifera* have rarely been studied. The influence of the different cultivars on the total phenol content (TPC) and its antioxidant activity has not been reported. The aim of this study was to investigate *in vitro* the antioxidant capacities of the methanol extracts from two endemic cultivars from the region of Laghouat (Algeria). In the present study, methanol extracts were prepared from dried and powdered plant material. The TPC and flavonoid contents were measured from plant extracts. Furthermore, the scavenging activity was also tested.

Material and Methods

Plant material

Our study focused on two cultivars of date palm with good quality of fruits which are endemic to the Laghouat region: the cultivar Tadala (TAD) and the cultivar Tizzaout (TIZ). Samples of leaves and roots were taken from female plants in the spring of 2020 and then carefully dried.

Chemicals and reagents

All solvents and reagents used in the experiments were of the highest purity. ethanol, methanol, ethyl acetate, petroleum ether, Folin–Ciocalteu reagent, Na2CO3, gallic acid, aluminum chloride, quercetine,1-diphenyl-2-picrylhydrazyl (DPPH) radical, vitamin C.

Sample preparation and extraction

The plant material was air dried in darkness at room temperature and milled into a fine powder using a coffee-grinder. Several grams of fine powder was heated under reflux in 100 ml methanol:water (80:20, v/v) for 6 h. The crude preparation was filtered. The filtrates of hydro-alcoholic were combined. After removing the alcohol using a rotary evaporator at 45°C, the aqueous phases were treated with petroleum ether to eliminate the pigments. The phenolic compounds were extracted twice with ethyl acetate (20 ml). The residual water in the ethyl acetate was eliminated with anhydrous sodium sulphate and filtered using filter paper, and then evaporated to dryness using a rotary evaporator. The extracted phenolics were dissolved in 10 ml of methanol. Methanolic solutions of phenolic compounds were kept frozen until analysis.

Determination of TPC

The amounts of TPC in plants extracts were determined with the Folin–Ciocalteu reagent using the colorimetric method of Singleton and Rossi (Singleton and al., 1965). Briefly, reaction mixture contained one 100 micro l of methanolic extracts (three replicates), 500 micro l freshly prepared dilute (1:10) Folin–Ciocalteu reagent, and 2 ml of Na2CO3 (20% w/v). Mixtures were shaken and left to stand at room temperature for 30 min before measuring the absorbance at 760 nm using a spectrophotometer (UV-1601; UV–visible spectrophotometer; Shimadzu). The TPC was determined from standard gallic acid curve and expressed as milligrams of gallic acid equivalents per 100 g of dry weight plant material (mg GAE/ 100 g dw).

Determination of TFC

The total flavonoid content (TFC) was determined according to the aluminum chloride colorimetric method described by Chang et al. (Chang et al., 2002) with slight modifications. This method is based on the quantification of yellow color produced by the interaction of flavonoids with AlCl3 reagent. To 1 ml of each sample (three replicates), 1 ml of 2% (w/v) AlCl3 in methanol solution and incubated for 20 min in the obscurity at the room temperature. The absorbance of all samples was measured at 409 nm (UV-1601; UV–visible spectrophotometer; Shimadzu). TFC was determined from standard quercetine curve and expressed as mg of quercetine equivalents per 100 g of dry weight plant material (mg QU/100 g dw).

DPPH radical scavenging activity

Radical scavenging activity of the extracts against stable DPPH. (2-diphenyl-2-picrylhydrazyl hydrate) was determined using the method of Brand-Williams et al. (Brand-Williams et al, 1995). This method was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light yellow) were measured at 517 nm on a UV–visible light spectrophotometer (UV-1601). The solution of DPPH in methanol was prepared daily, before the measurements. Various concentrations of 1 ml of sample solution diluted methanol were added to 1 ml of the DPPH radical solution. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The decrease in absorption was measured at 517 nm. Absorption of a blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The antioxidant activity of the extract was expressed as an IC_{50} value defined as the concentration (mg/l) of the extract that inhibited the formation of DPPH radicals by 50% and compared to the IC_{50} of vitamin C. The percentage of DPPH scavenging effect was calculated by following equation.

DPPH scavenging effect (%)/% Inhibition=A0-A1/A0 × 100

Where A0=The absorbance of control.
A1=The absorbance of standard.

Statistical analysis

The results were presented as the mean values ± SEM. Data were statistically evaluated by two-way ANOVA. Correlation analyses were carried out using the correlation and regression in the EXCEL program.

Results and discussion

Total phenolic content

The results showed very important levels of TPCs in the leaves and roots of date palm with a predominance in the leaves (Fig. 4). TPC were determined using standard gallic acid curve (Fig. 1). The amount of TPC varied significantly in the date palm extracts investigated and ranged from 332,9 ± 1 to 825,63 ± 3 mg GAE/100 g dw (Table 1). Among extracts, extremely high TPC was detected in TIZ leaves (825,63 mg GAE/100 g dw). The lowest TPC was detected in TAD roots (332,9 mg GAE/ 100 g dw). The order of TPC in DP extracts is: TAD r<TIZ r<TAD l<TIZ l.

These results of TPC in leaves are clearly superior to those found in Algerian Oued-Souf cultivars where the maximum was 215.24 mg EAG/100g dw in the cultivar "Ghars" (Laouini, 2014). Also, for those found in cultivars of Ghardaia where the maximum was 24.45 mg GAE / 100g dw. In this case the richest cultivars in TPC were Seba'Bedra, Ghar and Takarmoust (Trichine, 2010). The TPC of the experiment are also superior to those found in mal leaves where the maximum was 233,42 ± 1,42 mg GAE/100g dw (Berramdane et al., 2020).

For the comparison of TPC in roots, one study was found. The levels of TPC were very low and not exceeding 1,5 mg GAE/100g dw (Gaseb-Terak, 2010). However, the results of the experience are very high even compared with leaves of other cultivars specially for the cultivar TIZ. The difference of TPC may be explained by the variation in growing conditions, the genetic variation or the methods of extraction.

Total flavonoid content

The results showed very important levels of TFCs in the leaves and roots of date palm with a predominance in the leaves (Fig. 5). TFC were determined using standard quercetin curve (Fig. 2). Significant differences in TFC were observed among the date palm extracts ranging from 11.24 to 70.41 mg (QE) / 100g dw (Table 1). TAD leaves had the highest TFC (70,41 mg (GAE) / 100g dw). The order of TFC in DPF extracts is: TAD r <TIZ r<TIZ l< TAD l.

Table 1. Total phenolic content (TPC), total flavonoid content (TFC)

	TPC **Gallic acid eq. (mg/100 g)**	**TFC** **Quercetin eq. (mg/100 g)**
TAD l	**619,99 ± 1**	**70,41 ± 0,62**
TAD r	**332,90 ± 1**	**11,24 ± 0,62**
TIZ l	**825,63 ± 3**	**51,32 ± 0,74**
TIZ r	**600,43 ± 1**	**17,62 ± 0,24**

Values are presented as mean ± SD (n=3)

These results of TFC in leaves are clearly superior to those found in Algerian Oued-Souf cultivars where the maximum was 101,09 mg E Ca /100g dw in the cultivar "Ghars" (Laouini, 2014). Also, for those found in cultivars of Ghardaia where the maximum was 6.86 mg E C/ 100g dw. In this case the richest cultivars in TPC were Akerboucht and the TFC in Ghar was high too (Trichine, 2010). For the comparison of TPC in roots, none studies were found. The difference of TPC may be explained by the variation in growing conditions, the genetic variation or the methods of extraction.

Antioxidant activity

In the current study, the ability of test samples to scavenge DPPH radical was assessed on the basis of their IC_{50} values, defined above as concentration methanolic extracts of DP to decrease the absorbance at 517 nm of DPPH radical solution to half of its initial value. These IC_{50} values were obtained using a calibration curves prepared by plotting percent inhibition values calculated as a function of concentration of test samples. IC_{50} values of the methanolic extracts of DP are given in Table 2.

Results were compared to the curve of ascorbic acid (Fig. 3). The curve of vitamin C was obtained by preparing 9 concentrations of solutions ranging from 0.0025 to 0.0225 mg / ml. These solutions were prepared from a stock solution of 0.025 g / l.

Table 2. Antioxidant activities in the different samples

	IC50 **DPPH (mg/l)**	**AE**
TAD l	**0,089**	**11,24**
TAD r	**0,161**	**6,21**
TIZ l	**0,069**	**14,49**
TIZ r	**0,036**	**27,78**
Vit C	**0,016**	**62,5**

Values are presented as mean ± SD (n=3)

The extracts showed significant anti-free radical efficacy when compared with vitamin C (one of the most powerful antioxidant). It can be seen from this table that IC_{50} values of methanolic extracts of DP ranged from 0,036 to 0,161 mg/l. The lowest value of IC_{50} was detected in TIZ r and it corresponds to the highest antioxidant activity; while the highest value of IC_{50} was detected in TAD r. The antioxidant efficiency (AE=1/IC50) in the methanolic extracts of DP decreases in the order TIZr<TIZ l<TAD l<TAD r. In this study, there was a significant difference between the cultivars of palm date. This indicates that the difference in the cultivar of DP influences the antioxidant activities.

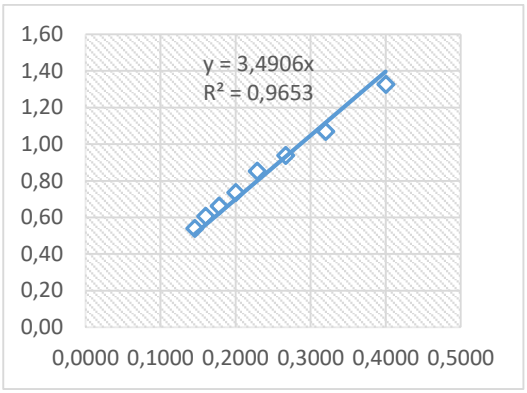

Fig. 1 Standard Gallic Acid Curve

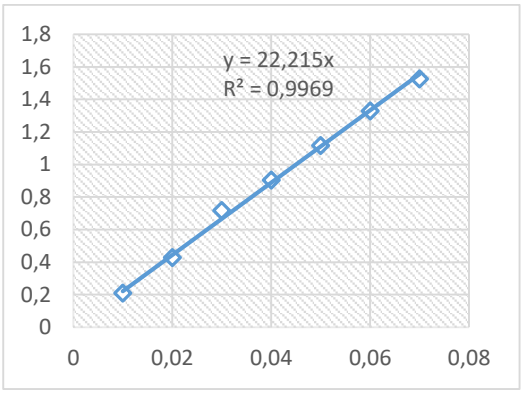

Fig. 2 Standard Quercetin Curve

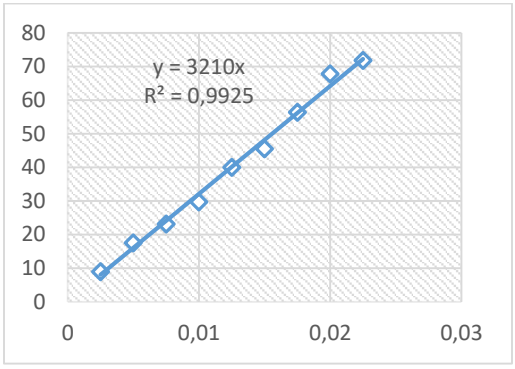

Fig. 3 Scavenging activity Curve for vitamin C

The results of this study are presented in Fig. 8. From this figure, we can see that the antioxidant activity of methanolic extracts of DP does not vary univocally and it is independent from TPC and TFC. However significant correlation was found between TPC and TFC. The antioxidant activity in date palm parts is likely influenced by the extraction, the analytical methods, the genetic and the environmental factors. All extracts of date palm of the experience contain a remarkable quantity of TPC and TFC, and show significant antioxidant activities. The, IC_{50} values are different for all the extracts, despite the variation in the TPC. Variable results have been reported on the relation between TPC and antioxidant activity of different natural products. Some authors found a correlation between TPC and the antioxidant activity (Adom et al., 2002; Gruz et al., 2011) while others did not (Zielinski et al., 2000; Hahkonen et al., 1999).

These results can be explained by the fact that the date palm contains an important quantity of ascorbic acid which varies according to the different cultivars, that interferes in the quantification of TPC values. In fact, the Folin–Ciocalteu method is reported to detect all phenolic compounds present in the extract, this reagent also measures other constituents besides phenolic compounds, such as peptides and amino acids. On the other hand, according to some studies, the antioxidant activity depends on the structural conformation of phenolic compounds. The latter is generally influenced by the phenolic compounds in the samples and by the different mechanisms involved in the radical–antioxidant reactions. These compounds may have a wide variety of chemical structures that could react with radicals by hydrogen donation and/or by electron transfer. Phenolic compounds can potentially also act synergistically in a positive or a negative way.

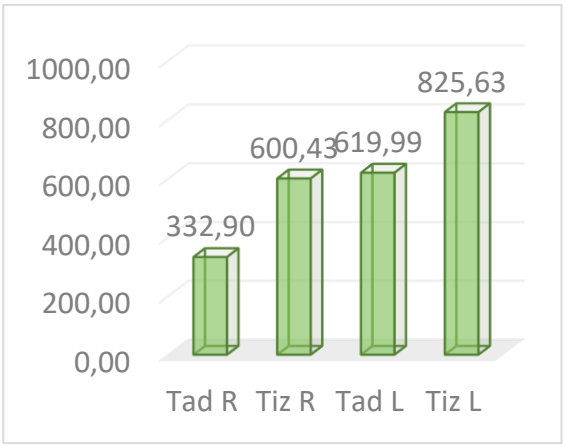

Fig. 4 Phenolic content in the exacts

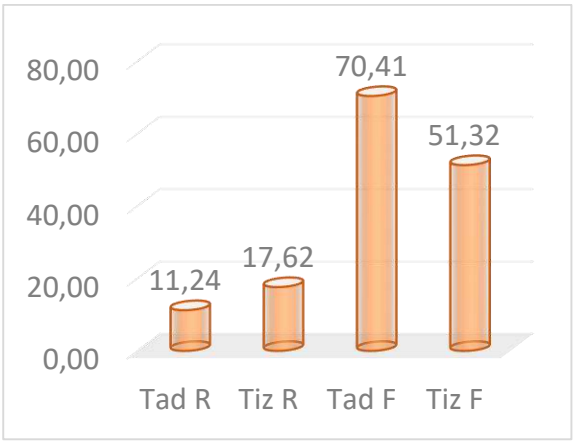

Fig. 5 Flavonoid content in the exacts

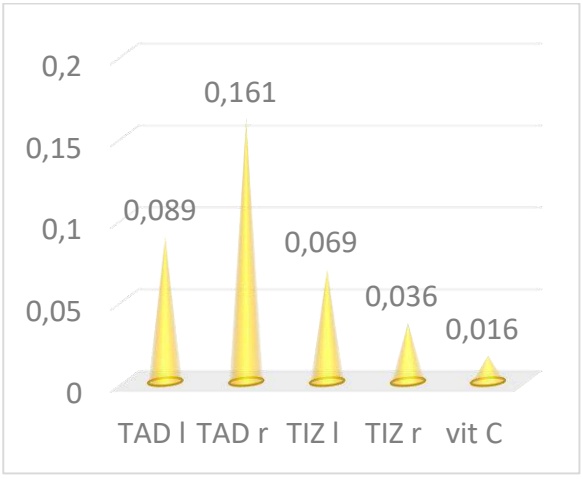

Fig. 6 IC_{50} of the exacts

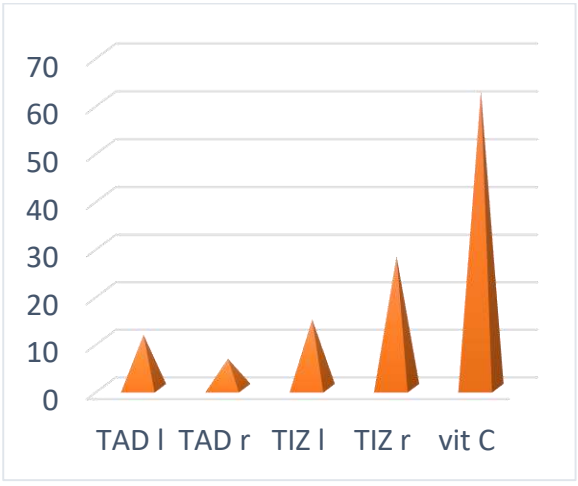

Fig. 7 AE of the exacts

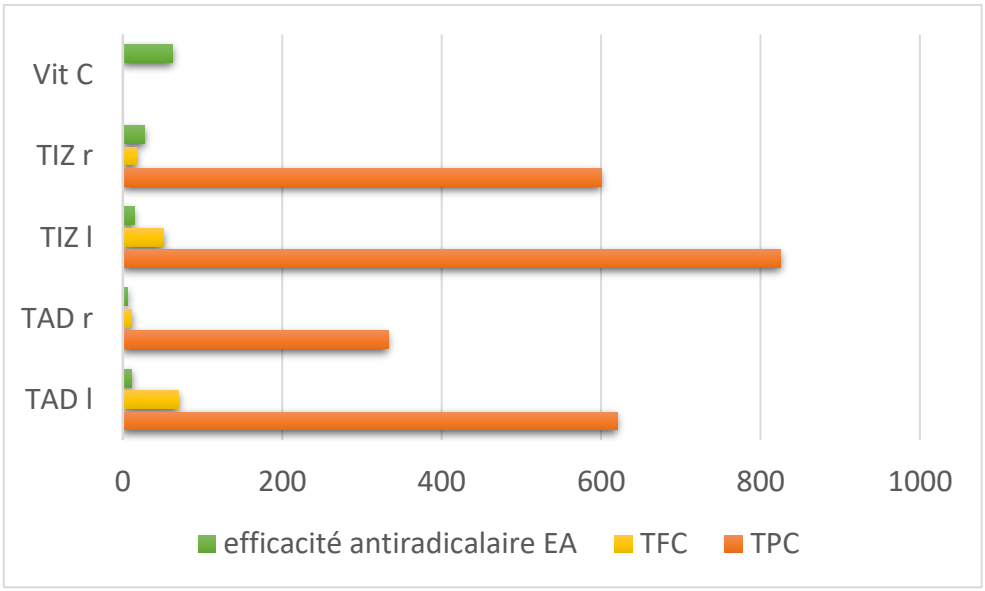

Fig. 8 Comparison between EA, TPC and TFC

Conclusion

The contents of phenolic compounds and the antioxidant activity of methanolic extracts of date plam cultivars from the Laghouat region in the South of Algeria were evaluated for the first time. In this study, it was demonstrated that the methanol extracts of DP contain a considerable quantity of phenolic compounds and possess a good antioxidant activity which may be associated with their alleged health benefits in traditional medicine. The broad range of antioxidant activity of the extracts indicates the potential of the DP parts as a source of natural antioxidants or nutraceuticals with possible applications to reduce oxidative stress and provide health benefits. The above results indicate that cultivars of date palm from the same region could lead to significant differences both in the content of bioactive compounds and in their bioactivities. Further studies are planned for the isolation and identification of individual phenolic compounds. Moreover, *in vivo* studies could clarify antioxidant properties and mechanism of action.

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CHANGES IN PHYSICAL AND QUALITY CHARACTERISTICS OF SWEET CORN VARIETIES DURING STORAGE

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ABSTRACT

Researh was established in Isparta University of Applied Sciences, Faculty of Agriculture, Field Crops laboratory in 2020, to determine effects on physical and quality of sweet corn varieties during storage. The study was established in completely randomized plot design with 3 replications. In study, two hybrid sweet corn (Batem Tatlı and Kompozit Şeker) were used. In experiment, sweet corn varieties were stored in modified atmosphere bags (MAP) in the refrigerator (+4^{o}C) for different periods (0, 5, 10, 20, 30 and 40 days). In the study, weight loss, dry matter content, color parameters (L* and C*), total soluble sugar content, ash ratio, crude protein ratio and soluble solids matter content were investigated according to storage time. When the sweet corn cultivars were stored for different periods, at the end of the 40th day, the ash content, crude protein content, total soluble sugar content, soluble solids content, brightness (L*) and vitality (C*) values decreased; weight loss and dry matter ratio increased were determined. As a result, sweet corn varieties should be consumed fresh after being harvested, and it can be recommended to be consumed within 5 days at most when they need to be kept at +4^{o}C. After the 5th day, it has been determined that there will be great losses in terms of quality and physical properties.

Keywords: Sweet corn, storage, physical properties, quality properties, sugar content

INTRODUCTION

Sweet corn is a culture crop grown fresh for human consumption. Sweet corn varieties have different varieties in terms of grain color (yellow, white or bi-color), maturation time (early, mid-late and late) and sugar content [standard (su-1), sugar content increased (se), super sweet (sh-2)]. Sweet corn is a source of fiber and vitamin B9. In also, it has 70% moisture and 23% total carbohydrate content and 27% of its total carbohydrate content consists of starch (Lertrat and Pulam, 2007). On the other hand, among the maize subspecies, sugar corn has higher sugar content than the others when harvested at the end of the milking period (Zadoks 73 - 75 - 77 - 79). In also, at the end of this period, it was determined that sweet corn had higher protein content and oil content in the grain (Coşkun et al., 2006).

There are not enough statistics about the cultivation area and production amount of sweet corn in our country. However, the consumption of sweet corn is increasing in our country and the cultivation areas are increasing year by year, especially in Çukurova, Aegean and Marmara regions. In also, it has been reported that even in the northern regions of our country, sweet corn varieties with a short vegetation period will increase the profitability of the farmers in their cultivation as the main and second crop (Özata et al. 2016). Sweet corn cobs can be consumed fresh (boiled and roasted directly), as well as canned or frozen food made from the grains separated from the cobs (Başçiftçi et al., 2012).

Sweet corn is included in perishable product groups due to high respiratory rate, rapid loss of sugar after harvest and quality deterioration (Dayı, 2011). As a matter of fact, it can lose approximately 60% of its sugar content at 30°C and 6% at 0°C in one day. Sweet corn, which loses sugar very quickly at room temperature, rapid cooling and low temperatures after harvest storage can delay the loss of sugar (Dayı, 2011). On the other hand, modified atmosphere packages (MAP) are used to preserve the color, brightness and greenness of the stems and to reduce the weight loss and spoilage of fruits and vegetables during storage and marketing. By changing the air composition surrounding the product during storage with MAP, the O_2 level of environment decreases, accordingly, respiration, enzymatic and oxidative degradation reactions slow down, and microbiological spoilage can be delayed (Çavuşoğlu, 2018). In addition, these packages reduce weight loss by creating high relative humidity in the environment surrounding the product during storage, transportation and distribution of products (Karaca and Şen, 2014). In the direction of this information, it was aimed to determine the effects on the physical and quality of sugar corn varieties during storage.

MATERIAL AND METHOD

Material and Trial Installation

The sweet corn cob samples used in the research were obtained from the sugar corn trials conducted in Isparta University of Applied Sciences, Faculty of Agriculture, Department of Field Crops in 2020 and where traditional care procedures were performed. In the study, Batem tatlı and Kompozit Şeker sweet corn varieties were used as trial material.

In the study, sweet corn varieties harvested during the milk production period were brought to the laboratory without losing time. Then, homogeneous and unharmed corn cobs were selected. The cob leaves of the selected sweet corn were peeled and placed in modified atmosphere bags (MAP). MAP are modified atmosphere bags that have water vapor and gas permeability at certain rates. Sweet corn varieties put in MAP were closed with clips and stored in the refrigerator at +4^{o}C at 90±5% relative humidity for different periods (0, 5, 10, 20, 30 and 40 days). The study was set up in a completely randomized plot design with 3 replications and 6 cobs in each replication.

Measurement and Analysis

Weight loss was determined as percent (%) of the samples whose weights were determined before storage, after they were taken out of the storage, by weighting them with a balance with an accuracy of ±0.05 g. Grain color in the cobs was measured with a colorimeter (Minolta CR-400) from three different points from the equatorial region of each cob, and the Croma (C*) value was calculated to determine the color changes (McGuire, 1992). After extracting the juice of the grains with the help of a juicer, the amount of soluble solid matter content (SSMC) was measured with a hand refractometer (Atago) and expressed as %. The total soluble sugar content was determined according to the phenol-sulfuric acid method and a reading was made in the spectrophotometer at a wavelength of 540 nm (Dubois et al., 1956).

For the dry matter ratio, grains were separated from the cob and weighed 100 g, and when they reached a constant weight in the oven at 65^{o}C, it was weighed again. Then, the ratio of the first weighing to the last weighing was calculated as %. For the ash and crude protein content, grains were first dried in an oven at 65^{o}C until they reached a constant weight and ground in a mill with a sieve diameter of 1 mm and made ready for analysis. The ash content was kept in an incubator at 65°C until the weight of the ground grains was stabilized in order to lose moisture first. Then, for each sample, 3 g samples were processed in a ash furnace at 550°C for 5 hours, and the value obtained was expressed as %. Again, the nitrogen content of

the ground grains was determined by the Kjeldahl method and the crude protein content of the grains was calculated as % by multiplying the value found with the coefficient of 6.25 (Bremner, 1965).

Statistical analysis

The data obtained from the study were evaluated in the TOTEMSTAT statistical package program in accordance with the Completely Random Plots Trial Design, and the differences between the averages were determined according to the Duncan multiple comparison test ($p<0.05$).

RESULTS

Weight Loss and Dry Matter Content

Weight loss refers to the change in weight of the product to be marketed during storage, and the effect of storage time in the study was found to be statistically significant ($P<0.05$). Weight loss of sweet corn varieties increased with increasing storage time. The highest weight loss was determined on the 40th day, and there was no statistical difference between the 40th and 30th days. On the other hand, it was determined that the weight loss of Kompozit Şeker variety (2.23%) was higher than that of Batem Tatlı (2.15%) (Table 1).

Table 1. Means values of weight loss and dry matter content properties as a result of storage of sweet corn varieties for different periods

	Weight Loss (%)			Dry Matter Content (%)		
Time / Type	**Batem Tatlı**	**Kompozit Şeker**	**Means**	**Batem Tatlı**	**Kompozit Şeker**	**Means**
1st day	0.00	0.00	**0.00 D[1]**	27.21 e	28.07 c	**27.64 D[1]**
5st day	0.60	0.74	**0.67 CD**	27.88 e	28.21 c	**28.05 D**
10st day	0.67	1.49	**1.08 C**	31.92 d	31.31 b	**31.62 C**
20st day	2.48	2.21	**2.35 B**	34.90 c	31.52 b	**33.21 BC**
30st day	4.09	4.32	**4.21 A**	36.88 b	31.88 b	**34.38 B**
40st day	5.05	4.64	**4.84 A**	40.66 a	33.71 a	**37.19 A**
Means	**2.15**	**2.23**		**33.24 A**	**30.78 B**	

[1]The difference between the means given with different letters in the same column and row is not significant.

The change in the dry matter content of the sweet corn cultivars during the storage period, the sweet corn cultivars and storage time x sweet corn cultivars interaction were found to be statistically significant ($P\leq0.05$). During storage, dry matter content increased continuously with the moisture and weight loss of sweet corn varieties. As a matter of fact, the lowest dry matter content during the storage period was determined on the 1st day (27.21% and 28.7%, respectively) when it was harvested in Batem Tatlı and Kompozit Şeker sweet corn varieties, and the highest on the 40th day (40.66%, 33.71%, respectively). During the storage period, the dry matter content of Batem Tatlı variety (33.24%) was higher than that of Kompozit Şeker (30.78) (Table 1).

Ash Content and Crude Protein Content

Ash is the unburnable particles that remain after the dry material has been burned at high temperatures for a certain period of time. The sweet corn varieties, storage time and, interaction of storage time × sweet corn varieties ash content were found to be statistically significant ($p<0.05$). In parallel with the increase in storage time, the ash content of sweet corn varieties

decreased. The highest ash content (2.52%) ash rate was determined on the 1th when the sweet corn varieties were harvested (Table 2). On the other hand, the lowest was determined on the 40th day of the storage period and the highest ash content was determined in Kompozit Şeker corn variety with an average of 2.36%.

The change in crude protein content of sweet corn varieties during storage and the interaction of storage time x variety were found to be statistically significant (P≤0.05). In terms of crude protein content, Kompozit Şeker variety (11.40%) was found to have higher values than Batem Tatlı variety (10.29%). During storage, the crude protein content varied between 9.70 and 11.88%, the highest value was determined on the 1st day and the lowest on the 40th day. Crude protein content decreased with the increase of storage time in both corn varieties (Table 2).

Table 2. Means values of ash content and crude protein content properties as a result of storage of sweet corn varieties for different periods

	Ash Content (%)			Crude Protein Content (%)		
Time / Type	**Batem Tatlı**	**Kompozit Şeker**	**Means**	**Batem Tatlı**	**Kompozit Şeker**	**Means**
1st day	2.46 a	2.58 a	**2.52 A[1]**	11.42 a	12.34 a	**11.88 A[1]**
5st day	2.34 b	2.48 b	**2.41 B**	11.06 ab	11.42 b	**11.24 B**
10st day	2.26 c	2.37 c	**2.32 C**	10.85 b	11.39 b	**11.12 B**
20st day	2.14 d	2.32 cd	**2.23 C**	10.00 c	11.21 b	**10.61 C**
30st day	2.03 e	2.25 d	**2.14 D**	9.89 c	11.12 b	**10.51 C**
40st day	1.86 f	2.13 e	**2.00 E**	8.49 d	10.90 b	**9.70 D**
Means	**2.18 B**	**2.36 A**		**10.29 B**	**11.40 A**	

[1]The difference between the means given with different letters in the same column and row is not significant.

Total Soluble Sugar Content and Soluble Solids Content

In the study, the change in total soluble sugar content of sweet corn varieties during storage, interaction of storage time x variety were found to be statistically significant (P≤0.05). In terms of total soluble sugar content, it was determined that Batem Tatlı variety (12.11 mg g^{-1}) had higher values than Kompozit Şeker (11.00 mg g^{-1}). During storage, total soluble sugar content varied between 9.50-13.50 mg g^{-1}. During the storage period, the highest value was determined on the 1st day and the lowest on the 40th day, and the storage days of the 5th and 10th days were included in the same statistical group. The decrease in total soluble sugar content of Batem Tatlı variety during storage was higher than that of Kompozit Şeker variety (15.49% and 13.18% respectively) (Table 3).

The amount of soluble solids matter content of the sweet corn cultivars during storage and interaction of storage time × variety were found to be significant ($p<0.05$). During storage days, soluble solids matter content values of both of sweet corn cultivars generally decreased compared to the initial values. Soluble solids matter content varied between 23.33% and 28.33% during storage, highest soluble solids matter content was determined on the 1st day when the sweet corn was harvested, and lowest on the 40th day. It was determined that the soluble solids matter content of Batem Tatlı cultivar (27.33%) was higher than that of Kompozit Şeker (24.83%). On the other hand, the proportional decrease in soluble solids matter content of Kompozit Şeker variety was higher than that of Batem Tatlı (19.74%, 15.73% respectively). The high proportional decrease in the soluble solids matter content of the Kompozit Şeker variety can be associated with the higher water loss of this variety (Table 3).

Table 3. Means values of total soluble sugar content and soluble solids content properties as a result of storage of sweet corn varieties for different periods

Time / Type	Total Soluble Sugar Content (mg g^{-1})			Soluble Solids Content (%)		
	Batem Tatlı	Kompozit Şeker	Means	Batem Tatlı	Kompozit Şeker	Means
1st day	14.33 a	12.67 a	**13.50 A[1]**	29.67 a	27.00 a	**28.33 A[1]**
5st day	13.00 b	11.67 b	**12.33 B**	28.00 b	27.00 a	**27.50 B**
10st day	12.687 b	11.65 b	**12.17 B**	27.6 b	26.00 b	**26.83 C**
20st day	12.00c	11.00 c	**11.50 C**	27.00 c	24.33 c	**25.67 D**
30st day	10.67 d	10.00 d	**10.33 D**	26.67 c	23.00 d	**24.83 E**
40st day	10.00 e	9.00 e	**9.50 E**	25.00 d	21.67 e	**23.33 F**
Means	**12.11 A**	**11.00 B**		**27.33 A**	**24.83 B**	

[1]The difference between the means given with different letters in the same column and row is not significant.

Grain Color (L and Croma values)

In the study, the change in color values (L* and C*) of sweet corn varieties during storage and the interaction of storage time x variety were found to be statistically significant ($P \leq 0.05$). Color values (L* and C*) of both sweet corn varieties decreased during storage. L* value represents the brightness of the products, and it was determined that Kompozit Şeker variety (72.44) had higher values than Batem Tatlı variety (71.76). While the highest L* value was determined on the 1st day (73.46) during the storage period, there was no statistical difference between the 1st day with the 5th and 10th days. The smallest L value was found on the 40th day (69.09). On the other hand, the Croma (C*) value, which shows vividness in color, varied between 37.50-54.46 during storage. The highest C value was detected on the 1st day and the lowest on the 40th day. C* value of Kompozit Şeker variety (47.26) was higher than Batem Tatlı variety (44.57) as well as L* value (Table 4).

Table 4. Mean values of L* and C* traits as a result of storage of sweet corn varieties for different periods

Time / Type	L*value			C* value		
	Batem Tatlı	Kompozit Şeker	Means	Batem Tatlı	Kompozit Şeker	Means
1st day	73.57 a	73.34 a	**73.46 A[1]**	52.02 a	56.90 a	**54.46 A[1]**
5st day	73.46 a	73.04 a	**73.25 A**	51.03 a	47.66 b	**49.35 B**
10st day	72.94 a	73.01 a	**72.98 A**	47.92 b	46.98 b	**47.45 BC**
20st day	72.62 a	72.42 a	**72.52 AB**	45.88 b	45.89 bc	**45.89 C**
30st day	70.29 b	72.35 a	**71.32 B**	37.26 c	44.42 c	**40.84 D**
40st day	67.67 c	70.50 b	**69.09 C**	33.30 d	41.69 d	**37.50 E**
Means	**71.76 B**	**72.44 A**		**44.57 B**	**47.26 A**	

[1]The difference between the means given with different letters in the same column and row is not significant.

DISCUSSION

In this study, weight loss increased as the storage time increased, and the maximum weight loss occurred on the 40th day in both cultivars (Table 1). Although there was an increase in weight loss of sweet corn varieties during storage, when the weight loss exceeds 5% (4.84%) at the end of the storage period under controlled conditions, it can cause perishable vegetables to wilt and shrivel (Xie et al., 2017). On the other hand, the high relative humidity in MAP plays an important role in limiting the water loss of the products. It has been reported in different studies that the weight loss of sweet corn decreases during storage when combined with storage

conditions or packaging materials (Liu et al., 2021). The data obtained with the literature sources examined are in harmony.

In both of the sweet corn varieties stored under controlled conditions, the dry matter ratio increased with the increase in storage time (Table 1). When the literature was examined, Kara and Şahin (2012) stated that the dry matter ratios of sweet corn varieties stored under controlled conditions (+4^{o}C) increased with the increase in storage time. Because of its high sugar and moisture content, sweet corn is one of the species with the highest respiration rate among all fresh consumable vegetables (Becerra Sanchez and Taylor, 2021). As a matter of ratio, an increase in the dry matter ratio of the sweet corn varieties was observed during the storage period, and it is predicted that this increase was caused by the high respiration rate of the sweet corn and the moisture loss in the grains.

In the study, it was determined that the ash content decreased with the increase in the storage time (Table 2). Liu et al. (2013), stated that the ash content decreased with the increase of storage time in their study in which they stored corn for different periods (0, 3, 6 and 12 months) without application. Bello and Oluwalana (2017), stored huskless corn cobs for 4 days and stated that the ash content decreased from 3.92% to 3.43%. Raw ash contains macro and micro minerals, which have many effects such as the presence of nucleoproteins, which have an important role in terms of their effectiveness on the cell functions of plants, and the intercellular transfer of oxygen. Considering the study, it is in agreement with the data we obtained.

Crude protein content decreased with increasing storage time (Table 2). In the literature, Bello and Oluwalana (2017), in their study in which they determined the effects of packaging on the storage of corn, reported that the crude protein content of husked cobs varies between 10.84-13.10% and the crude protein content decreases with the increase of storage time. The data obtained are in harmony with the literature study examined.

Parallel to the increase in storage time, the change in total soluble sugar content decreased gradually in both sweet corn varieties (Table 3). In the study, it was observed that the decrease in total soluble sugar content of sweet corn varieties preserved with MAP during storage was below 20%, slowing down the sugar content, which is an important disadvantage of sweet corn after harvest, for a certain period of time. Sweet corn is a type of corn with a high respiration rate. If pre-cooling and low temperature storage is not done immediately after harvest, sugar loss and some quality deterioration occur (Olsen et al., 1991; Dayı, 2011). Sugar content decreases with the formation of sucrose and some organic compounds during the storage of sweet corn (Karande et al., 2014). During storage, the amount of sugar decreases and the amount of starch increases (Dayı, 2011). It is reported that the sugar content decreases by 25% in the first 24 hours after the sugar corn is harvested (Kün, 2004). Brecht (2004) reported that sweet corn loses 60% of its total sugar at 30°C and 6% at 0°C in one day. Kara and Şahin (2012) determined that the decrease in sugar content of Lumina F_1 and Sakarya Kompozit sweet corn varieties at the end of the 12th day was between 77.89-80.05% at room temperature and 37.02-35.90% at +4°C, respectively. It has been reported in different studies that the total soluble sugar content of sweet corn decreased significantly with the increase in storage time (Liu et al., 2021). The literature studies examined are in agreement with the findings we obtained.

Soluble solids matter content of sweet corn varieties decreased in general during the storage period (Table 3). During storage, the amount of soluble solids content decreases with the use of sugars in respiration (Koyuncu et al., 2018). A similar decreasing trend in the amount of soluble solids matter content during storage in sweet corn has been reported by many researchers (More et al., 2017).

L* value is a color parameter that is also affected by the darkening reactions of the products and the pigment density, hence the darkening of the meat color (Rosaj-Graü et al., 2006). Post-harvest discoloration occurs in most of the freshly cut products, and this color change has many causes and degrees. The most common color change is browning, which is found in fresh cut products due to oxidation of phenolic compounds. In the study, as the storage time increased, L and C* values decreased and a darkening in the color of the cobs was observed (Table 4). The C* value represents the saturation of the color (0=matte, 60=saturated) (Karan, 2015). Karan (2015) found decreases in L and C* values with the increase in storage time of black nut types. These reviewed literatures are in agreement with this sweet corn study.

CONCLUSIONS

In the study, the physical and chemical properties of the cobs of post-harvest sweet corn varieties were evaluated during storage. When the sweet corn cultivars were stored for different periods, at the end of the 40th day, the ash content, crude protein content, total soluble sugar content, soluble solids matter content, brightness (L) and vitality (C*) values decreased; weight loss and increase in dry matter ratio were determined. When it is desired to store sweet corn at +4^{o}C; when stored for 5 days after harvesting, it was determined that there was no difference in terms of physical properties, and statistical differences were determined in terms of quality. For this reason, sweet corn varieties should be consumed fresh after harvesting, and when it needs to be kept at +4^{o}C, it can be recommended to be consumed within a maximum of 5 days. After the 5th day, it has been determined that there will be great losses in terms of quality and physical properties.

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EFFECTS OF QUINCE ROOTSTOCKS ON VEGETATIVE GROWTH AND YIELD OF 'HAFIF ÇUKURGÖBEK' LOQUAT CULTIVAR

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ABSTRACT

In the research, it was aimed to determine the effects of different quince rootstocks on vegetative growth and yield in intensive loquat cultivation.'Hafif Çukurgöbek' loquat budded on three quince rootstock (BA-29, A, and C) were evaluated in 2018 and 2019 in Hatay, Turkey. The experiment was arranged according to a completely randomized designed with 5 replications and 6 plants were used in each replicate. Vegetative growth parameters [annual shoot length (cm), scion and rootstock trunk diameters(mm) etc.] and yield characteristics of the cultivar/rootstock combinations in the study were determined. Quince-C rootstock gave higher values in terms of annual shoot length, scion and rootstock diameter compared to Quince-A and BA-29 rootstocks. The differences between the rootstocks in terms of vegetative parameters were found to be statistically significant at 1% level. The highest yield values (640 g plant-1 and 0.921 g mm-2, 1.33 ton da-1, respectively) in terms of all three yield per plant, yield per unit trunk cross-sectional area and yield per decare were obtained from the BA-29 rootstock. This was followed by Quince-C rootstock with values of 632 g plant-1, 0.895 g mm-2 and 1.26 ton da-1. Quince-A rootstock gave the lowest values in terms of all three yield elements. This difference between the rootstocks was found to be statistically significant at 1% level. Our preliminary data indicate that dwarfing quince rootstocks can be used in intensive plantings of loquat. In this study, BA-29 and Quince C rootstock performed better than Quince-A. However, it is necessary to continue the work in order to make a definite judgement as to which rootstock is the most suitable.

Key words: loquat, quince rootstocks, vegetative growth, yield

INTRODUCTION

Loquat seedlings, quince, and hawthorn can be used as rootstocks for loquat (Ochse et al., 1961; Hızal et al., 1982; Polat, 1995). Loquat seedlings are preferred over quince or pyracantha rootstocks under most conditions. Loquat trees are 5-10 m tall and form a large crown with high cultivation and harvesting costs. The most effective method of controlling plant height is the use of dwarfing rootstocks.

Dwarfed trees reduce costs of pruning, spraying, thinning and harvest and with high populations have high yields with excellent fruit quality (Polat et al., 2003, 2004). 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 (Polat and Kaşka, 1992a, b; Polat, 1995).

Although quince rootstocks have dwarfing effect on loquat, the effects of quince rootstocks on the fruit yield and quality have not been evaluated on Turkish cultivars. The present study evaluated yield and vegetative growth of 'Hafif Çukurgöbek' loquat budded on Quince-A, Quince-C and BA 29 rootstocks. Here, the first results were presented.

MATERIAL AND METHODS

Material

This study was carried out in Hatay (36º12′E, 36º52′N, 80 m.a.s.l.), Turkey during 2018 and 2019. The experiment area has a typical Mediterranean climate; the yearly average temperature is 18.3ºC, with 1168 mm precipitation which primarily falls during winter and spring. The soil is alkaline (pH:7.76), with very little lime (% 2.4), moderate salt (EC microsiemens: 446) and sandy-loam (%57.37 sand, %25.32 loam and %12 clay).

One-year old 'Hafif Çukurgöbek loquat trees budded on BA-29, Quince-A and Quince-C quince rootstocks were planted at spacings of 1.0 x 0.5 m in January 2017, drip irrigated, with standard cultural practices. Trees were trained according to open-centre system.

The BA-29, Quince-C and Quince-A rootstocks used in the experiment are rootstocks were selected at the East Malling Research station, UK. These rootstocks limit the vegetative growth of budded cultivars and generally trees grow slowly.

'Hafif Çukurgöbek' is an early cultivar with medium-sized, orange-colored fruit, very tasty and sweet was selected in Turkey (Demir, 1987). It is self-fertile and resistant to black spot incited by *Spilocaeae eriobotryae.*

Method

The experiment was arranged according to a completely randomized designed with 5 replications and 6 plants were used in each replicate. The following measurements and analyzes were made in the study.

Vegetative Growth

Vegetative growth of trial plants was measured at three month intervals starting from February 2018. *Annual shoot length (cm):* Four shoots from each plant were measured from 4 sides of the plants. *Trunk diameter (mm):* Scion and rootstock trunk diameters (5 cm below and above of bud union) were measured in all plants with a digital caliper sensitive to 0.01 mm. *Bud union-first branching (cm)*: The distance between the bud union and the first branching on the scion trunk was measured. *First branching - longest shoot (cm)*: The distance between the first branching and the top of the longest shoot on the scion trunk was measured. *Bud union-longest shoot (cm):* The distance between the bud union and the top of the longest shoot on the scion trunk was measured.

Yield

Yield per tree (kg plant^{-1}): Fruit weight of each plant was determined. *Yield per trunk unit cross-sectional area (g mm^{-2}):* Stem cross section was measured 5 cm above the budding point in May of each year. *Yield per area (ton/ha):* Considering the planting distances in the experiment, the yield for area-basis was calculated by multiplying yield per tree with the number of plants.

Data Analysis and Statistics

Analysis of variance (Anova) was used based on a completely randomised design, and the means were separated by Tukey's HSD multiple comparison test at 0.01(Steel and Torrie, 1980). The percentage values were transformed to increase normality by the angle transformation before submitting the data to the analysis of variance.

RESULTS AND DISCUSSION

Vegetative Growth

In terms of annual shoot length, scion and rootstock trunk diameters, Quince-C rootstock showed stronger growth and gave higher values than Quince-A and BA-29 rootstocks (Table 1).

Table 1. Average annual vegetative parameters of 'Hafif Çukurgöbek' loquat budded on quince rootstocks(average 2018 and 2019 years).

Rootstock	Annual shoot length, (cm)	Scion trunk diameter (mm)	Rootstock trunk diameters (mm)	Bud union-first breanching (cm)	First breanching-longest shoot (cm)	Bud union - longest shoot (cm)
Quince-A	39.64 c	18.79 b	23.41 b	24.67 a	54.73 b	79.31 b
Quince-C	74.13 a	28.05 a	32.77 a	18.48 b	87.72 a	106.19 a
BA-29	63.67 b	25.12 ab	30.92 ab	22.92 ab	79.11 a	102.02 a
$HSD_{\%1}$	9.45	7.39	8.49	5.28	9.80	6.20

(x): Means followed by different lowercase letters indicate significant difference by Tukey's test at $p < 0.01$ level.

The Quince-A rootstock had lower values than the other two rootstocks except for the distance between the budding point and the first branching of the stem. In terms of the distance between the budding point and the first branching of the trunk, Quince-A gave the highest value, followed by BA-29, and Quince-C gave the lowest value (Table 1). The differences of rootstocks in terms of vegetative parameters were found to be statistically significant at 1% level.

In the study, it was determined that the first branching in the plants budded on the Quince-C rootstock was lower than the other rootstocks, while it also formed larger plants compared to the plants budded on other rootstocks. Quince-A rootstock, which has the lowest values, has been found to form smaller plants than other rootstocks.

In previous studies, Polat and Kaşka (1992a) reported the shoot growth as an average of 23.4 cm in buddings on the Quince-A rootstock. Polat (1995), in the measurements made between 1993 and 1995, measured the scion diameter in the loquat (cvs. Akko-XIII and Armut Şekilli) saplings whose rootstocks were Quince - A as 18.24 mm, 30.15 mm and 36.39 mm and the sapling height as 74.3 cm, 120.2 cm and 124.4 cm, respectively. The values obtained from our study were lower than the values measured by Polat and Kaşka (1992a) and Polat (1995). It is thought that this is due to the effect of the difference in the cultivar and rootstocks of the trial material plants as well as the age difference.

Yield

The yields of Hafif Çukurgöbek loquat on quince rootstocks are given in Table 2.

Table 2. The effects of quince rootstocks on fruit yield of Hafif Çukurgöbek loquat (average of 2018-2019)

Rootstock	Yield (g $plant^{-1}$)	Yield per unit trunk cross-sectional area (g mm^{-2})	Yield (ton da^{-1})
Quince-A	279.35 b(x)	0.690 b	0.558 b
Quince-C	632.50 a	0.895 a	1.265 a
BA-29	640.00 a	0.921 a	1.330 a
HSD	**	**	**

(x): Means followed by different lowercase letters within a column are indicate significant

difference by Tukey's test at $p < 0.01$(**) level.

According to the two-year averages, the highest yield values (640 g plant^{-1} and 0.921 g mm^{-2}, 1.33 ton da^{-1}, respectively) in terms of all three yield per plant, yield per unit trunk cross-sectional area and yield per decare were obtained from the BA-29 rootstock. This was followed by Quince-C rootstock with values of 632 g plant^{-1}, 0.895 g mm^{-2} and 1.26 ton da^{-1}. Quince-A rootstock gave the lowest values in terms of all three yield elements. This difference between the rootstocks was found to be statistically significant at 1% level (Table 2).

Only four references of high density loquat orchards have been found in the literature. Insero et al. (2004) compared ten cultivars grafted on BA-29 quince and spaced 4 × 2 m. Average cumulative yield from 4th to 8th season was about 45 t/ha. With similar tree density but spacing loquats at 3 × 3 m, Polat et al. (2004) have compared 'HÇG', 'Sayda' and 'Golden Nugget' budded on loquat seedlings. They obtained a mean productivity of 7165 kg/ha during the first three harvests. After two additional seasons, Polat et al. (2005) have published that orchard productivity had raised to 9311 kg/ha/year. In the study by Hueso et al. (2007), to check suitability of loquat to extreme intensification it was designed an orchard of 'Magdal' budded on quince C at a distance of 2.5 × 1.7 m (2353 trees per ha). First yield was reached an average of 2.8 kg/tree on season 2003/2004. Second yield in April 2005 was reached a worthy level of 10.8 kg/tree (25 t/ha). Third yield was limited to 13.0 kg/tree (30 t/ha).

CONCLUSION

The tall and wide crown structure of loquat suggests that high populations of dwarfed trees achieved with dwarfing rootstocks would increase yields per unit area and facilitate cultural practices including harvest. However, studies of dwarfed loquat produced with quince rootstocks are rare in Turkey. In this study, the effects of three quince rootstocks (Quince A, Quince B, and BA-29) on vegetative growth and fruit yield of 'Hafif Çukurgöbek' loquat spaced 0.5 x 1.0 m and planted in January 2017 were compared in 2018 and 2019.

Yield per plant were highest with Quince-C, followed closely by B-29 but yields with Quince-A rootstock were much lower. Yield per unit trunk cross-sectional highest with Quince C and B-29 followed by Quince A. Shoot length, scion and rootstock diameter were greatest with Quince-C. Our preliminary data indicate that dwarfing quince rootstocks can be used in intensive plantings of loquat. In this study, BA-29 and Quince C rootstock performed better than Quince-A. Considering yield, BA-29 rootstock seems the best choice.

Also, in further studies that will be planned, it should be carry out more detailed some studies by adding dwarf rootstocks as well as pyracantha rootstock that is drought-tolerant and loquat seedling rootstock as control for all these rootstocks.

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THE EFFECTS OF COLD STORAGE ON VIABILITY AND GERMINATION LEVELS OF QUINCE POLLEN

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Abstract

In hybridization breeding studies, one of the most important factor determining the success of parent combinations, whose flowering dates do not overlap with each other, is the storage of pollen under appropriate conditions. Knowing the germination and viability of pollen under different storage conditions is extremely important for the process and success of hybridization studies. In this study, the pollen germination and viability of 3 quince genotypes (Genotype 2152, Genotype 2423, Quince A) were determined directly after detonation of anthers in artificial light (fresh pollen) and after storage at +4 °C for 1 month (cold-stored pollen). The interaction of genotype, application and genotype x application was found significant in both examined parameters. Pollen viability rates were determined between 80.89-85.35% in fresh pollen, and between 50.37-81.71% in cold-stored pollen. Only viability of Quince A pollen did not affect after cold storage and had the same statistical level with fresh pollen. On the other hand, pollen germination rates varied between 31.55-58.15% in fresh pollen, while this rate was observed as 6.02-6.82% in cold-stored pollen. Pollen germination rates of genotypes were lower than pollen viability in both fresh and cold-stored pollen. In addition, when the relationship between pollen viability and germination rates was examined regardless of the applications, a positive (R=+0.442) and significant (P≤0.0005) correlation was found between the two parameters. It was determined that the germination rates of the cold-stored pollen decreased at higher rates than the viability rates. Low germination rates will negatively affect the success of hybridization studies. It will be important to determine the most appropriate method with the studies to be done by adding different times and temperatures.

Keywords: *Cydonia oblonga* M., pollen storage, pollen viability, pollen germination.

INTRODUCTION

Cydonia oblonga Miller. is the third important species in terms of world pome fruit production (FAO, 2020). The increase in quince production brings with the increase of new quince varieties and rootstocks with features for different sectors. In recent years, studies on the breeding of quince varieties and rootstocks have increased both in our country and in the world (Stančevic, 1990; Ercan et al., 1992; Bobev et al., 2009; Anonymous, 2015; Şahin et al., 2020 a,b).

Aegean Agricultural Research Institute (AARI) is the first degree responsible institution for the protection of "Turkey Quince Genetic Resources". Quince selection, breeding and adaptation studies were initiated between 1967-2000 within the scope of the "Plant Genetic Resources Project". As a result of these studies, Ege 2, Ege 22, Ege 25 varieties were registered

in 1990 and Altın 35, Zeybek 35 varieties and Quince B-35 rootstock were registered in 2015 (Ercan et al., 1992; TTSM, 2015; Şahin and Mısırlı, 2016).

In 2016, resistance breeding studies were started with selection breeding method with the project "Fire Blight Tolerant Quince Breeding (Project number TAGEM/BBAD/16/A08/P03/04)" (Şahin et al., 2016) and the second 5-year period of the project "Fire blight disease (*Erwinia amylovora* Burril.) tolerant quince breeding (II- part)" (Project number TAGEM/BBAD/B/21/A1/P3/2697) continues with crossbreeding studies (Şahin et al., 2021). Within the scope of this project, it is planned to obtain a total of 29.000 hybrid individuals from 40 different hybridization combinations, determined according to their resistance to fire blight, and to proceed to the 2nd stage of selection with 1.000 individuals with high disease resistance (Şahin et al., 2021).

The most important factor especially in crossbreeding breeding studies for disease resistance breeding is that the flowering dates of the selected parents coincide with the same time. The high genetic diversity of quince genotypes requires pollen storage due to the asynchronous blooming period among the genotypes. Flowering in quince occurs between March and May, depending on the genotype and geographical region. When the effective flowering period is examined, it is seen that there is a period of approximately 20-30 days between quince genotypes. For parents whose flowering period does not overlap in crossbreeding combinations, it is important to store pollen under suitable conditions without losing viability and germination. So knowing the pollen viability and germination rates under different storage conditions is important for success of hybridization.

There are many studies on pollen viability and germination in different fruit species by applying different media and concentrations (Eti et al., 1998; Dorukoğlu and Aslantaş, 2013; Eroğlu and Mısırlı, 2016; Aksoy and Dalkılıç, 2019; Luo et al., 2020). In order to keep the vitality and germination rates of the pollen at sufficient levels for pollination and fertilization during the hybridization studies, pollens are kept at different temperatures. Studies on the storage of pollen under cold conditions have increased in recent years. When the studies are examined, it is seen that storage is gathered under two main headings; 1- short-term storage that is done at especially 0 °C, + 4 °C and room temperature, and 2- long-term storage that is done at especially -20 °C, - 70 °C, - 80 °C, and -196 °C (cryo-stored) in different time periods (Martínez-Gómez et al., 2002; Aiqin et al., 2010; Dutta et al., 2013; Novara et al., 2017; Mesnoua et al., 2018; Özcan, 2020).

In this study it was aimed to determine the possibilities of using quince genotypes with early flowering characteristics as paternal parents to genotypes with late flowering characteristics by using short-term pollen preservation (+4 °C for 1 month) method.

MATERIAL AND METHOD

Plant material and pollen collection

The experiments were carried out with using 3 quince genotypes (Genotype 2152, Genotype 2423, Quince A (rootstock) that located at Quince Field Gene Bank of Aegean Agricultural Research Institute at Menemen/İzmir in March, 2021. Quince flower buds at pink balloon stage were collected approximately between 9:00 and 10:00 a.m. For each genotype nearly 75 flower buds were collected from 3 trees and quickly brought to the laboratory in a cooler container. Using forceps, anthers were transferred to petri dishes and dehisced under an incandescent lamp during overnight (Hesse, 1975). In the next morning, the pollen grains were collected in Eppendorf tubes.

Pollen storage conditions

To test the storage conditions, collected pollens were divided into two samples: fresh pollen and cold-stored (+4 °C for 1 month) pollen. Pollen viability and germination was assessed immediately after collection and after 1-month storage.

Pollen viability and germination tests

TTC (2,3,5-triphenyl tetrazolium chloride) test was used to determine pollen viability (Oberle and Watson, 1953; Norton, 1966; Eti, 1991) and 1% TTC solution was added on the slide, and the pollen was sprinkled with a brush and then covered with a coverslip. After 2 hours, counting was made under the microscope (Carl Zeiss ERC 5) at 20X magnification, and those that were stained pink were considered alive, and light-colored and unstained pollen were considered inanimate.

Pollen germination tests were all carried out *in vitro* using the agar-in-petri method, which consisted of 1% agar + 15% sucrose (Sütyemez and Eti, 1995) and pH adjusted to 5.4 by using a digital pH-meter. Pollens planted in germination medium in petri dishes were kept at 24-25 °C for 6 hours to germinate and then counted under the microscope. Pollens with tubes exceeding their radius were noted as germinated.

Statistical analysis

The study was set up in a randomized plot design. For pollen viability tests, 2 slides for each genotype and 10 randomly selected areas were counted on each slide, and for pollen germination tests, 2 petri dishes and 10 areas in each petri were counted.

All data analysis was performed using JMP version Pro 13.0 statistical software. The effects of the genotype and application were analyzed using two-way ANOVA. Angle transformation was applied to the obtained data and the analysis of variance was compared with the LSD test (Steel and Torrie, 1980; Yurtsever, 1984). Pairwise correlation analysis was applied to determine the relationship between pollen viability and germination.

RESULTS AND DISCUSSION

Genotype, application and genotype x application interaction were found significant in pollen viability and germination parameters (Table 1). Since the genotype x application interaction was important, main effects were not considered, and comments were made on interactions.

Table 1. Mixed factorial design showing the effect of cold-storage temperatures on pollen viability and germination rate

Source	**Pollen viability**		**Pollen germination**	
	F Ratio	**Prob > F**	**F Ratio**	**Prob > F**
Genotype	13.8978	**<.0001***	8.0587	**0.0009***
Application	300.8703	**<.0001***	30.3661	**<.0001***
Genotype x Application	12.3048	**<.0001***	6.7836	**0.0024***

Pollen viability rates were determined between 80.89-85.35% in fresh pollen, and between 50.35-81.71% in cold-stored pollen. Only viability of Quince A pollen did not affect after cold storage and had the same statistical level with fresh pollen (Table 2). Genotype 2152 had the lowest viability after storage +4 °C for 1 month. In terms of this parameter, the difference between two applications is clearly seen in Figure 1.

Table 2. Effects of genotype and application on pollen viability of quince

Genotype	**Application**	
	Fresh pollen	**Cold-stored pollen (+4 ºC for 1 month)**
2152	80.89 a	50.37 b
2423	85.35 a	60.77 b
Quince A	83.08 a	81.71 a

Pollen viability was observed significantly different among genotypes and pollen storage temperatures. This is an expected result within studies clearly demonstrated that the viability rates vary according to the fruit species, genotype, year, cold storage, and method (Oberle and Watson, 1953; Parfitt and Ganeshan, 1989; Bolat and Pırlak;. 1999; Chaudhury et al., 2010; Dalkiliç and Mestav. 2011; Bükücü et al., 2018; Luo et al., 2020; Normasiwi et al., 2020).

Pollens have main role in sexual reproduction of seed-bearing plants and its viability is essential for successful pollination and fertilization (Boavida et al., 2005; Mao et al., 2019). Different staining methods can be used to estimate pollen viability like TTC, blue ink dyeing, methylene blue, I2-KI, acetic carmine.

Luo et al. (2020), were used 9 staining methods for determining pollen viability for *Castanea mollissima* and *Castanea henryi* and only Benzidine-H_2O_2 and 2,5-diphenyl mono tetrazolium bromide (MTT) dyes were found to be suitable. When the studies on quince were examined, it was observed that IKI (Dalkiliç and Mestav, 2011), aniline blue (Radović et al., 2020) and like our study TTC (Erdem and Çekiç, 2016) were used successfully.

Pollen features of Nonpareil, Ne Plus Ultra, Sonora, and Peerless almond cultivars was evaluated and after 2 months' storage at 4 °C pollen viability of decreased nearly 80% (Martínez-Gómez et al., 2002). In another study in which viability was determined by the IKI test, it was determined that there was a statistically significant difference in the viability rate of between 7 quince genotypes. While the average viability rate was 95.3 % in fresh pollen, it decreased to 91.3 % in cold-stored pollen (4 °C for 14 days) (Dalkiliç and Mestav, 2011).

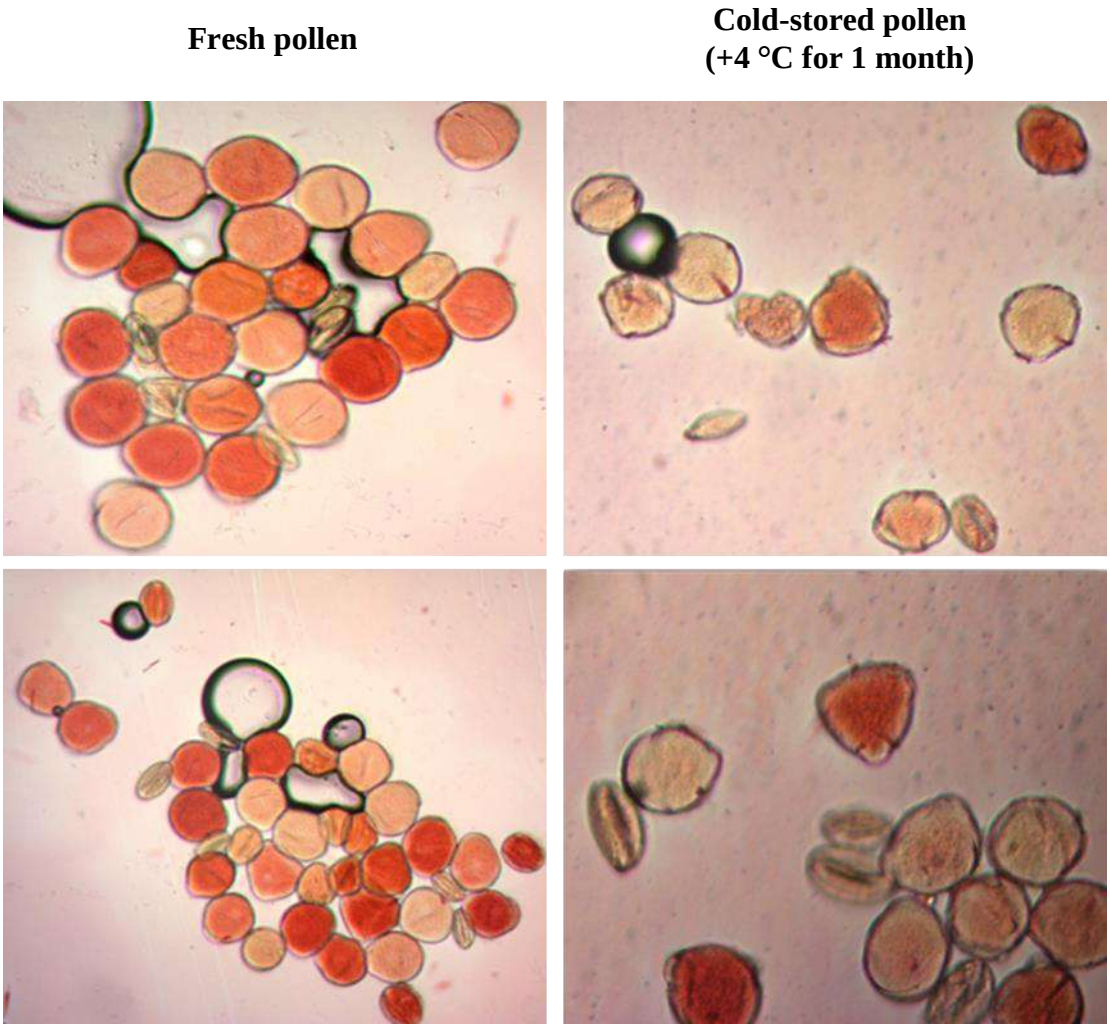

Figure 1. Differences between fresh and cold stored pollen viability on Genotype 2152

In addition to pollen viability, pollen germination was significantly different among genotypes and pollen storage temperatures (Table 3). While pollen germination rates varied between 31.55-58.15% in fresh pollen, this rate was observed as 6.02-6.82% in cold-stored pollen. The high percentage of germination was recorded at Genotype 2152 for fresh pollen. The lower ratios were observed at cold stored pollen of all studied genotypes at same statistical level. It was also determined that the germination rates of cold-stored pollen decreased at higher rates than the viability rates.

Table 3. Effects of genotype and application on pollen germination of quince

Genotype	**Application**	
	Fresh pollen	**Cold-stored pollen (+4 °C for 1 month)**
2152	58.15 a	6.83 d
2423	41.35 b	6.26 d
Quince A	31.55 c	6.02 d

Quince A had the lowest germination after storage +4 °C for 1 month with the same statistical level with other two genotypes. The difference between the two applications of Quince A is clearly seen in Figure 2.

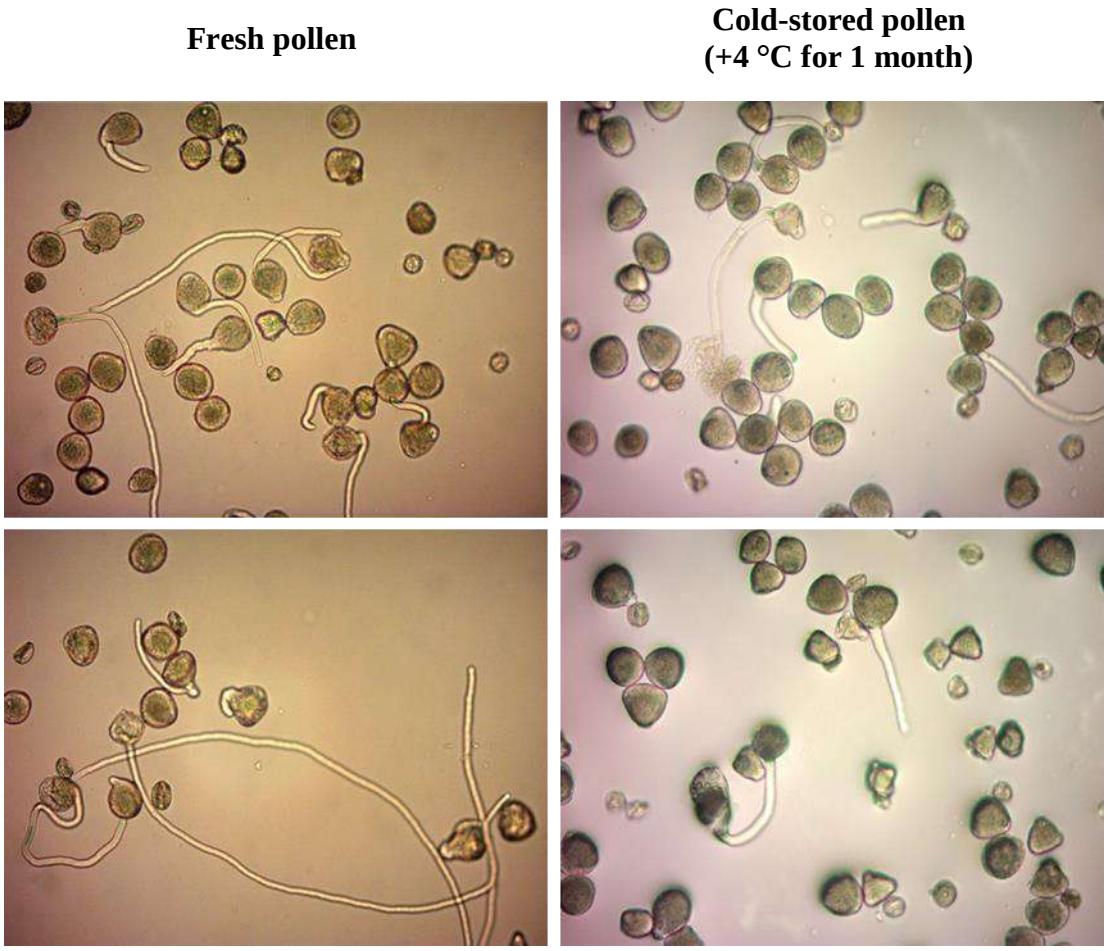

Figure 2. Differences between fresh and cold stored pollen germination on Quince A rootstock

Like pollen viability, pollen germination is also depending on species, cultivars, storage conditions, method, medium, and chemical concentration of ingredients (Bolat and Pırlak, 1999; Dutta et al., 2013; Martínez-Gómez et al., 2002; Ćalić et al. (2021). The pollen germination rates of fresh pollens ranged between 57.83-84.42 % in apricot cultivars and 52.40-66.60% in sweet and sour cherry cultivars at 15% sucrose concentration of agar-in-petri method (Bolat and Pırlak, 1999). In another study using the same method (15% sucrose concentration, agar-in-petri method), germination rates of quince and apple varieties were determined between 55.47- 71.95 % in fresh pollens that collected at flower buds at early balloon stage (Erdem and Çekiç, 2016). In studies conducted on *Diospyros kaki*, it has been determined that the pollen germination rate varies between 3.4 and 68% (Yakushiji et al., 1995; Krisanapook et al., 2004; Evrenosoglu et al., 2011; Sağır et al., 2012).

Ćalić et al. (2021), were observed pollen germination ratios of fresh and cold stored pollen (storage +4 °C for 1 month) by using modified hanging drop technique in four autochthon apple cultivars. Like our study, their results showed that germination rates significantly decreased (up to 43.33%) depending on apple cultivars after cold storage.

Six date palm cultivars under three different storage temperature (+4 °C , +20 °C , -20 °C) conditions were studied and germination rates of fresh and cold stored (2 months at +4 °C) pollens were ranged 87.6-97.0% and 44.6-98.6%, respectively. As seen, while the germination of some cultivars decreased in cold storage, contrary to our study, an increase was observed in some cultivars (Mesnoua et al., 2018).

When we looked at Table 2 and 3, it was seen that the viability rates were higher in both fresh and cold stored pollen compared to germination rates. The result obtained in the present study was in agreement with results obtained by other authors (Bolat and Pırlak, 1999; Dalkiliç and Mestav, 2011; Eroğlu and Mısırlı, 2016; Özcan, 2020).

In addition, when the relationship between pollen viability and germination rates, regardless of the applications, was examined, a positive (R=+0.442) and significant (P≤0.0005) correlation was found between the two parameters (Table 4).

Table 4. Correlation coefficients between pollen viability and pollen germination rate

Variable	by Variable	Correlation	Signif Prob
Pollen viability rate	Pollen germination rate	+0.442	0.0005*

In a similar study on peach, a significant and positive correlation was determined between both IKI and TTC tests and TTC and germination tests (Eroğlu and Mısırlı, 2016). Also in *Rubus* spp. pollen germination and viability was positively correlated (R=+0.537) with ($p < 0.01$) significant level (Normasiwi et al., 2020).

CONCLUSSION

Its known that storage temperature of pollen varies significantly on the basis of species and even cultivars. As a result of the storage of pollen at +4 °C, although the viability rates are within acceptable limits, low germination rates will adversely affect the success of hybridization studies. Since there are no reports of on long term storage of quince pollen, this report aimed at providing information for best storage conditions and necessity of testing storage at -20 and -80 degrees have been put forward. It will be important to determine the most appropriate method by adding different times (1, 2, 3, and 4 week) and temperatures (-20 °C, -80 °C).

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MICROPLASTICS IN FRESHWATER ECOSYSTEMS: BIODEGRADATION AS POTENTIAL SOLUTION

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ABSTRACT

Using plastics in our daily activities increases the accumulation of microplastics (MP) in the environment and become major pollutants due to their resistance to the environmental factors. All plastics with <5 mm in size is defined as MP. They are primary MP when they are manufactured within such size, and secondary microplastics when they are generated by the fragmentation of plastic under physical or other degradation ways. Their distribution over the environment has an impact on public health. They have been reported even in some pregnant women placentas. Even though their sources are still unclear, water consumption or a food chain system may be one such source. Freshwaters constitute an important key in human life. Because they are constituting sources of drinking waters and shelter different organisms consumed by human and which also contribute to different phenomena like climate change and the depollution of the environment. However, the presence of microplastics in lakes, rivers and other freshwater ecosystems has been reported with effects on the *in-situ* organisms. Through the food chain system or water consumption, organisms from other ecosystems, including humans, can also be affected. Furthermore, MP can act as vectors of pathogenic microorganisms or other parasites from freshwaters to other ecosystems (terrestrial and marine). In order to reduce these pollutants, their biodegradation can be a potential solution. In fact, this study will generate a discussion on the impacts of microplastics on freshwater ecosystems before elaborating in detail the question of their biodegradation, especially that ensured by micro-organisms (bacteria and fungi). With that, the study contributes to the evolution of research on the problem of microplastics in freshwater ecosystems for a rapid and potential solution to this major global problem.

Keywords: Biodegradation, freshwater ecosystems, Microplastics, Primary MP, Secondary MP

INTRODUCTION

Plastics are widely used in the production of different items in this modern society. Starting from electronic items until our daily used items such as food/water and cosmetic containers. The global plastic production reached about 348 million tons in 2018 (https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics_the_facts_2018_AF_web.pdf). By 2050, plastic wastes are estimated to reach up to 26 billion ton (Ru et al., 2020). The increase of polymer production is related to its consumption. The high consumption of these polymers and their physic-chemical properties such as slow biodegradation rate cause their accumulation in the environment and become one of the major global ecological problems. Management of plastics is based on their recycling; however, this solution is until know not enough to avoid the presence and the ubiquitous distribution of these polymers in the

environment. Although the large plastic pieces can be recycled, they may after that generate toxic materials as well as microplastics (plastics <5mm) which cause other environmental problems. On the other hand, there are the primary microplastics or nano-plastics that are responding unaffected by the recycling using tools (Yang et al., 2021).

The plastic debris observed in the environments are significantly divers. It is due to the variety of the used plastic materials such as polyethylene, polypropylene, polyurethane, as well as their size and shape. All these properties should be considered during the study of these pollutants ending up. Because their distribution and impacts on the environment are also depending on the previous parameters (Frere et al., 2017). The distribution of microplastics over the deep of the ocean and high mountains is approved and main sources are thought to be wind, rivers, rain, or anthropogenic activities (Miri et al., 2022). Even-though the freshwaters are among the microplastic sources of marine ecosystems, negative impacts of MP in freshwater ecosystems should not be neglected. These ecosystems housing aquatic biota that are also consumed by human, the impact on that fauna can indirectly through the food chain pathway affect the human being. On other hand, some of these freshwaters constitute main sources of drinking waters. The presence of microplastics in drinking waters is approved and reported for the first time in 2017 and a failed treatment of the sources is thought to be one of the causes (Eerkes-Medrano et al., 2019).

Although the study on MP in freshwater systems are limited comparing to those of the marine ecosystems (Sarijan et al., 2020), the occurrence and distribution of these particles in different freshwater systems over the word has been reported through different studies. Sarijan et al., (2020) have reviewed data and reported that the most studied freshwater systems were in Asia especially in China. These data do not indicate the most contaminated areas, since studies are very limited in some areas like in the biggest continent in the world (Africa) (Khan et al., 2018). From the water surface to the sediments, plastic particles are distributed depending to their chemical and physical compositions (Sarijan et al., 2020; Pico et al., 2018). It is also noted that the environmental chemical and physical properties such as the organic matter present in the sediment of water surface are also affecting the distribution of microplastics in the environment (Pico et al., 2018).

In the Lake Ulansuhai in China, the surface waters have been reported to be contaminated with microplastic concentrations ranging from 1760 ± 710 to $10{,}120 \pm 4090$ n/m3 with a heterogeneity of the distribution (Wang et al., 2019). Authors shown also that the main type of microplastics observed in this water surface was coloured, fibers, and smaller than 2 mm constituted of polyethylene, polystyrene, polypropylene, and polybutylene terephthalate. Another study shown an abundance of 0.21 to 19.1 particle/m3 of microplastics recorded in Mlwaukee River to Lake Michigan (Lenaker et al., 2019). Along the water colon and sediment, the distribution was observed to be dependent to the particle density and shapes (fragment, pellet/bead, fiber/line, film, or foam). In the water surface, the main observed polymers consisted of black foams with lower density values. On the other hand, the water subsurface was dominated by fiber or line particles with low- or high-density values. The black foams with high density values dominated the sediment samples. However, polymers with lower density have been few detected in sediment samples. In European countries, the Rhine-Main River in Germany was found to housing the highest microplastic concentration in sediments with 4000 particles/Kilogram (Sarijan et al., 2020; Klein et al., 2015). In the North of Africa, seven streams located at Bizerte City in Tunis have been analysed and results showed that the abundance of microplastics contaminating these areas was ranged from 2340 ± 227.15 to 6920 ± 395.98 particles/Kilogram (Toumi et al., 2019).

Both primer and seconder microplastics are contaminating the natural environment through human activities. The primer microplastics are mainly come from cosmetic and hygienic products such as microbeads present in face cleansers or toothpastes. These particles together with fibers from textiles are transported through domestic effluents to soil, freshwater, and marine ecosystems. The wastewater treatment plants (WWTP) are known to be an important source of microplastics in the environments. The filtered microplastics and other waste particles (sludge) are applied on the land as fertilizers, however they are accumulated and transported through rivers or rainfall to other aquatic ecosystems such as lakes and other water bodies(https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics_the_facts_2018_AF_web.pdf ; Mahon et al., 2017; Rolsky et al., 2020). Freshwater systems near factories (highly activated areas) and high people density are estimated to be more contaminated by microplastics (Browne et al., 2011; Zhou et al., 2020).

IMPACTS OF MICROPLASTICS IN FRESHWATER ECOSYSTEMS

The microplastics as well as the nano-plastics have low affinity to penetrate the tissues or cells of some aquatic organisms. Once organism's uptake these small pieces, tissues or organs may be teared or become injured; in this way, the path for other pieces or organisms (can be pathogens) to enter the organism /cells is obviously open and can also cause death of some organisms (Scherer et al., 2018; Mateos-Cárdenas et al. 2021). Sarijan et al., (2020) have reported from 14 experimental studies that different type and shape of microplastics have been detected in more than 50 species of freshwater fishes from different freshwater ecosystems in the world. These particles have been detected through different detection methods in the gastro-intestinal tract, the head, and other organs, and tissues. The adsorption of MP on the surface and in the internal of plants present in freshwaters has been reviewed and literature shown the presence of different microbeads in different plant roots like roots of *Lepidium sativum* (Mateos-Cárdenas et al., 2021; Bosker et al., 2019) and *L. minor* (Mateos-Cárdenas et al., 2021; Kalčíková et al., 2017a, 2020). Authors also shown that MP are also fragmented by amphipods in the freshwater ecosystems. On the other hand, MP can be a vector of chemicals or pathogens which can have negative impacts on the freshwater biota. In addition, during the degradation of monomers/plastic additive materials, toxic compounds can be released and affected the *in-situ* organisms (Jeyavani et al., 2021).

The MP present in freshwater ecosystems is affecting not only the contaminated ecosystems but other like terrestrial and marine ecosystems are also impacted. Through the food chain, human may consume fish or aquatic fauna that have uptake MP. However, it can be through a contaminated food chain or contaminated drinking water. In vitro experiment shown that 10 mm and some nanometres of microplastics cause cytotoxicity and oxidative stress in human brain cells and epithelial cells (Schirinizi et al., 2017; Fu et al., 2020). Deng et al., (2017) have been also shown the possibility of accumulation of microplastics in the kidney, gut, and liver of mice which cause oxidative stress and neurotoxicity in the animal. Fu et al., (2020) have reported that plastic particles >150 mm are speculated to can be excreted from the body, while those <150 mm may translocate enter the circulatory system. These particles can cause cell damages and organs damages like in the aquatic organisms. Besides, the other chemical or microorganisms which are adsorbed by or attached to the microplastics may also cause negative effects on human health (Liao and Yang, 2020). There is still many studies to be done to understand the factors that affect the introduction of microplastics in human body as well the toxicological effects that can cause on the human health.

In addition, MP of the freshwaters can migrate to the marine ecosystems through runoff of rivers or other freshwaters. During this migration, MP can also acquire on their surfaces some pathogens, *ex-situ* microorganisms or, toxic chemicals to the marine ecosystems that will

impact on the marine biota (Jeyavani et al., 2021). On other hand, using freshwater systems as source of water irrigation may constitute a source of microplastic contamination on soils (Xu et al., 2020). In this context, microplastics accumulated on the soils, due to their hydrophobic property, inhibit the soil filtration of water that induce the degradation of soils. Besides, as it was observed in many studies on the impacts of MP on soils and terrestrial ecosystems, microplastics can enter to soils through water irrigation and interact with the soil biota that can cause ecotoxicity of these organisms (Ambumani and Kakkar, 2018). Panno et al., (2019) highlighted the presence of microplastics in two karst aquifers in Illinois, USA with high concentration (>16 particles per litre). Here, the contamination of ground waters is highlighted. It was mentioned that 25% of the global drinking water in Illinois is supplied by the Karst aquifer (ground waters) alone (Wong et al., 2020). It is important to note that most of the inland/ground waters are used as potable water sources and are also crucial for the local economy and development, since most industrial and agricultural sectors are depending to those waters. The contamination of these waters by microplastics may induce both economic and social crisis.

BIODEGRADATION OF MICROPLASTICS

The accumulation of microplastics on natural environment is due to that these particles are undegradable. Degradation tools have been designed but unfortunately this contamination still inestimably increase in the nature. Beside the inefficacity of the physical and chemical degradations, these methods release other environmental dangerous materials. Investigations are rewarded to find natural, completely and environment friendly methods. In this context, algae, fungi, and bacteria are studying for their ability to use plastic materials as growth nutrients, for energy production or, for the synthesize of new macromolecules for cellular protection. Microorganisms are known to use these strategies in the presence of organic materials (pollutants) such as hydrocarbons to faith against the stress present in their environment (Othman et al., 2021). The biodegradation of these materials consists of enzymatic reactions series activated by the presence of substrates in the environment where these organisms are inhabiting (Sarijan et al., 2020).

Biodegradation mechanism starts by the contact of microorganisms and the surface of the MP piece: this contact induces secretion of extracellular enzymes which will adhere on the surface and cause the cleavage of the polymer main chain. This process ending with the production of low molecular weight fragments: the fragmentation step. These fragments will be used by the microorganisms for their growth or energy production and after that some of their products may be assimilated in their cells and biomineralized (Alshehrei, 2017; Miri et al., 2022). Different and specific enzymes are involved in each step of the biodegradation. However, few of these enzymes are known with limited information on their mechanisms.

Microorganisms isolated from different ecosystems have been studied and we expect that the most of those that can degrade these hydrocarbons are still undiscovered. We should note that different microorganisms may acting together or successively for the degradation of one polymer particle. Since some microorganisms have the biofilm formation ability on the MP and upon reducing the hydrophobicity of the particle, those which are uncapable to attach their cells on the microplastic continue the degradation of the different fragments. By this fact, a cocktail of microorganisms may be produced for a complete biodegradation. Shimoa et al., (1986) have reported that a strain of *Pseudomonas putida* can degrade poly(vinyl-alcohol) in the presence of another strain of this species. It is due to the secretion of the growth factor (Pyrroloquinoline Quinone) in the presence of this polymer by the non-degrading strain for the degrading bacteria. The degradation of microplastics by microorganisms isolated from freshwaters was reviewed by Sun et al., (2021). Authors confirmed at that time that

microorganisms with microplastics degradation ability are not yet identified from freshwater ecosystems. However, even the studies are limited, the effects of bacteria isolated from freshwater fish species on MP as well as the interaction between MP and freshwater microalgae have been studied and biodegradation reaction has been demonstrated (Amadi and Nosayame, 2020; Adian et al., 2019).

One of the most used plastic types is the polyethylene. The biodegradation of this polymer was observed to involve a diversity of bacteria isolated from different sources like marine sediments, worm's guts, aquatic organisms, and soils. *Enterobacter asburiae* YT1 and *Bacillus sp.* YP1 isolated from the gut of the larvae of *Plodia interpunctella* (waxworm) have been observed to degrade polyethylene pieces after 28 days of incubation (Yang et al., 2014). The results demonstrated by Atua et al., (2017) shown the ability of the two mangrove isolated Bacillus species (*B. cereus* and *B. ghotheitii*) to participate in the removing of plastics including polyethylene, polypropylene, and polystyrene from the environment. Some bacteria like Pseudomonas species that contain *alkB* genes which encode enzymes for hydrocarbon degradation have shown a decrease effect on the polyethylene weight loss (Ru et al., 2020). In addition, bacteria like *Rhodococcus ruber* C208 have been reported with the laccase enzyme activity which play a role in the biodegradation of LDPE (low density polyethylene) films. Fungi species like *Aspergillus oryzae*, *Aspergillus fumigatus*, *Aspergillus nidulans,* and *Zalerion maritimum* isolated from soil and marine ecosystems have been observed for their impacts on polyethylene particles (Paço et al., 2017; Muhonja et al., 2018).

Polypropylene is another type of plastic material frequently found in the natural environment as accumulated pollutants. Its biodegradability by microorganisms was found to be limited than the other plastic types. Ru et al., (2020) have noted that no enzyme has not yet determined for the degradation of polypropylene microplastics. However, some bacteria, even microalgae have been reported to decrease PP microplastics weights with a modification of their chemical structure. Among the bacteria, *Bacillus lentus*, *B. licheniformis* and *S. epidermidis* isolated from the organs of a fish harvested from Ohiakwu estuary in Rivers State in Niger have been determined by Amadi and Nosayame, (2020) as PP decomposer. On other hand, the interaction of the microalgae *Spirulina sp* the microplastics (polypropylene and polyethylene terephthalate) in freshwater environment was evaluated in laboratory. This study resulted in a decrease of the tensile strength of PET and PP. However, the when the concentration of microplastics increased, the growth of the microalgae decreased (Adian et al., 2019). This indicates the ability of the *Spirulina sp* to decompose microplastics but with a certain contaminating concentration, these particles affect the life of these microorganisms.

Polyethylene terephthalate are common plastic materials used in the production of water bottles, clothes fibers and others. This polymer is considered as a relatively low biodegradable MP (Miri et al., 2021). Enzymes involving in the MP biodegradation are mainly determined for PET degradation. Extracellular enzymes involved in the PET degradation including PETase, hydrolases, MHETase, TfH, FsC and, Tcur0390 (Miri et al., 2021). These enzymes are isolated from microorganisms including bacteria and fungi species. The *Ideonella sakaeinsis* has been identified for its ability to adhere to PET and produces the extracellular enzyme (PETase) that hydrolyses the polymer to produce the Mono(2-hydrxyethyl) terephthalic acid (MHET) and the terephthalic acid (TPA). This bacterium produces the MHET hydrolysing enzyme MHETase that hydrolyse MHET to TPA and ethyl glycol that are both PET monomers which are then transported to the cytoplasm of the bacterium to be mineralized (Miri et al., 2021; Yoshida et al., 2016). Other microorganisms that have shown PET degradation ability including *Alteromonas macleodii, Celeribacter neptunius, Pseudoalteromonas citrea, and Oleispira antarctica* RB-8 (Miri et al., 2021; Webb, 2012., Danso et al., 2018b, a).

Either using the whole cells or microbial enzymes, the biodegradation de MP can be limited by numbers of factors. The environmental conditions such as temperature and pH during the treatment can affect the enzymatic reactions (Alshehrei, 2017). In this context, studies in the biodegradation of microplastics should be done from different environments especially the freshwater ecosystems in which still far from terrestrial and marine ecosystems in term of studied environments. Other factors including the microbial diversity available in the polluted areas (Alshehrei, 2017; Shabbir et al., 2020). As we previously said, microorganisms can complete each other for a complete microplastic degradation. On the other hand, it is shown through different studies that the chemical or physical pre-treatment of MP improve the biodegradation mechanism, since these treatments improve the hydrophilicity of the polymers (Shabbir et al., 2020).

CONCLUSION

In summary, microplastics are in growing contaminate all the natural and artificial environments due to the overuse of plastics as well as the non or low degradability property of these polymers. Anthropogenic activities are the main source of these contaminants in the different ecosystems. From the land activities, MP are transported through rivers, domestic and industrial effluents, and rainfalls to freshwater ecosystems. Inside the freshwater source, activities like fishing in lakes and rivers are also contribute to the input of MP into those ecosystems. These MP are interacted with the fauna and flora of these ecosystems and cause different negative impacts on that biota. As the freshwaters constitute many sources of drinking waters, in addition to the food web chain humans are directly or indirectly affected by these polymers. Techniques for MP management are multiplied, and biodegradation seems the best solution for a complete degradation of these polymers. However, studies are limited especially in freshwater ecosystems. Environment friendly pre-treatments of MP before their incubation with enzymes of hole microorganism may introduced in the studies for improving biodegradation methods.

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SEASONAL CHANGES IN AN ECOLOGICALLY IMPORTANT WETLAND, IMBROS LAGOON BETWEEN 2016 AND 2020: EVIDENCE FROM HIGH RESOLUTION SATELLITE IMAGERIES

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ABSTRACT

The Imbros Lagoon is known to be one of the tree coastal salty lagoons in Turkey. Due to its geographic location, vegetation and water related properties, the lagoon serves as a precise zone especially for migrating bird species, in different times of the year. Increasing of evaporation in summer season manipulates the status of this specific zone and as result of the process the salt became visible whereas it becomes a hotspot for tourism activities in dry season. However, environmental issues that threat the wetland are reported whereby the most highlighted one is the impacts of climate change which would affect not only water but also biodiversity in return. In this context the study aimed to investigate the alternations in surface water area of the lagoon in respect to different seasons between 2016 and 2020 using Sentinel 2 imageries. Different water related indices and their combinations were used to identify the most discriminative one. Findings revealed that there are obvious changes in water area of the lagoon in all seasons in coherency with meteorological data.

Keywords: Ecology, High Resolution Satellite, Imbros Lagoon, Seasonal change, Wetland.

INTRODUCTION

Wetlands are known to have significant functions in the environmental systems such as water storage, nutrient processing, and these areas are considered to be one of the most vulnerable ecosystems against human activities (Milennium Ecosystem Assessment, 2005; Ramsar Convention, 2016; Slagter et al., 2020). Moreover, wetlands provide living habitats for different kinds of plant, animal and microorganisms, and thus, being a supporter of biodiversity in a certain area (Mosime and Tesfamichael, 2017). The functions and statuses wetlands are reported to be strongly related with weather conditions whereby they provide water in dry seasons by storage function in the wet seasons by also helping to avoid flooding by excessive rains (Vilardy et. al., 2012, and Torres-Bejarano et al., 2020). One of the most important threats for maintanence of wetland existence seems the consequences of climate change. Several studies have demonstrated that water levels of many wetlands have a reduction tendency due to changing climatic situations (Inalpulat and Genc, 2019). Therefore monitoring of dynamic alternations in wetlands provides valuable information for supporting the conservation of

ecosystems. In this context, facilitating from remote sensing data and image processing techniques provides fast and reliable results. On the other hand, there are some limitations for identification of coastal wetlands which are usually located between fringe zone of sea and land due to difficulties on differentiation between complicated land cover and land use types and objects of wetlands that exhibiting mixed spectral characteristics due to variance in water characteristics such as level, salt, and vegetation existence, as cited by Zhang et. al (2019).
In present study, it was aimed to determine whether there are seasonal changes in water surface area of Imbros Lagoon, one of the third salty coastal lagoons in Turkey, were evaluated between 2016 and 2020. The study was mainly focused on the discrimination between wet, high moisture, low moisture, and dried areas. The performances of different indices derived from bands of Sentinel-2 imageries for this discrimination process were evaluated.

MATERIAL AND METHOD

The study was conducted in Imbros Island, which is located in the Aegean Sea, Canakkale Province, Turkey (Figure 1). The area one of the three nationally important salty coastal lagoons is valuable in many terms such as, fauna and flora biodiversity. The area provides the salt for animal needs. In addition, the area is important touristic place due to activities like surfing and bird watching, and also natural properties of salty lagoon. Also, it is an ecologically important in many terms but especially for migrating bird species such as flamingos. It was reported that the number of bird species and number or birds are decreasing gradually in time. This situation is expected to source from different reasons including population pressure particularly in warm seasons, increased pollutants in the area, and decreasing water level against alternations in climatic parameters.

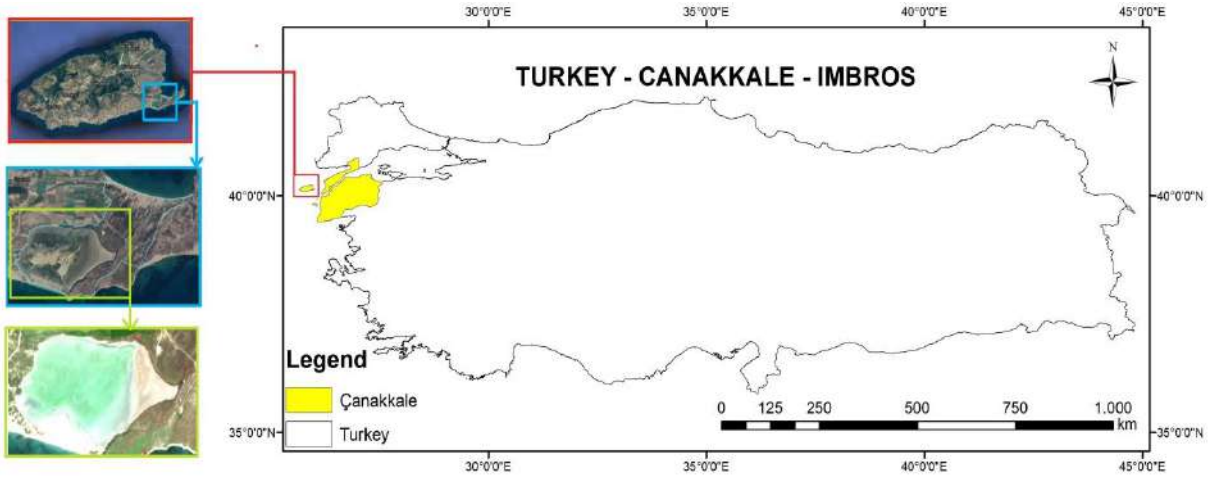

Figure 1. Location of Imbros Lagoon in Imbros, Canakkale Province, Turkey

The Sentinel 2 imageries acquired in the four seasons of 2016 and 2020 years were used as the main data source in the study (USGS, 2020). The acquisition dates of imageries were selected from the same months of each year to eliminate the differences between illumination conditions and to minimize the variances due to probable alternations in vegetation existence within the season. The band properties of Sentinel 2 are given in Table 1. The dates of the used imageries can be seen in Figure 2 for all seasons.

Figure 2. Acqusition dates of used Sentinel-2 imageries

The various water-sensitive indices were tested in preliminary study. In present study, the performance of Normalized Difference Water Index (NDWI), Modified Normalized Different Water Index (MNDWI), and Land Surface Water Index (LSWI), and their combinations of NDWI&MNDWI, NDWI&LSWI, MNDWI&LSWI, and NDWI&MDNWI&LSWI were evaluated. The formulas of the indices are given below, respectively (Eq 1-3).

$$NDWI = (B_G - B_{NIR}) + (B_G + B_{NIR}) \quad \text{(Eq. 1)}$$

$$MNDWI = (B_G - B_{SWIR}) + (B_G + B_{SWIR}) \quad \text{(Eq. 2)}$$

$$LSWI = (B_{NIR} - B_{SWIR}) + (B_{GNIR} + B_{SWIR}) \quad \text{(Eq. 3)}$$

The meteorological data related to temperature was taken from the data service of Turkish State Meteorological Service (TSMS, 2020). The change patterns of monthly average temperatures in 2016 and 2020 were compared with each other and with the long-term records.

RESULTS AND DISCUSSION

According to the results of the study, it was seen from all seven images that there were notable changes in wetness level of the lagoon between 2016 and 2020 in all seasons. Moreover, depending on the visual analysis and in-situ records, among the seven the composed index images and their combination images it was seen that even the small differences in wetland level could be identified bu using MNDVI&LSWI combined image in each date (Figure 3). The analysis gave satisfactory results for discrimination of wet-dry areas with different moisture contents. On the other hand, the suggested image combination of MNDWI and LSWI indices should be supported with instantaneous in-situ measurements to improve the study findings.

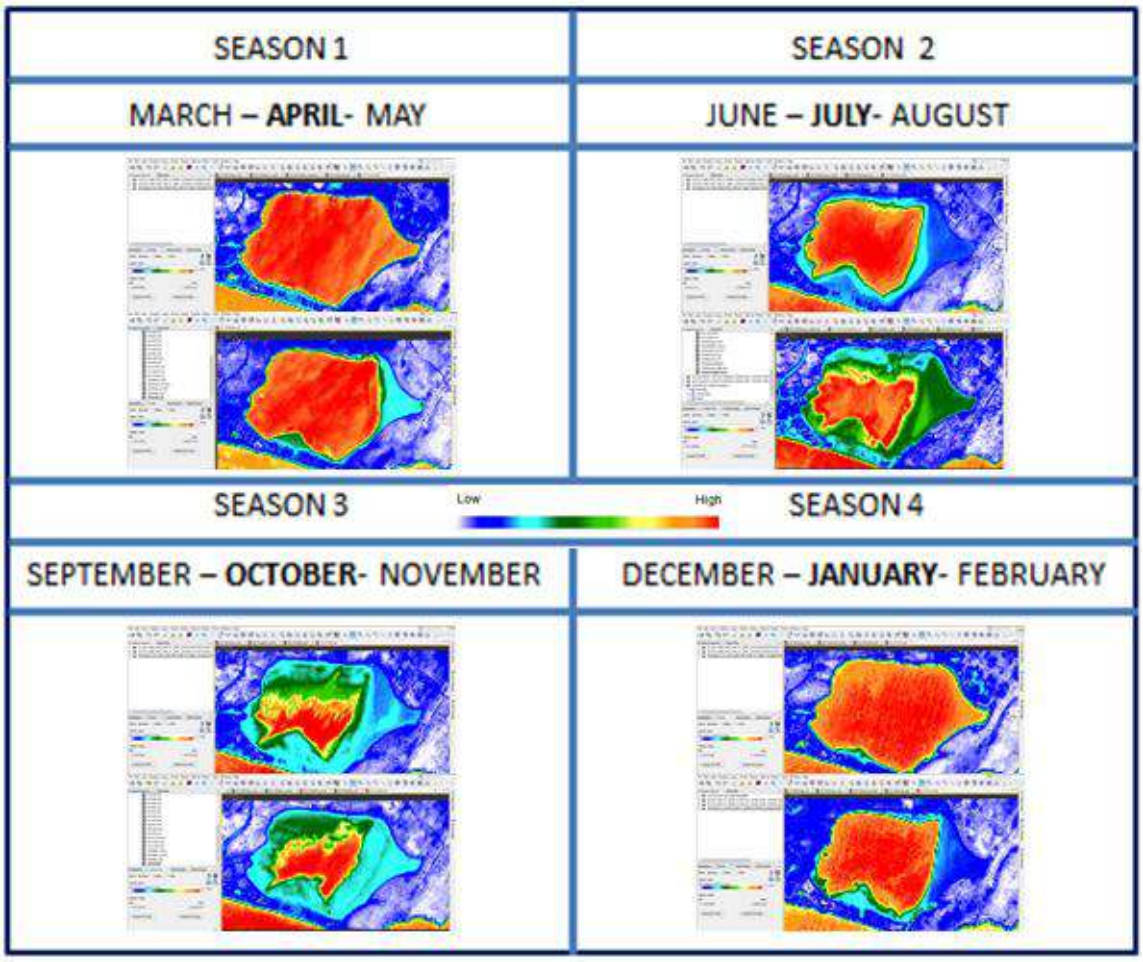

Figure 3. Water level differences identified from MNDVI&LSWI

Assessment of the temperature data has revealed that there were observable changes in monthly change patterns of long-term average, 2016 and 2020 year (Figure 4). There were increase tendencies in almost all months, except the sharp decreases in December in both years. Continuous trend of the situation is expected to impact the natural ecosystems in further steps.

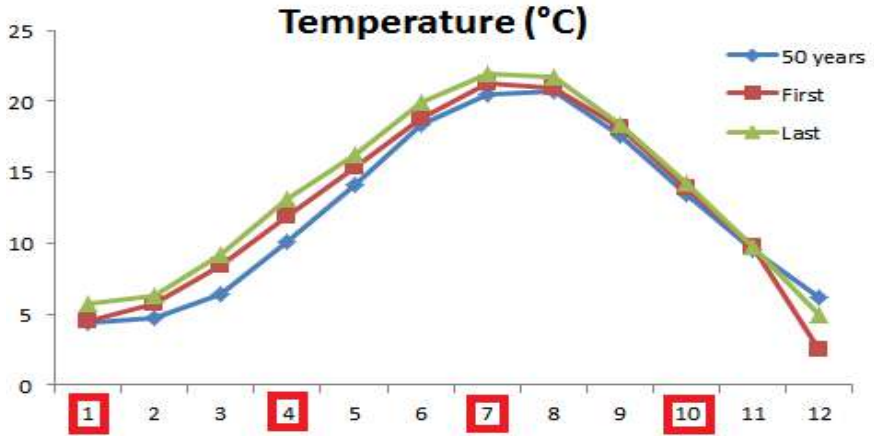

Figure 4. Change patterns of temperature data in the first and second study year, and longterm average

CONCLUSIONS

The study presents the evaluation attempts of three different water-sensitive images and their combinations for identification of water level in the salty coastal lagoon located in Imbros, Turkey. In brief, it was concluded that, Sentinel 2 imageries may assist to ecosystem monitoring in the Imbros Lagoon even in the rainy season, long before the salt was noticeable due to evaporation. On the other hand, there is a need for periodically monitoring of the changes at least monthly-level together with biological diversity indicators to determine the impacts on the different species. Therefore ongoing study is focused on determination and forecasting of the short-term changes and fore more precise evaluation of climate change impacts.

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***The initial results of the study were presented in MEDRIN Capacity Building Workshop.**

INVESTIGATING SPECTRAL CHARACTERISTICS OF MUCILAGE THROUGH HYPERSPECTRAL REMOTE SENSING METHODS

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ABSTRACT

Mucilage is known to be an organic matter produced by different kinds of microorganisms whereas it became a significant issue for maintenance of not only marine ecosystems, but also public health around Marmara and North-Aegean regions. The process has anticipated to be come into the frame as result of various climatic, ecologic and human-induced factor interactions. There is an urgent need for determination of its formation mechanism, underlying or accelerating factors, disposal methods and usability potentials in appropriate areas. Moreover, monitoring of mucilage covered area in different time periods provides precious information on its horizontal change in terms of amounts and directions. The study focused on evaluation of spectral characteristics of mucilage samples that were collected from the same coordinates in North-Aegean coast of Canakkale, Turkey, in different time periods. A hand-held spectroradiometer with a 325-1075 nm wavelength range, 1.4 nm sampling (bandwidth), 3.5 nm resolution was used to monitor the change in spectral reflectance patterns. The research enabled derivation of Mucilage index (MI), which is likely to be valuable for UAV and satellite-based monitoring of mucilage.

Keywords: Hyperspectral Data, Mucilage, Remote Sensing, Spectral Characteristics.

INTRODUCTION

Mucilage is one of the most recent environmental problems in marine ecosystems particularly in Marmara Sea, Bosphorus, and Dardanelles. The first mucilage case in Marmara region has reported to be occurred in 2007, whereas it repeated occasionally (Aktan et. al., 2008; Tüfekci et al., 2010; Tas et. al., 2020). The mucilage generation process occurs as a result of overgrowth of a sea algae type, and it is reported to be related with natural factors such as increased temperature of sea water, and human induced factors like increased level of pollutants, inadequate treatment applications, and enormous fishing activities (Savun-Hekimoglu and Gazioglu, 2021). As it's well known, the most severe mucilage generation has eventuated in the previous month of the present year. Mucilage cover can reach to very large extensions over the sea surface and it can lead to death of living organisms at various life stages

by preventing oxygen transfer, while it also has a potential to host different kinds of harmful viruses or bacteria that may threat flora and fauna by clogging the gills of underwater creatures, which reported to be adversely impact fishery and tourism activities in further steps (Yetilmezsoy, 2021). Therefore, urgent and instantaneous determination of coverage area and its density have become one of the major concerns for many researchers from various disciplines. Using remotely sensing data from different platforms offers rapid, reliable and relatively economic results even in large scales. There are some studies conducted with satellite-based remote sensing data around Marmara Sea, for instance Ozsoy (2021), and Acar et al. (2021). However, there was no sea-level hyperspectral measurement that reported to be conducted around the area. Present study aimed to determine hyperspectral characteristics of mucilage samples collected from the same location of Dardanelles Strait in different time periods using a hand-held spectroradiometer.

MATERIAL AND METHOD

Mucilage samples were collected from Dardanelles Strait on 09 June, 14 June, and 18 June of 2021. The sample collection site and mucilage cover is given in Figure 1. Collected samples have immediately transferred to the laboratory, and measurements were conducted in temperature and light controlled conditions. For the measurement processes, a halogen lamp fixed at 45° angle was used as the energy source for spectral measurements whereas spectral data was collected using FieldSpect hand-held spectroradiometer that equipped with 10° FOV (ASD Inc. USA) (Figure 2). The spectroradiometer has wavelength coverage between 325-1075 of electromagnetic spectrum, with 1.4 nm sampling (bandwidth) and with 3.5 nm resolution. The samples were placed at the same position in each measurement and spectroradiometer was calibrated with a plate made of barium sulphate-plate before each measurement. The representation of spectral measurement process can be seen on Figure 3.

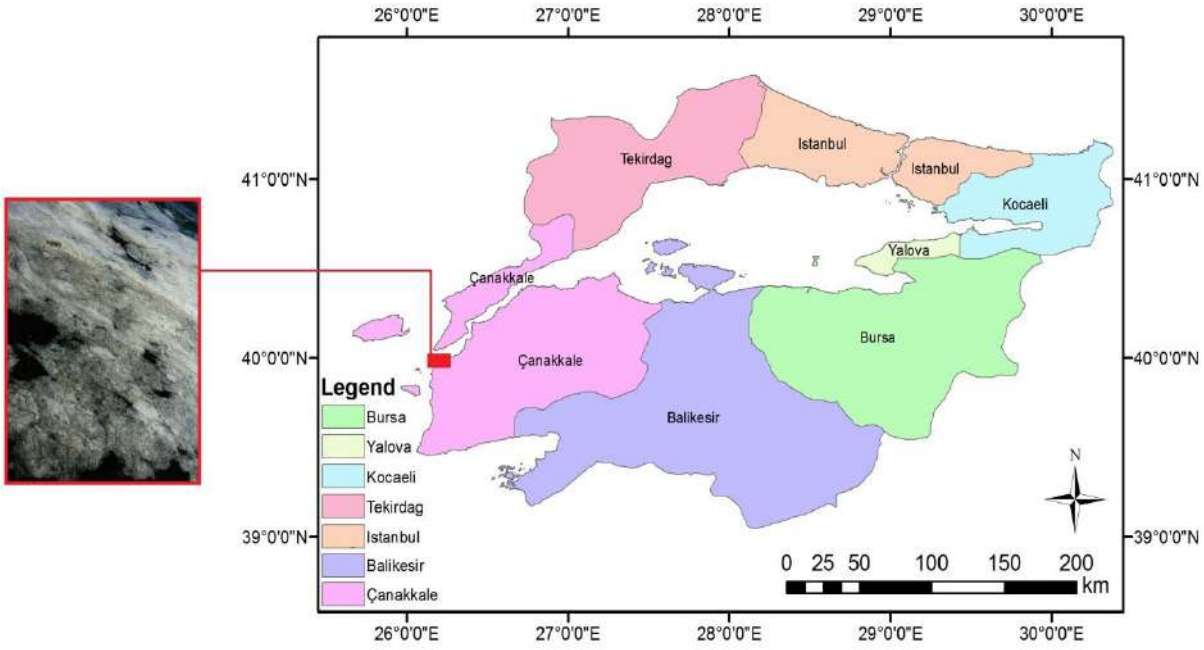

Figure 1. Location of sample collection site, and mucilage cover

Figure 2. The portable hand held FieldSpec spectroradiometer (ASD Inc., USA)

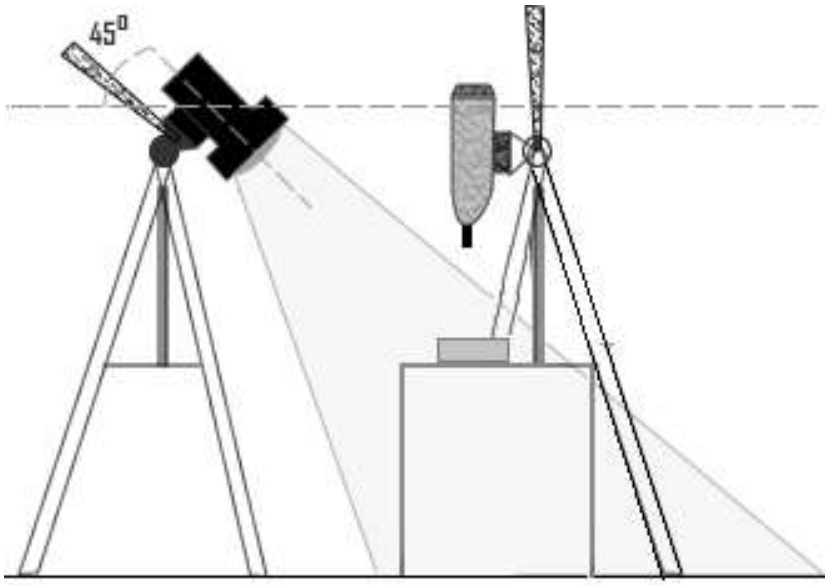

Figure 3. Representation of spectral measurements at controlled conditions

The indices of Normalized Difference Vegetation Index (NDVI) (Rouse et. al., 1973), Pigment Specific Simple Ratio (PSSRa) (Blackburn, 1998), Inverted Red Edge Chlorophyll Index (IRECI) (Frampton, et al., 2013), Mucilage Index (MI) were calculated using spectral data for each date and equations are given below, respectively (Eq. 1-4).

$$NDVI = (r_{NIR} - r_{RED})/(r_{NIR} + r_{RED}) \quad \text{(Eq. 1)}$$

$$PSSRa = r_{NIR}/r_{RED} \quad \text{(Eq. 2)}$$

$$IRECI = (r_{NIR} - r_{RED})/(\frac{r_{RE1}}{r_{RE2}}) \quad \text{(Eq. 3)}$$

$$MI = r_{665}/r_{710} \quad \text{(Eq. 4)}$$

RESULTS AND DISCUSSION

The findings of the study have demonstrated that spectral characteristics of the sampled mucilage cover showed different reflectance values but similar patterns while the reflected amounts were differed noticeably due to changing density and concentration of mucilage within in ten-day period (Figure 4). In all dates, the spectral reflectance curves have tent to increase continuously in the blue and green regions Therefore, the wavelengths between 460 and 576 seemed useful for mucilage detection. There were absorption trends around the center wavelength of 655 nm of each measurement. This situation expected to be resulted from chlorophyll content of mucilage samples (Flander-Putrle, 2008), since the band range represents the chlorophyll absorption region (Jensen, 2000). The peaks of each reflectance curve were captured around the 702 nm wavelength of red edge as it was a recommended band for other studies related to chlorophyll (Smith et al, 2004; Cao et al, 2020). Another characteristic change seemed to be occurred around center wavelength of 760 nm, which is well known to be the one of the two most sensitive bands in NIR region (Peng et al., 2013). On the other hand, the situation was more observable in initial measurements,. After a slight increase in reflectance patterns, a continuous decreasing tendency started from 815 nm, with different slope values. The SWIR region could not be evaluated in the study due to properties of FieldSpec device. MODIS-based MI was offered by Vescovi et al. (2003) and it was modified latter for Sentinel-2 by using identical bands (Kavzoglu et. al., 2021), whereby the considered broad-band intervals were 458-523 nm, 543-578 nm 855-875 nm, and 1565-1655 nm of electromagnetic spectrum for Sentinel-2. Thus, the findings of the study were in coherency between band selections of previous satellite-based studies. Moreover, depending on the narrow range due to wavelenght-based monitoring capability instead of broad-band representation, the study expected to serve a baseline for further satellite- or aerial-based studies with different spatial and spectral resolutions for selecting the useful band intervals.

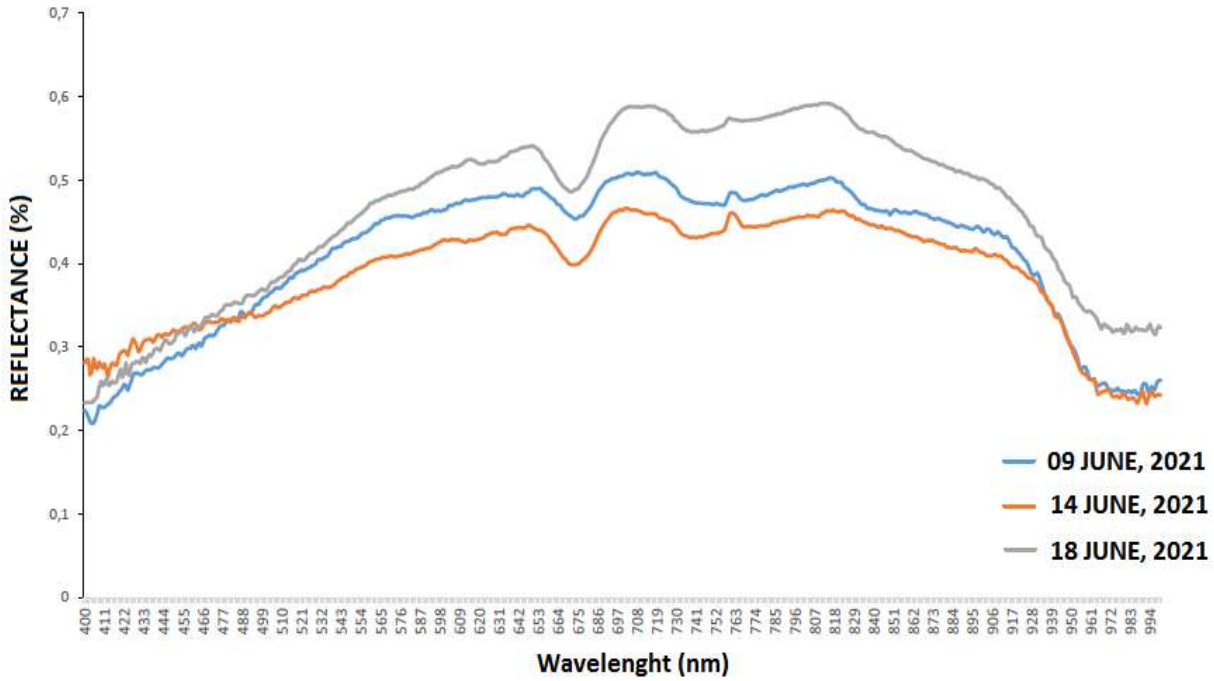

Figure 4. Spectral reflectance curves of mucilage samples under controlled conditions

In addition to the above mentioned characteristics of individual wavelengths, the changes in the NDVI, IRECI, PSSRa, and MI are presented in Figure 5 a-d. It was seen that NDVI values were increased during the study period (Figure 5a). The IRECI values of samples seemed to be almost stable between 09 and 18 June, 2021 (Figure 5b). The change pattern of PSSRa values were similar as expected (Figure 5c). In contrast, values of MI were decreased in both periods with an higher decrease rate in between 09 July and 14 July, 2021. The reduced values of MI have sourced from increased difference levels between the selected wavelengths, as it is evident from Figure 4. However, deeply analysis of mucilage chemical composition simultaneously with spectral measurements seemed as an helpful tool for better understanding of reasons for changes in the reflectance characteristics even in individual bands.

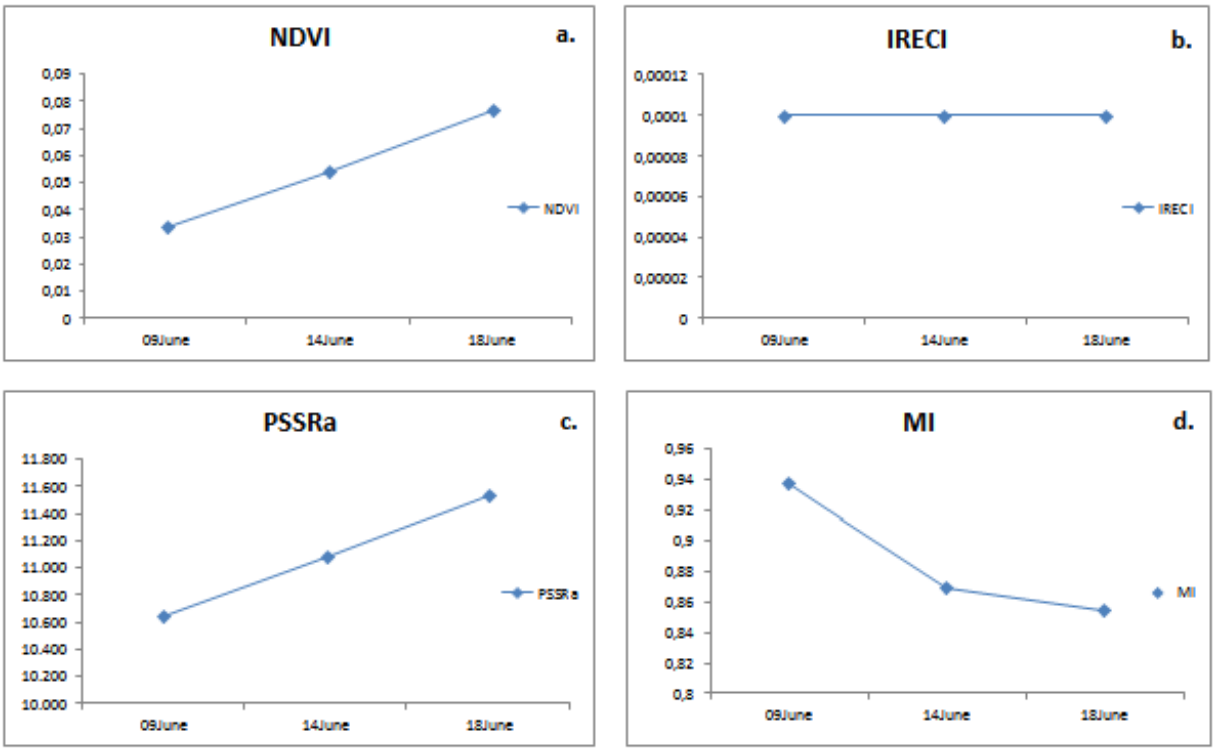

Figure 5. Change patterns of a. NDVI, b. IRECI, c. PSSRa, and d. MI

CONCLUSIONS

The measurement in mucilage samples in controlled conditions represented that there were considerable changes in spectral characteristics of mucilage even though the samples for each date were visually similar. The regions that were sensitive to mucilage existence were determined through wavelenght based interpretation. In brief, the results seemed coherent with the previously conducted satellite-based studies. Moreover, results can provide underlay for band selection in further studies. However, it was concluded that daily monitoring of mucilage coverage with in-situ hyperspectral measurements, and supporting the results with laboratory analysis of chlorophyll content or other chemical properties of mucilage will have a great capability of improving the findings for finer predictions in further steps by linking the mentioned data to each other.

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DETERMINATION OF ANTIOXIDANT POTENTIAL OF AGARICUS MACROSPORUS AND RUSSULA VESCA MUSHROOM EXTRACTS

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ABSTRACT

The aim of this research was to determine the content of bioactive compounds phenols and flavonoids in two extracts (aqueous and ethanolic) of two wild mushroom species: *Agaricus macrosporus* and *Russula vesca* collected from the Republic of North Macedonia. Moreover, their antioxidant potential was determined through the ability to capture free DPPH radicals, as well as chelating iron ions. Generally, aqueous extracts showed slightly higher, statistically significant ($p<0.05$) antioxidant activity, compared to the ethanolic extract. Aqueous extract of *Agaricus macrosporus* was characterised with statistically significant ($p<0.05$) higher content of phenols, compared to the same extract of *Russula vesca*. On the other hand, both tested extracts of *Russula vesca* had statistically significant ($p<0.05$) higher content of flavonoids, compared to those of *Agaricus macrosporus*. Therefore, it can be concluded that the aqueous extracts of both tested mushrooms showed good antioxidant properties that can be a substitute for some of the synthetic antioxidants used for industrial purposes. According to that, this study can be a novel starting point for future research in which mushroom extracts can be used in various fields such as food industry, pharmaceutics, medicine or cosmetics.

Keywords: wild mushrooms, extracts, antioxidant potential.

INTRODUCTION

Mushrooms have long been considered to have medicinal value. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as a source of food (Chang and Miles, 2004). Several important compounds including bioactive polysaccharides (lentinan), dietary fiber, ergosterol, vitamins B_1, B_2, C, phenols, flavonoids and minerals have been isolated from the fruiting body, mycelia, culture medium of the mushrooms, as well as their extracts (Gursoy et al., 2010).

Phenolic compounds are aromatic hydroxylated compounds, possessing one or more aromatic rings with one or more hydroxyl groups. They include a large number of subclasses, such as flavonoids, phenolic acids, including hydroxybenzoic acids and hydroxycinnamic acids, stilbenes, lignans, tannins, and oxidized polyphenols, displaying a great diversity of structures (Palacios et al., 2011).

Flavonoids show antioxidant activity typical of polyphenolic compounds, through the ability to donate hydrogen with the formation of a resonant stabilized radical, as well as the ability to chelate transition metal ions. Flavonoids have a comparable or several times better

ability to neutralize free radicals in aqueous solution compared to tocopherols and ascorbic acid (Dubost et al., 2007 *qtd in* Stojanova et al., 2020).

Agaricus macrosporus (F.H. Muller and Jul. Schäff.), commonly known as the white button mushroom, is one of the most economically important edible mushrooms. It is considered as a valuable health food with high contents of polyphenols, ergothioneine, vitamins, minerals and polysaccharides (Dubost et al., 2007; Tian et al., 2012). Moreover, this mushroom has been demonstrated to possess various valuable biological properties including antitumor, anti-aromatase, antimicrobial, immunomodulatory, anti-inflammatory as well as antioxidant activities.

Russula vesca (Fr.) is a common and widespread edible mushroom on mainland Europe and North America. This mushroom appears in summer or autumn and grows primarily in deciduous forests. *Russula vesca* is considered edible and good, with a mild nutty flavour. In some countries, including Russia, Ukraine and Finland it is considered entirely edible even in the raw state (Dahlberg, 2019).

Numerous studies have shown that regular consumption of certain mushroom species as either a regular food or as extracted compounds is effective in both preventing and treating specific diseases, mainly through immunopotentiation and antioxidant activity. Thus, the intake of mushrooms and their extractable bioactive compounds appears to be effective in cancer prevention and growth inhibition. Another important fact is the certainty that mushroom extracts, compared with other drugs, show a very low toxicity when regularly consumed, even in high dosages (Reis et al., 2014).

The aim of this research was to determinate the antioxidant potential of aqueous and ethanolic extracts of the wild mushroom species *Agaricus macrosporus* and *Russula vesca*.

MATERIAL AND METHOD

In this research, as a work material two types of mushrooms collected from the territory of the Republic of North Macedonia were used: *Agaricus macrosporus* and *Russula vesca*. The collected fresh mushrooms were chopped into thin slices. The mushroom pieces were dried in a chamber dryer with hot air at a temperature of 40 ºC for 6–7 h. Dried mushrooms were first ground to a fine powder and then, extracted in two ways, with water and ethyl alcohol as extragens.

Preparation of aqueous extract

Aqueous extract was prepared by Sławińska et al. (2013) and Ribeiro et al. (2015) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with about 200 mL of distilled water, and after that was extracted on a boiling water bath for 1 h. To determine the yield of the extract, the mass of empty evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

Preparation of ethanolic extract

Ethanol extract was prepared by Vidović et al. (2011) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with 100 mL of 50% ethanol and extract was covered for 40 minutes on an ultrasonic bath at 45 °C. To determine the yield of the extract, the mass of the evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

Determination of total phenolic compounds

The content of total phenols was determined by Singleton et al. (1999) method, adapted for microplates. The method is based on the reaction of phenol with Folin-Ciolcateu reagent where a colored complex is formed. The phenol content was calculated on the basis of the calibration curve (concentration-dependent absorbance function) of a standard gallic acid solution.

Equivalent gallic acid (GAE)/g dry matter values were obtained according to the following formula:

mg eq. GAE/gd.m. = read concentration GAE (µg/mL) / working concentration x 1000

The results are expressed as the mean of the three measurements.

Determination of total flavonoid content

The flavonoid content was determined by a modified method of Chang et al. (2002), adapted for microplates. The principle of the method is based on the properties of flavonoids and flavoglycosides to give appropriate complexes with metal ions, and the Al-complex is especially important. It is a simple method based on the construction of a colored complex, whose absorption maximum is 430 nm.

The flavonoid content was calculated based on the calibration curve (concentration-dependent absorption function) of a standard quercetin solution.

Equivalent quercetin (QE)/g dry matter values were obtained according to the following formula:

mg eq. QE/g d.m. = read concentration QE (µg/mL) / working concentration x 1000

The results are expressed as the mean of the three measurements.

Determination of antioxidant potential

Ability to capture DPPH radicals

The ability to capture DPPH radicals was determined by Brand-Williams et al. (1995) method.

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100\%$$

The radical scavenging capacity of the samples was calculated as IC_{50} values (inhibitory concentration of extract reducing the absorbance of DPPH solution by 50%) by regression analysis:

$$IC_{50}\ (mg/mL) = (50 - b)/a^*$$

(*a – slope; b – intercept)

BHT and α-tocopherol were used as positive controls.

The results are expressed as the mean of the three measurements.

Ability to chelate iron ions

The chelating ability of iron was determined by Dinis et al. (1994) method. The chelating ability of iron ions is calculated by the formula:

$$\text{Ability to chelate iron } \% = [(A_{blank} - As_{ample})/A_{blank}] \times 100\%$$

The ability to chelate iron ions of the samples was calculated as IC_{50} values by regression analysis:

$$IC_{50}\ (mg/mL) = (50 - b)/a^*$$

(*a – slope; b – intercept)

Citric acid and EDTA were used as positive controls.
The results are expressed as the mean of the three measurements.

Statistical analysis

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained results was used the Independent Sample t-test (p = 0.05).

RESULTS AND DISCUSSION

The content of phenols and flavonoids is thought to greatly contribute to the antioxidant activity of mushroom extracts. In general, the presence of phenols is associated with the ability of mushrooms to chelate metals, inhibit lipid peroxidation, and capture free radicals, while the presence of flavonoids correlates with the antioxidant, anti-inflammatory, antiviral, and anticancer activity of mushrooms (Selvakumar and Sankar, 2015 *qtd in* Stojanova et al., 2020).

Table 1. Total phenols and flavonoids content in aqueous and ethanolic extracts

Aqueous extracts	**n**	**Total phenols (%)**	**Total flavonoids (%)**	**Ethanolic extracts**	**Total phenols (%)**	**Total flavonoids (%)**
		$\bar{x} \pm$ SD	$\bar{x} \pm$ SD		$\bar{x} \pm$ SD	$\bar{x} \pm$ SD
Agaricus macrosporus	3	$4.95^{aA} \pm 1.05$	$2.26^{aA} \pm 2.03$	*Agaricus macrosporus*	$4.17^{aB} \pm 1.61$	$3.14^{aB} \pm 0.57$
Russula vesca	3	$3.30^{bA} \pm 0.97$	$2.79^{bA} \pm 0.93$	*Russula vesca*	$2.98^{bB} \pm 0.78$	$3.19^{aB} \pm 1.00$

a, b - values of the same extract of different fungal species marked with different letters, have a statistically significant difference ($p<0.05$).
A, B - values of different extract of the same fungus, marked with different letters, have a statistically significant difference ($p<0.05$).

According to data presented in Table 1, can be seen that aqueous extracts were characterized with higher content of total phenols, while the ethanolic extract had higher flavonoid content. So, the aqueous and ethanolic extract of *Agaricus macrosporus* had statistically significant ($p<0.05$) higher total phenols content (4.95% and 4.17%), compared to the aqueous extract of *Russula vesca* (3.30% and 2.98). On the other hand, both tested extracts of *Russula vesca* had higher content of total flavonoids (2.79% and 3.19%), compared to the both tested extracts of *Agaricus macrosporus* (2.26% and 3.14%).

The values for phenolic content of ethanolic extract of *Agaricus macrosporus* are higher compared to the methanolic extract of *A. bisporus* (3.4 mg GAE/g dw) from Spain (Palacios et al., 2011) methanolic extract of *A. bisporus* (4.5 mg GAE/g dw) from Spain (Ramirez-Anguiano et al., 2007), and ethanolic extract of *A. bisporus* (8.0 mg GAE/g dw) from the United States (Dubost et al., 2007). Moreover, Barros et al. (2008) found that the content of flavonoids in methaolic extract of *A. bisporus* is 1.73 mg/g. On the other hand, Buruleanua et al. (2018) pointed out that the water extract of *Russula vesca* have 11.13 mg GAE/g d.w. totap phenols, while the content of total flavonoids was found as 3.93 mg QE/g d.w, where the water-ethanol mixture exhibited a better extraction of flavonoids than water, as in the case in this study.

Based on data presented in Figure 1 and Figure 2, can be seen that both aqueous extracts showed better antioxidant potential in terms of the ability to capture free DPPH radicals,

compared to the ethanolic extracts. From this point of view, aqueous and ethanolic extracts of *Agaricus macrosporus* showed better ability for capturing DPPH radicals (55.95% ie 52.61% at a concentration of 10 mg/mL), compared to both extracts of *Russula vesca* (51.32% ie 49.26% at a concentration of 10 mg/mL). Nevertheless, can be seen that both tested extracts of *Agaricus macrosporus* and *Russula vesca* were competitive with the BHT, as a positive control, at a final concentration of 5 mg/mL. However, none of the tested extracts was not competitive with the second positive control (alpha-tocopherol).

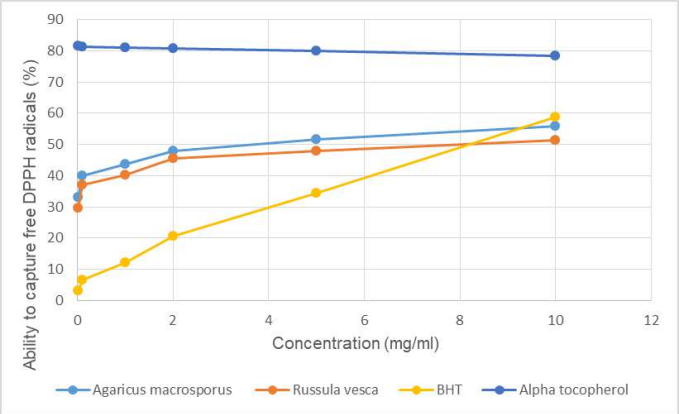

Figure 1: Ability of aqueous extracts to capture DPPH radicals

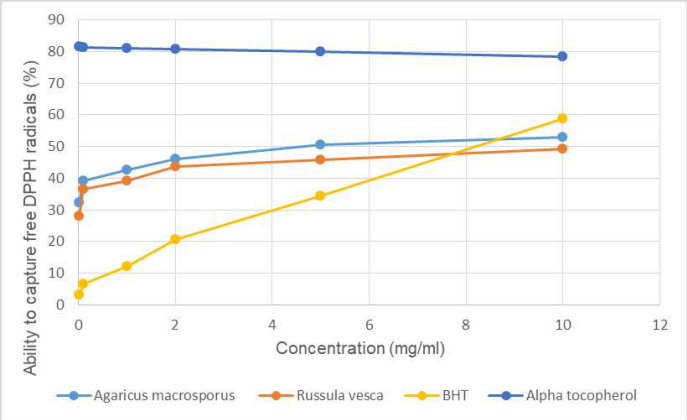

Figure 2: Ability of ethanolic extracts to capture DPPH radicals

According to the results in Figure 3 and Figure 4, can be noticed that both aqueous extracts had better antioxidant potential in terms of chelation of iron ions compared to the ethanolic extracts. Moreover, all of the analysed extracts were competitive with the citric acid in all of the tested concentrations. On the other hand, none of them had better ability for chelating iron ions compared to the EDTA. In this case, extracts of *Russula vesca* showed higher antioxidant potential (60.05% ie 58.14% at a concentration of 10 mg/mL), compared to the both extracts of *Agaricus macrosporus* (56.29% ie 53.97% at a concentration of 10 mg/mL). Expressed through IC_{50} values, from data shown in Table 2, can be seen that this values are in accordance with the antioxidant activity of the mushroom extracts. Thus, with a statistically significant ($p<0.05$) lowest IC_{50} values for the DPPH test were characterized both extracts of *Agaricus macrosporus* (5.51 mg/mL, ie 6.65 mg/mL), compared to the same extracts of *Russula vesca*. On the other hand, aqueous and ethanolic extract of *Russula vesca* showed statistically significant ($p<0.05$) lower IC_{50} values for the ability to chelate iron ions (2.95 mg/mL, ie 2.27 mg/mL) compared to the same extracts of *Agaricus macrosporus*.

Table 2: IC_{50} values of tested extracts

Aqueous extract	n	IC_{50} (mg/mL)		Ethanolic extract		
		DPPH	Chelating Fe^{3+} ions		DPPH	Chelating Fe^{3+} ions
		$\bar{x} \pm SD$	$\bar{x} \pm SD$		$\bar{x} \pm SD$	$\bar{x} \pm SD$
Agaricus macrosporus	3	$5.51^{aA} \pm 0,09$	$3.56^{aA} \pm 0,10$	*Agaricus macrosporus*	$6.65^{aB} \pm 0,07$	$4.00^{aB} \pm 0,06$
Russula vesca	3	$7.73^{bA} \pm 0,05$	$2.95^{bA} \pm 0,31$	*Russula vesca*	$8.90^{bB} \pm 0,03$	$2.27^{bB} \pm 0,06$

a, b - values of the same extract of different fungal species marked with different letters, have a statistically significant difference ($p<0.05$).
A, B - values of different extract of the same fungus, marked with different letters, have a statistically significant difference ($p<0.05$).

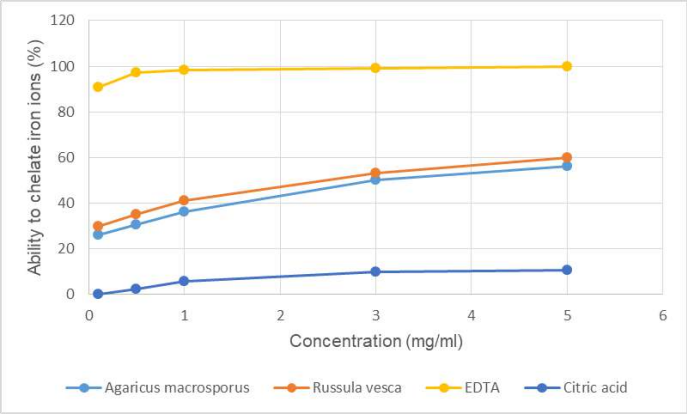

Figure 3: Ability of aqueous extracts to chelate iron ions

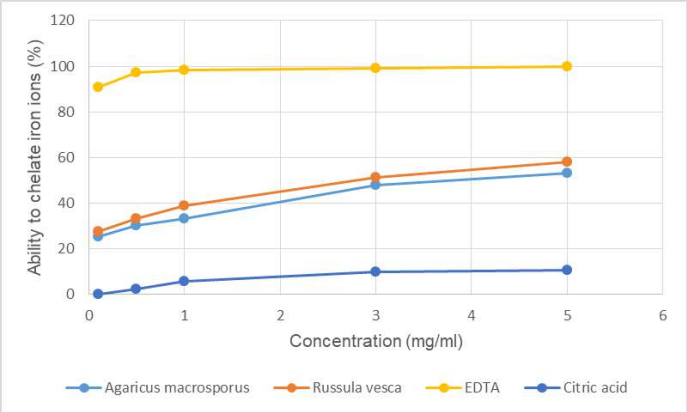

Figure 4: Ability of ethanolic extracts to chelate iron ions

The scavenging antioxidant activities of aqueous and ethanolic extract increased with the increase of concentrations (Liu et al., 2013), as in the case of this study.

The results for IC_{50} values from the DPPH test obtained in this research were higher compared to the same values for ethanolic extract *A. bisporus* (3.13 mg/mL) from Portugal (Reis et al., 2012), ethanolic extract of *A. bisporus* (0.52 mg/mL) from Spain (Ramirez-Anguiano et al., 2007), methanolic extract of *A. bisporus* (1.77 mg/mL) from France (Savoie et al., 2008) and methanolic extract of *A. bisporus* (9.61 mg/mL) from Portugal (Barros et al., 2008), and methanolic extract of *A. bisporus* (18 mcg/mL) from Turkey (Elmastas et al., 2007).

Buruleanua et al. (2018) reported that the water extract of *Russula vesca* have 40 to 60% antioxidant activity based on the DPPH test, which is in accordance with the results from this research. Ruiz-Rodriguez, Santoyo (2009) and Soler-Rivas (2009) pointed out that the results concerning the antioxidant properties of many mushroom species varied from author to author, probably because of different environmental conditions, cultivation methodologies, developmental stage, and genetic variation within strains.

In addition, water as a polar extraction agent probably proved to be better due to the higher value of most of the chemical components that are thought to be responsible for antioxidant activity (e.g., total phenols) than in ethanolic extracts of examined mushroom strains. Moreover, ethanolic extracts have a higher content of flavonoids, which is considered to contribute to the antioxidant activity of the extracts (Stojanova et al., 2020).

CONCLUSIONS

Based on the presented data, can be concluded that both tested extracts of *Agaricus macrosporus*, as well as *Russula vesca*, showed moderate to good antioxidant activity. Slightly higher values were obtained in the aqueous extracts compared to the ethanolic extracts. Both analysed extracts of *Agaricus macrosporus* had higher phenolic content, while extracts of *Russula vesca* had higher flavonoids content.

Therefore, it can be concluded that the aqueous extracts of both tested mushrooms showed good antioxidant properties that can be a substitute for some of the synthetic antioxidants used for industrial purposes. According to that, this study represents a novel starting point for future studies in which mushroom extracts can be used in various fields such as food industry, pharmaceutics, medicine or cosmetics.

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VALORIZATION OF AQUATIC BY-PRODUCTS IN BIOMEDICAL APPROACHES

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ABSTRACT

The use of marine by-products has become one of the major challenges in recent years. There has been a need to modify these materials by using sterile, non-hazardous and ecological procedures. Biological by-products from marine organisms are known renewable as highly volatile materials which can be easily tunable in different composite substances production. The easily changeable properties of marine biomaterials have led to an increase in the usage of these materials n biomedical approaches. Chitosan from shellfish and gelatin or collagen from fish skin has gained importance over the recent years owing to their admirable benefits. With a better understanding of the importance of the usage of natural and tunable materials in bio-medical approaches, marine organism derived materials have started used commonly. The yields, shapes, sizes, and other physical-chemical characteristics of marine-derived by-products vary depending on the used species, extraction process and the target utilization procedure. The exhaustively of manufactured marine by-products have capacitated future uses for biomedical applications such as tissue engineering and tissue regeneration. This study summarizes the existing approaches for the production of marine byproducts from plants, shellfish and fish species. The valorisation strategies of by-products, the differences of material biomass, biopolymers, and their stabilization capacities were compared. The current status and potential problems related to large-scale industrial-scale production. All the collection, processing of marine by-materials, production of biomaterials and utilization of these biomaterials in biomedical approaches have been highlighted. This work may investigate deeply several biomaterials from marine by-products for novel admirable sources for biomedical approaches.

Keywords: biomaterial, aquatic by-product, valorization, alginate,chitosan, biomedical, gelatin.

INTRODUCTION

Biomaterials with practical challenges in biomedical research in terms of sustainability and safety. The state-of-the-art cell biology and cell-surface interactions incorporate various fields in advanced polymer chemistry, reproduction of resorbable characterized polymers, porous scaffolds, and evaluate cell responses (Pappalardo et al.,2019). Engineered tissues are designed to repair and replace the damaged tissues to solve organ failures, putting thousands of people on donor organs waiting for lists. Tissues may be built by growing suitable cells on a scaffold that offers structural stability and tissue integrity and most of the scaffolds seek to recapitulate the arrangement, form, and role of the extracellular matrix (ECM) that the cells are usually enveloped by *in vivo (*Ahadian et al.,2018). Obregón, R. et al. (2014) reported that skin, cartilage, bone, blood vessels, and heart and muscle tissues are the pioneers in designed

tissues and organs.

Gelatin and collagen based material

The skin,head and scale of aquatic animals accepted as main sources of collagen and its derivates. The utilization of collagen and gelatine obtained from several species has been subjected to a wide range of research recently. A wide range of fish species have an important place in the world as a source of collagen and gelatin (Aksun Tumerkan, E. T., 2021a). Fish skin structured from dermis and epidermis layers with similar features to human skin and the differences are mostly related to adaptation to the aqueous environment; scales instead of hair, secretion from cells on the skin surface and a lack of a keratinized layer (Le Guellec et al.,2004; Aksun Tumerkan, E. T., (2021a). The adaptation properties ledto the integrity of the skin, the biomaterial derived from fish skin can be used as scaffold materials, which demonstrate good degradable capacity and usage in human body (Yamada, et al.,2014;.Lima-Junior,et al. 2019; Aksun Tumerkan,2021a). Especially, decellularized fish skin offers several benefits as fish skin does not risk disease and contains more bioactive compounds which promote the eliminating foreign body reactions in tissue engineering and increases its biocompatibility (Magnússon,. et al. (2017).It has been reported that decellularized fish skin can offer several benefits such antimicrobial, antiviral capacity which can be specified in the natural and synthetic alternatives (Yang, et al.,2018).

These excellent properties make fish collagen and its derives alternative sources to mammalian-derived ECM or collagen. The functional characteristics of collagen such as melting point, foaiming capacity,amino-acid profile, thermal stability and thereof utilization capacity vary depending on the used wasteobtained from which or where species, and applied extraction techniques.For example, warm water species offer more thermallystable myofibrillar proteins than cold-water species that could be related to differences in the hydroxyproline contents of cold-water fishes (Aksun Tumerkan, 2021a). The proline and hydroxyproline level skin or scale of fish and therefore collagen or gelatine obtained from them play an important the transition capacity of fish collagen and gelatine from gel to sol occurs below the ambient temperature (Kwak, Shin, Lee, Yun, Song, Yang, et al., 2017). Besides the skin or scales of various warm-water species such as catfish, tilapia, and tuna, salmon, Alaska Pollack and cod are also utilized for the production of collagen and gelatine (Du., 2016; Aksun Tumerkan,2021a). Collagen, which enables the ECM structure of the skin to be three-dimensional and is one of the mostly found proteins in the structure, is one of the most effective molecules in this process. It offers resilience and elasticity to collagen tissue and these properties lead to usage in several approaches from drug delivery systems to tissue engineering(Ambati, 2013; Ibusuki, 2007) The physiochemical characteristics of collagen has important advantages owing to its excellent biocompatibility, low antigen capacity, high biodegradabile property and cell growth ability in the ECM structure (Hayashi et al.,2012). Also, the low degenerative temperature of the collagen, which protects of the three-dimensional structure in the fish skin ECM, is fastly biodegradable when it comes into contact with the human body (Hayashi et al.,2012).

The utilization of fish collagen and fish gelatine in the biomedical application such as tissue engineering or organ on a chip systems have driven by the physicochemical properties

and the product yield has played important role in the industrialization of these biodegradable materials in biomedical application, the collagen and gelatine yield differs among species between 5.00 to 15.00 %, which can be depending on the protein profile and amino acid composition of the relevant species (Aksun Tumerkan et al.,2019; Aksun Tumerkan, 2021a). The physicochemical properties and functionality of fish-derived gelatine and collagen can be well qualified by using enzymes or other acidic treatments (Moosavi-Nasab et al.,2020;Aksun Tumerkan, 2021b). Fish collagen and gelatin-based hydrogels usage is an alternative approach in the regeneration of bone as a reconstruction based on skull and prosthetic heart valves (Aksun Tumerkan, 2021a). As a natural bio-polymer, fish-derived collagen and gelatine have offers some advantages such as the regulation task in the cell division, adhesion and migration capacity of cells for bone in the tissue engineering approaches (Ranganathan, Balagangadharan, & Selvamurugan, 2019; Aksun Tumerkan, 2021a).

The fish collagen and its derivate have utilized as an ECM in biomedical approaches especially in OCC Systems. The extraction methods and used raw material have also impacted on the types of collagen or gelatin and therefore utilization in OOC systems. Especially, collagen types IIV considered as the main element of the native ECM and are the main proteins as a part of structures of most of the connective tissues (Hassan, Heinrich, Cecen, Prakash, & Zhang, 2020). Functional characteristic of fish collagen such as biocompatibility, low immunogenicity, and enzymatic degradation capacity lead to utilization capacity of collagen in OOCs. Li et al. (J. Li, Wang, Qiao, Tian, Liu, Qin, et al., 2018) highlighted that collagen obtained from the skin of the tilapia prepared by tissue engineering methods with three-dimensional biomaterials can be prepared in regenerative medicine.

Chitin and chitosan based biomaterial

Similar to fish species, the huge amount of by-products are generated during shellfish and crustacean species such as shrimps, lobsters and oyster processing. Chitin and its derivates chitosan is the main by products from shells and scales of these animals. While the chitin can be derived from molluscs, insects and insects, the seafood by-products such as shrimp and crab shells accepted as the main sources of industrial production for chitin and therefore its derivates chitosan are the waste and by-products of seafood processing (Inanli, Tümerkan, Abed, Regenstein, & Özogul, 2020; Tayel, Moussa, Salem, Mazrou, & El-Tras, 2016;Özoğul et al.,2021). While the hydroxyl groups in the C2 position are replaced by acetamide groups during the chitin derivation process, chitin is a non-soluble polymer and this property limit its usage and industrialization (de la Caba, Guerrero, Trung, Cruz-Romero, Kerry, Fluhr, et al., 2019; Bandara et al.,2020).Chitosan derivates after deacetylation, chitosan, is soluble in acidic medium, and the antimicrobial properties related to amine groups the presence of amine groups which led to commonly usage of this polymer (de la Caba, Guerrero, Trung, Cruz-Romero, Kerry, Fluhr, etal., 2019).

Chitosan has some advantages than other biomaterial such as being nontoxic, immunological characterisctics and higher biocompatible properties (Sun, Sun, Chen, Niu, Yang, & Guo, 2017). Chitosan as a biodegradable and antimicrobial natural polymer has utilized in biomedical approaches. With increasing utilization of natural-originated polymers in the tissue engineering application; chitosan has used extensively over the recent years. Chitosan also known as the exceptional natural polysaccharide with positively charging capability

(Pavinatto, Caseli, & Oliveira, 2010). Frequently, glycosaminoglycans (GAGs) derived from marine animal waste is of better quality to that of terrestrial sources (Valcarcel, Novoa-Carballal, Pérez-Martín, Reis, & Vázquez, 2017; Sharma et al.,2019). Owing to chitosan has linear polysaccharide structure same as glycosaminoglycans (GAGs) and it can be moulded into several sizes and shapes, chitosan has used a biopolymer in several types of native cartilage production (Alves da Silva, Crawford, Mundy, Correlo, Sol, Bhattacharya, et al., 2010). These similarities can offer easily interaction between the material for ECM and specific signalling proteins(Nettles, Elder, & Gilbert, 2002).

Zhao et al (2019) and Sinha and Bit (2020) stated that the in vitro tests demonstrated the achievements of synthesized photo-cross-linked scaffolds contained porous chitosan and gelatin agglomerate usage in chondrocyte culture and this biopolymeric material. can be used as an option for cartilage tissue engineering application thanks to its excellent biocompatibility and the capability of encouragement the growth of chondrocytes. Chtiosan had also used in the composite nanoparticle synthesis with silver nanoparticles with the antimicrobial impact Escherichia coli and relatively less ctotocitiy had approved by Vrana et al.,(2020) and Ynah et al.,(C.-H. Yang, Wang, Chen, Huang, Li, Lin, et al., 2016). The achievements of chitosan microfibers in chip systems is also reported by in another research: chitosan microfibers were fabricated using poly- methyl-methacrylate (PMMA) microfluidic chip and chitosan solution and sodium tripolyphosphate (STPP) solution by cross-junction micro structured channel and utilization of the chitosan microfibers offer simply and less expensive solutions for cells growth (Yeh, Lin, & Lin, 2009).Freeze drying and freeze gelation approaches usually used for chitosan-based scaffolds with explosion level and exploitation ability in the human intervertebral disk (Reshmy et al.,2020).Furthermore, chitosan accepted as one of the most suitable materials for 3D printing owing to its advantageous physicochemical characteristics and essential properties for cell adhesion with lead to utilization of it as ECM (Jiankang, Dichen, Yaxiong, Bo, Hanxiang, Qin, et al., 2009; Sahranavard, Zamanian, Ghorbani, & Shahrezaee, 2020).

Beside these properties, similarity of chitosan to GAG-like material and fibrous structured lead to the adaptation of amine and hydroxyl groups (Da Silva, Kumar, Choonara, du Toit, & Pillay, 2020).The extrusion process of viscous chitosan to printable end products demand some thermal and chemical applications (Kamdem et al.,2021). The increasing of acidic treatment or extension time cause to harsh situation for chitosan and the restricted of further utilization (Ang, Sultana, Hutmacher, Wong, Fuh,Mo, et al., 2002; Carrow, Kerativitayanan, Jaiswal, Lokhande, & Gaharwar, 2015).The achievements of 3D printed and chitosan-contained material reported by several research;the usage of UV in chitosan lead to fabrication of transdermal curing hydrogel due to its cross linking ability (B. Li, Wang, Xu, Gang, Demirci, Wei, et al., 2015).Similarly, Elviri et al.(Elviri, Foresti, Bergonzi, Zimetti, Marchi, Bianchera, et al., 2017) reported that the benefits of 3D-printed chitosan scaffold as alternative. Using of chitosan with some materials such as poly (caprolactone)-diacrylate/poly (ethylene glycol)-diacrylate and gelatin/hydroxyapatite and in 3D-printed ECM and scaffold has also reported (Mobaraki, Ghaffari, Yazdanpanah, Luo, & Mills, 2020).

Other aquatic sources based biomaterial

Collagen and gelatine had most commonly derived from fish species, but frog skin has been used as alternative for animal sourced gelatine and collagen production. Frog leg commonly consumed as luxury food item and the skin part of frog wasted, the novel re-

earch revealed that frog skin is an admirable collagen/gelatine source as a result of physic-chemical characteristics (E. T. Aksun Tumerkan, Cansu, Boran, Regenstein, & Ozogul, 2019) . Zhng and Duan (J. Zhang & Duan, 2017) investigated that reported that as a model for amphibian species, frog skin utilized for acid-soluble collagen (ASC) and pepsin-solubilised collagen (PSC) production and they reported that the properties of frog skin collagen very similar to aquatic collagen and potentially be used in biomaterial.

Within increasing demand for natural ECMs and some religious and health problems caused by aquatic vertebrate sources, natural materials derived from aquatic invertebrate animals have became an alternative for natural ECMs. Jellyfish has more than 60% collagen and thus the potential of utilization of this collagen and its derivates gelatin is very important (Arslan, Ozudogru, Sezgin Arslan, Derkus, Emregul, & Emregul, 2019). More recently, jellyfish have emerged as an alternative safer source of collagen and its derivates gelatin due to lack of health risk originated from virus and BSE and its admirable properties when compared to bovine sources (Mortimer, Widdowson, & Wright, 2018). Similarly, Hoyer et al., reported that the fibrillized collagen from jellyfish R. esculentum. used for porous 3-D collagen scaffolds and they demonstrated that the similarity of jellyfish collagen to mammalian collagen type II and and collagen from jellyfish can be used as ECM matrix.

Lately, Fernández-Cervantes et al (Fernández-Cervantes, Rodríguez-Fuentes, León-Deniz, Alcántara Quintana, Cervantes-Uc, Herrera Kao, et al., 2020) indicated that scaffolds from another jellyfish species Cassiopea andromeda (C. andromeda), using a decellularization process. Following the feasibility tests, DNA quantification and the biocompatibility, the results revealed that scaffold containedjellyfish collagen which has lower DNA content as a result of decellularization process, the macro and microstructure of the scaffold allowed good proliferation and adhesion rate thatrepresent an admirable ECM for skin tissue engineering.

Aquatic plant sourced biomaterials

Algae known as a plant-like structured and comprise of very large and distinctive group of aquatic organisms.Owing to the algae comprise a considerable amount of the world's biodiversity and act as an oxygen source, and thereby they accepted as primary source in the chain of marine food (Tierney, Croft, & Hayes, 2010). Most of the species of algae have resistance capability to extreme environmental conditions and have their specific survival mechanisms, which are absent in terrestrial plants (Varshney et al.,2015).These unique algae-specific characteristics make them one of the richest and promising sources of natural and useful products used a wide range of purpose from the pharmaceutical industry to food and cosmetic sectors; Rahelivao et al (Rahelivao, Gruner, Andriamanantoanina, Andriamihaja, Bauer, & Knölker, 2015) reported that approximately one in ten (10%) of total bio-active compounds from marine organisms are obtained from algae group and approximately 15 % of total drugs obtained from algae approved by Food and Drug Administration (FDA) in the United States (Rengasamy, Kulkarni, Stirk, & Van Staden, 2014). Natural hydrogels are preferable alternatives to intervene cell delivery process and 3D microenvironment cells thanks to their closest similarities to extra cellular matrices (Bidarra, Barrias, & Granja, 2014). The signaling capacity of hydrogels differ depending on obtained from which source and targeting usage. (Gasperini, Mano, & Reis, 2014; K. Y. Lee & Mooney, 2012).In addition to utilization of algae itself in ECM or other materials production used tissue engineering and biomedical approaches, biomaterials derived from algae have became more popular than algae(Cesário et al.,2018; Reshmy, et al.,2020). Due to adaptive mechanisms of algae species to the harsh environmental conditions, availability of these sources is promising approach for sust

ainability of raw material for biomaterials.

Alginate accepted as a natural biopolymer obtained from marine sources, commonly found in the cell wall of seaweed and brown algae (Phaeophyceae) and commonly extracted by alkali extraction methods (K. Y. Lee & Mooney, 2012). Due to alginate being a part up to 45% of the dry weight of brown algae, it is accepted that as major polysaccharide source and it is responsible to this biomaterials mechanical and structural response such as easily ionic exchange facility (Lalzawmliana, Mukherjee, Kundu, & Nandi, 2019). The structure of alginate is containing both α-L-guluronic acid and β-D-mannuronic acid residues which lead to unique properties of this copolymer such as biocompatibility, biodegradability, non-toxicity, gelation ability (T. H. Silva, Alves, Ferreira, et al., 2012).The gelation mechanisms of alginate and its products proposed in the 1970s and has revised within technological advance and experimental confirmations.The divalent cations binding ability of alginate cause to excellent hydrogel formation in cross linked polymeric scaffolds and the presence of O-acetyl groups cause to rising in the solubility of biopolymer influential physicochemical characteristics as molecular mass and visco-elasticity (Mørch, Donati, Strand, & Skjåk-Bræk, 2006; Windhues & Borchard, 2003). Since alginate can be derived from several algae species, kind of alginate produced with various viscosities, degradation capability, molecular weights and which are key factors for its functional properties and commercialization process (Bidarra, Barrias, & Granja, 2014). Alginate let to the hydrogels creation with water and the gelling procedure which offer easy cell encapsulation and entrapment under various physiological situations such as pH and temperature (Bidarra, Barrias, & Granja, 2014). The bioadhesive capacity of alginate (Puertas-Bartolomé, Benito-Garzón, Fung, Kohn, Vázquez-Lasa, & San Román, 2019). The easily accessibility and lower cost make alginate based biomaterials to a promising bioink aletnatives in 3D bio-printing applications. Pahlevanzadeh et al (Pahlevanzadeh, Mokhtari, Bakhsheshi-Rad, Emadi, Kharaziha, Valiani, et al., 2020) approved that alginate and alginate containing biomaterials can be used in biomedical approaches by several printing techniques.

In contrast to above mentioned benefits of alginate usage as biomaterial, there are two main challenges in the bioprinting of it; alginate is challenging to print 3D cell-laden alginate-containing scaffolds with entirely interconnected pores as a result of handling issue during the gelation processes(Kim and Lee, 2016). the weak structure of alginate based material might be risk for interaction potential with water and thereby variation in viscosity of biomaterial limiting the fabrication of them(Carrow, Kerativitayanan, Jaiswal, Lokhande, & Gaharwar, 2015; Pahlevanzadeh, et al., 2020). The surface properties can cause tosignificant cell damage as a result of flow problem during printing. The micro channel integration ability can be restricted by relatively lower resolution of alginate included hydrogel material (Hernández-González, Téllez-Jurado, & Rodríguez-Lorenzo, 2020; Zhu, Chen, Liu, Mu, Zhang, Wang, et al., 2018). However, these issues can be easily solve by various chemical and industrial adjustment, for example textural characteristics of alginate and its products can be modified by the presence of sodium and calcium chloride (S. Liu, Zhang, Hu, Shen, Rana, & Ramalingam, 2020). With the exception of these advantages and disadvantages ,alginate and alginate based biomaterials accepted as robust material in this area.

Carrageenans are known as a natural water soluble polysaccharides obtained from several species belonging to red seaweeds (Rhodophyceae) such as Irish moss (Chondrus crispus), Mastocarpus stellatus and Eucheuma cottonii (Kim and Lee, 2016; Pereira,

Amado, Critchley, van de Velde, & Ribeiro-Claro, 2009). The sulphated construction is formed from its long linear chains of D-galactose and D-anhydrogalactose. This polysaccharide can be classified as three major families based on the number and position of sulfate mechanisms; kappa (κ), iota (ι), and lambda (λ) (Kim and Lee, 2016). While κ-carrageenan has most actively been explored for cell therapy thanks to its specific properties, usage of carrageenan alone or combined with other bio- based polymers have also recently take interest over the last years (Oun & Rhim, 2017; Rhim & Wang, 2014;Kim and Lee, 2016). The thermo-stabile mechanism of carrageenan-based biomaterial give chance to effective usage of them and thereof easily production. The utilization of carrageenan in cartilage tissue engineering has also another promising approach owing to its similar structure with glycosaminoglycans which are the key elements constituting the ECM as a part of the cartilage tissue ((Kim and Lee, 2016; Bhattacharyya, Liu, Zhang, Jam, Dudeja, Michel, et al., 2010; T. H. Silva, Alves, Popa, Reys, Gomes, Sousa, et al., 2012).The achievement of carrageenan hydrogels in the cartilage tissue within proliferation and cell viability tests reported by Rocha et al.,(Rocha,Santo, Gomes, Reis, & Mano, 2011).The excellent properties of carrageenans such as being antitumor and antioxidant give chance to using of them in several approaches from wound-healing and tissue engineering (Venkatesan et al.,2015;Salehi et al.,2019).Agarose known as a linear structured polysaccharide polymer derived from red algae species and most frequently used for cartilage printing (Rahul, Nair, & 2016). Mouw et al.(Mouw, Case, Guldberg, Plaas, & Levenston, 2005) reported that agarose contained scaffold had highest glycosaminoglycan (GAG) level and the highest similarity to native cartilage determined in the comparsion of different Bovine articular chondrocytes including polyglycolic acid (PGA), collagen and alginate. Owing to the thermo-settable ability of agarose based biomaterial, this material can be used as a functional extracellular matrix in different cartilagetissue approaches (Buckley, Thorpe, O'Brien, Robinson, & Kelly, 2009).Tan et al., (Tan, Dong, Ateshian, & Hung, 2010) claimed the reparative ability of agarose hydrogel including chondroces resulted in limited interaction and low adherence in the agarose hydrogel, the global market size of agarose and its derivates agar agar was rised from 214.98 million USD to 219 million USD and is expected to rise in next years (Khrunyk, Lach, Petrenko,& Ehrlich, 2020).

CONCLUSIONS

Non-toxic, comparatively cheaper and eco friendly approaches accepted as biomaterial synthesis. Several techniques for the synthetization of aquatic by product using this new technology within sustainability opportunities were explored, along with their proposed synthesis mechanisms and possible applications. Furthermore, there is an increasing demand for additional research to thoroughly identify the toxicity and processes in the biological by-product sector. This review evaluated that aquatic by products utilization in biomedical approaches within advantages and risks.

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THE EFFECTS OF NITROGEN DOSES AND HARVEST STAGE IN FORAGE COWPEA AND SOYBEAN GENOTYPES ON THE YIELD AND SOME FEATURES

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ABSTRACT

In this study, the effects of different nitrogen doses (4-6-8-10 N kg/da) and different harvest dates (pod setting and seed formation) on yield and some agricultural characteristics were determined in forage cowpea (Ülkem and A Genotype) and forage soybean (Yemsoy and Yeşilsoy) genotypes. The research was conducted with 4 replications according to split-split plot design, on the experimental area of Ondokuz Mayis University, Faculty of Agriculture in 2020. Plant height, main stem thickness, fresh forage yield and hay yield were determined. It was determined that there are statistically significant differences between the examined features. The highest plant height was determined in the Yeşilsoy cultivar (124.42 cm) at N10 dose and pod setting period while the lowest plant height was determined in the Ülkem cultivar (33.00 cm) at N4 dose in the pod setting period. The main stem thickness of the genotypes varied between 6.21-11.15 mm in the pod setting period and between 6.97-11.19 mm in the seed formation period. The highest fresh forage yield was obtained in Yemsoy and Yeşilsoy cultivars (5886.88-6319.38 kg/da) at N6 dose in the pod setting period, respectively. In the seed formation period, the highest fresh forage yield was obtained for Yemsoy cultivar (7681.25 kg/da) at the N6 dose. In terms of hay yield, in the pod setting period, the highest yield was obtained in Yeşilsoy and Yemsoy cultivars at N6 dose (1994.83-2051.87 kg/da), respectively. Similarly, in the seed formation period, the highest yield was obtained in Yeşilsoy and Yemsoy cultivars at N6 dose (2045.48-2511.06 kg/da), respectively. According to the results of the one-year data, it was concluded that forage soybean varieties were more suitable to be grown by giving 6 kg nitrogen dose per decare in terms of high yield under irrigated conditions in the environmental conditions of Samsun.

Keywords: forage cowpea, forage soybean, nitrogen, yield, harvest stage.

INTRODUCTION

Soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.) plants, belonging to the legume family, are annual crops cultivated in the summer period. Both soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.) are important nutritional sources in terms of high protein, carbohydrate, fat and vitamins. The seeds of the soybean plant are widely used in the oil industry, as they consist of 18-24% fixed oil and 35-42% protein (Arıoğlu, 2014). After the oil is removed, the remaining pulp can be used for animal feeding. The pulp consists of 40-46% crude protein, 1-7% fat, 23-27% carbohydrates, and 5-7% cellulose. Soil selectivity of cowpea plants is low. In addition, the cowpea plant is resistant to heat and drought, and its straw contains

11% protein and is highly digestible (Çulha and Bozoğlu, 2016). Considering all these features, soybean and cowpea plants have an important role in human and animal diets.

Fertilization is one of the most important cultural practices that affect the yield and quality of the product obtained from the unit area. When there are not enough macro and micronutrients in the soil, fertilizers contribute to the development of plants. Adequate fertilizer dose contributes positively to the growth and development of plants and increases the amount of product per unit area. In order to obtain the desired benefit from fertilization, it is necessary to make conscious and technical fertilization. Optimum fertilizer dose varies according to a number of factors such as plant genus-species and varieties, climate, and soil characteristics.

The applied nitrogen dose is an important factor in terms of quality and yield. Excessive nitrogen fertilizer applications trigger excessive vegetative development. Therefore, some issues occur in plants such as lodging and late maturation. Due to these reasons, yield, and quality decrease. In addition, a small amount of nitrogen fertilizer application negatively affects vegetative growth and yield. In other words, this condition causes a decrease in terms of plant yield. In legumes, the protein ratio, which is the most important quality parameter, decreases (Kılınç and Arıoğlu, 2018). For this reason, it is of great importance to determine the optimum fertilizer dosage for plant development and to implement fertilization programs by taking these determined amounts into account.

Considering the existence of animals, quality roughage resources are extremely limited in our country, which has a constant shortage of quality roughage. Especially in the summer season, as the yields of the pastures decrease due to the temperatures, there is a greater need for forage crops. However, the number of forage plants grown in the summer season is limited. In terms of forage crops that can be grown in summer, corn plant belonging to the grass family is considered first. However, the corn plant alone is insufficient to meet the demand of roughage and nutritions. Therefore, it is necessary to include legumes in the rations along with corn.

It is important to determine the agronomic desires of these plants in order to obtain the expected yield. For this purpose, two forage soybean and cowpea cultivars were grown in Samsun conditions at 4 different nitrogen (N) doses (4-6-8-10 kg/da) in 2020 and harvested in two different growing stage (pod setting, seed formation). Plant height, stem thickness, fresh and hay yields values were determined in the harvested plants.

MATERIAL AND METHOD

The research was conducted with 4 replications according to split-split plot design, on the experimental area of Ondokuz Mayıs University, Faculty of Agriculture in 2020. The soil

properties of the study area include 45.16% clay, 28.43% silt, 26.41% sand, and the texture class is clay. It was determined that pH 6.30 (slightly acid), electrical conductivity 0.37 dS/m (without salt), 1.88% lime (low lime) and organic matter level 1.88%.

The average temperature of the growing season (between April and August) was 19.6°C, while it was recorded as 18.8°C for long years. The total amount of precipitation between April and August was 222.9 mm in terms of the long-term average, and 136.8 mm in the sowing year. The trial year had higher temperatures and less precipitation compared to the long-term average (Anonymous, 2020).

In the study, two forage soybean varieties (Yemsoy and Yeşilsoy) developed by the Eastern Mediterranean Agricultural Research Institute and two forage cowpea genotypes (Ülkem cultivar and Siyah breeding line) developed by Ondokuz Mayıs University were used. In the experiment, which was established according to the split-split plots experimental design, the harvest time in the main plots, the varieties in the sub-plots and the nitrogen doses in the sub-sub-plots.

The experiment was established on 20.05.2020. The sowing ratio was 10 kg of seeds per decare. The row spacing was 40 cm, and the plot size was 1.2 m wide and 4 m long. With sowing, 8 kg of phosphorus and 4 kg of nitrogen were applied to each plot. Ammonium sulfate was used as a nitrogen source. The remaining part of the nitrogen fertilization was applied on 08.07.2020 when the plants were approximately 25 cm tall.

After emergence of the seedlings, weeds were controlled by hand and irrigation was done on 10 June 2020. Sprinkler irrigation was used as the irrigation method, the plants were irrigated regularly once a week.

The plots divided into two parts for harvest, while one part at the pod settingand the other part at the seed formation period were harvested. Harvest dates of the plants are given in the table.

Genotypes	Pod setting	Seed formation
Ülkem	11.08.2020	18.08.2020
Siyah	29.07.2020	4.08.2020
Yemsoy	9.09.2020	17.09.2020
Yeşilsoy	1.09.2020	8.09.2020

Plant height, main stem thickness, fresh forage and hay yields were calculated in the harvested plants. Plant height values were recorded as the distance between the soil and the top of the plant in soybean varieties, while cowpea varieties were measured as natural plant height because they developed in a horizontal form. The main stem thickness was measured between the second and third nodes of the plant in 5 randomly selected plants from each plot with the help of a calliper. Fresh forage yield was calculated by converting the harvested parcel size to decare. 500 g samples were taken from each harvested parcel and dried at 60 ˚C until reaching constant weight. The dried samples were weighed and then the hay yields were determined.

The results obtained from the experiment were analyzed using the SPSS 22.0 V. Statistical Package program, according to the Split-Split Plots at Randomized Block Design. The differences between the means were evaluated using the Duncan Multiple Comparison Test (Açıkgöz, 1993).

RESULTS AND DISCUSSION

Plant Height (cm)

In terms of plant height, the effects of different nitrogen doses were examined in forage cowpea and soybean genotypes harvested at different harvest periods. According to the results of the statistical analysis, there are significant differences between harvest dates and genotypes and also the interaction of harvest date x genotype was significant ($p \leq 0.001$). As an average of the N doses, the highest plant height was obtained from Yeşilsoy and Yemsoy (110.71-105.70 cm) cultivars. According to the harvest date x genotype interaction, the highest plant height was determined as 118.85 cm in Yeşilsoy cultivar in the pod setting period (Table 1). Plants start to mature their seeds after the pod setting period. During this period, plants tend to lodging more, but the degree of lodging was varied between the genoptyes. Therefore, the interaction of harvest time x genotype was found significant. For example average plant height of the Siyah cowpea plants was 51.14 cm in the pod setting period, it decreased to an average of 42.72 cm in the seed maturation stage period. While similar development was observed for Yeşilsoy soybean cultivar, plant height of the other genotypes were increased from pod setting to seed formation period (Table 1).

Table 1. Average plant height values of cowpea and soybean genotypes grown at different nitrogen doses*

		Plant Height (cm)				
Harvest stage		Ülkem	Siyah	Yemsoy	Yeşilsoy	Average
Pod Setting	N4	33,00	52,41	98,33	123,33	76,77
	N6	36,25	52,33	101,00	115,75	76,33
	N8	33,08	48,50	105,50	111,92	74,75
	N10	41,92	51,33	109,25	124,42	81,73
	average	36,06 d	51,14 c	103,52 b	118,85 a	77,40 a
Seed Formation	N4	35,33	36,42	112,25	93,17	69,29
	N6	36,92	47,00	109,92	103,83	74,42
	N8	37,08	44,67	102,92	105,75	72,60
	N10	38,08	42,83	106,50	107,58	73,75
	average	36,85 d	42,72 d	107,89 b	102,58 b	72,51 b
Average of Dozes	N4	35,94	48,69	104,98	114,57	76,04
	N6	36,59	47,79	106,91	111,32	75,65
	N8	36,73	46,51	107,23	109,66	75,03
	N10	37,57	45,57	107,39	108,93	74,86
Average of cultivars		36,70 c	45,93 b	105,70 a	110,71 a	74,69

* There is no difference between the means shown with the same letter in the same column ($p<0.01$).

Soybean and cowpea absorb a large amount of nitrogen from the soil during the growin period. Absorbed nitrogen promotes vegetative development in the plant and affects many physiological activities, product quantity and quality. The amount of nitrogen applied during the growing period caused an increase in plant height. The results obtained in this study resemble with the findings of researchers conducting similar studies (Güneş, 2006; Çalışkan et al., 2008; Werder, 2016).

The main stem thickness (mm)

In terms of main stem thickness, the effects of different nitrogen doses were examined in forage cowpea and soybean genotypes harvested at different harvest periods. According to the results of the statistical analysis, there is a statistically significant difference among the genotypes ($p\leq0.001$). As the average of the cultivars, the highest main stem thickness was determined as 10.51 mm in Yemsoy variety and the lowest main stem thickness was 7.29 and 7.94 mm in cowpea genotypes (Ülkem and Siyah), respectively. According to the doses, the main stem thickness varied between 7.33-10.54 mm in the pod setting period and between 7.26-10.49 mm in the seed formation period. As the nitrogen dose increases, there is a partial increase

in stem thickness because nitrogen fertilizer triggers vegetative growth. Similar results were obtained in some studies (Can and Akman, 2014; Özyazıcı et al., 2020).

Table 2. The mean stem thickness values of cowpea and soybean genotypes grown at different nitrogen doses*

		Main Stem Thickness (mm)				
Harvest stage		Ülkem	Siyah	Yemsoy	Yeşilsoy	Average
	N4	6,21	8,56	11,15	10,29	9,05
	N6	7,04	8,35	9,75	9,01	8,54
	N8	7,41	7,81	10,82	9,18	8,80
Pod Setting	N10	8,67	7,53	10,43	9,80	9,11
	average	7,33	8,06	10,54	9,57	8,88
	N4	7,38	7,77	11,19	9,00	8,84
	N6	6,97	8,20	10,63	9,26	8,76
Seed	N8	7,77	8,10	10,24	9,39	8,87
Formation	N10	6,90	7,23	9,90	8,02	8,01
	average	7,26	7,82	10,49	8,92	8,62
Average of						
Dozes	N4	6,80	8,17	11,17	9,64	8,94
	N6	7,01	8,28	10,19	9,13	8,65
	N8	7,59	7,95	10,53	9,28	8,83
	N10	7,79	7,38	10,17	8,91	8,56
Average of						
cultivars		7,29 c	7,94 c	10,51 a	9,24 b	8,75

* There is no difference between the means shown with the same letter in the same column ($p<0.01$).

Fresh Forage Yield (kg/da)

The effects of different nitrogen doses were examined in forage cowpea and soybean genotypes harvested at different harvest periods in terms of green yield. According to the statistical analysis results, there are significant differences between genotypes and nitrogen doses, and also harvest stage x genotype interaction was significant ($p\leq0.001$). As an average of N doses, the highest fresh forage yield was determined as 5832.50 kg/da in the Yemsoy variety. Ülkem and Yeşilsoy varieties were also included in the same statistical group. In the Siyah genotype, lower leaves turned to yellow and droped after the pod formation period. Thus, fresh forage yield was decreased at the seed formation period. As the average of the genotypes, the highest fresh forage yield was determined as 5367.10 kg/da in the N6 nitrogen dose, while the N4 dose was included in the same statistical group (Table 3).

Table 3. Mean fresh forage yield values of cowpea and soybean genotypes grown at different nitrogen doses*

		Fresh Forage Yield (kg/da)				
Harvest stage		Ülkem	Siyah	Yemsoy	Yeşilsoy	Average
	N4	5596,25	1970,50	5434,38	5585,63	4646,69
	N6	5481,25	2596,25	5886,88	6319,38	5070,94
	N8	5471,25	2066,63	4710,00	5020,00	4316,97
Pod Setting	N10	4632,50	1992,13	4652,50	4620,00	3974,28
	average	5220,31 b	2156,37 c	5170,93 b	5386,25 b	4502,22
	N4	6499,38	1347,50	7709,38	5086,25	5160,63
	N6	6287,50	2308,13	7681,25	6376,25	5663,28
Seed	N8	6797,50	1836,25	5606,88	5271,25	4877,97
Formation	N10	5710,00	1550,00	4978,75	5220,63	4364,84
	average	6323,59 a	1760,46 c	6494,06 a	5488,59 b	5016,68
Average of						
Dozes	N4	6047,81	1659,00	6571,88	5335,94	4903,65 ab
	N6	5884,38	2452,19	6784,06	6347,81	5367,10 a
	N8	6134,38	1951,44	5158,44	5145,63	4597,46 bc
	N10	5171,25	1771,06	4815,63	4920,31	4169,56 c
Average of						
cultivars		5809,45 a	1958,42 b	5832,50 a	5437,42 a	4759,45

* There is no difference between the means shown with the same letter in the same column ($p<0.01$).

Hay yield (kg/da)

In terms of hay yield, the effect of different nitrogen doses on forage cowpea and soybean genotypes harvested at different harvest periods was examined. According to the statistical analysis results, there are significant differences between genotypes and nitrogen doses, and also harvest stage x genotype interaction was significant ($p\leq0.001$). As an average of N doses, the highest hay yield was determined as 1975.22 kg/da in the Yemsoy variety. As the average of the genotypes, the highest hay yield was determined as 1534.50 kg/da at the N6 nitrogen dose and the N4 dose was included in the same statistical group (Table 4). In the Siyah cowpea genotype, more dry grass was obtained during the pod setting period than the seed formation period. This condition can be explained by the shedding of leaves as the plant development progresses.

Table 4. Mean hay yield values of cowpea and soybean forage grown at different nitrogen doses*

		Hay Yield (kg/da)				
Harvest stage		Ülkem	Siyah	Yemsoy	Yeşilsoy	Average
Pod Setting	N4	960,75	644,49	1807,22	1629,09	1260,39
	N6	1018,29	707,86	2051,87	1994,83	1443,21
	N8	975,62	641,99	1659,86	1711,64	1247,27
	N10	976,96	541,64	1758,47	1608,45	1221,38
	average	982,90 d	633,99 e	1819,35 b	1736,00 b	1293,06
Seed Formation	N4	1342,01	354,86	2511,06	1809,64	1504,39
	N6	1368,20	583,51	2506,02	2045,48	1625,80
	N8	1388,99	463,47	1849,13	1274,27	1243,97
	N10	1209,46	445,18	1658,12	1692,08	1251,21
	average	1327,16 c	461,75 e	2131,08 a	1705,36 b	1406,34
Average of Dozes	N4	1151,38	499,67	2159,14	1719,36	1382,38 ab
	N6	1193,24	645,69	2278,95	2020,16	1534,50 a
	N8	1182,30	552,73	1754,49	1492,96	1245,61 bc
	N10	1093,21	493,41	1708,30	1650,26	1236,29 c
Average of cultivars		1155,03 c	547,87 d	1975,22 a	1720,68 b	1349,70

* There is no difference between the means shown with the same letter in the same column ($p<0.01$).

CONCLUSIONS

In this study, which was carried out to determine the yield and some yield characteristics of forage soybean and cowpea genotypes grown at different nitrogen doses in two harvest periods, the effect of different nitrogen doses on these properties was determined that it is significant. In a very small amount of fertilization, the activities of *Rhizobium* bacteria decrease due to factors such as heat and drought and the high clay content in the soil, and vegetative growth is declined as the nitrogen amount needed by the plants cannot be met. According to one-year results, it was determined that 4 kg/da nitrogen fertilization applied to both cowpea and soybean varieties was insufficient, and more yield was obtained with 6 kg nitrogen application. While preparing the fertilization program, climatic conditions, soil conditions, and economic analysis should be done. In order to obtain healthier results, the study should be carried out in different ecological conditions and for at least two years.

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POSSIBLE EFFECTS OF GLOBAL CLIMATE CHANGE ON TURKEY

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ABSTRACT

Conditions such as the use of fossil fuels, deforestation and agricultural activities have led to a significant increase in the emissions of natural greenhouse gases such as methane and carbon dioxide, especially with the industrial revolution. This increase in greenhouse gas emissions in the atmosphere has caused and continues to cause the deterioration of the natural greenhouse effect and warming of the atmosphere. The negative effects of this situation on the environment by disrupting the climate balance within the cause-effect relationship are quite large. With the effect of possible threats brought by its geography, Turkey may face problems arising from climate change in the near future. The potential effects of global climate change, which are known to have some global and regional effects; it focuses on clean water resources, agriculture, forest, sea level, energy, tourism, human health and biodiversity. It is estimated that Turkey is among the risk group countries in terms of the possible effects of global climate change, and that especially the Mediterranean and Central Anatolian Regions will be more affected by climate change in the future. In this study, the problems that may be encountered due to climate change in Turkey have been examined in general.

Keywords: Ecosystem, Greenhouse effect, Climate change, Turkey

INTRODUCTION

With the industrial revolution in the 1860s, the increase in anthropogenic activities, the unceasing demand of people for "more", rapid population growth and industrialization, unplanned settlement and urbanization, wrong land use, deforestation and rapid destruction of the natural environment destabilized the natural climate change. As a result, a complex process of "global warming and global climate change" based on anthropogenic activities, which is difficult or even impossible to recycle, has been entered.

The fact that the unlimited negative effects of climate change are not given enough attention means that these effects will increase rapidly in all areas. Plants, which are indispensable for all living things because they are the basic building blocks of the food chain, are greatly affected by climate change. In particular, the variable and unpredictable climate pattern, extreme weather events, high temperatures, drought and damagingly excessive precipitation occur. In these cases, the 10% reduction risk faced by plant species increases the sensitivity of plants gradually and poses a serious threat to living things (Haşlak, 2007; Lane and Jarvis, 2007).

Turkey has climatic diversity due to its location in the middle latitudes. According to the Köppen Climate Classification System, the climate classes in Turkey are as follows (Türkeş, 2016);

• Subtropical steppe in the central part of the Central Anatolia Region and in the Van-Iğdır part of the easternmost part of Eastern Anatolia,

• Humid subtropical without dry season in the Black Sea Region, except for the west,

• Marmara, Aegean, Mediterranean and Southeastern Anatolia Regions and the Mediterranean in the western and southern parts of Central Anatolia,
• In a wide belt extending generally in the central northern parts of the Central and Eastern Anatolian Regions, the summer is arid humid continental,
• In a relatively narrow area in the north of Northeast Anatolia (Erzurum-Kars section) and Central Anatolia, humid terrestrial without a dry season.

As stated above, agriculture has been practiced since ancient times in Turkey, where different climate systems exist in different geographies. However, the global temperature increase experienced since the end of the 19th century has affected the flora in Turkey as well as in the whole world today. Changes in plant species and communities primarily manifest themselves in areal distributions (Erlat, 2014).

Turkey, located in latitudes where the warming trend observed in global average surface temperatures is high, will have a complex climate structure in the future according to climate projections. The increasing warming trend may lead to radical changes over time in areas such as water, agriculture, biodiversity and ecosystem in Turkey (Demir, 2009). The global surface temperature increase, which was 0.3 °C-0.5 °C in the 1990s and has reached 1 °C today, will increase to 1.4 °C-5.8 °C according to the forecasts for the year 2100 (Demir, 2009; Türkeş, 2008). This situation reveals that Turkey, like all the countries of the world, will face serious problems in plant breeding. One of the most important steps in the fight against climate change is the estimation of the impact of changing climatic conditions on Turkey's crop production (agricultural product pattern).

Turkey has a very rich structure in terms of agricultural products due to the diversity of its topographic structure and its location in the semi-arid and semi-humid mid-latitude region. This abundance and diversity in agricultural products is highly affected by climatic parameters. A crop or plant can grow at suitable temperatures and precipitation. These temperature and precipitation compatibility ranges vary from plant to plant. As a result, the response of each plant to the change of climatic parameters is different, and with the continuation of these parameters, it will be inevitable for the plant to migrate or have to adapt.

It has been determined that even if the areas suitable for growing corn, millet and cotton crops increase relatively, they will not change significantly compared to today, the availability of aspirin will expand significantly and suitable areas for canola will be replaced. The main agricultural product of Turkey, which will be subject to both spatial changes and shrinkage, is wheat, which loses significantly here. Öztürk (2002) also states that wheat will experience weakness due to climate change in the future. In addition to this, it is seen that the determinations made in the study on wheat are confirmed by some local projects.

Aydın and Sarptaş (2018) determined that the areas where some crop plants can grow today may become unsuitable in the future, while the unsuitable regions may become suitable in the future due to the changes in temperature and precipitation patterns due to climate change. It is stated that changes in the precipitation regime will cause a decrease in agricultural production, and relatively increases in precipitation in arid and semi-arid regions may lead to increases in the amount of product. In addition, there is a possibility of an increase in the amount of production for some products in mid and high latitudes (Başoğlu and Telatar, 2013).

It is thought that the significant temperature increases seen in Turkey after the 1990s precipitate the phenological periods of field and garden plants. In a climate index study, it was determined that the length of the growing season in Turkey increased by an average of 21 days per century (Şensoy et al., 2013). Increasing temperatures accelerate plant growth in mid-northern latitudes (Kadıoğlu and Şaylan, 2000). This gives us the signals that there will be significant shifts in the phenological periods of plants towards the end of the century. The shortened development period will have negative effects on grain fullness, number of grains per ear and grain weight. Early flowering of fruit trees may also increase late frost damage, the

quality of early maturing products will deteriorate and their market values will decrease (Türkoğlu et al., 2014).

In the studies carried out within the framework of climate models, significant increasing trends were found in the regions located in the south and southwest in Turkey's average air temperatures. While a temperature increase of 2-3 ^{o}C is predicted throughout the country, it is estimated that this increase will be between 2 ^{o}C in winter and between 3 and 4 ^{o}C in the western regions compared to the eastern regions in summer. Particularly, the warming trend in average summer temperatures is more evident than other seasonal increase trends and shows significant differences in the western and southern regions. While the warming trend observed in the spring season is effective in the Mediterranean, Southeastern Anatolia and Marmara regions, the weak warming and cooling trends observed in the autumn season do not provide spatial integrity (Türkeş, 2008a; Türkeş and Erlat, 2008).

Another situation that will affect the climate of Turkey is possible changes in the precipitation regime. Climate models generally predict a decrease in precipitation along the Aegean and Mediterranean coasts, and an increase along the Black Sea coast. The precipitation, which was generally within the normal limits between the 2001-2006 periods, remained below the long-term averages in many regions of Turkey in the winter, spring and summer months of 2007, causing a new series of drought events. The last drought events that took place between the years 2006-2007 were especially effective in the Aegean, Marmara, Central Anatolia, Western Mediterranean, Western and Central Black Sea regions in Turkey (Türkeş, 2008a; Türkeş, 2008b; Türkeş and Erlat, 2008). Turkeş et al., (2007) the decreasing trends observed in the Black Sea precipitation regime region show that the drought trends in Turkey are gradually shifting towards northern latitudes.

According to the predictions for determining the trend of change in temperature, although the predictions for precipitation are more unstable and unsteady, it increases by 10% to 25% especially in winter in the northern regions of Turkey, and decreases by 20% to 60% in the south (Gao and Giorgi, 2008). Especially when the annual total precipitation amounts are examined, the precipitation increases in the Black Sea coasts, and the decrease in precipitation increases as one goes south in all other regions (Evans, 2009; Apak et al., 2007).

Considering the data on precipitation and temperature changes, it is clear that any change in the climate will change the amount of precipitation, evaporation, runoff and usable water in the soil. Changes in seasons and annual precipitation are very important in terms of both storing water resources and regulating the moisture regime in the soil (Türkeş et al., 2007; Türkeş, 2008a). Water deficiency that may occur during flowering, pollination, fruit formation and grain filling of plants will significantly affect the range and life span of natural ecological species. Due to the increase in temperatures, evaporation in the soil and transpiration in the plant will increase, especially in regions such as South East and Central Anatolia, which are under the threat of desertification. For this reason, since the plant will be stressed, it will be inevitable for the non-drought resistant plant species to disappear, while the emergence or development of new drought resistant plant species will be inevitable.

Undoubtedly, it is an inevitable fact that climate change will disrupt the composition and productivity of natural ecological systems in Turkey, which has rich biological diversity and ecosystems, reduce biological diversity, and cause changes in the natural habitats of plants, animals and microorganisms. The structure, composition, productivity and geographical distribution of many ecosystems will be disrupted, as the response of species to changes in climate and deteriorating climate regimes will be at different levels and in different ways. However, many of these anticipated ecological changes may be delayed for decades to centuries following changes in climate. As the habitats of fauna and flora change, there may be local increases in biodiversity due to new arrivals. However, increased adverse events (epidemics and fires) can also lead to decreases in biodiversity and increases in unwanted species,

fragmentation in habitats and new barriers to the migration of climate-dependent species. In this case, many plant, insect, bird, etc. species may disappear, and their local populations may decrease or increase.

The development of natural ecosystems on fragile, damaged and not very rich soils, especially in Turkey, makes ecosystems more sensitive to climate change, except for the mountainous areas and coastal zone of the Black Sea region. This sensitivity; Regions at high latitudes and regions at low latitudes will not have the same response and adaptation process, with some ecosystems responding quickly and others slowly. For example, cool/humid climate meadows in high areas and steppes such as Erzurum-Kars region may migrate to respond to changes in the amount and seasonal distribution of precipitation. On the other hand, the survival of some species and forest types may be endangered due to the movements of climate zones at rates faster than the predicted migration rates of species (Türkeş, 2008b).

Turkey's low flood/delta and coastal plains are also under threat from climate change. Projected temperature increases and precipitation decreases may lead to the drying up of wetlands and shallow lakes in deltas or inland areas, resulting in the weakening or extinction of the species living in these areas, and the biodiversity in general (Türkeş, 2008b).

Climate change creates a big problem especially for endemic species. The fact that Turkey is a high and mountainous country with an average altitude of approximately 1130 m, and is rich in forest species and especially endemic plant species in these areas, is an indication that the impact and potential impact of climate change will be high (Türkeş, 2008b). Species that are only regional, endemic and have a narrow distribution area may be at greater risk or may disappear altogether, depending on the increasing temperature and decreasing precipitation. There are more than 3000 endemic species in Turkey, where approximately 12 thousand plant species have been identified, and some of them have a narrow distribution area. The Lakes region contains 900 endemic species, of which 48 are threatened with extinction. Another plant group under potential risk in our country is the bulbous plants called geophytes. Nearly 300 of 600 bulbous plant species are endemic. These plants that bloom in winter and early spring and the ecological chain in which these plants are located are adversely affected by the decrease in winter precipitation and especially the increase in winter temperature.

The effect of climate change emerges faster in aquatic ecosystems than in terrestrial ecosystems. In this context, especially wetlands, marine and lake ecosystems are in great danger. Since the 1940s, the effect of global climate change has started to show itself in the seas of Turkey. The most important proof of this is that the creatures originating from the Indian Ocean and the Red Sea reached the Turkish seas via the Suez Canal. It is stated that 90 out of 650 fish species known to be found in the Mediterranean are not endemic species, and 59 of them enter the Mediterranean via the Suez Canal (Öztürk, 2002). However, it has been determined that the bleaching and peeling seen in the colonies of soft corals (*Eunicella cavaloni* and *Eunicella singularis*) living in the Aegean Sea are related to the increase in temperature (Figure 1a, b). 25% whitening was detected in corals. This phenomenon is clearly seen especially in Kaş and Kemer/Antalya regions. Apart from this, the fact that salpa, kupes and kingfishes have started to be caught in the Black Sea especially in İğneada, Kıyıköy and Şile regions in recent years is explained by the increase in the Black Sea surface water temperature (Çelik et al., 2002).

a)  b) 

Figure 1. Soft coral colonies a) *Eunicella cavaloni* b) *Eunicella singularis*

Precipitation irregularities caused by global warming have led to the drying up of many rivers and lakes used as fisheries resources, thus leading to habitat loss. In some cases, fish stocks that can be caught in lakes and rivers have been lost due to heavy rains and floods. For example, anchovy fish, which form a flock depending on the water temperature, have been delayed in arriving at our shores or could not form a flock due to the lack of snowfall and the water temperature not falling to 16-17 °C. This adversely affected the production of anchovy caught by purse seine method. With the increase in water temperature, changes in the migration behavior and paths of anchovy should be expected. Anchovy herds coming to the Turkish shores for feeding may prefer to stay in the northern waters instead of the warmer southern Black Sea, as the northern Black Sea waters warm and food production increases. This means that the source that provides 80% of fish production will disappear and fishermen will lose their jobs (Sağlam et al., 2008).

It is an inevitable fact that sea turtles spawning on the southern coasts will lose their traditional spawning grounds with the rise of sea water. With the increase in maritime trade, sea snails began to be seen in the Black Sea at the end of the 1940s. The excessive use of inorganic fertilizers and the increase in animal husbandry have increased the amount of nutrients in the Black Sea and accelerated the transport of organic pollution to the Black Sea via rivers. In short, the eutrophication that has occurred has led to extensive destruction of marine life by overfishing and the introduction of invasive species such as the scalloped medusa into the ecosystem. As a result of the combination of these factors with global climate change, the extent of the damage has increased (Anonymous, 2005).

The increase in water temperature, eutrophication caused by agricultural pollution and waste waters, along with the increase in water level in inland waters and seas, have led to an increase in aquatic plants, the problem of terrestrialization and oxygen deficiency in the water. For example, mass fish deaths occasionally seen in Sakarya River and Izmit Bay can be attributed to this reason (Düzgüneş, 2007a; b).

The destruction of forests, meadows and pastures, which are one of the basic elements of ecological balance, and the insufficient protection of national parks may cause major problems for Turkey in the future (Öztürk, 2002). In recent years, there has been an increase in fires due to temperature increase as well as man-made fires, especially in the destruction of forest areas. Hundreds of thousands of tree species, plant, insect and microorganism species as well as millions of decares of forest land have been destroyed and continue to disappear as a result of fires that have occurred due to the increase in temperature in the Mediterranean region in recent years.

There are strong data showing that drought, air pollution and acid rain are the primary causes of disasters such as tree drying and pest epidemics, which have started to be seen frequently in Turkey's forests due to climate change in recent years. Between 1993-1994 alone, approximately 2 million m^3 of tree wealth was cut due to insect destruction. In addition, due to

the drier-than-normal conditions that have been effective in the Mediterranean Basin, perhaps starting from the 1970s, visible tree drying is observed in the Aegean and Mediterranean regions, although not massively.

Turkey's biological richness is based on mountain habitats and mountainous ecologies. These ecologies, which are rich in endemic species, may encounter migration and adaptation problems. Climate change may cause the melting of mountain glaciers, exposing species here to migration or extinction. The best example of this is seen in the Kaçkar Mountains of the country. In the last two years, glaciers have started to melt in the Kaçkar mountains. Along with the melting of the glaciers, changes were detected in the composition of the living forms unique to this region. In addition, in Kızılcahamam National Park, where ecological studies have been carried out for 20 years, it has been determined that while there were 20 species of liverworts, whose lives depend entirely on ecological environments with water or moisture, there are now 4 species (Çetin, 2007).

Considering that the country will have a drier climate as a result of the effects of global climate change along with population growth, it is estimated that the amount of water per capita in Turkey in 2050 will be around 1,200 m^3 per year. In other words, considering the changing climate and rapidly increasing population, it is predicted that Turkey will be a water-poor country in 2050. In addition, in Turkey, water is limited, the amount and distribution of precipitation is irregular except in some regions, water is limited in big cities and agricultural production, the quality of drinking, utility and irrigation water decreases as a result of environmental pollution resulting from increasing industry and other activities, and the global climate The effects of change are known to increase. For this reason, it is obvious that in the very near future, the severity of the drought will be felt much higher than today.

In Turkey, adaptation policies against the effects of climate change have focused on the increase in water scarcity and the drought problems accordingly. Drought ranks first in the ranking of climate-related disasters in the country. It is tried to establish disaster warning policies and systems related to drought, and to create realistic water policies. Drought is a disaster that occurs in a slow and time-consuming process, the effects of which occur in the long term. These characteristics of drought show that the measures to be taken against drought should be such that they will eliminate the long-term effects of drought before it occurs and the effects of drought for years. Therefore, risk management is much more important than last-minute measures against drought such as crisis management in order to keep the long-lasting effects of drought under control in drought management. For this reason, drought planning, which is based on risk management in the country, should be continued on a national and regional basis (Turan, 2018).

The most important factor determining the water potential of a region is precipitation and climate. Therefore, changes in climate directly affect water resources. Climate instability means unseasonable precipitation. In this case, the agricultural sector will carry great risk. The decrease in snowfall and the occurrence of floods and floods in the summer season in Turkey are indicators of climate change. The water problem will affect not only agriculture and energy production, but also the planning and management of water resources, including irrigation, drinking water and other hydrological systems.

It is estimated that the decrease in precipitation will be more noticeable in the spring and autumn months. According to the simulation results of a water balance model, there will be a 20% reduction in surface waters by 2030 (UNDP, 2007). If these forecasts for temperature increases and decreases in precipitation are realized, it is clear that Turkey will be affected by the environmental and socio-economic effects of climate change in the upcoming period. These effects will cause destruction on environmental factors and worsening on socio-economic factors, thus hindering Turkey's sustainable development efforts.

The tourism sector is one of the economic sectors that is extremely sensitive to climate change and negatively affected. Since tourism relies heavily on natural resources, Turkey is a country that will be most affected by the direct effects of climate change and is at risk. Winter sports tourism, especially coastal tourism, etc. types of tourism are affected by climate change and this effect is expected to increase further in the future. For this reason, determining the risks of tourism in Turkey arising from climate change and developing measures for this, that is, adaptation of the sector to climate change is a mandatory and urgent situation (UNWTO, 2018). For this reason, mitigation and adaptation efforts against the effects of climate change are of vital importance for the tourism sector in Turkey. Only in this way can the threats of climate change on the sector be turned into opportunities (Türkeş et al., 2000).

Şen (2013), according to the findings of the number of disasters such as heavy rain, storms, heat waves, forest fires and floods has increased recently in Turkey. A temperature increase of 5 ºC is expected across the country in the future. Parallel to this expectation, it is predicted that there will also be an increase in the number of disasters such as drought, heat waves, forest fires and floods in the future. Considering that such climate disasters will have some negative effects on settlements, it is obvious that cities should take some adaptation measures against the effects of climate change. Cities will most likely suffer from the problems arising from the effects of climate change. For example, according to the findings of Çobanyılmaz and Yüksel (2013), Ankara has a high degree of vulnerability to the effects of climate change.

RESULTS AND DISCUSSION

Global climate change is a chain reaction and burning buried carbon resources is the turning point of the process. It is not possible for humanity to give up the benefits and conveniences of the industrial revolution. The chain of disasters caused by natural events and climate change, showing a rapid increase trend by leaving their natural, normal cycle, heralds the necessity of taking precautions.

To date, no legal regulation has been made in Turkey to directly reduce and/or control the greenhouse gas emission volumes that cause climate change. On the other hand, there are many legal regulations and measures (laws, regulations, announcements, etc.) aimed at indirectly reducing greenhouse gas emission volumes, such as protecting the natural environment and saving energy in general (Ministry of Environment, 2002). However, these efforts are not sufficient for the continuity of Turkey's sustainable development process.

Possible developments after 2020 in the global climate change regime, to which Turkey is a party, make urban climate governance extremely important in Turkey. Developed countries invite all countries to take responsibility for post-2020 climate change. Municipalities in Turkey have an important potential in terms of mitigation and adaptation measures. With the powers of energy efficiency, renewable energy, waste management, urban transportation and zoning management (urban planning and zoning control), municipalities can become key actors in the implementation of climate change mitigation and adaptation policies in Turkey. In this respect, improving the climate change governance capacity of municipalities in Turkey has become an important agenda item in local politics. In terms of developing local governance capacity in Turkey, providing municipalities with access to additional financial resources and developing horizontal and vertical cooperation opportunities with other organizations are of great importance.

Climate change and global warming are closely related to Turkey, both because of its geographical location surrounded by seas and its transition location between the undeveloped parts of the Asian Continent and the developed European Continent. Analyzing the risks it may face with an appropriate assessment, Turkey is among the countries that have taken a pioneering stance in the fight against climate and global warming from the very beginning. In fact, Turkey

is a developing country, so its greenhouse gas emissions are far behind industrialized countries. On the other hand, the possible environmental and social crises that Turkey may face are much bigger than most of the industrialized countries with high emission rates.

It is estimated that the first irreversible, preliminary consequences of global warming and climate changes will be on arable land and fresh water resources. Such a development would bring along numerous social, economic and security risks for Turkey. As a matter of fact, Turkey has been experiencing transboundary water problems with its two southern neighbors, Iraq and Syria, in the last decades. In case of a decrease in water resources, Turkey may have to reduce the amount of water flowing outside the borders of the Euphrates and Tigris Rivers for its own needs. In this case, there may be disagreements with the two southern neighboring states, possibly leading to water wars, with other southern states that will join this coalition. The phenomenon of migration from these countries to Turkey is a cross-border problem that cannot be prevented even under current conditions. Extreme heat, water scarcity, lack of arable land will cause millions of people living in this region to migrate en masse to Turkey, which is cooler, democratic, more prosperous, and has water and arable land.

Physical infrastructure services such as land leveling, drainage, and land consolidation should be developed in order to save water and ensure its effective use. In addition, it is necessary to minimize water losses, improve water management, create a rational irrigation water pricing model, develop agricultural support policies that support production, and raise awareness of farmers about water use (Çakmak and Gökalp, 2011).

The main objective of Turkey's environmental policy is to protect and improve the environment along with sustainable development. The main principle of this policy is to ensure sustainable development on the condition of rational management of natural resources, protection of human health and natural balance, and leaving a livable natural, physical and social environment to future generations (Ministry of Environment, 2002). However, at the last point reached today, climate change has started to play a determining role on the living standards of future generations by threatening the sustainable development process of our country with both its environmental and socio-economic effects. The decrease in rainfall in recent years due to climate change has led to a decrease in agricultural production potential and a decrease in the water level in dams.

Although it does not make sense to fight alone or in cooperation with the countries in the region, Turkey will have to meet the disasters or risks brought by global climate changes to a large extent and find solutions. For this reason, special attention should be paid to education and raising awareness, active representation should be continued in international initiatives to control global warming, and solution models should be created for possible disasters in advance (Köse, 2018).

Considering that the pressure of climate change has begun to be felt widely, especially in Turkey, which has rich biological resources, the magnitude of the danger is remarkable. Necessary measures should be taken to prevent the irreparable and possible damages of life support units, which are indispensable elements of aquatic and terrestrial ecosystems such as forests, wetlands, lake and sea diversity, resulting from climate change. Even if there is no question of replacing extinct species or habitats with measures to be taken and effective and sustainable policies to be implemented, at least the current situation can be stabilized. Within the framework of national and international agreements, it is extremely important to find realistic and fair solutions with a common but differentiated understanding of responsibility, to implement sustainable policies, to protect and sustain biological resources, and to fulfill the obligations arising from the Kyoto Protocol (Şanlı et. al., 2017).

Due to climate change, tourism centers, tourist products, tourism demand, tourism season and number of tourists change (Giles and Perry, 1988). In the future, it is foreseen that the holiday periods will be extended, the demand for the south will turn to the north and the holiday

centers will expand towards the north. According to this possibility, the review of the roads in the Eastern Black Sea region and the construction of additional roads are among the measures that should be taken. Tourism authorities and investors need to be motivated, informed and directed towards areas that will suffer less from climate change. For a sustainable highland tourism, public authorities, entrepreneurs, host communities and tourists should adopt an environmentally friendly way of thinking.

In the upcoming period, Turkey needs to take measures at national and international level to ensure the continuity of the sustainable development process by eliminating the effects of climate change on environmental and socio-economic factors (Kılıç, 2009). As can be seen, the climate change, which is exacerbated by the human, harms the human being again. It is among the expectations that this and similar studies will shed light on the problems that may occur in the future, and that people will develop the awareness that they are not the owner of this nature, but a part of it.

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MISCONCEPTIONS IN BIOLOGY TEACHING AND THEIR REASONS

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ABSTRACT

The aim of this study is to determine the misconceptions in the basic subjects of biology and the reasons for these misconceptions. Misconceptions are an educational problem that can be encountered at every stage of life from a very young age. Studies have shown that even those who teach have misconceptions about the subjects they are expected to be experts in. Since the formation process of misconceptions spans a very large period of time, the reasons for their occurrence should be carefully examined, and measures should be taken to detect and eliminate them at every stage of education. In order to solve a problem completely, first of all, the sources of the problem should be investigated and learned. As long as the teaching techniques do not correct the misconceptions of the students, they cause new misconceptions to emerge. Therefore, misconceptions should be tried to be eliminated by using different teaching methods other than traditional methods. Studies conducted in our country in recent years show that concept maps and conceptual change methods are effective teaching methods in eliminating misconceptions. Research shows that children learn biology better with a constructivist approach.

Keywords: Biology education, Student, Teacher, Misconceptions

INTRODUCTION

Concept; It is an information form that represents the changeable common features of different objects and phenomena that are meaningful in the human mind. Concepts are the building blocks of knowledge. Relationships between concepts constitute scientific principles. It enables people to classify and organize what they have learned (Kaptan, 1999). Concepts are the unit of thought. For this reason, concepts and their names are learned in childhood, then the concepts are classified and the relationships between them are found. Using these concepts, new concepts and information are produced. This learning takes a lifetime.

Most of the information taught consists of concepts and theories. The main thing here is to ensure that students understand the concepts correctly and use these concepts in solving the problems they encounter. In this regard, the duty of teachers is to help and guide their students (Demir and Sezek, 2009).

Biology course is among the courses that students have difficulty because it contains abstract concepts. Biology appears in different forms in different areas of our lives. Students' ideas can sometimes differ from scientific facts (Palmer, 1999). The wrong preliminary information that students have causes them to misunderstand.

It is only possible for students to establish a connection between their old knowledge and their new knowledge through meaningful learning. Since the most important thing in concept teaching is to detect misconceptions and correct these misconceptions, students' prior knowledge should be determined. Misconceptions are explained as information that prevents the teaching and learning of concepts that are formed as a result of personal experiences, contradict scientific truths and have been verified by science. In addition, misconceptions are

ideas that are resistant to traditional teaching methods, are fixed, and are widely opposed to scientific concepts (Gülev, 2008). According to Şeker (2010), misconceptions are information that is contrary to scientific truths and thoughts that have emerged as a result of personal experiences and that prevents meaningful learning.

Without learning any subject and concept in the course content, it is very difficult to learn other subjects and concepts related to this subject and concept. Studies have shown that students have misconceptions about biology and these misconceptions make it difficult for them to learn new subjects (Gülçiçek, 2005). This situation requires the identification of misconceptions about biology subjects and the use of conceptual change strategies to eliminate these misconceptions.

In studies examining the effectiveness of conceptual change strategies, it is seen that students' misconceptions are significantly reduced after experimental applications. However, even when the number of misconceptions decreased significantly in students, it was observed that a large number of misconceptions still remained.

It is of great importance for individuals to know the prior knowledge they have created in their minds in learning concepts. Today, most research in educational sciences focuses on the identification of the first concepts that individuals form beforehand and have a great impact on their learning (Griffiths et al., 1988). Concept teaching and learning has an important place in terms of education. This fact can be better understood if the place of concept teaching for education is determined well and in a planned way.

Effective biology teaching is achieved by comprehending the given concepts accurately and effectively. While teaching the concept, it is necessary to be aware of the students' prior knowledge or the concepts they have learned before. These learnings also affect their subsequent learning. It is difficult for an individual with a misconception to reach the next level of learning in a healthy way. For this reason, many studies have been made and are being carried out today to detect and correct misconceptions in biology teaching.

Students learn new information every day in the environment they live in. However, they tend to interpret the information they have learned in the light of the beliefs and thoughts they have previously developed through intuition. As a result, students' perceptions of scientific events begin to be gradually restructured. Since most of this information, which students reconstruct based on their own interpretations, is against scientific facts, it constitutes an important obstacle in biology teaching (Driver, 1989). Students usually develop the knowledge expressed as pure theories, intuitive beliefs, preconceptions, children's science, personal concepts, alternative frameworks, alternative concepts or misconceptions, as a result of their own interpretations or some inconsistent explanations made in and out of school environments in the early stages of their school years (Wandersee et al., 1994).

In some cases, teachers' thoughts or expressions in textbooks cause misconceptions in students or reinforce existing misconceptions. Misconceptions are quite common in formal teaching and are highly resistant to change, comprehensive, stable and fixed (Westbrook and Marek, 1991). If these cannot be detected and compensated, they continue for many years and constitute important obstacles in the learning process. In some studies on biology education in Turkey, it has been determined that students have misconceptions in different fields of biology (Aşçı et al., 2001).

It is strongly supported by the fact that one of the most important reasons for misconceptions stems from the difference between the language used scientifically and the language used in daily life. Examples of this situation are concepts such as "seal" and "seal fish", "respiration" and "breathing". Respiratory is used synonymously with breathing in daily life. For this reason, it is not surprising that many people conclude that respiration takes place in the lungs. On the other hand, the fact that the units are closely related to each other is another reason for students' misconceptions. Students who cannot relate the subjects with each other

correctly have difficulty in understanding some basic concepts (Novak, 1970). Textbooks should be re-evaluated in this respect and integrity between the subjects should be ensured.

Students' real-world concepts play a critical role in their view of the world. Their real-world concepts are often difficult to change. Learning science requires more than just adding new concepts to knowledge. Learning science requires realism and the construction of new ideas that may conflict with previous ideas. Piaget, one of the proponents of scientific theory, defined such changes as harmony. Students use old ways of thinking and create new ways of thinking based on new knowledge they find useful. When students succeed in concept change, they will have the ability to understand, explain and predict real-world events and the thinking that will carry them to accepted scientific teachings (Aykurt and Akaydın, 2009). Many students cannot correctly encode biology concepts into their brains, resulting in misconceptions.

The misconceptions that students have are not at a certain level of education and can continue throughout life, starting from pre-primary education. Piaget stated that misconceptions are like a structure and continue by adding to each other. According to Ausubel, meaningful learning occurs when a correct relationship is established between the concepts that students have just learned and the concepts they have learned before. Thus, when new and old information are correlated, both correct information is obtained and knowledge is developed (Tekkaya et al., 2000).

One of the most important aims of biology education is to enable students to learn biology concepts meaningfully without memorizing them and to use these concepts in line with their needs. When the studies were examined, it was concluded that the students' prior knowledge in the learning process was effective in their learning (Hawsen et al., 1998). It is possible for students to establish a relationship between their previous knowledge and their new knowledge, provided that they construct it in their minds in a way that does not conflict with their learned knowledge. New information has to be associated with existing information, otherwise it is difficult for students to adopt new information.

Traditional approach methods used in biology education are insufficient in teaching students concepts. It guides students to memorization and prevents them from learning the misconceptions that students have on subjects that require definition, explanation and guessing (Sönmez and Geban, 2001). Studies show that children learn biology better with a constructivist approach.

When the teacher wants to identify the misconceptions of her students, she can identify the misconceptions by using the concept tests in the relevant literature or simply from the verbal and written statements of the student during the course process. Asking students questions that encourage them to think about explaining the cause of events, instead of results-based tests, may reveal the misconceptions of the student. Misconceptions can be detected with open-ended questions asked to students and brainstorming activities. In addition, assignments that provide logical explanations are very useful in detecting students' misconceptions.

According to Tekkaya et al. (2000) conducted interviews with faculty members in order to identify the misconceptions that students have and to determine what their causes might be. As a result of their research, they determined that the reasons for the misconceptions were the teacher's lack of knowledge about the subject, the insufficient teaching techniques they used in the classroom, the preference for rote-based education, the lack of any connection between the subjects and the fact that they were not associated with daily life. In addition, they stated that students' lack of foreknowledge and non-scientific prejudices, and the differences in textbooks, language used in daily life and scientific language cause misconceptions.

Various misconceptions about the structure and functions of the cell, cell division, comparison of plant and animal cells have been identified in the unit "The basic unit of life - cell" in high school biology textbooks. It supports the view that similar misconceptions and

understanding difficulties in biology subjects may also arise from textbooks in high school students (Dikmenli and Çardak, 2004).

The fact that students' misconceptions stem from textbooks has led educational research in Turkey and other countries to work on this subject. Studies in the field of biology education in recent years have shown that students have misconceptions in many subjects. These; cell structure and function (Marek, 1986), osmosis and diffusion (Zukerman, 1994; Odom and Barrow, 1995; Westbrook and Marek, 1991), photosynthesis (Amir and Tamir , 1994), genetics (Stewart et al., 1990; Stewart and Dala, 1989; Clough and Wood-Robinson, 1985), growth and development (Smith and Anderson, 1984), food web (Griftiths and Grant, 1985), ecology (Adeniyi, 1985), theory of evolution (Bromby, 1984), living and non-living things (Looft, 1974), respiration (Sander, 1993), cell metabolism (Storey, 1991), classification (Trowbridge and Mintzes, (1988), homeostasis (Westbrook and Marek, 1992), human circulatory system (Amaudin and Mintzes, 1985).

In addition, in the field of biology education, there are many studies conducted in our country on subjects such as cell division (Yılmaz, 1998), diffusion and osmosis (Tarakçı et al., 1999; Tekkaya et al., 1999), photosynthesis (Çapa, 2000). These studies draw attention to the prevalence of misconceptions among Turkish students.

For effective biology education, it is extremely important to learn basic biology concepts completely and accurately. Because these concepts form the basis for learning other related concepts and more advanced biology concepts. Therefore, new information becomes meaningful when it is associated with previous concepts. Incorrect or incomplete learning of basic concepts can cause new knowledge to be learned incorrectly (Aydın and Balım, 2013). For an effective biology teaching, students' misconceptions about biology should be known. In order to eliminate misconceptions and realize meaningful learning, students' knowledge that cannot be accepted as scientifically correct must be replaced with correct information in a way that will adapt to new information, which is the process of conceptual change.

Internalizing the ideas represented by the concepts and thinking of these concepts with their correct meanings, assimilating them in the mind by moving them beyond the memory, is the most indispensable necessity of reaching the upper steps in biology teaching. Misconceptions that may occur in primary education can create future problems in biology teaching (Eyidoğan and Güneysu, 2002). In biology teaching, conceptual change texts, concept maps, mind maps, concept cartoons, analogies and models can be used to eliminate misconceptions and provide conceptual change in students.

With conceptual change texts, misconceptions that students may have are written and it is emphasized why these concepts are insufficient or wrong. With scientific explanations and examples, students are tried to gain the right concept. In conceptual change texts, students are asked questions and their prior knowledge is activated and then students are made to feel that this prior knowledge is wrong and insufficient (Köseoğlu et al., 2003).

Concept maps graphically show concepts related to a topic and relationships between concepts. It is a visual tool used to understand how students perceive concepts and establish relationships between concepts, to determine their preliminary concepts and alternative concepts, and to evaluate their conceptual understanding (Aydın et al., 2007).

In mind maps, a central figure, key words, codes and symbols are used. Mind maps consist of a central thought that connects to related concepts. The map consists of a central thought and 5-10 secondary concepts related to it. 5-10 third level concepts can be drawn for each of these concepts (Zhao, 2003).

In concept cartoons, three or more characters' mutual questions or ideas about a daily event are presented in the form of speech bubbles (Uğurel and Moralı, 2006). Generally, there is a picture expression of the discussion of three or more characters on a subject (Şaşmaz Ören, 2009). In this discussion, each character in the painting is advocating a different idea.

Analogy is a technique used to explain an unknown concept with a known concept. Analogies make the new idea logical and plausible by associating it with familiar knowledge. Analogies can be used to establish meaningful relationships between prior knowledge and new knowledge (Kesercioğlu et al., 2004). If new topics that are difficult to comprehend are conveyed to the student by analogy with the topics they already know or by establishing a relationship with them, learning will take place more quickly (Sağırlı and Macaroğlu Akgül, 2004).

Models are scientific and mental activities used to facilitate people's understanding of seemingly complex events (Canpolat et al., 2004). If models are used correctly and appropriately, they will lead students towards conceptual models accepted by scientists. Enabling students to make and critique their own models provides conceptual development in learning.

It can be said that teaching with conceptual change strategies based on constructivist approach is more effective in eliminating misconceptions in learning concepts and relations between concepts. In the studies carried out; Özkan, Tekkaya and Geban (2001), Gökçe (2002), Balcı, Çakıroğlu, and Tekkaya (2006) found that conceptual change texts in their studies; Güçlüer (2006) concept maps; Bilgin and Geban (2001) analogies; Kabapınar (2005), Saka, Akdeniz, Bayrak, and Asilsoy (2006), Ekici, Ekici, and Aydın (2007) concept cartoons; Özyılmaz Akamca's (2008) analogies and concept cartoons; Yılmaz (1998) and Tekkaya (2003) use conceptual change texts and concept maps together; Glyn and Takahashi (1998) and Pabuçcu and Geban (2006) found that the use of analogies in conceptual change texts is effective in eliminating misconceptions.

The topics of "Cell Division and Heredity" include many concepts that are not often encountered in daily life. Concretizing and structuring the basic concepts of genetics, which are abstract and complex, is difficult for learners. Determining the misconceptions about Cell Division and Heredity is of great importance in terms of knowing how concept teaching takes place and arranging learning activities in a way that does not cause misconceptions (Aydın and Balım, 2013).

In a study, it was seen that the students in the control group could not establish the size relationship between the concepts of nucleotide, DNA, and chromosome, and as a result, they had misconceptions such as nucleotide contains chromosomes and DNA forms nucleotides. On the other hand, Unal et al. (2001) in their study on teaching the subject of mitosis with a model, they found that many students had difficulty in establishing a relationship between the concepts of gene, DNA, chromosome and cell division. Şahin and Parim (2002) also stated in their study that the relationship between the concepts of gene and chromosome was not clearly understood, and students said that genes are larger than chromosomes. Researchers have stated that learning the chromosome-gene relationship is difficult for students in the 14-15 age group who are in the phase of transitioning from concrete to abstract in their cognitive development. From the students in the control group, It was determined that the students in the control group had the misconception that although the chromosome numbers were the same, the reason for the differences between people was that the phosphate of the genes was different. They also identified the misconception that the chromosome numbers of living things carried by the students in the experimental and control groups are directly proportional to the level of development.

In the study conducted by Aydın and Balım (2013), a misconception was found in one of the students in the control group that an accident when they were young can cause color blindness. Again, the expression of conjoined ears and hereditary disease, which was said by one of the students in the control group, shows that the student could not structure the concepts of hereditary trait and hereditary disease in her mind well.

Clough and Wood-Robinson (1985), in their study with 84 middle school students, encountered the misconception that a kitten whose parents' tails were cut off is born with a cut tail and the kittens with a short tail continue to be born for generations. In their study, the researchers asked students how long the tails of their offspring would be if the rats' tails were cut off. The students stated that the tails of the offspring will be of normal length, but if the tails of the mice are continued to be cut for generations, tailless offspring will be born after 4-5 generations and this will become a hereditary feature. Similarly, it was seen that the students in the control group had the misconception that accidental blindness becomes hereditary more than the students in the experimental group.

Similarly, Berthelsen (1999) found in her study that students have the misconception that inherited characters continue to be acquired throughout the life of living things. In the study, hereditary diseases determined to be present in one of the students in the control group are carried by dominant genes; Topçu (2004) also revealed in his study that they have the misconception that blood clotting (hemophilia) is controlled by dominant genes.

It is better for students to have no knowledge about a subject than to have wrong information about it. Because misconceptions on a subject prevent learning new subjects and concepts related to that subject. The fact that students have misconceptions in biology lessons, which includes a large number of subjects and concepts and the relations between them, makes it difficult for them to learn new relevant subjects. For this reason, it is of great importance to identify students' misconceptions in biology lessons and to use conceptual change strategies (conceptual change texts, concept maps, and mind maps, concept cartoons, and analogies, models) to eliminate these misconceptions.

RESULTS AND DISCUSSION

It was determined that the research on misconceptions in biology education was more in 2004-2005 compared to other years. It was determined that the misconceptions were investigated more in the subjects of environment/ecology and cell, more quantitative research methods were used as a method, and more undergraduate students were preferred as the sample group. It has been determined that the sample size mostly varies between 101-300 people, achievement tests and alternative evaluation tools are mostly used in the studies, and quantitative data analysis is at the forefront as a data analysis method (Gül and Özay Köse, 2018).

The analyzes showed that most of the pre-service teachers had difficulties in understanding some basic concepts and had misconceptions. The misconceptions detected are in parallel with the misconceptions obtained from studies conducted abroad (Sanders and Cramer, 1992; Seymour and Longdon, 1991).

Studies in the field of biology education in our country show that there are misconceptions in secondary education and university level students (Çapa, 2000; Tarakçı et al., 1999; Tekkaya et al., 1999). Identifying misconceptions is very important as it will provide opportunities for teacher candidates to realize and correct their misconceptions during their education. For this reason, teacher candidates and teachers should be informed about the misconceptions identified in the field of biology and the reasons for these misconceptions, how to prevent them and how to eliminate them, through activities such as in-service training seminars. In this way, teachers and prospective teachers can understand whether students' mental models are compatible with scientific ones. Thus, it can be ensured that students learn about biology subjects and concepts that have an important place in daily life.

It has been concluded that traditional teaching methods are not sufficient in concept teaching and in eliminating misconceptions, and it is necessary to use teaching methods and techniques based on the constructivist approach. When the methods used in concept teaching

are investigated; It has been determined that teaching methods and techniques based on constructivist approach such as inquiry-based teaching, project-based teaching, computer assisted teaching, material assisted teaching, 5E-6E learning model, concept maps, concept cartoons, conceptual change texts, vee diagrams, and argumentation are used and effective in concept teaching (Ecevit and Özdemir Şimşek, 2017; Hodson, 2014; Stein and Galili, 2014).

In addition, textbooks should be prepared and arranged in a way that contributes to students' concept teaching. Biology lesson hours can be increased in order to create teaching environments where students can learn by doing and experiencing, and to use special teaching methods. In addition, teachers can identify the dominant intelligence areas of their students according to the theory of multiple intelligences or the learning styles of the students and eliminate their misconceptions, if any, by using activities that will address this intelligence area. The conceptual changes to be made in this way can be understood in a much shorter time and more permanent learning can be achieved. The quality and quality of biology teaching in our country can be increased if teachers are informed about this issue by not only detecting misconceptions but also working on how to eliminate these misconceptions.

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EXTRACTION OF PHENOLIC COMPOUNDS FROM FENUGREEK SEEDS USING DIFFERENT EXTRACTION TECHNIQUES

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ABSTRACT

Food materials having biomaterials such as polyphenols and flavonoids have great importance for both food industry and consumers. *Trigonella-foenum graecum* (fenugreek) is a plant known with its medicinal properties. Fenugreek seeds are the most used parts of the plant having high amount of phenolic compounds. Thus, in this study, extracts rich in phenolic compounds were obtained from fenugreek seeds using different extraction techniques such as Soxhlet, maceration and ultrasonic-assisted extraction. Moreover, the effects of the different solvents such as ethanol, methanol, ethyl acetate, hexane and distilled water or their mixtures and fenugreek seed:solvent ratios on total phenolic compounds, total flavonoids and antioxidant activity of the extracts were investigated. Results showed that the highest antioxidant activity was obtained with 50% mixture of ethanol and distilled water for the maceration, whereas the extracts obtained using hexane and ethyl acetate had the lowest antioxidant activity values. Among all extraction techniques, ultrasonic-assisted extraction gave the best results. 16.96 mg gallic acid/g dry sample of total phenolic compounds, 11.82 mg quercetin /g dry sample of total flavonoids and 5.52 mg trolox/g dry sample of antioxidant activity were achieved at the conditions of 55% ultrasonic amplitude, 60 minutes of extraction time and 50% solvent mixture of ethanol-distilled water.

Keywords: Fenugreek seeds, ultrasonic-assisted extraction, ultrasonic amplitude, phenolics, antioxidant activity

INTRODUCTION

Due to their health benefits, seeds are of remarkable interest by consumers and researchers. Recently, consumers are more prone to consume foods that are beneficial to health and this has led to an increase in the production of functional foods. Thus, it is becoming increasingly important to obtain extracts from products such as seeds rich in phenolic compounds in order to produce foods with functional properties. In recent years, the importance of plants and seeds which are rich in secondary metabolites such as phenolic acids, flavonoids, and alkaloids are shown by some researchers. They claimed that the extracts obtained from these plants and seeds can be used to avert or treat chronic diseases (Alara et al., 2018). One of the seeds having this potential is fenugreek seeds.

Trigonella-foenum graecum (fenugreek) is a medicinal plant and it has been used for centuries as prevalent and natural medicine in Asia, the Middle East, and some European countries (Akbari et al., 2019). Fenugreek seed extracts can be rich in phenolic acids and flavonoids and they have high antioxidant capacity because they contain flavonoids such as vitexin, quercetin, and luteolin (Pająk et al., 2019). The phenolic compounds existed in fenugreek seeds can be used for the treatment of different diseases (Chan et al., 2017). Therefore, it is important to determine the true extraction technique, extraction conditions and solvents for the highest recovery of these compounds (Akbari et al., 2019).

Extraction of phenolic substances from plants is carried out using conventional and novel techniques (Alara et al., 2018). Maceration and Soxhlet extraction can be considered as the most frequently used conventional extraction techniques due to their simplicity, low cost, and being suitable for total recovery of the extracts. On the other hand, these techniques require very long extraction times and considerable amount of solvents (Oreopoulou et al., 2019). In order to overcome these shortcomings, novel extraction techniques can be used. Ultrasonic-assisted extraction (UAE) technique is a novel and green method to obtain polyphenols from plant materials (Bhargava et al., 2021). In the UAE method, numerous bubbles are produced in a liquid medium. Explosively growing bubbles collapse eventually and as a result, the turbulency of the liquid phase will be increased by ultrasound waves, which considerably enhance the extraction process (Zhao et al., 2007).

Some researchers have studied the extraction of phenolic compounds by Soxhlet extraction, and maceration from fenugreek seeds (Wani et al., 2016). However, UAE technique was not frequently used for the extraction of polyphenols from fenugreek seeds. Al-Juhaimi et al. (2016) extracted phenolic substances from fenugreek seeds and they used different concentrations of ethanol as solvents. As far as we know, there is no study comparing the different extraction techniques for the extraction of polyphenols from fenugreek seeds in literature.

In this study, polyphenolic extracts of the fenugreek seeds were obtained using three different extraction techniques namely maceration, Soxhlet extraction and UAE. Moreover, the effects of the parameters such as extraction time, seed to solvent ratio, and solvent type were investigated.

MATERIALS AND METHODS

Material

The fenugreek seeds were bought from a local market found in Tokat, Turkey. Firstly, the seeds were combed out and then cleaned seeds were grained by a rotary grinder, Sinbo SHB 3020. Then the grained seeds were sieved using a sieve having 630 μm pore diameter, following that the samples under the sieve were collected. The final sample had moisture content of 5.79% (wet base).

Soxhlet extraction

Three g of the seed sample was weighed into a Soxhlet cartridge and extraction was carried out using 200 mL of ethanol. 6, 12, 18, and 24 hours of extraction times were applied. The extracts containing ethanol obtained after remaining extraction times was subjected to evaporation (Buchi RE121 Rotavapor with Buchi 461 Water Bath, Germany), and then the extract sticked on the glass flask was recovered with 50 mL of ethanol, which made the last seed to solvent ratio 60 g/L (See et al., 2016).

Extraction with maceration technique

Firstly, the same seed to solvent ratio was used as Soxhlet extraction (60 g/L) and only 100% ethanol was chosen as the solvent. 1-12 hours of extraction times were applied to the prepared seed-ethanol mixtures using 400 rpm of agitation speed at 25°C. Then, only distilled water was used as solvent and 1-6 hours of extraction times were applied. In order to find out the effect of the seed to solvent ratio, 2-60 g/L concentrations were chosen (distilled water used as solvent and 2 hours of extraction time was applied). Another study for maceration technique was carried out to determine the effect of the solvent types and different solvent mixtures. Ethanol, methanol, ethyl acetate, hexane and distilled water were chosen as different solvents. Pure, 50% aqueous forms, and total mixture of the solvents were used.

After the required extraction times were expired, samples were centrifuged for five minutes at 6000 rpm and filtered using a coarse filter paper. If an extract contained an organic solvent, it was evaporated with rotary evaporator and the dry extract was taken with distilled water considering the sample to solvent ratio. These samples were used for the chemical analyses.

Ultrasonic-assisted extraction

The UAE process was conducted using a laboratory scale sonicator (Q Sonica Q 500, 500 W, 20 kHz, USA) having a 13 mm diameter probe. α value was set to 0.8 which was calculated as $\alpha = t_{open}/(t_{open}+t_{closed})$. This α value was determined by preliminary tests and it was aimed to prevent overheating of the probe and the liquid medium. Here, t_{open} indicates the time (s) that sonication is active, and t_{closed} indicates the time (s) that sonication is passive (Chan et al., 2017). An ice bath under the sample carrier was used to keep the extraction temperature at ~25°C. For 50% mixture of ethanol and distilled water, 55% of ultrasonic amplitude was used and 1 hour of extraction time was applied. For the only distilled water as solvent, 80% of ultrasonic amplitude and 0.5, 1, and 2 hours of extraction times were applied. All samples had the seed to solvent ratio of 10 g/L. Centrifugation and filtering processes were applied as same as the maceration technique.

Chemical analysis

The total phenolic compounds (TPC), total flavonoids (TFL) and the antioxidant activity of the all-different extracts were determined.

The TPC of the samples was determined using Folin-Ciocalteau method (Singleton and Rossi, 1965). Absorbance of the samples were measured at 725 nm wavelength (PG Instruments T80, United Kingdom). The TPC were presented as mg gallic acid/g dry sample.

The TFL of the samples was determined with aluminum chloride method. The absorbance of the samples was read at 510 nm of wavelength, and TPC was expressed as mg quercetin/g dry sample (Belguith-Hadriche et al., 2013).

Antioxidant activity of the samples was determined using DPPH method. 50 µl of sample was mixed with 1.95 mL of DPPH (0.1 mM) and this mixture was incubated for 30 minutes. After incubation time, the absorbance of the samples was determined at 515 nm wavelength. Antioxidant activity of the fenugreek seed samples was expressed as mg trolox/g dry sample (Pająk et al., 2019).

Statistical analysis

The data were evaluated by univariate analysis with a Duncan significant difference test (significance level of 95%) using SPSS 22.0 statistics package program (IBM Corp., New York, USA).

RESULTS AND DISCUSSION

The results of Soxhlet extraction of phenolic compounds from fenugreek seeds were shown in Table 1. TPC, TFL and antioxidant activity results followed a similar trend and the highest results were obtained at 18 hours of extraction time. When 18 hours of extraction time exceeded, the results were reduced by 20-25%. The results are following the Fick's second law of diffusion. Final equilibrium will be reached between the solvent and the sample at a certain time of the extraction, after this time, the yield may tend to decrease beyond this equilibrium state (Alara et al., 2018). Similar results were obtained by Chew et al. (2011), they extracted

polyphenols from *Centella asiatica* and after 120 minutes of extraction time, TPC values were decreased.

Table 1. Soxhlet extraction of phenolic compounds

Extraction time (hours)	Total Phenolic Compounds (mg gallic acid/g dry sample)	Total Flavonoids (mg quercetin/g dry sample)	Antioxidant activity (mg trolox/g dry sample)
6	15.97 (±0.86)^{d}	10.30 (±0.13)^{d}	0.52 (±0.01)^{d}
12	23.33 (±0.81)^{b}	15.22 (±0.15)^{b}	0.75 (±0.01)^{b}
18	24.75 (±0.72)^{a}	17.20 (±0.22)^{a}	0.79 (±0.02)^{a}
24	18.51 (±0.36)^{c}	13.52 (±0.18)^{c}	0.60 (±0.01)^{c}

$^{a-d}$ Means with uncommon superscripts within a column are significantly different (p<0.05).

Table 2 shows the results of the extraction of phenolics by maceration using solvent as 100% ethanol, which was the same solvent for Soxhlet extraction process. After 8 hours of extraction time, TPC values did not differ, significantly (p>0.05). The highest TPC values were obtained at the 8-12 hours of extraction time for maceration and these values were approximately 26% lower than that of the highest values of Soxhlet extraction.

Table 2. Extraction of phenolic compounds by maceration using only ethanol as solvent

Extraction time (hours)	Total Phenolic Compounds (mg gallic acid/g dry sample)	Total Flavonoids (mg quercetin/g dry sample)	Antioxidant activity (mg trolox/g dry sample)
1	10.28 (±0.63)^{f}	7.15 (±0.15)^{g}	0.32 (±0.00)^{g}
2	12.17 (±0.28)^{e}	8.47 (±0.10)^{f}	0.40 (±0.01)^{f}
3	14.30 (±0.51)^{d}	9.92 (±0.21)^{e}	0.47 (±0.00)^{e}
4	15.36 (±0.09)^{c}	10.81 (±0.12)^{d}	0.51 (±0.00)^{d}
6	16.53 (±0.20)^{b}	11.68 (±0.03)^{c}	0.54 (±0.00)^{c}
8	17.51 (±0.28)^{a}	12.19 (±0.10)^{b}	0.57 (±0.00)^{b}
12	18.30 (±0.26)^{a}	13.37 (±0.15)^{a}	0.61 (±0.01)^{a}

$^{a-g}$ Means with uncommon superscripts within a column are significantly different (p<0.05).

In Table 3, the results of the extraction of the phenolic materials from fenugreek seeds by maceration using solvent as 100% distilled water were shown. The results were enhanced 7-fold higher than at the same extraction time of the maceration with only ethanol. The highest TPC value of maceration with only ethanol was at the 12th hours, however, when the maceration technique was done with only distilled water, at 6th hour the highest TPC, TFL and antioxidant activity values were obtained. In general, ethanol, which has low polarity, is not capable of extracting more bioactive compounds (Akbari et al., 2019). This phenomenon is mostly related with the polarity of the bioactive components such as phenolics and flavonoids (Amid and Mirhosseini, 2012). Thus, a polar solvent such as distilled water may enhance the extraction yield and the amount of the TPC. The results showed that fenugreek seed aqueous extracts had higher TPC and TFL than ethanolic extracts, which led to having higher antioxidant activity values (Table 3). Moreover, 2 hours of the extraction time was chosen as the equilibrium point of the aqueous maceration, because there was no significant difference between 2, 3, and 4 hours of extraction time in terms of TPC values (p>0.05). After this point, 2 hours of extraction time was used for other maceration extractions.

Table 3. Extraction of phenolic compounds by maceration using only distilled water as solvent

Extraction time (hours)	Total Phenolic Compounds (mg gallic acid/g dry sample)	Total Flavonoids (mg quercetin/g dry sample)	Antioxidant activity (mg trolox/g dry sample)
1	70.95 (±4.61)^{c}	49.27 (±0.08)^{f}	2.30 (±0.13)^{e}
2	109.67 (±5.12)^{b}	76.68 (±0.33)^{e}	3.54 (±0.11)^{d}
3	114.01 (±0.68)^{ab}	81.97 (±0.25)^{d}	3.69 (±0.04)^{cd}
4	116.42 (±1.02)^{ab}	84.07 (±0.25)^{c}	3.79 (±0.04)^{bc}
5	118.11 (±0.68)^{a}	85.35 (±0.41)^{b}	3.91 (±0.02)^{ab}
6	120.16 (±0.85)^{a}	86.51 (±0.40)^{a}	3.98 (±0.02)^{a}

$^{a-f}$ Means with uncommon superscripts within a column are significantly different (p<0.05).

Another important parameter for the extraction processes is sample to solvent ratio. The food matrix should be completely immersed to the solvent to obtain higher recovery of the target compounds during extraction processes and usually higher volume of the solvent increases the extraction yield (Alara et al., 2018). Table 4 shows the effect of the seed to solvent ratio on the total phenolics, total flavonoids and the antioxidant capacity at aqueous maceration. According to the results, at a constant extraction time of 2 hours and distilled water concentration of 100%, the highest values of TPC, TFL and antioxidant activity were obtained 10, 5 and 2 g/L. It can be seen in Table 4 that the TPC, TFL and antioxidant activity values tend to increase when seed to solvent ratio decreases. When TPC values were considered, there was no statistical difference between 10, 5 and 2 g/L (p>0.05), so that 10 g/L seed to solvent ratio was chosen for further extractions.

Table 4. Effect of the seed to solvent ratio on the total phenolics, total flavonoids and the antioxidant capacity at aqueous maceration extraction

Seed powder:solvent (g/L)	Total Phenolic Compounds (mg gallic acid/g dry sample)	Total Flavonoids (mg quercetin/g dry sample)	Antioxidant activity (mg trolox/g dry sample)
60	69.53 (±0.11)^{b}	47.29 (±0.74)^{d}	2.25 (±0.01)^{d}
30	74.24 (±5.69)^{b}	51.94 (±0.75)^{c}	2.37 (±0.07)^{d}
20	76.24 (±1.79)^{b}	53.46 (±0.41)^{c}	2.49 (±0.03)^{cd}
10	109.67 (±5.12)^{a}	76.68 (±0.33)^{b}	3.54 (±0.11)^{b}
5	113.19 (±3.41)^{a}	79.70 (±0.66)^{ab}	3.62 (±0.04)^{ab}
2	111.09 (±2.56)^{a}	77.72 (±2.47)^{a}	3.45 (±0.05)^{a}

$^{a-d}$ Means with uncommon superscripts within a column are significantly different (p<0.05).

Due to being safe, ethanol is the one of the mostly preferred organic solvents for the food industry. In this study, the highest recovery of the TPC and TFL was obtained when 50% aqueous ethanol was used (Table 5). These results are consistent with the findings of Kalia et al. (2008). They extracted phenolic antioxidants from *Potentilla atrosanguinea* using different solvents and their concentrations, and they have found that 50% aqueous ethanol was the most effective solvent for the extraction process.

In literature, methanol, ethyl acetate and hexane were also used for the extraction of polyphenols from fenugreek seeds. Belguith-Hadriche et al. (2013) extracted phenolic substances from fenugreek seeds by maceration and methanol, hexane, ethyl acetate, and dichloromethane were chosen as solvents. TPC values were in the range of 23.2 and 78.1 mg gallic acid/g sample and highest TPC yields were obtained when methanol and ethyl acetate used as solvents. On the other hand, hexane and ethyl acetate gave the lowest TPC, TFL and antioxidant activity results in our study. This phenomenon can be related with the origin of the samples and other extraction parameters such as temperature, seed to solvent ratio and the extraction time.

Table 5. Effect of the different solvents on the total phenolics, total flavonoids and the antioxidant capacity at maceration extraction

Solvent	Total Phenolic Compounds (mg gallic acid/g dry sample)	Total Flavonoids (mg quercetin/g dry sample)	Antioxidant activity (mg trolox/g dry sample)
Ethanol (100%)	20.17 (±1.71)^{h}	13.88 (±0.15)^{i}	0.66 (±0.02)^{h}
Methanol (100%)	52.61 (±0.85)^{f}	28.72 (±0.82)^{g}	1.64 (±0.02)^{f}
Ethyl Acetate (100%)	7.14 (±0.34)^{i}	5.40 (±0.03)^{j}	0.24 (±0.00)^{i}
Hexane (100%)	4.73 (±0.34)^{i}	4.59 (±0.18)^{j}	0.20 (±0.00)^{i}
Ethanol-Methanol (50-50%)	34.88 (±0.68)^{g}	24.42 (±0.49)^{h}	1.22 (±0.02)^{g}
Ethanol-H_2O (50-50%)	137.05 (±0.17)^{a}	93.18 (±0.82)^{a}	4.39 (±0.11)^{a}
Methanol-H_2O (50-50%)	123.78 (±2.56)^{b}	68.86 (±1.32)^{c}	4.02 (±0.01)^{b}
Ethyl Acetate-H_2O (50-50%)	82.65 (±3.41)^{d}	58.96 (±1.48)^{e}	2.71 (±0.08)^{d}
Hexane-H_2O (50-50%)	77.34 (±0.34)^{e}	50.35 (±1.15)^{f}	2.49 (±0.05)^{e}
Ethanol-Methanol-Ethyl Acetate-Hexane (25%)	85.91 (±4.26)^{d}	62.34 (±0.33)^{d}	2.78 (±0.04)^{d}
Ethanol-Methanol-H_2O (33%)	105.21 (±1.88)^{c}	73.63 (±1.15)^{b}	3.43 (±0.02)^{c}

$^{a-j}$ Means with uncommon superscripts within a column are significantly different (p<0.05).

The solvent of 50% aqueous ethanol had better results than 100% distilled water at 55% ultrasonic amplitude and 1 hour of extraction time, and the highest values of TPC, TFL and antioxidant activity were obtained among all our trials. These results may be related with the effect of both solvent and ultrasound. Ethanol-H_2O (50-50%) mixture had highest results at maceration (Table 5), and this mixture can be applied to the UAE system to achieve the highest yield of TPC, TFL and antioxidant activity (Table 6). Similar to our findings, Del Hierro et al. (2018) extracted polyphenols and saponins from fenugreek seeds using UAE and they reported that highest TPC content was obtained when ethanol-H_2O (50-50%) mixture was used as solvent.

The results at ultrasonic amplitude of 80% showed that highest TPC, TFL and antioxidant activity values were obtained for 1 hour of extraction time. Compared to the 1-hour extraction time, TPC, TFL and antioxidant activity values were 5% lower for half-hours of extraction time and 12% lower for two-hour extraction time (Table 6). These results indicated the importance of determination of optimum extraction time for UAE. At different rates of ultrasonic power or ultrasonic amplitude, the most appropriate extraction time to obtain highest amount of phenolic compounds may differ. If the extraction time is kept shorter than the optimal time, sufficient extraction may not be performed, or if the extraction time is longer, phenolic substances may be damaged due to the intense effect of the ultrasound, as a result, the yield of TPC and antioxidant activity may decrease.

Table 6. Extraction of phenolic compounds using ultrasonic-assisted extraction

Solvent	Extraction Time (hours)	Ultrasonic Amplitude (%)	Total Phenolic Compounds (mg gallic acid/g dry sample)	Total Flavonoids (mg quercetin/g dry sample)	Antioxidant activity (mg trolox/g dry sample)
Ethanol-H_2O (50-50%)	1	55	169.62 (±1.88)^{a}	118.20 (±1.32)^{a}	5.52 (±0.04)^{a}
H_2O (100%)	1	55	138.98 (±0.17)^{c}	94.69 (±2.30)^{d}	4.45 (±0.04)^{d}
H_2O (100%)	0.5	80	165.03 (±1.19)^{a}	101.68 (±0.66)^{c}	5.15 (±0.07)^{b}
H_2O (100%)	1	80	166.84 (±5.46)^{a}	105.75 (±0.82)^{b}	5.41 (±0.07)^{a}
H_2O (100%)	2	80	148.03 (±3.07)^{b}	93.41 (±0.16)^{d}	4.78 (±0.12)^{c}

$^{a-d}$ Means with uncommon superscripts within a column are significantly different (p<0.05).

CONCLUSIONS

In this study, phenolic compounds were extracted from fenugreek seeds using different extraction techniques and the effect of the different extraction times, seed to solvent ratios and solvent types on TPC, TFL and antioxidant activity were investigated. Soxhlet extraction and maceration extraction gave similar results in terms of TPC, TFL and antioxidant activity values when only 100% ethanol was used as solvent. On the other hand, when distilled water used as solvent, all values were significantly enhanced. Among all different solvents, 50% aqueous ethanol gave the best results for maceration. When all different extraction methods were compared, UAE ensured the highest TPC, TFL and antioxidant activity values. This study confirmed that UAE technique could be a simple and a rapid method to obtain extracts having high amount of polyphenols. Further, an optimization study may be carried out to find out the best conditions for obtaining the richest extract in terms of polyphenols.

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IDENTIFYING THE TOXIC EFFECTS OF CAR TIRE RUBBER LEACHATES ON ZEBRAFISH (DANIO RERIO) LARVAE AND DAPHNIA MAGNA

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ABSTRACT

Progressive fragmentation and weathering processes of synthetic polymer-based plastics found in the aquatic ecosystem result in leaching of additive chemicals. It is thought that these leaching of may lead to toxic impact in aquatic organisms. This research investigates the toxic effects of leachates car tire rubber on *Danio rerio* embryos and *Daphnia magna.* In this study, the toxic effects of 24, 48, 72 and 96 hours leachates of rubber at 7.5 g/L concentration on *D. rerio* and *D. magna* were determined. While no mortality was observed in the 24th and 48th hour leachates process, mortality (20.8% and 41.7% respectively) was observed in the 72nd and 96th hour leachates process in *D. rerio*. Mortality rates were determined as 54.3%, 62.9%, 94.3% and 97.1 respectively at 24th, 48th, 72nd and 96th hours of leachates application in *D. magna.* In the study, growth retardation was observed in both *D. rerio* embryos and *D. magna* exposed to the test solution. In addition, various malformations and heart rate decreased were observed in *D. rerio* embryos. According to the results of the study, *D. magna* was more sensitive to rubber leachates than *D. rerio* larvae.

Keywords: Rubber, Leachates, *Daphnia magna, Danio rerio,* Toxicity

INTRODUCTION

Plastics are synthetic ingredients composed of organic polymers. These products are used to extensively around the world owing to their resistant, powerful, light weight, and low thermal conductivity (Meng et al., 2020). Microplastics (MPs) are plastics that are smaller than 5 millimeters long. Progressive fragmentation and weathering processes of these synthetic polymer-based plastics found in the aquatic ecosystem result in leaching of additive chemicals (Capolupo et al., 2020).

The continuing research on effects of plastic particles and microplastics (MPs) in the aquatic ecosystem has raised the attention that organic pollutant arrive the aquatic ecosystem not only in dissolved, but also in particle form (Wagner et al., 2018). Studies on MPs have only focused on thermoplastic particles such as polyethylene and polystyrene and did not take into account elastomers like rubber. Car tire rubber (CTR) releases wear particles due to mechanical wear. Many research have reported that these wear particles are a significant source of MPs in the environment (Kole et al., 2017). Several additives such as benzothiazoles, phthalates and phenols leach from the CTR. Morever these additives have been shown to adversely affect aquatic organisms in literature (Canesi and Fabbri, 2015, Silva et al., 2016). There are a several studies on the effects of MPs on aquatic organisms, but most data available were on direct effects of MPs on organisms (Silva et al., 2016).

The aim of this study is to investigate the toxic effects of car tire rubber leachate on *Danio rerio* embryos and *Daphnia magna.*

MATERIAL AND METHOD

Testing organisms

Adult *D. rerio* colony used in our study was obtained from in Inonu University, Faculty of Arts and Science, Department of Biology Environmental Toxicology Laboratory using Zebrafish Aquatic System (ZebTec Active Blue, Tecniplast, Italy) (pH 7.30, conductivity 720 µS/cm, temperature 28.2 ℃, and 14:10 hours light and dark photoperiod) *D. rerio* larvae were obtained from AB population through a filtered breeding system (iSpawn, Tecniplast, Italy). The fertilized eggs were collected within 3 hours and the in E2 medium (E2 medium: 15 mM NaCl, 0.5 mM KCl, 1.0 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$ ve 1.0 mM $MgSO_4$, pH 7.2) (Westerfield, 2007) was kept in an illuminated incubator at 28 ºC (Kimmel vd., 1995).

Leachate preparation

Leachates of car tire rubber were generated in two different media; embryo water (E2 medium) for *D. rerio* larvae and daphnia culture medium for *D. magna*. The rubber-water ratio was kept constant at 7.5 g/L rubber. The leachates process was performed according to Capolupo et al. 2020. The samples were shaken on shaker (150 rpm) at 25 °C temperature for 24, 48, 72 and 96 h in the dark. The pH of the rubber leachates was measured. After the leaching process, samples were centrifuged at 2,000 rpm for 5 minutes, and the upper organic phase was taken and transferred to a glass bottle.

Characterization of the rubber

Structural characterization of microplastic structures used in the study was defined by FT-IR spectroscopy. FT-IR analyzes were performed on the Perkin Elmer Spectrum Two device in the wavenumber range of 400-4000 cm^{-1}. Particle size analyzes were carried out by dispersing 0.01 g of rubber in 1 ml of toluene using the Malvern Zetasizer Nano-ZS model device.

Exposure Protocols and Microscopic Observation

The rubber solution was prepared as 7.5 g/L in embryo water for *D. rerio* and in daphnia culture water for *D. magna.* D. rerio embryos were subjected to rubber leachates in 96 well microplates with 250 µL of solution and 24 embryos were exposed to each process and 96 hours. *D. magna* neonates were exposed to rubber leachates for 48 hours in a 50 ml beaker. Mortality of the embryos and neonates was observed for 24-hour intervals with a stereo-microscope. The developmental malformation data and size of the surviving embryos were noted. Body lengths were measured with Euromex Image Focus 4.0 software.

Statistical Analysis

The statistical analysis of the collected data was conducted with SPSS and GraphPad Prism 5 (SPSS Inc., USA). The results that did not comply with the required hypotheses for parametric tests were processed with the Mann-Whitney U and Kruskal Wallis test.

RESULT AND DISCUSSION

When looking at the FT-IR spectrum, aliphatic C-H stretching vibrations are seen as two intense peaks in the range of 2790-2950 cm^{-1}. It is seen that C-C aliphatic stretching vibrations occur at 1550 cm^{-1}, aliphatic C-H stretching vibrations on the polymer chain at 825 cm-1 and 525 cm^{-1}. The broad band at approximately 1000 cm^{-1} is due to Si-O and Zn-O structures and is due to

the additive. In the range of 3100-3600 cm^{-1}, we see the peaks originating from the surface – OH groups of these additives. S-S disulfide bonds are 500-540 cm^{-1} (Figure 1).

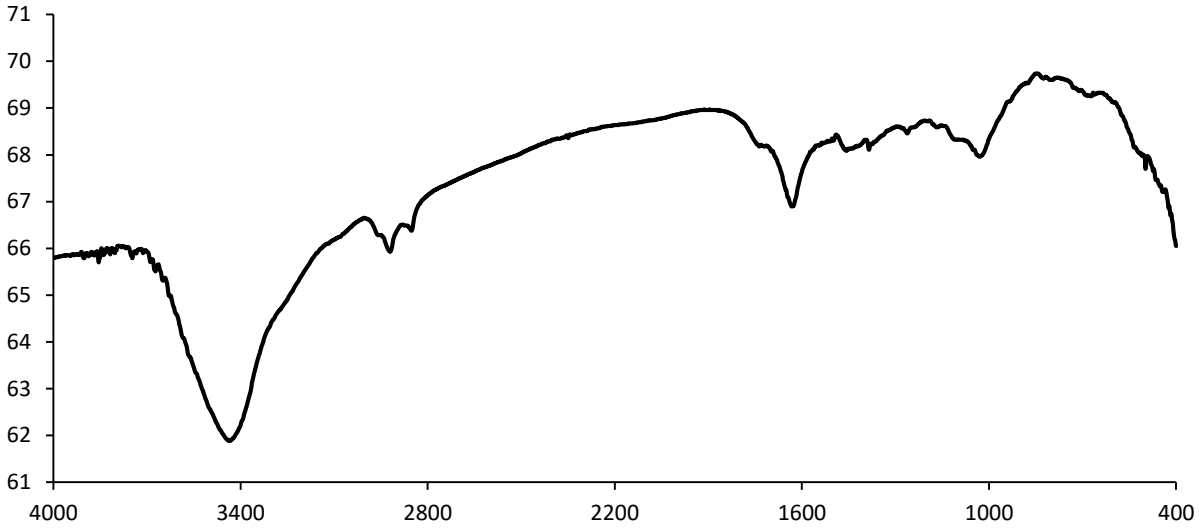

Figure 1. FT-IR spectra of rubber

The rubber used as a microplastic source within the scope of the study has a particle size distribution range of 100 nm and 1000 nm.

In *D. rerio* juveniles exposed to rubber leachates, no mortality was observed at the 24th and 48th hours in the leaching application, while mortality was observed at the 72nd and 96th hours (Figure 2).

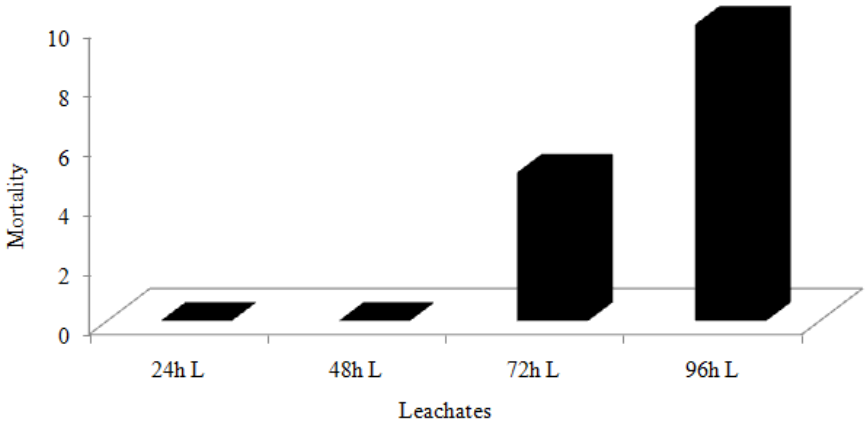

Figure 2. According to hours of leachates processes mortality rates for *D. rerio* juveniles

As shown in Table 1, 48h, 72h and 96h leachates processes resulted in significant inhibition of embryonic growth for *D. rerio* juveniles ($p < 0.05$). Many studies reported growth inhibition in *D. rerio* juveniles exposed to pollutants (Liu et al., 2018, Qui et al., 2020, Turhan et al., 2021).

Table 1. Lengths and death levels in *D. rerio* juveniles exposed to rubber leachates for 96 hours

Concentration (g/L^{-1})	Hours of leachates processes	Living individual	[a]Lengths (mm) Mean±SD			
Control	24	24	3.11	±	0.06	
	48	24	3.08	±	0.07	
	72	24	3.09	±	0.08	
	96	24	3.12	±	0.06	
7.5	24	24	3.08	±	0.08	
	48	24	2.99	±	0.21	***
	72	19	2.81	±	0.19	***
	96	14	2.45	±	0.22	***

24 individuals were exposed for each leachates processes

[a]Lengths are expressed as mean ± standard errors. These values were obtained from the lengths of the surviving individuals

*Indicates groups that are significantly different from the control ($p < 0.05$)

In zebrafish juveniles exposed to rubber, heart rate increased at 24 and 48 hours, but decreased at 72 and 96 hours (Figure 3). Similar to the results of this study, Mersereau et al., 2015 found that in zebra fish juveniles exposed to cocaine, heart rate increased at low doses and decreased at high doses.

Malformation was observed in 12.5% of *D.rerio* juveniles exposed to rubber leachates processes. Spinal curvature, tail malformation, pericardial edema, and yolk sac edema were determined in embryos. Pericardial edema is the most common type of malformation.

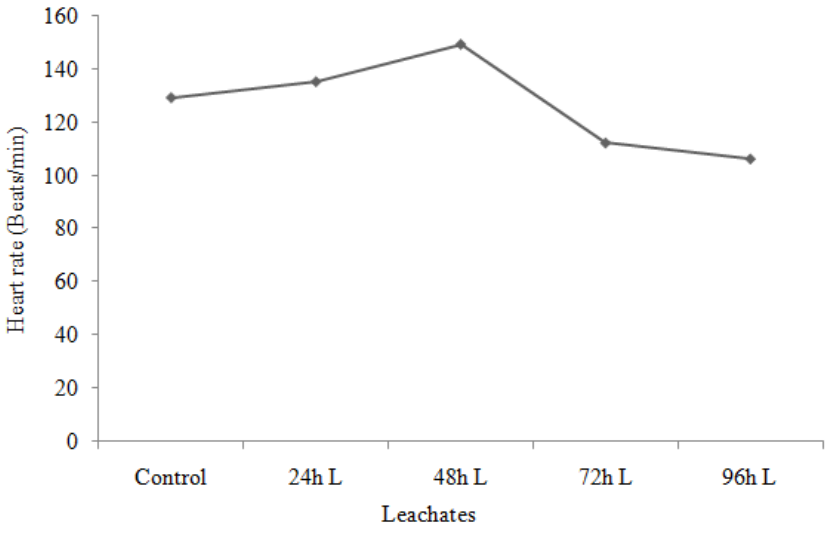

Figure 3. Heart rate in 96h *D. rerio* embryos exposed to rubber leachates

Contrary to *D. rerio* juvenils, mortality was observed at 24 and 48 hours in *D. magna* neonats exposed to rubber leachate. However, there was an increase in mortality rate at 72 and 96 hours, similar to *D.rerio* results (Figure 4).

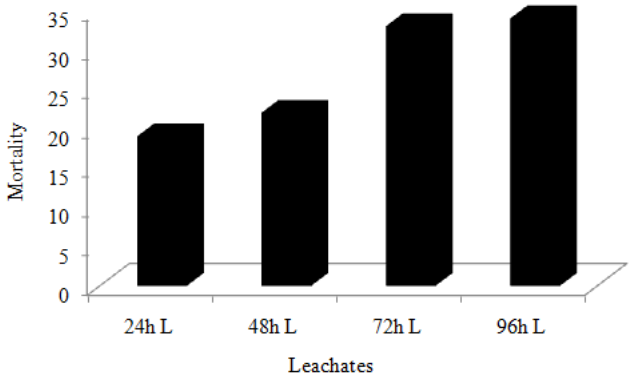

Figure 4. According to hours of leachates processes mortality rates for *D. magna* neonates

As shown in Table 2, 48h, leachates processes resulted in significant inhibition of growth for *D. magna* neonates ($p < 0.05$).

Table 2. Lengths and death levels in *D. magna* neonates exposed to rubber leachates for 48 hours

Concentration g/L^{-1}	Hours of leachates processes	Living individual	[a]Lengths (mm) Mean±SD			
Control	24	35	1.11	±	0.12	
	48	35	1.09	±	0.09	
	72	35	1.07	±	010	
7.5	24	16	1.08	±	0.13	
	48	13	0.97	±	0.10	*

35 individuals were exposed for each leachates processes

[a]Lengths are expressed as mean ± standard errors. These values were obtained from the lengths of the surviving individuals

*Indicates groups that are significantly different from the control ($p < 0.05$)

CONCLUSIONS

This research provides knowledge on the chemical characterization of rubber and toxicological effects of leachates from rubber on *D. rerio* and *D. magna.* According to the results of this study, 72nd and 96th hour leachates processes are more effective on both species. Individuals of the D. magna species are more susceptible to rubber leachate. Car tires are generally formed as a result of vulcanization of styrene-based styrene butadiene rubber as a mixture of natural rubber and many additives. Since these microparticles have been proven to be toxic, their amount released into the environment should be reduced.

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INVESTIGATION OF THE EFFECT OF GERMINATION AND FERMENTATION PROCESS APPLIED TO CEREAL AND LEGUME GRAINS ON MICROBIAL FLORA

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ABSTRACT

Grains, classified as legumes and cereals, are global food sources that have been consumed since the beginning of humanity and create significant health benefits. Recently, especially in this period when the interest in functional foods is increasing, people want to provide health benefits while providing their nutritional needs. The interest in these grains, which have high nutritional values, is increasing day by day. Being an alternative to protein sources of animal origin makes them valuable in terms of environmental sustainability, meanwhile lowering the risk of being affected by cardiovascular diseases, they are also a favourable source of dietary fiber and antioxidant phytochemicals. However, the digestibility and bioavailability of these grains are limited due to some antinutrient components they contain, and their usability can be limited in some cases technologically compared to their counterparts and cannot satisfy the desired quality criteria. In order to eliminate these disadvantages, some pre-treatments such as heat treatment, germination, fermentation are applied to cereals and legumes and their technological and nutritional values are increased. Microorganisms involved in the fermentation process can break down the anti-nutritional substances in the grains with the enzymes they produce, and can provide higher digestibility, richer nutritional, sensory, and technological profiles and improve antioxidant properties. Although the germination of legumes and cereal is an old and acknowledged method, it has latterly become the focus of attention, especially because it significantly increases the nutritional and bioactive content and also improves the flavor. The germination process triggers the enzymatic activity in the grain and provides the deterioration of starch and non-starch polysaccharides and proteins, while increasing the amount of reducing sugar content, soluble dietary fibers, peptides and amino acids, and can provide the release of release of the insoluble phenolic compounds covalently bound to cell wall polysaccharides. This changing nutritional profile can also conduce changes in the microbial flora, and it is important to investigate the microflora formed especially in systems where fermentation and germination processes are used in combination. It is thought that these environments may be rich in lactic acid bacteria, which can enable the development of functional products with various techno-functional properties.

KEY WORDS: Lactic acid bacteria, Legumes, Cereals, Germination, Fermentation, Functional Foods

INTRODUCTION

The Food Safety and Security Group Working Paper (2019) defines the concept of "food security" as the state of all people at all times having physical and economic continuous access

to sufficient, healthy, safe and nutritious food to meet their nutritional needs for an active and healthy life. Nevertheless, the world population has been increasing at alarming levels since the beginning of the 20th century and it is informed that in the report published by Revision of the United Nations Population Estimates and Projections in 2015 that this number will reach 9.6 billion by 2050 and 10.9 billion in 2100 (Bessada et.al, 2019). Nevertheless, the world does not make sufficient progress to provide accessibility of safe nutritious and adequate food all year round for the population, which tends to increase continuously, or to eliminate malnutrition. Continuing conflicts, climate changes and economic fluctuations in the global framework slow down this progress and this is especially felt in places where unequalness is high. The COVID-19 pandemic has made progress even more difficult and has increased the number of people affected by hunger in the world in 2020. The prevalence of malnutrition (PoU), which was virtually unaffected from 2014 to 2019, rose from 8.4 percent a year ago to 9.9 percent, according to the State of Food Security and Nutrition in the World, 2021 report. In terms of population, it is reported that between 720 and 811 million people worldwide are facing hunger in 2020 (Figure 1).

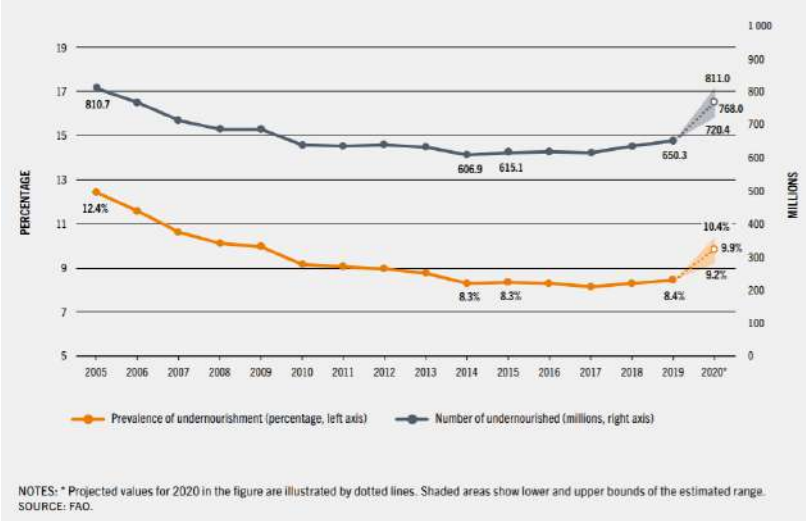

Figure 1 Statistical data on the increase in the number of people exposed to malnutrition in the world by 2020.

It is stated that the disequilibrium in food production due to the increasing population around the world and especially climate change will cause food shortages in the future, but of the moment, a remarkable number of trends on the planetary scale jeopardize the sustainability of food and agriculture systems Bessada et.al, 2019; Fasolin et.al, 2019). Within this scope, it is necessary to take on alternative and sustainable food and water resources as well as global agricultural production management in order to feed people and prevent malnutrition. Since April 2016, the main goal of The United Nations Decade of Action on Nutrition is to "eliminate all forms of malnutrition" and within the scope of adopted at the Second International Conference on Nutrition in 2014, is to continue to develop "sustainable and resilient food systems for healthy diets" (Fasolin et.al, 2019).

One of the most critical public health problems in many industrial countries is Protein energy malnutrition (PEM) (Bessada et al, 2019). As the main source of protein intake, meat and meat products occupy the first place and maintain their importance (33.14%), followed by cereal products and milk and dairy products. Fish and other seafood and other plant-based products such as eggs or legumes are also among the protein sources. Considering the last 50 years, it is seen that there has been an undeniable scale up the consumption of products of animal origin, which represents more than half of the daily protein supply (Bonnet et.al, 2020; Garrido-Galand et.al, 2021). The amount of water required for animal protein production requires 100% more water than for the same amount of plant protein production, which is both expensive and unsustainable. (Bessada et.al., 2019). Artificial meats produced in vitro, which are among the available alternative protein sources, have been in a trend recently as good quality proteins that help to reduce the carbon footprint during production, while eliminating the problems related to animal diseases and slaughter. On the other hand, insect proteins are seen as another alternative source, not only with their high nutritional value, but also because they can be grown in environmentally friendly conditions (Garrido-Galand et.al., 2021; Montowska et.al., 2019). However, since such products are not widely accepted by consumers, their production and consumption are controversial. Abovementioned information, the presence in the diet of plant-based foods such as legumes, cereals and seeds are seen as the strongest alternative for environmentally sustainable protein sources, both as a source of lower greenhouse gas emissions and substantial protein and other nutritional components (Fasolin et.al., 2019; Garrido-Galand et.al., 2021). It has been informed that there is a positive relationship between a healthy lifestyle and plant-based diets, and according to scientific evidence, legumes can provide significant benefits in the way of weight control and gastrointestinal health, reducing the risk of being affected by diseases such as cardiovascular disorders, metabolic syndrome and type 2 diabetes (Patel et.al., 2017). Harvard Health Publishing and nutritionists advised a "Healthy Nutrition Plate" as a guide for a balanced diet and stated that legumes and pulses, which are taken in consideration healthy and miscellaneous protein sources, should be included in the proteins that should make up ¼ of our plates (Margier vd, 2018). The origin of the word "Cereal" comes from "Ceres", the name of the ancient Roman goddess of harvest and agriculture (Petrova & Petrov, 2020). Cereal grains are important in terms of providing carbohydrates, proteins, vitamins, micronutrients, and dietary fibers required for human growth and development in their daily diet (Samtiya et.al., 2020). Grains in the cereal group form an important part of a balanced diet because they ensure notable amounts of most essential nutrients. Recent research has shown that cereal grains contain antioxidants and disease-preventing factors that have beneficial effects on human health. However, the nutritional quality of grains and the sensory properties of the products are sometimes lower or weaker when compared to some product groups. The reasons for these are (i) low protein content, (ii) deficiency of some essential amino acids (lysine), (iii) low starch availability due to swelling of macronutrient starch during cooking, (iv) certain anti-nutritional components (phytic acid, tannins, Polyphenols), (v) low content and bioavailability of micronutrients such as iron and zinc, and (v) coarse structure of the grains. Various methods such as cooking, germination, milling, fermentation are used to improve the nutritional qualities of cereals (Singh et.al., 2013). The pods or fruits of plants in the botanical family Leguminosae (or Fabaceae), which includes more than 13000 species, are gone by the name of legumes. According to the classification made by FAO, edible dried seeds with low oil content, called pulses, containing protein, vitamins, and minerals, which are a basic food and harvested for their dry grains (except peanuts and soybeans), constitute the pulses group (FAO,2016; Bessada et. al., 2019). Other than this

definition, products harvested as green such as peas and broad beans are alternatively classified as "vegetable products", while products used for oil extraction or planting purposes are not included in the "pulses" group (Figure 2) (FAO,1994). Legumes, once considered poor men' meat, have now emerged to be splendid food sources, the year 2016 was declared as the Year of Pulses (IYP) by the Food and Agriculture Organization of the United Nations (FAO) to highlight the important nutritional properties and low environmental impact of pulses. (Giusti et.al., 2017; FAO,2016). After cereals, the most economically important products are pulses. 11 major pulses groups dry beans, dry broad beans , dry peas by FAO, chickpeas, dry cowpeas, pigeon peas, lentils, bambara beans, vetches, lupins, and minor pulses accepted Legumes intended for human consumption include lentils, chickpeas, beans, peas , green peas, broad beans and soybeans. (FAO, 2016; Havemeier et.al., 2017). In terms of nutritional profile, pulses can meet 33% of the daily dietary protein, are rich in carbohydrates and dietary fibers, some essential vitamins, and minerals, and also contain important bioactive components (Bessada et.al., 2019; Temba et.al., 2016).

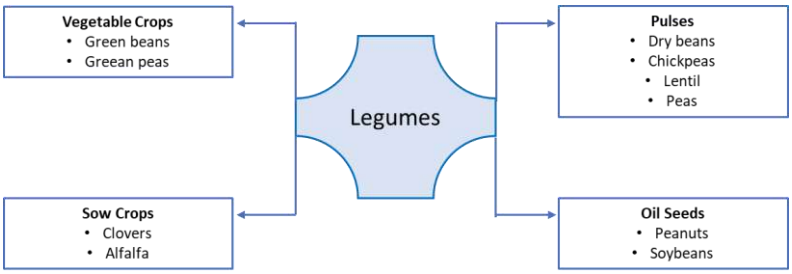

Figure 2: classification of legumes

Cereals and legumes can be used as is or milled, as well as by germination, fermentation, or selective heat treatment, improving their nutritional profile or increasing their usability. Germination of these grains is an old and well-known practice, but recently it has attracted attention as it significantly increases the nutritional and bioactive content and also contributes to the development of flavor. During the germination process, the reserves in the storage tissues of the grains are hydrolyzed to low molecular weight compounds and immobilized to support growth (Bewley et.al., 2001; Montemurro et.al., 2019). When seeds break dormancy, they synthesize many bioactive compounds such as riboflavin, thiamine, biotin, pantothenic acid, niacin, vitamin C, tocopherols and phenolic compounds as protective responses and improve their availability (Donkor vd, 2012). In germinated grains, hydrolytic enzymes gain their activity and enzymes are synthesized. This process helps to improve the nutritional profile by causing the deterioration of starch and non-starch polysaccharides and proteins, an increase in the amount of reducing sugars, soluble dietary fibers, peptides and amino acids, and the release of insoluble phenolic compounds that covalently bond with polysaccharides in the cell wall. Many fermented foods produced around the world encompass a wide variety of substances and microorganisms. One of the most important criteria showing the suitability of the substrate to be used in fermentation technologies is the percentage of starch and reducing sugar present in the medium. Lactic acid bacteria (LAB) are known as microorganisms that tend to be nutritionally fastidious as they often need specific amino acids, B vitamins and other growth factors for their growth. Many LAB species and other food-associated bacteria have a long

historical association with human food and are often considered safe (GRAS) bacteria (Hung et.al., 2012; Liptáková et.al., 2017).

This research study aims to examine the nutritive and anti-nutritional profiles of legumes and cereal grains in general and to evaluate the possible effects on microflora of using biological methods alone or in combination, which lead to positive changes by improving the functional and nutritional properties of whole grains and legumes and their flours.

Evaluation of Nutritional Profile

The United Nations Food and Agriculture Organization (FAO) recommend legumes as a staple food to meet basic protein and energy needs in human nutrition. Pulse grains are important because they are low-fat protein and carbohydrate sources and are in the gluten-free products category (Margier et.al., 2018). Cereal grains, another important group of plant origin, are also recognized as one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fiber for people all over the world, but their low protein content, lack of some essential amino acids (lysine), low starch availability, depending on the presence of certain anti-nutritional components and the coarse structure of the grains, the nutritional quality and sensory properties may sometimes be lower and weaker than substitutes obtained from different sources (Blandino et.al., 2002). Grain derivatives are also considered as an ingredient in the formulation of functional foods, due to the nutritional factors they provide, such as dietary fiber, proteins, energy, minerals, vitamins, and antioxidants, which are important for human health. They are also used as fermentable ingredients for the growth of probiotic microorganisms (Charalampopoulos et.al., 2002).

Legumes have comparatively low energy density, low fat content, digestible protein, and slowly digestible high carbohydrate content depending on the cooking method. They also contain soluble and insoluble dietary fiber, while being a source of mono- and polyunsaturated fats and plant sterols as other health-important components. Looking at the amino acid profile of legumes, they contain higher levels of lysine and threonine and essential amino acids such as leucine, aspartic acid and arginine compared to other plant-based protein sources, while the ratio of other sulfur-containing amino acids such as methionine, tryptophan and cysteine is low. Although legumes are considered as a low-quality protein source due to the lack of some amino acids, when consumed with cereals, a balance can be achieved between the two and quality protein intake can be achieved. They also have a rich content of micronutrients such as vitamin A and vitamin E, selenium, thiamine, niacin, folate, riboflavin, pyridoxine, zinc, iron (Mudryj et.al., 2014; Havemeier et.al., 2017). Grains are mainly sources of carbohydrates, which make up about 2/3 to 3/4 of the dry matter and are ultimately good sources of energy. Starch, which is the main component in the structure, is found in the endosperm as granules of different sizes. Cereals are good sources of proteins, the proportion of which can vary depending on the genotypic characteristics as well as different production methods and environmental conditions. Lipids exist as minor components in the grain structure and the amount can vary between 1.7% and 7.0% in dry matter, depending on the type of grain. Along with most B vitamins, they contribute significantly to vitamin E intake. The mineral content in the structure of whole grains is between 1.0-2.5% and contains trace elements such as selenium at a lower rate, while it contains significant amounts of magnesium, calcium, iron and zinc (Liptáková et.al., 2017).

It is important to prevent colonic protein fermentation, which causes toxic products and colon carcinogenesis, during the transition from meat-based diet to plant-based diet, and therefore, it

is necessary to ensure the intake of macro and micronutrients, especially in the stomach and small intestine, and to ensure efficient digestion of proteins, which are a macro component. The bioavailability of plant-based ingredients may be limited in some cases. Components called anti-nutritional (ANF) substances can seriously affect the bioavailability of nutrients in foods. Although legumes are nutritionally rich, the presence of adequate amounts of anti-nutrients can reduce their nutritional value (Robinson et.al., 2019; (Rosa-Sibakov et.al., 2018). ANFs; It can be divided into two classes as non-protein compounds such as tannins and saponins, phytic acid, α-galactosides and alkaloids, and proteinaceous compounds such as lectins, trypsin, chymotrypsin inhibitors (Garrido-Galand vd, 2021).

Phytic acid salts are found in plant seeds, cereal grains, nuts, legumes, and oilseeds (1-5% by weight). Cereals contain the highest amount of phytic acid (Priyodip, Prakash, & Balaji, 2017); Sharma et.al., 2020). The non-protein anti-nutritional component, which is also abundant in legumes, is phytic acid (or phytate). Phytic acid, known as inisitol hexakisphosphate, is the phosphate and inositol storage form of dicotyledonous seeds, and can exist in free form as a calcium/magnesium salt of phytic acid (phytin) or as a calcium salt of phytic acid (phytate), depending on pH and metal ions. Phytic acid has a high concentration of negatively charged phosphate groups and forms quite constant complex structures with mineral ions such as calcium, zinc, iron, and magnesium, preventing their absorption from the gut. The chelation of mineral elements acting as enzyme cofactors cause a decrease in the activity of digestive enzymes and thus to the formation of insoluble complexes that are not suitable for absorption from the small intestine. In addition, phytates can also form complexes with proteins at different pH ranges, change their structure and cause results such as lower protein solubility, enzymatic activity and proteolytic activity, and they reduce food digestibility by binding to digestive enzymes (lipase, amylase, pepsin, trypsin and chymotrypsin. While ruminants can hydrolyze phytic acid to myo-inositol and inositol phosphates (IP1-IP5) via the phytase (myo-inositol hexaphosphate phospho-hydrolase) enzyme in their polygastric digestive system, humans and monogastric animals lack enough enzymes to enable this breakdown (Bessada et.al., 2019; Robinson et.al., 2019; Rosa-Sibakov et.al., 2018).

Oxalic acid [(COOH)2] is another anti-nutritional component that chelates with minerals, reducing their absorption and inhibiting their bioavailability, and its salts are known as oxalates. Oxalates are classified in two different ways as oxalic acid or sodium, potassium and ammonium oxalate in water-soluble form and as calcium salt in water-insoluble form, and since they do not provide any benefit to humans, they must be excreted from the body with urine. (Shi et.al., 2018).

Polyphenolic compounds of legumes; It consists of phenolic acids, flavonoids and tannins, and although it is known that it is proportionally more in darker colored varieties with high pigment, they are important sources of polyphenolic compounds in certain proportions. (Rehman et.al., 2014; Garrido-Galand et. al., 2021). Proteins, amino acids and/or polyvalent cations can form complex compounds with polyphenols due to their positive charge. Among polyphenolic compounds, especially tannins precipitate proteins, reducing their digestibility and reducing the redundance of usable amino acids. In addition, tannins are important in that they form a complex with salivary glycoproteins, exhibit astringent properties and cause a decrease in flavor and a sensory limitation (Garrido-Galand et.al., 2021; Bessada et.al.,2019).

Saponins are secondary metabolites of legumes with a steroidal or triterpenoid structure, containing an aglycone-linked carbohydrate moiety (mono/oligosaccharide). Most of the saponins exist as large complex micelles, form insoluble complexes with 3-d hydroxysteroids, interact with bile acids and cholesterol, causing a decrease in the absorption rate of cholesterol and free fatty acids. Moreover, they form saponin-mineral complexes in insoluble form. They are generally characterized by their bitter taste and capability to foaming, while their ability to hemolyze red blood cells is a result of their amphiphilic properties (Rehman et.al., 2014; Garrido-Galand et.al, 2021; Parca et.al., 2018).

Fermentable Oligo-, Di- and Monosaccharides and Polyols (FODMAPs), known as short-chain carbohydrates that are not digested in the body for energy, are characteristically not considered an anti-nutritional factor. Mostly, α-galactosides are oligosaccharides such as raffinose, stachyose, and verbascose that have an α-1-6-galactosyl residue attached to the sucrose end and are found in legumes. (Rehman et.al., 2014; Garrido-Galand et.al., 2021). Normally, when α-galactosides are absent, the sugars are hydrolyzed by passing from the small intestine to the large intestine unchanged. When these compounds are selectively fermented by bacteria in the colon microflora, they release CO2, H2 and sometimes methane gas along with short-chain fatty acids, which causes flatulence in the intestines. In addition to gas formation due to excessive intake, diarrhea and abdominal pain may also occur as symptoms of discomfort (Thirunathan & Manickavasagan, 2018; Bessada et.al., 2019).

In the group of enzyme inhibitors, which are found in almost all legumes and cereals and are known as important anti-nutritional components, those that act upon trypsin and α-amylase activity are the two most important members of this group. The enzyme α-amylase is effective in the breakdown of carbohydrates into oligosaccharides, and its inhibition delays the digestion of carbohydrates and increases the absorption time. As a result, the rate of glucose absorption decreases depending on the prolongation of the digestion time and the normal postprandial plasma glucose level is affected by this situation. Trypsin inhibitors, on the other hand, affect the digestion of proteins taken into the body, reducing the availability of amino acids, which causes a decrease in growth rate (Samtiya et.al., 2020; Bhutkar & Bhise, 2012).

Protein-based protease inhibitors are found in high amounts in plants for various purposes in nature and are divided into two main groups as Kunitz-type, which contain at least nine trypsin and chymotrypsin inhibitors and Bowman-Birk-type protease inhibitors.These inhibitors, which are commonly found in pulses, reduce the digestibility of proteins by inhibiting hydrolases that are effective in breaking down dietary proteins. (Garrido-Galand et.al., 2021). Protease inhibitors are resistant to pepsin enzyme and stomach acid, they are not digested in the small intestine, they increase stool activity by binding proteases. For this reason, the availability of sulfur-containing amino acids in legume grains is low (Bessada et.al.,2019; Parca et.al., 2018).

Lectins (agglutinins / hemaagglutinins / phytohemagglutinin) are glycoproteins that form complex structures with some special sugars and proteins, ultimately inhibiting the bioavailability of nutrients. These components interact with some sugars in the cell membrane structure and cause agglutination of red blood cells, thus reducing the absorption of nutrients as they pass through the intestinal walls (Garrido-Galand et.al., 2021; Bessada et.al.,2019; Rehman et.al., 2014; Shi et.al., 2018).

Alkaloid compounds, which cause sensory limitations such as tastelessness and bitter taste, are unacceptable and toxic for human and animal consumption, are generally found in lupine. Similarly, aldehydes such as hexanal found in pea proteins can cause unwanted taste and aroma formation due to their presence (Garrido-Galand et.al., 2021; Kasprowicz-Potocka et al., 2018; El Youssef et.al., 2020).

Antinutrient compounds can cause adverse effects when consumed in large quantities regularly for long periods of time. However, some of the bioactive substances in legumes mentioned above can be ambivalent and interestingly, similar to dietary fibers in fruits, vegetables and grains, they can exhibit positive effects such as lower blood sugar and hormonal response, reduction in blood lipids and lowering the risk of cancer in starchy foods (Martine M.-J. Champ., 2002; Margier et.al. 2018). It has been proposed that low intake of phytate may reduce the risk of colon cancer by enabling the indigestible starch to reach the intestines with the prebiotic activity they provide in relation to the antioxidant effects of phytates and their capability to bind enzymes such as amylase (Margier et al., 2018). Otherwise, saponins, have been stated to have a lowering effect on lipid intake by affecting the absorption of dietary lipids, cholesterol, and bile acids. They can show their effects in different ways; (i) binding to dietary cholesterol and preventing its absorption (ii) binding to bile acids, lowering cholesterol (iii) interfering with enterohepatic circulation (iv) increasing fecal excretion. As a result, there is a balanced increase between the increase in bile acid excretion and the bile acid synthesized from cholesterol in the liver, and plasma cholesterol decreases (Martine M.-J. Champ., 2002). Although tannins exhibit anti-nutritional properties, they are also attribute to as “double-edged swords” in biology due to their high antioxidant activity and their anti-HIV and anti-cancer activity in in vivo and in vitro studies on rodents (Robinson et.al., 2019).

Another classification of anti-nutritional compounds found in legumes; (i) those that affect protein digestion (trypsin and chemotrypsin inhibitors), (ii) those that reduce mineral absorption (lectins, phytates and oxalates), (iii) those that affect starch digestibility (amylase inhibitors and saponins). While some of these ingredients, such as protease inhibitors or lectins, are heat labile and can be inactivated during cooking, milder heat treatments are not sufficient for heat stable ones like tannins, phytic acid or saponins. Depending on the inactivation or reduction of the amount of these ANF components, the bioavailability of macro and micronutrients in legume grains can be increased with the appropriate and sufficient processes, and at the same time, flavor increase can be achieved by inhibiting the undesirable flavor components (Bessada et. al., 2019; Shi, Arntfield, & Nickerson, 2018). For this purpose, processes applied alone or in combination can be grouped as physical processes, heat treatments, biological applications (germination/sprouting, fermentation) and enzymatic applications (such as phytase enzyme). In order to advance the nutritional profile of cereal grains, in addition to methods such as genetic improvement methods, protein concentrates or the addition of protein-rich legumes or de-oiled oilseed meal, cooking, germination, milling and fermentation practices are used, similar to legumes (Bessada et al., 2019; Blandino et al., 2002).

Biological methods for inactivation of anti-nutritional components and improvement of nutritional profile

Soaking in water

Soaking legumes and cereal grains in water for a short or longer period of 15-20 minutes softens their texture and facilitates their processing, while decreasing the concentration of anti-

nutritional components can increase their nutritional value. Phytate, which is frequently found in these grains, has a water-soluble structure and a significant amount of it can be removed during soaking, while this process also allows the phytate enzyme found in grains (legumes and cereals) to become naturally active. The hydrolysis of phytate in the structure depends on pH and temperature, and the optimum values are 5.6-6.0 and 45-65 ^{0}C, respectively (Rehman et al., 2014). For instance, it has been notified that the redundance of phytic acid in chickpea can be reduced by 8.26% as a result of a 12-hour soaking process, while it is stated that the amount of other water-soluble substances such as vitamins, minerals and phytochemicals can also decrease in this process for grains (Samtiya et al., 2020). Similarly, α-galactosides, which have a water-soluble structure, can pass into the soaking water via osmosis, and this process can activate the natural process in legumes and enable further degradation of α-galactosides. The rates of removal of these components from the structure were associated with the seed coat and matrix (Thirunathan et al., 2018). In a study, it was stated that in a soaking solution containing sodium bicarbonate, α-galactosides could diffuse more easily from the seed due to the change in shell permeability associated with the high pH environment (Vijayakumari, Pugalenthi, and Vadivel, 2007). At the same time, rise in the temperature of the soaking water also increases the permeability of the seed coat and can provide more α-galactoside leach out. In addition to this, more sugar degradation occurs due to the increase in α-galactosidase activity in the grains (Thirunathan et al., 2018).

Germination and Malting

Germination is essentially a biological stage that takes place at the beginning of the period that allows seeds to germinate and develop into their constituent plants and causes some nutritional, biochemical and sensory changes. Malting, on the other hand, is the application that includes the processes in which the grains are first brewed and then subjected to the germination process. In the germination method, optimal environmental conditions are deliberately created for plant seeds to sprout, and the grains are pre-soaked to absorb moisture beforehand. The germination method is one of the most suitable methods used to lower the effectiveness of non-nutritive factors that can be found in the structure of foods with plant content. With the resulting changes, higher nutrient levels, lower anti-nutrient content, and positive changes in protein and starch digestibility are observed in germinated seeds or sprouts compared to ungerminated seeds, related to the activation of inactive enzymes in the structure (Thirunathan et al., 2018; Zhang et al., 2015; Nkhata et al., 2018; Samtiya et al., 2020).

The germination process, which has been used throughout human history, softens the core structure of grains, increases their nutritional value, and reduces anti-nutritional factors. During the malting and germination process, a decrease in the amount of starch in the structure for energy production and an increase in the amount of simple sugar are observed due to the activation of enzymes in inactive form, which are inactive in the raw product and have an effect on carbohydrates. In addition, the breakdown of starch increases its digestibility. Raw grains generally contain low levels of glucose and fructose sugars, but the level of these two soluble sugars increases significantly with the activation of the enzyme invertase during germination (Singh et al., 2013; Zhang et al, 2015; Oghbaei & Prakash, 2016; Nkhata et al., 2018).

The effect of germination and malting on proteins is contradictory. While it was stated that the protein content increased depending on the type of grain/seed, some researchers stated that the amount of protein decreased despite the increase in the amount of lysine, tryptophan, and

methionine amino acids after germination (Nkhata et al., 2018; Bhathal & Kaur, 2015). The proportional change in protein in the structure can be associated with the amount of amino acids synthesized during germination as well as dry weight loss due to the use of carbohydrates and fats during respiration.The decrease in protein amount is associated with degradation due to the activity of protease enzymes during germination. Most cereal grains contain trypsin inhibitors, which affect the digestibility of proteins and maintain their stability at high temperatures by resisting heat. However, the activity of trypsin inhibitors is greatly reduced in the germination process (Nkhata et al., 2018).

The increase in the amount of crude fiber composed of cellulose, lignin and hemicellulose is one of the changes observed during germination. This increase in structure is a desired result, and dietary fibers slow down glucose release for patients with diabetes, and dietary fiber increases the feeling of satiety by forming gels that slow down starch digestion and gastric emptying in the stomach (Nkhata et al., 2018; Yu et al., 2014).

While the phytate found in plant foods is heat-stable, the phytase enzyme naturally found in plants is heat-labile and high temperature application may cause its inactivation. During germination, phytate is broken down by the natural phytase enzyme, and this fragmentation improves the flavor and improves the nutritional value by increasing the availability of minerals. Legumes are rich in anti-nutritional elements in terms of phytic acid, which causes poor absorption and digestibility of nutrients, but is destroyed by malting due to endogenous phytase activity. In ground and soaked malted grains, complete degradation of phytate is also observed when optimum conditions are set (Rehman et al., 2014; Nkhata et al., 2018).

The ratio of various vitamins such as tocopherols, riboflavin and total niacin found in grains and legumes increases due to their synthesis by new shoots. Plants and animals can synthesize vitamin C using glucose, mannose, and galactose. In the malting and germination process, starch is hydrolyzed enzymatically by amylases and diastases, resulting in their increased availability in vitamin C synthesis due to the increased amount of glucose. (Nkhata et al., 2018; Kim et al, 2012; Zilic et al., 2015)).

Fermentation

Derived from the Latin verb "Fevere" meaning "to boil", the concept of fermentation is a food processing method that dates back to the Neolithic period. Among other food processing methods, fermentation offers advantages such as preserving food, improving food safety, increasing nutritional value, reducing anti-nutritional components, increasing dietary diversity, increasing palatability and acceptability, and in some cases providing enhanced functional properties (Singh et al., 2013; Tsafrakidou et al., 2020). Fermentation as a biotechnological method; *Alcohol fermentation*, in which CO_2 and ethanol are formed as the primary product and carried out by yeasts, *Acetic fermentation* in which acetic acid is produced as the primary product by the genus *Acetobacter*, *Lactic acid fermentation* in which lactic acid bacteria (LAB) is produced and lactic acid is produced as the primary end product, and ammonia developed by the use of protein substrates or alkaline fermentation, consists of a number of sub-categories in which different metabolites are produced. The common aspects of these subcategories that make up the biochemical fermentation system are the metabolic pathways used by microorganisms, and in the lack of exogenous oxidizing agents, they obtain energy from organic compounds. Thus, any raw material comprising organic compounds can be fermented by microorganisms with the necessary enzymatic systems as suitable carbon resources that can

provide energy and used to obtain energy. Plant-derived raw materials can provide environments that encourage microbial growth. During fermentation, many biochemical changes occur that affect the properties of the product such as bioactivity and digestibility (Singh et al., 2013). In this process, changing conditions enable the activation of existing enzymes, while selectively increasing the activity of particular nzymes such as amylases, proteases, hemicellulases and phytases, depending on pH. Microbial metabolites and enzyme-induced changes bring their effects on the techno-functional and nutritional properties of fermented grain-based foods. Fermentation helps reduce indigestible carbohydrates, improves digestibility, taste and aroma, improving food quality in all while providing a wealth of essential amino acids, vitamins and minerals. (Ray et al., 2016; (Singh et al., 2013).

Fermentation is presumably the most appropriate way for improving the nutritional profile of grains. During the natural fermentation of grains, reductions at the carbohydrate level and some indigestible poly and oligosaccharides can be observed. In this process, protein quality improves due to the increase in the amount of amino acids such as lysine, while the amount and availability of B group vitamins scales up in proportion to the activities of their LABs. The pH change occurring in the fermentation medium may cause the enzymatic breakdown of phytate, which is in complex with iron, zinc, calcium, magnesium and proteins and restricts their bioavailability, and this may increase the soluble mineral ratio due to the decrease in the amount of phytate. An increment in the amount of folate, free phenolic acids, total phenolic compounds, lignans and alkylresorcinols is also observed. Cereals are also appropriate substrates for the advance of foods comprising probiotic microorganisms. Indigestible components in their matrix provide them with prebiotic properties (Blandino et al, 2002; Singh et al., 2013).

Grain-based fermented products are the focus of increasing global attention because they contain fermentable sugars (prebiotics), beneficial microorganisms called probiotics, as well as nutritional components such as low fat/cholesterol, high minerals, dietary fiber and phytochemicals, and being the source of some microbial enzymes that help digestion. (Ray et. al., 2016).

Relationship with Lactic acid bacteria

The diversity in fermented products is related to the variety in the raw materials or substrates where fermentation is carried out, or the multifarious process factors applied, and this diversity is emerged in the diversity of microorganisms, which are the fundamental factors of the fermentation. Self-fermented foods are promising resources for the discovery of previously uncharacterized microorganisms, due to their wide diversity and high microbial richness yet to be identified. Especially fermented foods, where microbial communities are poorly characterized, create environments rich in unused microbial diversity (Wuyts et al., 2019; Tamang et al., 2016).

Lactic acid bacteria (LAB) are common in most foods and beverages with natural fermentation, and major genera such as *Alkalibacterium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella* were isolated from these environments (). LAB represent a widespread and heterogeneous species that can produce lactic acid as a consequence of sugar metabolism and acidify the pH of the medium up to 3.5 (Tamang et al.; Holzapfel and Wood, 2014; Liptáková et al, 2017). LABs reduce the pH of the environment with the lactic acid they produce and do not allow the development of other microorganisms (Thirunathan et al., 2018).

LABs are fastidious microorganisms, regardless of the specific requirement of the species, and fermentable carbohydrate sources, amino acids, B group vitamins, nucleic acids and minerals must be present in the medium for their growth. As a result of fermentation of carbohydrates for cellular energy production, mainly lactic acid production takes place. The microflora that develops in any and every fermented food varies ups to conditions such as pH, salt concentration, water activity, temperature, and composition of the food matrix. (Liptáková et al, 2017).

In most fermented foods, fermentation is substantially carried out by LAB. Cereal grains are usually subjected to soaking before fermentation after cleaning. This process naturally provides the formation of a microbial flora in which LAB can dominate in the environment. Grains are excellent environments where they can perform LAB fermentation in terms of their macro and micronutrient components (Peyer et al., 2016). Endogenous grain amylases, which are activated in fermentation medium, produce fermentable sugars that can be an energy source for lactic acid bacteria (Blandino et al., 2002).

The bioavailability of bound nutritional components in the form of starch and protein can be improved by adding malt grains or by adding hydrolytic enzymes. Peyer et. al. (2016) in a study, it was stated that fermentable sugars in the form of glucose, fructose, maltose, and sucrose and free amino acids were found to be at significantly higher levels in liquid barley malt medium, and it was shown that LAB grew better than raw barley and raw wheat medium and another study Perri et al. (2020), where they compared the microflora of germinated grain, pseudo grain and leguminous flours with the raw flours of these grains, they stated that the consumption capacity of carbohydrate, polymer, amine, carboxylic acid and amino acid sources was highest in the flours belonging to germinated grains. They also found that all sprouted flours had a dissimilar microbiome and greater number of LAB strains, and bacterial density was positively correlated with free sugar concentrations in flours. In a research with germinated and ungerminated barley grains, it was shown that germination affects the microbial flora, and the LAB number varies between 2.8 and 4.6 log cfu/g in ungerminated grains and between 4.9 and 6.3 log cfu/g in germinated grains (Østlie et al., 2021).

Studies have suggested that germination increases the nutritional and bioactive components of grains such as vitamins, protein, amino acids and sugar and, therefore, encourages the development of probiotic bacteria (Singh et al., 2013). Montemurro et al. (2019) stated in a exploratory, they conducted with germinated grain flours that, thanks to the fermentation performed by LAB, sprouted grain flours further improved their nutritional and functional properties by promoting the set free of peptides, free amino acids, phenolic compounds, and soluble fibers and and a significant reduction in the amount of some ANFs. The inclusion of germinated cereals and leguminous grains in bread is recommended for improving nutritional quality and seems to be easefully feasible due to the favorable sensory impact of germination on grains (Montemurro et al, 2019; Singh et al., 2013; Mäkinen et al, 2015; Kaukovirta et al, 2004).

CONCLUSION

Within the framework of changing world conditions, the need for the existence and continuity of sustainable systems to ensure food security is undeniable. In addition, due to the increase in people's tendencies towards a healthy lifestyle, the interest in products that are less processed in daily nutrition, functional and meeting special nutritional needs is gaining more importance day by day. In this context, cereals and legumes, which have been in our lives since the history of humanity and whose importance is increasing day by day, have features that can meet almost all demands. Their nutritional profile can be improved by various physical, thermal, or biological treatments or enzymatic applications applied to these grains, or by using a few of these applications in combination. Depending on the changes, while the bioavailability of the nutritional components they have increases for humans, the use of these components in the microflora naturally found in the grains can be facilitated. Herein, environments consisting of the combination of different processes applied to grains with fermented systems, have a rich microflora for the isolation of different genera of LAB species, which can increase the nutritional value of the products, improve their techno-functional properties, contribute to their shelf life and are accepted in GRAS status. It is important to characterize the existing bacterial population by investigating the natural microflora, especially in the fermentation environments created by the diversity of cereals and legumes and the existence of different processing parameters.

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THE QUALITY CHARACTERISTICS OF DRIED RED CAPIA PEPPER

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ABSTRACT

Convective drying (CVD) at 50, 60 and 70 ºC was applied to obtain dried red capia peppers. The effects of CVD on color values (L^*, a^*, b^*), rehydration capacity, and selected chemical properties including dry matter, pH and titratable acidity of seven dried red capia peppers were compared. In addition, the effects of the cutting types (ring and cube) on the final quality of pepper samples were determined at room temperature. The samples dried at 60 ºC and cut in ring forms (6-R samples) resulted with high-quality dried peppers. 6-R samples showed the highest dry matter, pH, titratable acidity and rehydration capacity (87.06%, 5.69, 807.15 mg citric acid/100 g, 6.72%, respectively). The closest L^*, a^*, b^* values to the fresh pepper samples were achived for 6-R samples (L^*: 29.87, a^*: 12.83, and b^*: 17.03). In overall, dried red capia peppers in ring forms at 60 ºC can be used to acquire a high-quality food material along with an enhanced color, physical and chemical properties.

Keywords: Color, Convective drying, Red capia pepper, Rehydration capacity, Titratable acidity

INTRODUCTION

Pepper belongs to the Capsicum genus of the Solanaceae family and grows in the United States, South America, Peru, Bolivia, Costa Rica, Mexico and almost all Southern European countries (Kumari, 2012). In addition to being rich in vitamins (especially A and C), minerals, phenolic components and carotene, it also has antioxidant properties (Karaağaç and Balkaya, 2010). Capia pepper is a type of pepper with a long conical structure, meaty, red color and a sweet flavor and has been used as "paste" and "oiliness" for years (Demirel et al., 2012). Although Capia pepper is consumed fresh and dried, it is also used in the food industry in the production of canned, tomato paste, pickled peppers, frozen food and sauce. In addition to these, it has been reported that it is also used in the production of antibiotic raw materials, feed materials and dyes (Hekimoğlu and Altındeğer, 2009; Akgün, 2010). As many fruits and vegetables, peppers can also be preserved for a long time by drying process. Drying is one of the oldest methods known for the preservation of food. The purpose of drying is to remove free water from the product, and to store the products for a long time. So, the crops can be available in areas besides the harvest season. In addition, the volume and weight of dried products are reduced, making packaging and transportation easier with a low-cost. The most popular method of drying food is the convective drying method (Michalska et al. 2016). However, this method causes deterioration of taste, color and nutritional compounds in products (Calin-Sanchez et al. 2014). In this sense, the importance of the temperature applied during the drying process is

understood and it is wondered how these degrees will have an effect on the quality of the foods. This research, for this reason, has the primarily aim of contributing to the works of the use of convective drying on capia pepper quality. Specifically, the influence of the drying temperature and cutting type on color values (L^*, a^*, b^*), rehydration capacity, and selected chemical properties including dry matter, pH and titratable acidity of dried red capia peppers were compared.

MATERIAL AND METHOD

Sample preparation

The red capia peppers were obtained at a local market in Iğdır, Turkey and kept at 4 ± 0.5 ºC until the experiments. They were washed to clean the dust, chemical residuals and attached dirt. After removing the excess water from the surface of pepper samples by paper towel, the peppers were cut in two different forms (ring and cube) with a sharp knife. The beginning moisture ratio of the pepper slices was defined as 83.8 ± 0.54 % by drying at 105 ± 5 °C before reaching the stable weight via forced air convective oven.

Drying process

Convective drying was applied in a lab convective oven (Arçelik KMF 833I, Turkey) following the method proposed by İzli (2018). The ring and cube shaped capia pepper samples were located in a thin layer. Air velocity was well-set at 1 m/s with air temperatures of 50, 60, 70 °C for the drying process. The drying process was continued until 12% moisture content. The experiments were conducted with three replications. A short definition of the treatments is tabulated in Table 1.

Table 1. Treatments used in the study

Sample names	Treatments
Control	Fresh sample, no treatment
5-R	Capia peppers dried at 50 ^{O}C in ring forms
5-C	Capia peppers dried at 50 ^{O}C in cube forms
6-R	Capia peppers dried at 60 ^{O}C in ring forms
6-C	Capia peppers dried at 60 ^{O}C in cube forms
7-R	Capia peppers dried at 70 ^{O}C in ring forms
7-C	Capia peppers dried at 70 ^{O}C in cube forms

Color measurement

The color changes of fresh and dried capia peppers were analyzed by a Konica Minolta (CR400, Japan) that is assembled with illuminant D 65 and 8 mm measuring scope in the CIE L^* a^* b^* color scale. Color parameters were described in a 3-dimensional L^*, a^*, and b^* color space, where L^* shows the lightness/darkness of the capia peppers, a^* demonstrates the redness/greenness, and b^* displays the yellowness/blueness (Yildiz, 2021).

Rehydration Capacity

The rehydration ratio (RR) of the dried capia pepper slices was determined according to Cemeroğlu (2009) by weighing ~10 g of CVD pepper samples in distilled water (100 mL) at room temperature for 24 hours. Subsequent to soaking, the extra water was taken off and the capia pepper samples were weighed. The calculation of RR of the pepper samples were determined as below:

Rehydration ratio (%) = m_3 / (m_1-m_2)
m_1: weight of water (fresh capia pepper), g
m_2: weight of water (dried capia pepper), g
m_3: weight of water (following rehydration), g

Dry matter

Homogenized capia pepper samples (5 g) were weighted in disposable aluminum dishes and dried at 105 ± 5 °C until reaching the stable weight via forced air convective oven (Cemeroğlu, 2009).

pH

Fresh samples put into blender directly and dried pepper samples were added with some water. The samples were homogenized, centrifuged and the clear supernatant was obtained. pH of the samples was measured using an Accumet Research AR15 pH meter (Fisher Scientific, USA) (Cemeroğlu, 2009).

Titratable acidity

Titratable acidity was determined following the procedure stated by Cemeroğlu (2009). While the fresh samples put into blender directly, dried pepper samples were added with some water. The samples were homogenized, centrifuged and the clear supernatant was obtained. Then, the samples were titrated with 0.1 N NaOH to the end point of 8.2. Titratable acidity was calculated by taking account of NaOH used.

Statistical data analysis

A factorial experiment (3 × 2: temperature and cutting type) with a randomized complete design was used to analyze the effect of three different temperature levels (50, 60, and 70°C) and two different cutting types (ring and cube). Three replications for each treatment were used for all measurements, unless otherwise stated. Statistical analyses were managed using a randomized plots factorial experimental design. The results were analyzed using the JMP (Version 7.0, SAS Institute Inc., Cary, NC, USA). Differences among the mean values were obtained by Fisher's least significant difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The color changes of fresh and dried capia pepper samples are tabulated in Table 2. It was observed that the L^* value of all the dried samples decreased significantly compared to the fresh capia pepper ($p < 0.05$). When compared with the pepper samples dried in cube forms, the samples dried in ring forms showed significantly higher L^* values (Table 2). While the highest L^* value (the closest to the fresh pepper sample) was found for the samples dried at 60 °C in ring forms (29.87 ± 0.63) and cube forms (28.75 ± 0.45), the lowest L^* value was determined for the samples dried at 70 ºC (Table 2). The lower lightness value means darker appearance of the convective-dried pepper slices at 70 ºC might be because of the non-enzymatic browning (Maskan, 2000). On the contrary to the L^* values, a significant increase in a^* values were observed for all dried samples compared to the fresh pepper samples. Among the dried pepper samples, the highest a^* value was observed for the samples dried at 70 ºC whereas the lowest a^* value was found for the samples dried at 60 ºC (Table 2). In brief, the pepper samples dried at 60 °C in ring forms showed the highest L^* value, and lowest a^* value. The preferred colors are those closest to the original color of fresh pepper samples. The capia pepper having higher lightness value can be evaluated as more favourable and marketable products with respect to color quality (Ergunes, and Tarhan, 2006). From this point of view,

drying at 60 °C in ring forms was suitable since it resulted with a lighter product color compared to other drying temperatures (50 and 70 °C) and closest color values to the fresh pepper sample.

Table 2. Color values of convective dried ring and cube shaped capia peppers

Color values	L^*	a^*	b^*
Control (Fresh sample)	38.55 ± 0.43^{a}	10.21 ± 0.98^{f}	18.12 ± 0.13^{b}
5-R	24.31 ± 0.12^{c}	19.24 ± 0.15^{d}	19.15 ± 0.34^{a}
5-C	22.26 ± 0.78^{cd}	23.45 ± 0.07^{b}	15.67 ± 0.72^{e}
6-R	29.87 ± 0.63^{b}	12.83 ± 0.16^{e}	17.03 ± 0.69^{c}
6-C	28.75 ± 0.45^{b}	12.74 ± 0.65^{e}	16.88 ± 0.18^{cd}
7-R	19.88 ± 0.03^{d}	21.18 ± 0.81^{c}	13.08 ± 0.04^{f}
7-C	15.63 ± 0.19^{e}	25.44 ± 0.27^{a}	15.54 ± 0.71^{e}

[a-f]: Means superscript with different alphabets in the same column differ significantly ($p < 0.05$).

Dry matter, pH, titratable acidity and rehydration capacity of convective dried ring and cube shaped capia peppers are demonstrated in Table 3. As shown in Table 3, dry matter of convective-dried pepper samples in ring forms at 60 °C (87.06%) were significantly higher compared to the fresh capia pepper and other dried pepper samples. On the other hand, the lowest dry matter was obtained for the pepper samples dried at 70 °C (Table 3). All dried pepper samples no matter if the samples dried in ring or cube forms or dried at 50, 60, and 70 °C showed a significant increase on dry matter compared to the fresh pepper samples. In addition, the pepper samples dried in ring forms showed a higher dry matter compared to the samples dried in cube forms. Dried pepper samples showed a significant decrease for pH, and a significant increase for titratable acidity compared to the fresh capia peppers (Table 3). As shown in Table 3, rehydration capacity convective-dried pepper samples in ring forms at 60 °C were significantly ($p < 0.05$) higher than fresh capia pepper samples, showing that dried pepper samples in ring forms at 60 °C were easier to recover by rehydration. The possible reason was that better porous structure and higher cell membranes permeability were formed in the dried pepper samples in ring forms at 60 °C. From the findings, it is obvious that the water gain is more noticeable in dried pepper samples at 60 °C ($6.72 \pm 0.11\%$ and $5.99 \pm 0.02\%$ in ring and cube forms, respectively) compared to the other capia peppers dried at 50 and 70 °C (Table 3). Similar results were observed in the study of İzli and Yildiz (2021). They've observed significantly higher rehydration ratio for the convective-dried quince samples at 60 °C compared the samples dried at 50 °C and 70 °C. In addition, each and every dried capia peppers in ring forms have better rehydration capacity compared to the convective dried capia peppers in ring forms.

Table 3. Dry matter, pH, titratable acidity and rehydration capacity of convective dried ring and cube shaped capia peppers

Samples	Dry matter (%)	pH	Titratable acidity (mg citric acid /100 g)	Rehydration Capacity (%)
Control	16.28 ± 0.96^{c}	5.78 ± 0.01^{a}	471.83 ± 0.65^{g}	0.18 ± 0.01^{e}
5-R	79.19 ± 0.64^{ab}	5.58 ± 0.02^{c}	656.37 ± 0.71^{d}	5.12 ± 0.07^{c}
5-C	77.34 ± 0.47^{ab}	5.54 ± 0.04^{c}	839.13 ± 0.48^{a}	5.05 ± 0.23^{c}
6-R	87.06 ± 0.34^{a}	5.69 ± 0.03^{b}	807.15 ± 0.67^{b}	6.72 ± 0.11^{a}
6-C	84.37 ± 0.65^{a}	5.66 ± 0.01^{b}	711.29 ± 0.94^{c}	5.99 ± 0.02^{b}
7-R	74.91 ± 0.03^{b}	5.63 ± 0.07^{bc}	565.65 ± 0.12^{e}	4.64 ± 0.07^{d}
7-C	73.15 ± 0.67^{b}	5.62 ± 0.02^{bc}	514.75 ± 0.73^{f}	4.58 ± 0.01^{d}

[a-g]: Means superscript with different alphabets in the same column differ significantly ($p < 0.05$).

CONCLUSIONS

The convective drying of capia peppers at 50, 60, and 70 ^{o}C in ring and cube forms was investigated. A significant development in the physical and chemical properties of ring-shaped convective-dried pepper slices at 60 ^{o}C was accomplished. A practical implication is that drying of capia pepper slices with an optimum condition by drying temperature and cutting type can be used to get a high-quality product with an improved color and better physical and chemical properties.

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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:

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 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].

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 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.

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 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.

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		Cumulative yield		Yield/trunk section area (kg/cm2)
		(kg/tree)	(t/ha)	(kg/tree)	(t/ha)	
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 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.

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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CHEMICAL PROPERTIES OF SOME WILD FRUIT SPECIES IN TURKEY

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Turkey is the gene center of many fruit species due to its geographical location and climatic characteristics. In addition to the fruits are grown by cultivation in Turkey, there are also fruit types that grow naturally. Determining the chemical properties and nutritional content of these fruits is an important stage in evaluating the diversity. In this study, it was aimed to determine some chemical properties of fruits belonging to hawthorn (*Crataegus* L.), apple (*Malus*) and jujube (*Ziziphus jujuba*) genotypes. The pH, TSC (total soluble content), acidity, vitamin C, sugar compounds, TPC (total phenolic contents), and phenolic compounds were investigated in fruits. As a result of the analyzes, pH, TSC, and acidity values of fruits belonging to hawthorn, apple, and jujube genotypes were determined to change between 2.8-4.22, 9.6-27.6 (%), and 0.26-1.1 (%), respectively. Among the genotypes, the highest vitamin C and TPC values were 33.9 mg/100 g and 1221.76 mg GAE/100 g, respectively. Phenolic compound determinations of fruit extracts were carried out by the HPLC-DAD system, and different amounts of gallic acid, catechin, epicatechin, epigallocatechin gallate, rutin, and caffeic acid components were determined.

Keywords: Wild fruits species, Phenolic compounds, HPLC, Vitamin C

INTRODUCTION

Turkey is located in the temperate climate zone and is very rich in plant biodiversity. In recent studies, it has been reported that Turkey has 12.000 plant taxa (Avcı, 2005). The rich plant diversity of Turkey is because it is located in three different flora regions: Europe-Siberia, Mediterranean and Iran Turan. Some of Turkey's plant richness consists of fruit species and varieties. Worldwide, 138 fruit species are grown, and 75 of these species are in Turkey. Along with the cultivated fruit species, there are also non-cultivated fruit species (Özbek, 1985). These wild fruit species grow naturally.

Wild fruit species have been used for nutritional, medicinal and other purposes in various periods of human history. Edible wild fruits have been an important source to meet a need basic agricultural foods in regions with food shortages and rural areas (Ercişli, 2017). These plants have also been frequently used to treat various diseases. Factors such as lifestyle, the industrial revolution, decreasing contact with nature, and the cultivation of a few numbers of cultivated plants in large areas have led to insufficient use of edible wild fruit species. However, many people in the world obtain some of their daily nutritional needs from wild fruits. Also, in modern societies, an increasing phenomenon has emerged regarding the use of wild fruits (Mahapatra and Panda, 2009).

Today, interest in wild fruits has increased due to their high nutritional value, use in alternative medicine, and the search for new tastes. The medicinal effects of the fruits used for this purpose are due to their bioactive compounds. Bioactive components are compounds that give to the plant color, taste, aroma and scent. Phenolic compounds are in this group and have the potential to increase the antioxidant capacity of the plant (Bostan and Islam, 2007). Fruits and vegetables containing high levels of flavonoids, carotenoids, and phenolic acids play an important role in reducing the risk of chronic diseases (Boyer and Liu, 2004). It is known that the antioxidant capacity of wild fruit species is higher than that of cultivated fruit species.

Wild fruits and other wild food crops represent versatile agricultural biodiversity and serve as a supplementary food and micronutrient sources. While wild edible fruits provide diversity in nourishment, they are important sources of nutrition and health safety (Mahapatra and Panda, 2009).

Nowadays, studies on wild fruit species are increasing and last for many years in some countries. Some of these fruit species have been cultivated. Many wild fruit species are traditionally consumed by the public in Turkey. It is very important to evaluate and determine the characteristics of the rich plant genetic resources we have.

In this study, it was aimed to determine the chemical properties of fruits of hawthorn (*Crataegus* L.), apple (*Malus*) and jujube (*Ziziphus jujuba*) genotypes that grow naturally in different locations.

MATERIAL and METHOD

Fruits belonging to three hawthorns, three apples, and three jujube genotypes were used as material. Fruit samples were collected from Malatya, Gümüşhane, and Bingöl provinces. Fruits of hawthorn, apple, and jujube genotypes were harvested in September and October 2019. The fruits were collected randomly and transported to the laboratory under refrigerated conditions. Fruit samples were stored at -85 °C until analysis. Fruit pictures of genotypes are presented in Figure 1.

In the fruit juices obtained from the samples, the pH, total soluble content (TSC), and acidity values were determined with a pH meter (Thermo Orion 2 Star), a refractometer (Atago), and titration method, respectively (Yamankaradeniz, 1983; Cemeroğlu, 1976). The acidity results of the samples are expressed as malic acid equivalents. In addition, vitamin C, sugar composition, total phenolic content (TPC), and individual phenolics were determined in fruits belonging to genotypes.

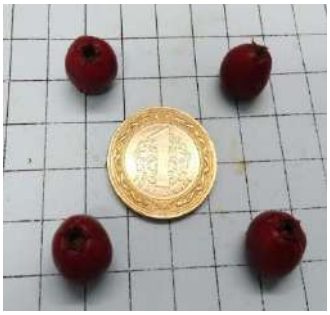
Vitamin C Analysis: Extraction of the samples was carried out according to Cemeroğlu (2007). The amount of vitamin C was determined in the HPLC-DAD system (Shimadzu). Ultrapure water adjusted to pH 2.2 was used as the mobile phase.

Sugar Analysis: The sugar composition of samples was determined in the HPLC-RID system (Shimadzu) by extraction with water. Acetonitrile: water (75:25 v/v) was used as the mobile phase.

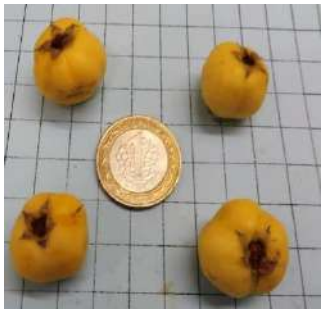
TPC Analysis: TPC was determined by the Folin-Ciocalteu method, and the results were expressed as gallic acid equivalents. UV/VIS Spectrophotometer (Shimadzu, Japan) was used for detection of TPC in fruit samples.

Phenolic Compounds Analyses: Determination of phenolic compounds was carried out by gradient elution in the HPLC-DAD system. 4.5% formic acid and acetonitrile were used as mobile phase.

Figure 1. Fruit Pictures Belonging to Genotypes



5 No



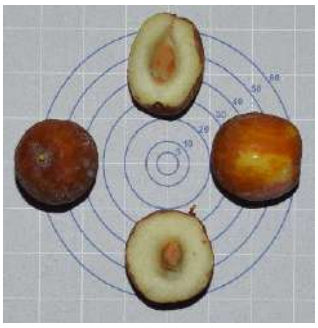
55-1

65-1

Bin. E.

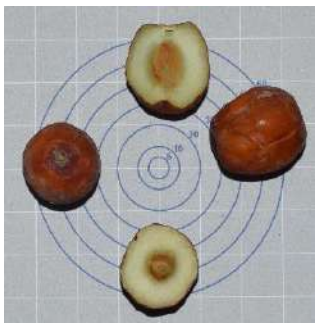
Ban.1.

Ban.2.



Hün.1.

Hün.2.



Hün.3.

RESULTS AND DISCUSSION

The pH, TSC, acidity, vitamin C, and TPC results of the fruit samples are presented in Table 1. The pH, TSC and acidity results ranged from 2.8 to 4.22, 9.6% to 27.6%, and 0.26% to 1.11%, respectively. Yanar et al. (2010) reported that the pH, TSC, and acidity values in hawthorn genotypes varied between 2.82 and 3.62, 6.40% and 16%, and 0.9% and 1.9%, respectively. Yaşa (2016) stated that pH, TSC, and acidity values were 2.5, 9.1%, and 3.06% in the study on jujube genotypes, respectively. The pH, SÇKM, and acidity values are important quality criteria during the processing and drying of fruits and vary greatly according to species, cultivar, genotype, growing conditions, and ecology.

The most important feature of fruits in terms of nutrition is that they are a source of vitamins. In our study, vitamin C values of genotypes ranged from 6 mg/100g to 33.9 mg/100g. In the literature, it was observed that vitamin C values in hawthorn species ranged from 3.86 mg/100g to 86.15 mg/100g (Türkoğlu, 2005; Liu et al., 2010). Sivakov et al. (1988) reported that vitamin C values ranged between 180.11 - 367.3 mg/100 g in their study on jujube

genotypes. Climate, genotype, species, and environmental factors have significant effects on the vitamin C concentrations of horticultural crops.

It was determined that there were notable differences between genotypes in TPC results of fruit samples. The genotype 55-1 (hawthorn) has the lowest TPC value (101.9 GAE/100 g) while genotype Ban.3 (apple) has the highest value (1221.76 GAE/100 g). In previous studies, it was stated that the total phenol content ranged between 66.2 - 211.9 mg/100 g in apple cultivars, 960 - 3626 mg GAE/100 g in hawthorn genotypes, and this value in jujube genotypes was 1968 mg GAE/100 g (Okatan et al., 2017; Yaşa, 2016).

T**a**ble 1. pH, TSC, Acidity, Vitamin C, and TPC Results of Genotype Fruits

Wild Fruit Species	Genotype Name	pH	TSC (%)	Acidity (%)	Vitamin C (mg/100g)	TPC (mg GAE/100 g)
Hawthorn	5	3.60	10.90	0.43	6.00	359.47
	55-1	3.11	10.53	0.92	ND*	101.90
	65-1	3.26	10.00	0.80	1.30	573.75
Apple	Bin. E.	2.80	9.60	0.86	8.10	638.00
	Ban.1.	3.71	10.00	1.11	17.50	1026.55
	Ban.2.	3.43	13.33	0.97	18.60	1221.76
Jujube	Hün.1.	4.18	24.80	0.27	22.60	281.68
	Hün.2.	4.60	22.27	0.26	18.60	388.55
	Hün.3.	4.22	27.60	0.30	33.90	321.27

*ND: Not dedection

Sugar compounds of fruits belonging to hawthorn, apple, and jujube genotypes were determined by using sucrose, glucose, and fructose standards, and the results are presented in Table 2. The amounts of sucrose, glucose, and fructose of fruit samples varied between 1.05 – 14.47 g/100g, 0.82 – 7.55 g/100g, and 0.13 – 10.42 g/100g, respectively. While sucrose was determined predominantly in jujube genotypes, it was determined that the amount of fructose was higher in hawthorn genotypes.

Table 2. Sugar Compounds of Genotype Fruits

Wild Fruit Species	Genotype Name	g /100 g fresh fruit		
		Sucrose	Glucose	Fructose
Hawthorn	5	ND*	2.68	3.08
	55-1	2.48	7.18	10.42
	65-1	8.11	7.55	9.27
Apple	Bin. E.	2.92	0.92	4.05
	Ban.1.	ND*	1.23	0.25
	Ban.2.	1.05	0.82	0.13
Jujube	Hün.1.	11.29	4.98	5.25
	Hün.2.	14.47	3.96	4.00
	Hün.3.	12.06	6.82	7.57

*ND: Not dedection

In the wild fruit samples, catechin, gallic acid, epicatechin, rutin, epigallocatechin gallate and caffeic acid components were determined in different amounts. It was determined that gallic acid, catechin, epicatechin, rutin, epigallocatechin gallate, and caffeic acid concentrations in fruits belonging to genotypes varied between 0.32 - 3.84, 0.65 - 308.77, 0.27 - 58.63, 1.44 - 41.65, 1.76 - 79.5, and 0.01 - 13.81 g/100 g, respectively. In a study in the Netherlands, the

amount of catechin in different apple varieties was determined between 7.11-11.54 mg/100 g (Arts et al., 2000). Alirezalu et al. (2020) determined the phenolic components of chlorogenic acid, vitexin, rutin, quercetin, isoquercetin, vitexin-2-O rhamnoside, and hyperoside in a study conducted in fifteen different hawthorn species. Phenolic compounds are secondary metabolites found in plants. These compounds have an important place in the human diet. Fruits, vegetables and beverages are the main source of phenolic compounds in the human diet. The antioxidant capacity of herbal products is due to the phenolic compounds they contain. These products prevent the formation of many diseases such as cancer, diabetes, Alzheimer's, and heart diseases by preventing the reactions caused by free radicals. It is known that wild fruits contain high amounts of phenolic compounds (Vrhovsek et al., 2004).

Table 3. Phenolic Compounds of Genotype Fruits

Wild Fruit Species	Genotype Name	mg/100g fresh fruit					
		Gallic Acid	Catechin	Epicatechin	Rutin	Epigallocatechin gallate	Caffeic Acid
Hawthorn	5	1,61	80,98	1,32	13,56	3,52	1,03
	55-1	0,32	15,10	2,33	6,62	0,75	0,01
	65-1	1,36	143,82	35,09	21,84	79,50	ND*
Apple	Bin. E.	0,78	162,31	9,43	1,44	1,76	ND*
	Ban.1.	3,84	223,44	12,69	28.00	41,13	9,96
	Ban.2.	3,67	308,77	58,63	41,65	46	13,81
Jujube	Hün.1.	ND*	0,82	ND*	2,77	ND*	ND*
	Hün.2.	1,26	11,75	4,84	12,74	ND*	0,17
	Hün.3.	0,49	0,65	0,27	5,28	ND*	ND*

*ND: Not dedection

CONCLUSIONS

In this study, chemical properties of fruits belonging to wild hawthorn, apple and jujube genotypes were determined. Wild fruit genotypes contain high levels of TPC and vitamin C. In addition, such fruits are rich in phenolic compounds. The secondary metabolite contents of wild fruit genotypes are higher than the cultivated species. Therefore, the nutritional value of these fruit types is high. They contribute to diet variety and taste. Today, studies on the wild fruit species are increasing. It is important to evaluate the plant genetic resources we have and to determine the characteristics of these wild fruit species.

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CONCLUSION

The monitoring of a forest allows to detect changes over time. Any living environment is in perpetual change. The study of the dendrometric characteristics of cork oak following the study plots showed that this stand is stable and regular with a complete and dense cover, characterized by trees of young and adult stage with a low rate of regeneration and mortality.

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CHANGES IN PHYSICAL AND QUALITY CHARACTERISTICS OF SWEET CORN VARIETIES DURING STORAGE

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ABSTRACT

Researh was established in Isparta University of Applied Sciences, Faculty of Agriculture, Field Crops laboratory in 2020, to determine effects on physical and quality of sweet corn varieties during storage. The study was established in completely randomized plot design with 3 replications. In study, two hybrid sweet corn (Batem Tatlı and Kompozit Şeker) were used. In experiment, sweet corn varieties were stored in modified atmosphere bags (MAP) in the refrigerator (+4^{o}C) for different periods (0, 5, 10, 20, 30 and 40 days). In the study, weight loss, dry matter content, color parameters (L* and C*), total soluble sugar content, ash ratio, crude protein ratio and soluble solids matter content were investigated according to storage time. When the sweet corn cultivars were stored for different periods, at the end of the 40th day, the ash content, crude protein content, total soluble sugar content, soluble solids content, brightness (L*) and vitality (C*) values decreased; weight loss and dry matter ratio increased were determined. As a result, sweet corn varieties should be consumed fresh after being harvested, and it can be recommended to be consumed within 5 days at most when they need to be kept at +4^{o}C. After the 5th day, it has been determined that there will be great losses in terms of quality and physical properties.

Keywords: Sweet corn, storage, physical properties, quality properties, sugar content

INTRODUCTION

Sweet corn is a culture crop grown fresh for human consumption. Sweet corn varieties have different varieties in terms of grain color (yellow, white or bi-color), maturation time (early, mid-late and late) and sugar content [standard (su-1), sugar content increased (se), super sweet (sh-2)]. Sweet corn is a source of fiber and vitamin B9. In also, it has 70% moisture and 23% total carbohydrate content and 27% of its total carbohydrate content consists of starch (Lertrat and Pulam, 2007). On the other hand, among the maize subspecies, sugar corn has higher sugar content than the others when harvested at the end of the milking period (Zadoks 73 - 75 - 77 - 79). In also, at the end of this period, it was determined that sweet corn had higher protein content and oil content in the grain (Coşkun et al., 2006).

There are not enough statistics about the cultivation area and production amount of sweet corn in our country. However, the consumption of sweet corn is increasing in our country and the cultivation areas are increasing year by year, especially in Çukurova, Aegean and Marmara regions. In also, it has been reported that even in the northern regions of our country, sweet corn varieties with a short vegetation period will increase the profitability of the farmers in their cultivation as the main and second crop (Özata et al. 2016). Sweet corn cobs can be consumed fresh (boiled and roasted directly), as well as canned or frozen food made from the grains separated from the cobs (Başçiftçi et al., 2012).

Sweet corn is included in perishable product groups due to high respiratory rate, rapid loss of sugar after harvest and quality deterioration (Dayı, 2011). As a matter of fact, it can lose

approximately 60% of its sugar content at 30°C and 6% at 0°C in one day. Sweet corn, which loses sugar very quickly at room temperature, rapid cooling and low temperatures after harvest storage can delay the loss of sugar (Dayı, 2011). On the other hand, modified atmosphere packages (MAP) are used to preserve the color, brightness and greenness of the stems and to reduce the weight loss and spoilage of fruits and vegetables during storage and marketing. By changing the air composition surrounding the product during storage with MAP, the O_2 level of environment decreases, accordingly, respiration, enzymatic and oxidative degradation reactions slow down, and microbiological spoilage can be delayed (Çavuşoğlu, 2018). In addition, these packages reduce weight loss by creating high relative humidity in the environment surrounding the product during storage, transportation and distribution of products (Karaca and Şen, 2014). In the direction of this information, it was aimed to determine the effects on the physical and quality of sugar corn varieties during storage.

MATERIAL AND METHOD

Material and Trial Installation

The sweet corn cob samples used in the research were obtained from the sugar corn trials conducted in Isparta University of Applied Sciences, Faculty of Agriculture, Department of Field Crops in 2020 and where traditional care procedures were performed. In the study, Batem tatlı and Kompozit Şeker sweet corn varieties were used as trial material.

In the study, sweet corn varieties harvested during the milk production period were brought to the laboratory without losing time. Then, homogeneous and unharmed corn cobs were selected. The cob leaves of the selected sweet corn were peeled and placed in modified atmosphere bags (MAP). MAP are modified atmosphere bags that have water vapor and gas permeability at certain rates. Sweet corn varieties put in MAP were closed with clips and stored in the refrigerator at +4ºC at 90±5% relative humidity for different periods (0, 5, 10, 20, 30 and 40 days). The study was set up in a completely randomized plot design with 3 replications and 6 cobs in each replication.

Measurement and Analysis

Weight loss was determined as percent (%) of the samples whose weights were determined before storage, after they were taken out of the storage, by weighting them with a balance with an accuracy of ±0.05 g. Grain color in the cobs was measured with a colorimeter (Minolta CR-400) from three different points from the equatorial region of each cob, and the Croma (C*) value was calculated to determine the color changes (McGuire, 1992). After extracting the juice of the grains with the help of a juicer, the amount of soluble solid matter content (SSMC) was measured with a hand refractometer (Atago) and expressed as %. The total soluble sugar content was determined according to the phenol-sulfuric acid method and a reading was made in the spectrophotometer at a wavelength of 540 nm (Dubois et al., 1956).

For the dry matter ratio, grains were separated from the cob and weighed 100 g, and when they reached a constant weight in the oven at 65ºC, it was weighed again. Then, the ratio of the first weighing to the last weighing was calculated as %. For the ash and crude protein content, grains were first dried in an oven at 65ºC until they reached a constant weight and ground in a mill with a sieve diameter of 1 mm and made ready for analysis. The ash content was kept in an incubator at 65°C until the weight of the ground grains was stabilized in order to lose moisture first. Then, for each sample, 3 g samples were processed in a ash furnace at 550°C for 5 hours, and the value obtained was expressed as %. Again, the nitrogen content of the ground grains was determined by the Kjeldahl method and the crude protein content of the

grains was calculated as % by multiplying the value found with the coefficient of 6.25 (Bremner, 1965).

Statistical analysis

The data obtained from the study were evaluated in the TOTEMSTAT statistical package program in accordance with the Completely Random Plots Trial Design, and the differences between the averages were determined according to the Duncan multiple comparison test ($p<0.05$).

RESULTS

Weight Loss and Dry Matter Content

Weight loss refers to the change in weight of the product to be marketed during storage, and the effect of storage time in the study was found to be statistically significant ($P<0.05$). Weight loss of sweet corn varieties increased with increasing storage time. The highest weight loss was determined on the 40th day, and there was no statistical difference between the 40th and 30th days. On the other hand, it was determined that the weight loss of Kompozit Şeker variety (2.23%) was higher than that of Batem Tatlı (2.15%) (Table 1).

Table 1. Means values of weight loss and dry matter content properties as a result of storage of sweet corn varieties for different periods

	Weight Loss (%)			**Dry Matter Content (%)**		
Time / Type	**Batem Tatlı**	**Kompozit Şeker**	**Means**	**Batem Tatlı**	**Kompozit Şeker**	**Means**
1st day	0.00	0.00	**0.00 D[1]**	27.21 e	28.07 c	**27.64 D[1]**
5st day	0.60	0.74	**0.67 CD**	27.88 e	28.21 c	**28.05 D**
10st day	0.67	1.49	**1.08 C**	31.92 d	31.31 b	**31.62 C**
20st day	2.48	2.21	**2.35 B**	34.90 c	31.52 b	**33.21 BC**
30st day	4.09	4.32	**4.21 A**	36.88 b	31.88 b	**34.38 B**
40st day	5.05	4.64	**4.84 A**	40.66 a	33.71 a	**37.19 A**
Means	**2.15**	**2.23**		**33.24 A**	**30.78 B**	

[1]The difference between the means given with different letters in the same column and row is not significant.

The change in the dry matter content of the sweet corn cultivars during the storage period, the sweet corn cultivars and storage time x sweet corn cultivars interaction were found to be statistically significant ($P\leq0.05$). During storage, dry matter content increased continuously with the moisture and weight loss of sweet corn varieties. As a matter of fact, the lowest dry matter content during the storage period was determined on the 1st day (27.21% and 28.7%, respectively) when it was harvested in Batem Tatlı and Kompozit Şeker sweet corn varieties, and the highest on the 40th day (40.66%, 33.71%, respectively). During the storage period, the dry matter content of Batem Tatlı variety (33.24%) was higher than that of Kompozit Şeker (30.78) (Table 1).

Ash Content and Crude Protein Content

Ash is the unburnable particles that remain after the dry material has been burned at high temperatures for a certain period of time. The sweet corn varieties, storage time and, interaction of storage time × sweet corn varieties ash content were found to be statistically significant ($p<0.05$). In parallel with the increase in storage time, the ash content of sweet corn varieties decreased. The highest ash content (2.52%) ash rate was determined on the 1th when the sweet corn varieties were harvested (Table 2). On the other hand, the lowest was determined on the

40th day of the storage period and the highest ash content was determined in Kompozit Şeker corn variety with an average of 2.36%.

The change in crude protein content of sweet corn varieties during storage and the interaction of storage time x variety were found to be statistically significant (P≤0.05). In terms of crude protein content, Kompozit Şeker variety (11.40%) was found to have higher values than Batem Tatlı variety (10.29%). During storage, the crude protein content varied between 9.70 and 11.88%, the highest value was determined on the 1st day and the lowest on the 40th day. Crude protein content decreased with the increase of storage time in both corn varieties (Table 2).

Table 2. Means values of ash content and crude protein content properties as a result of storage of sweet corn varieties for different periods

	Ash Content (%)			Crude Protein Content (%)		
Time / Type	**Batem Tatlı**	**Kompozit Şeker**	**Means**	**Batem Tatlı**	**Kompozit Şeker**	**Means**
1st day	2.46 a	2.58 a	**2.52 A[1]**	11.42 a	12.34 a	**11.88 A[1]**
5st day	2.34 b	2.48 b	**2.41 B**	11.06 ab	11.42 b	**11.24 B**
10st day	2.26 c	2.37 c	**2.32 C**	10.85 b	11.39 b	**11.12 B**
20st day	2.14 d	2.32 cd	**2.23 C**	10.00 c	11.21 b	**10.61 C**
30st day	2.03 e	2.25 d	**2.14 D**	9.89 c	11.12 b	**10.51 C**
40st day	1.86 f	2.13 e	**2.00 E**	8.49 d	10.90 b	**9.70 D**
Means	**2.18 B**	**2.36 A**		**10.29 B**	**11.40 A**	

[1]The difference between the means given with different letters in the same column and row is not significant.

Total Soluble Sugar Content and Soluble Solids Content

In the study, the change in total soluble sugar content of sweet corn varieties during storage, interaction of storage time x variety were found to be statistically significant (P≤0.05). In terms of total soluble sugar content, it was determined that Batem Tatlı variety (12.11 mg g^{-1}) had higher values than Kompozit Şeker (11.00 mg g^{-1}). During storage, total soluble sugar content varied between 9.50-13.50 mg g^{-1}. During the storage period, the highest value was determined on the 1st day and the lowest on the 40th day, and the storage days of the 5th and 10th days were included in the same statistical group. The decrease in total soluble sugar content of Batem Tatlı variety during storage was higher than that of Kompozit Şeker variety (15.49% and 13.18% respectively) (Table 3).

The amount of soluble solids matter content of the sweet corn cultivars during storage and interaction of storage time × variety were found to be significant ($p<0.05$). During storage days, soluble solids matter content values of both of sweet corn cultivars generally decreased compared to the initial values. Soluble solids matter content varied between 23.33% and 28.33% during storage, highest soluble solids matter content was determined on the 1st day when the sweet corn was harvested, and lowest on the 40th day. It was determined that the soluble solids matter content of Batem Tatlı cultivar (27.33%) was higher than that of Kompozit Şeker (24.83%). On the other hand, the proportional decrease in soluble solids matter content of Kompozit Şeker variety was higher than that of Batem Tatlı (19.74%, 15.73% respectively). The high proportional decrease in the soluble solids matter content of the Kompozit Şeker variety can be associated with the higher water loss of this variety (Table 3).

Table 3. Means values of total soluble sugar content and soluble solids content properties as a result of storage of sweet corn varieties for different periods

Time / Type	Total Soluble Sugar Content (mg g^{-1})			Soluble Solids Content (%)		
	Batem Tatlı	Kompozit Şeker	Means	Batem Tatlı	Kompozit Şeker	Means
1st day	14.33 a	12.67 a	**13.50 A[1]**	29.67 a	27.00 a	**28.33 A[1]**
5st day	13.00 b	11.67 b	**12.33 B**	28.00 b	27.00 a	**27.50 B**
10st day	12.687 b	11.65 b	**12.17 B**	27.6 b	26.00 b	**26.83 C**
20st day	12.00c	11.00 c	**11.50 C**	27.00 c	24.33 c	**25.67 D**
30st day	10.67 d	10.00 d	**10.33 D**	26.67 c	23.00 d	**24.83 E**
40st day	10.00 e	9.00 e	**9.50 E**	25.00 d	21.67 e	**23.33 F**
Means	**12.11 A**	**11.00 B**		**27.33 A**	**24.83 B**	

[1]The difference between the means given with different letters in the same column and row is not significant.

Grain Color (L and Croma values)

In the study, the change in color values (L* and C*) of sweet corn varieties during storage and the interaction of storage time x variety were found to be statistically significant ($P \leq 0.05$). Color values (L* and C*) of both sweet corn varieties decreased during storage. L* value represents the brightness of the products, and it was determined that Kompozit Şeker variety (72.44) had higher values than Batem Tatlı variety (71.76). While the highest L* value was determined on the 1st day (73.46) during the storage period, there was no statistical difference between the 1st day with the 5th and 10th days. The smallest L value was found on the 40th day (69.09). On the other hand, the Croma (C*) value, which shows vividness in color, varied between 37.50-54.46 during storage. The highest C value was detected on the 1st day and the lowest on the 40th day. C* value of Kompozit Şeker variety (47.26) was higher than Batem Tatlı variety (44.57) as well as L* value (Table 4).

Table 4. Mean values of L* and C* traits as a result of storage of sweet corn varieties for different periods

Time / Type	L*value			C* value		
	Batem Tatlı	Kompozit Şeker	Means	Batem Tatlı	Kompozit Şeker	Means
1st day	73.57 a	73.34 a	**73.46 A[1]**	52.02 a	56.90 a	**54.46 A[1]**
5st day	73.46 a	73.04 a	**73.25 A**	51.03 a	47.66 b	**49.35 B**
10st day	72.94 a	73.01 a	**72.98 A**	47.92 b	46.98 b	**47.45 BC**
20st day	72.62 a	72.42 a	**72.52 AB**	45.88 b	45.89 bc	**45.89 C**
30st day	70.29 b	72.35 a	**71.32 B**	37.26 c	44.42 c	**40.84 D**
40st day	67.67 c	70.50 b	**69.09 C**	33.30 d	41.69 d	**37.50 E**
Means	**71.76 B**	**72.44 A**		**44.57 B**	**47.26 A**	

[1]The difference between the means given with different letters in the same column and row is not significant.

DISCUSSION

In this study, weight loss increased as the storage time increased, and the maximum weight loss occurred on the 40th day in both cultivars (Table 1). Although there was an increase in weight loss of sweet corn varieties during storage, when the weight loss exceeds 5% (4.84%) at the end of the storage period under controlled conditions, it can cause perishable vegetables to wilt and shrivel (Xie et al., 2017). On the other hand, the high relative humidity in MAP plays an important role in limiting the water loss of the products. It has been reported in different studies that the weight loss of sweet corn decreases during storage when combined with storage

conditions or packaging materials (Liu et al., 2021). The data obtained with the literature sources examined are in harmony.

In both of the sweet corn varieties stored under controlled conditions, the dry matter ratio increased with the increase in storage time (Table 1). When the literature was examined, Kara and Şahin (2012) stated that the dry matter ratios of sweet corn varieties stored under controlled conditions (+4^{o}C) increased with the increase in storage time. Because of its high sugar and moisture content, sweet corn is one of the species with the highest respiration rate among all fresh consumable vegetables (Becerra Sanchez and Taylor, 2021). As a matter of ratio, an increase in the dry matter ratio of the sweet corn varieties was observed during the storage period, and it is predicted that this increase was caused by the high respiration rate of the sweet corn and the moisture loss in the grains.

In the study, it was determined that the ash content decreased with the increase in the storage time (Table 2). Liu et al. (2013), stated that the ash content decreased with the increase of storage time in their study in which they stored corn for different periods (0, 3, 6 and 12 months) without application. Bello and Oluwalana (2017), stored huskless corn cobs for 4 days and stated that the ash content decreased from 3.92% to 3.43%. Raw ash contains macro and micro minerals, which have many effects such as the presence of nucleoproteins, which have an important role in terms of their effectiveness on the cell functions of plants, and the intercellular transfer of oxygen. Considering the study, it is in agreement with the data we obtained.

Crude protein content decreased with increasing storage time (Table 2). In the literature, Bello and Oluwalana (2017), in their study in which they determined the effects of packaging on the storage of corn, reported that the crude protein content of husked cobs varies between 10.84-13.10% and the crude protein content decreases with the increase of storage time. The data obtained are in harmony with the literature study examined.

Parallel to the increase in storage time, the change in total soluble sugar content decreased gradually in both sweet corn varieties (Table 3). In the study, it was observed that the decrease in total soluble sugar content of sweet corn varieties preserved with MAP during storage was below 20%, slowing down the sugar content, which is an important disadvantage of sweet corn after harvest, for a certain period of time. Sweet corn is a type of corn with a high respiration rate. If pre-cooling and low temperature storage is not done immediately after harvest, sugar loss and some quality deterioration occur (Olsen et al., 1991; Dayı, 2011). Sugar content decreases with the formation of sucrose and some organic compounds during the storage of sweet corn (Karande et al., 2014). During storage, the amount of sugar decreases and the amount of starch increases (Dayı, 2011). It is reported that the sugar content decreases by 25% in the first 24 hours after the sugar corn is harvested (Kün, 2004). Brecht (2004) reported that sweet corn loses 60% of its total sugar at 30°C and 6% at 0°C in one day. Kara and Şahin (2012) determined that the decrease in sugar content of Lumina F_1 and Sakarya Kompozit sweet corn varieties at the end of the 12th day was between 77.89-80.05% at room temperature and 37.02-35.90% at +4°C, respectively. It has been reported in different studies that the total soluble sugar content of sweet corn decreased significantly with the increase in storage time (Liu et al., 2021). The literature studies examined are in agreement with the findings we obtained.

Soluble solids matter content of sweet corn varieties decreased in general during the storage period (Table 3). During storage, the amount of soluble solids content decreases with the use of sugars in respiration (Koyuncu et al., 2018). A similar decreasing trend in the amount of soluble solids matter content during storage in sweet corn has been reported by many researchers (More et al., 2017).

L* value is a color parameter that is also affected by the darkening reactions of the products and the pigment density, hence the darkening of the meat color (Rosaj-Graü et al., 2006). Post-harvest discoloration occurs in most of the freshly cut products, and this color change has many causes and degrees. The most common color change is browning, which is found in fresh cut products due to oxidation of phenolic compounds. In the study, as the storage time increased, L and C* values decreased and a darkening in the color of the cobs was observed (Table 4). The C* value represents the saturation of the color (0=matte, 60=saturated) (Karan, 2015). Karan (2015) found decreases in L and C* values with the increase in storage time of black nut types. These reviewed literatures are in agreement with this sweet corn study.

CONCLUSIONS

In the study, the physical and chemical properties of the cobs of post-harvest sweet corn varieties were evaluated during storage. When the sweet corn cultivars were stored for different periods, at the end of the 40th day, the ash content, crude protein content, total soluble sugar content, soluble solids matter content, brightness (L) and vitality (C*) values decreased; weight loss and increase in dry matter ratio were determined. When it is desired to store sweet corn at +4^{o}C; when stored for 5 days after harvesting, it was determined that there was no difference in terms of physical properties, and statistical differences were determined in terms of quality. For this reason, sweet corn varieties should be consumed fresh after harvesting, and when it needs to be kept at +4^{o}C, it can be recommended to be consumed within a maximum of 5 days. After the 5th day, it has been determined that there will be great losses in terms of quality and physical properties.

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THE IMPROVEMENT DIFFERENT FORMULATIONS OF FISH SOUP AND FISH SAUCE

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ABSTRACT

Fish based ready to eat products became popular because of their low price, easily prepared, besides being characterized by a high protein and omega 3 concentration, which attend the demand of consumers for healthier food. The aim of the present study was to investigate the using of rainbow trout (Oncorhynchus mykiss) in different ready-to-eat fish soup and fish sauce production and to determine nutritional components in different fish soup and fish sauce group. Rainbow trout (Oncorhynchus mykiss), vegetables, spices and other additives obtained from a local firm in Adana. Fish was individually headed, gutted, filleted and washed after appearing on the laboratory. Then the fillets divided into nine groups that five different fish soup (fish meatball soup, vermicelli fish soup, zucchini - dill fish soup, lemon fish soup, mushrooms fish soup) and four fish sauce formulations (tomato fish sauce, pepper fish sauce, potatoes fish sauce, zucchini dill fish sauce) obtained. After production of fish-based sauce and soup, products freeze- dried and pulverized. At the end of study; in soup groups, the highest and lowest protein content obtained in fish meatball soup and in lemon fish soup, respectively. Compared in terms of content and the lowest and highest lipid content of the fish sauce was seen in tomato and zucchini-dill fish soup and found values, respectively, 3.12% and 7.12%.In fish sauce group, the highest protein content was obtained from in potatoes fish sauce group as 37.98 %. Lipid rate varied from 13.42% to 18.49 % in fish sauce group. At the end of study, based on the nutritional composition of vegetables and additive elements content varies; in the types of products it is observed that the increasing use of fish meat nutritional quality fish soup fish ball soup, vermicelli fish soup, lemonade fish soup, mushroom fish soup.

Keywords: fish soup, fish sauce, formulation, lemon sauce, fish meat, proximate composition.

INTRODUCTION

Instant foods have becoming very popular food products that are prepared easily and quickly (Habib et al.,2011). Soup categorized as a liquid and heterogeneous food prepared from fish or meat vegetables, and other indigents using with water, juice or stock (Erkekoglu et al., 2009). Using different type of protein source for soup production is a novel approach for increasing nutritional value of instant soup. Due to having important vitamins, minerals, species-specific essential amino acids and fatty acids, fish meat can play a key role in the human diet (Cho and Kim., 2010).Instant soup has some advantages such as being almost ready to consume and taking less time to prepare (Islam et al., 2018). In the global market, there has been a big

demand of dry soup mixes prepared with animal protein source (Warang et al., 2008). With better understanding of the importance of fish and seafood importance in human diet, instant soup industry has tend to use fish and seafood in this industry like other industry.

Valorization of fish species in different fish soup or fish sauce also an economic approach for seafood processing industry. Furthermore using a high protein source such as fish meat in instant soup and sauce, which categorized as ready to eat food has some benefits for public health (Islam et al., 2018. Due to fish meat is very susceptible for spoilage, appropriate processing and storage techniques are essential and play key role for good quality of seafood products (Warang et al., 2008). Preparation method of soup is very important step for storage quality. Soup can prepared with mixing all used components such as meat, vegetables and food additives as solid, dried form, on the other way all of the component can mix as non-dried form and then homogen mixture will dry together(Kayode et al., 2005). Drying all components together after mixing as natural form, cause to advantage such as reflecting sensory and nutritional characteristic of main components to final product. Different drying methods applied for instant soup preparing step. Lyophilisation (vacuum freeze drying) is one of the popular methods for dehydration of frozen food materials by sublimation with vacuum (Hua et al.,2010).In this research, instant soup and instant sauce prepared with rainbow trout (Oncorhynchus mykiss) and different food additives, then nutritional value in terms of proximate composition was investigated.

MATERIAL AND METHOD

Rainbow trout (Oncorhynchus mykiss) were obtained from local fish suppliers and transported to laboratory. Then fish headed, gutted, filleted manually and fish meat cutted into small pieces and stored at 80 °C until soup and sauce preparation. Vegetables and food additives obtained from local market were frozen and lyophilized in lyophilizator (Teknosem-TRS 2/2V) and prepared as use for fish soup and sauce. Different formulations of fish sauce and fish soup were decided after preference test done by expert panellists. Further to preference test results, meatball soup, vermicelli fish soup, and zucchini - dill fish soup, lemon fish soup, and mushrooms fish soup chosen as soup groups. Similarly depends on preference test, tomato fish sauce, pepper fish sauce, potatoes fish sauce, zucchini dill fish sauce were chosen as fish sauce. Formulations of different fish soup and fish sauce were given in Table 1.

Table 1. Different formulations of fish meat enriched soup

Indigents (%)	Fish Meatball Soup	Vermicelli Fish Soup	Zucchini - Dill Fish Soup	Lemon Fish Soup	Mushrooms Fish Soup
Fish Meat	19,59	38,95	29,49	26,16	21,48
Onion	17,05	-	-	-	-
Garlic	0,88	-	-	-	-
Chickpea	17,03	-	-	33,31	-
Wheat	11,44	-	-	-	-
Flour	14,30	-	-	-	-
Tomato Sauce	13,56	20,86	12,32	-	-
Olive Oil	4,52	8,99	5,49	8,88	10,91
Tomato	-	12.98	11.94	-	-

Green Pepper	-	5,25	2,38	-	-
Red Pepper	-	-	5,51	-	10,24
Parsley	1,06	-	0,39	-	-
Carrot	-	-	5,82	-	16,04
Dill	-	-	2,76	-	-
Zucchini	-	-	23,23	-	-
Vermicelli	-	11,87		-	-
Mushroom	-	-	-	-	40,01
Lemon Sauce	-	-	-	10,93	-
Egg	-	-	-	19,64	-
Salt	0,55	1,09	0,67	1,08	1,33

Table 2.Different formulations of fish meat enriched sauce

Indigents (%)	**Tomato fish sauce**	**Pepper fish sauce**	**Potatoes fish sauce**	**Zucchini dill fish sauce**
Rainbow trout (Black muscle)	8,92	14,24	17,16	15,84
Rainbow trout (White muscle)	14,89	10,93	-	-
Onion	17,10	5,21	-	15,93
Garlic	0,47	0,74	-	0,29
Pepper pure	0,37	-	-	-
Olive oil	5,50	8,83	9,52	11,06
Tomato	21,01		10,87	11,29
Green pepper	6,46	8,86	-	-
Red pepper	6,46	27,25		
Parsley	-	-	2,58	-
Carrot	-	-	13,45	-
Dill	-		-	1,97
Zucchini	-	-	-	27,19
Potato	-	-	44,87	-
Mushroom	13,53	21,76		13,53
Tomato pure	3,42	-		
Basil	0,12	-		
Salt	0,93	1,23	0,47	
Black pepper powder	0,40	0,53	0,29	0,30
Red pepper powder	0,41	0,27	0,30	0,31
Thyme	-	0,15	0,16	-

Determination of Proximate composition:

Prepared soup and sauce based on different formulations located in Table 1 and Table 2. For determination of the proximate composition of fish soup and fish sauce groups, protein, lipid, moisture and ash content were quantified in triplicate. The crude protein was measured by a Kjeldahl technique (Association of Official Agricultural Chemists (AOAC, 1998). Lipid content was analyzed according to the Bligh and Dyer method (Bligh,and Dyer, 1959). Different fish soup and sauce samples were analyzed for moisture by oven drying of 5 g of sample at 105 °C (Method 950.46 AOAC, 1990). Finally, the ash content of fish soup and fish sauce was estimated by the method 938.08 (AOAC, 1990).

Statistical analysis

All measurements were carried out in triplicate and the results are given as the mean and standard deviation. A general linear model, was used to determine the statistically significant differences ($P<0.05$) among different formulation tuna pates stored in different packaging material applied by using the software SPSS version 19 (Chicago, Illinois, USA

RESULTS AND DISCUSSION

The protein, lipids, moisture and ash content of fish soup were shown in Table 3. The highest protein ratio was determined in fish meatball soup group as 57.50. This highest ratio could be caused by meatball done by only fish meat. Meatball known as a high protein source (Shibahara et al., 2013). The highest lipid content was found in lemon added fish soup as 18.31%. Lipid content of different formulated soup changes from 13.10 to 18.31%.The lower lipid levels were found in fish meatball soup and vermicelli fish soup as 13.33 and 13.15., respectively. All of the used fish meat chosen in one fish meat mixture, variations of soup caused by used food additives and indigents. In terms of moisture content, the highest and lowest level was determined in fish meatball soup (7.04) and mushroom fish soup (1.01), respectively. Moisture levels of food depend on used indigent's moisture content and influenced by additives and preparing methods. Using different indigents lead to proximate composition variations in homogen matrix foods such as soup (Shukla et al., 2014).These results are in agreement with many researches who stated that fat and moisture content of sardine (*Sardinella longiceps*) soup as 7.95 and 10.61 %.,respectively (Udari et al., 2015).

Table 3. Proximate composition results of different formulated fish soup

Proximate composition of different fish soup					
Parameter	**Fish Meatball Soup**	**Vermicelli Fish Soup**	**Zucchini - Dill Fish Soup**	**Lemon Fish Soup**	**Mushroom Fish Soup**
Protein	57.50	55.98	54.02	53.45	53.75
Lipid	13.10	13.15	16.02	18.31	17.93
Moisture	7.04	3.95	1.97	1.03	1.01
Ash	18.80	27.23	18.35	27.90	25.12

Table 4. Proximate composition results of different formulated fish sauce

Proximate composition of different fish sauce				
Parameter	**Tomato fish sauce**	**Pepper fish sauce**	**Potato fish sauce**	**Zucchini dill fish sauce**
Protein	37.50	34.98	37.98	35.39
Lipid	3.12	4.01	3.34	7.20
Moisture	60.03	54.97	54.02	51.04
Ash	6.23	5.19	4.20	6.18

The proximate composition of different fish sauce in terms of protein, lipids, moisture and ash content were shown in Table 4. The highest protein ratio was determined in potato fish sauce as 37.90%. Protein level is one of the most important parameter in determining the nutritional quality and physical quality such as texture of fish meat enriched food products. This effect cause by fish meat and its water holding capacity response (Udari et al., 2015). The highest lipid content was found in zucchini dill fish sauce as 7.20 %.The lipid contents were found between 3.12-4.01 % in the other fish enriched fish sauce groups. Cause of all of the used fish meat was taken from same fish meat dough, the highest lipid content caused by variations of used sunflower oil ratio. The lowest and highest moisture contents were found between 51.04 and 60.03 in Zucchini dill fish sauce and Tomato fish sauce, respectively. Highest moisture content could be caused the high water content of tomato. When compare the moisture content of fish enriched soup and sauce, due to fish sauce groups did not dried with vacuum freezer. The lowest ash content was found in Potato fish sauce (4.20), the highest ash content was found in tomato fish sauce (6.23). Nutritional value of fish enriched soup or fish enriched sauce is better than dried vegetarian soup enriched with supplemented with legumes carried out by Abdel-Haleem and Omran,(2014).On the other hand this study is a good model for using different plant or food additives in fish enriched foods. Similarly Rekha et al., (2010) reported that using plant in dried soup mix is goof approach for increasing nutritional value and sensory quality of soup. The results of proximate composition revealed that nutritional value of fish enriched soup or sauce are sufficient for human diet requirements. With better understanding of the importance of animal protein in human diet, fish enriched instant food alternatives will become more popular.

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CONCLUSIONS

Based on the study, fish meat enriched soup and sauce products gained nutritional benefits. Within better understanding of animal based protein sources, utilization of directly fish meat or bu-products will take attendance in food industry. The tested insane food will be a robust model for production of other fish-enriched products production. Due to the fish meat or by-products of fish are highly perishable, the processing and string steps should be perform carefully and the applications should carried out with food safety regulations.

The commercialization process is so important for any food items and sensory evaluation is key parameter for proximate composition. This research could be valuable for further research in the same area.

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DETERMINATION OF PHYSICO-CHEMICAL AND SENSORIAL PROPERTIES OF FUNCTIONAL KAVILCA FLOUR YOGURT

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ABSTRACT

It was aimed to examine the production and acceptability of yoghurts enriched with Kavılca flour in this study. For this purpose, yoghurts were produced using different concentrations of [1.5% (A1), 3% (A2) and 4.5% (A3)] kavılca flour-milk compositions and stored at 4°C for 21 days. The pH, titration acidity, serum separation, viscosity, colour values and sensory properties of yoghurt samples on the 1st, 7th, 14th and 21st days of storage were investigated. It was found that the pH values of yoghurt samples decreased and acidity values of samples increased with the addition of kavılca flour. As the content of Kavılca flour increased, there was a decrease in serum separation. Serum separation values of yoghurt containing 4.5% Kavılca flour during storage were lower than the control sample. Viscosity (20 rpm) values were affected by both the addition of kavılca flour and storage period. Kavılca flour additive the determined L* value, caused a decrease in the whiteness value. In addition, the value of a* was found to be positive on the 14th and 21st days of storage in the samples with the addition of Kavılca flour. As the amount of kavılca flour added to the yoghurts increased, b* value increased regularly. The effect of Kavılca flour addition and storage time on the sensory properties of yoghurts was found to be statistically significant ($p<0.01$). As the rate of Kavılca flour increased, it was determined that there was a decrease in the appearance scores of the samples, but when the general acceptability scores were examined, they received high scores by the panellists. In the light of the data obtained, it was concluded that by adding kavılca flour to yoghurt, a functional feature can be added to yoghurt, and considering the sensory properties, it can be recommended to use kavılca flour in yoghurt production.

Keywords: Kavılca flour, Functional food, Yoghurt

INTRODUCTION

Yoghurt is a fermented milk product from dairy product that has an important place in human nutrition and is frequently consumed all over the world, especially with its rich protein and calcium content. This situation has led to an increase in the production of new types of yoghurt in different formulations, taking into account consumer demands in the food industry. Some researchers investigated effects of various enriching agents to yoghurts such as walnut, hazelnut, almond and pistachio (Ozturkoglu-Budak et al., 2016)], iron oxide, zinc oxide and calcium phosphate nanoparticles (Santillán-Urquiza et al., 2017), cinnamon powder (Halal and Tagliazuacchi, 2018), *Lactobacillus acidophilus* and green tea powder (Çakmakçı et al., 2019), nigella sativa and honey (Okur et al., 2019), pea flour (Akın and Avkan, 2019), quince powder (Ürkek et al., 2019), clove, propolis and probiotic bacteria (Bayır et al., 2020), spade powder and propolis extract, (Bilici, 2017), black grape pulp (Demirkol, 2016), wheat germ and phytase (Yalçınkaya et al., 2003), sugar beet fibre (Saldamlı and Babacan, 1996), probiotic, pumpkin puree and raisins (Çağlayan, 2018), apricot fibre (Yedikardaş, 2010), inulini (Debon et al., 2010), avocado (Öner et al., 2020), *Hibiscus sabdariffa* L. flower marmalade (Arslaner et al., 2020) etc. Researchers have focused on reducing the risk of chronic diseases, protecting health, and providing consumers with safe and different functional products. For this purpose, it is important to evaluate yoghurt as a good tool to provide and improve daily intake of nutritional components that can prevent diseases and have positive effects on consumer health by being loved by most people (Gahruie et al., 2015). For this purpose, Kavılca flour was used to improve nutritional properties in yoghurt production. Kavılca wheat is a member of the grass family (Gramineae=Poaceae). Due to its low gluten content, it

is recommended to be consumed especially in diseases such as celiac. This feature is explained by the high gliadin and low glutenin ratio of kavılca wheat (Schober et al., 2006; Yüksel 2019).

Kavılca wheat has become popular especially in recent years due to the increasing interest in organic agriculture. In this study, it was planned to investigate the physico-chemical and sensory properties of yoghurts enriched with kavılca flour at different concentrations and to develop a new nutritious product.

MATERIAL AND METHOD

Materials

Commercially available pasteurized cow's milk was used to make yogurt. Starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) of control and Kavılca flour yoghurts were obtained commercially (Maysa Gıda, Adana, Turkey). Kavılca wheat was obtained from Akyaka Hacıpiri village of Kars and ground into flour in a traditional mill.

Production of Yoghurts

Pasteurized cow's milk processed in yoghurt is heated up to 45°C with controlled heating and is made into Control (K), 1.5% Kavılca flour (A1), 3% Kavılca flour (A2) and 4.5% Kavılca flour (A3). divided into 4 separate groups. Sterile Kavılca flours were added to the milk and then homogenized with ultra turrax (Daihan Scientific, Co., Ltd). Starter culture was inoculated and incubated at 43°C. After incubation, it was stored in a refrigerator at +4°C. Chemical, physical and sensory analyses were performed on the 1st, 7th, 14th and 21st days of storage (Tamime and Robinson, 1999). Yogurt production was carried out in 2 replications. Produced trial yoghurts are given in Figure 1.

Figure 1. Trial yogurt samples

Physical and Chemical Analysis

The pH values of the experimental samples were measured using a pH meter (Eutech PH 150 Model) (AOAC 1990). Titration acidity (lactic acid %) were determined according to Kurt et al. (2007). The viscosity determination of yogurts was analysed using a Brookfield viscometer (Model DV-1; Brookfield Engineering Laboratories, Inc., MA, USA) (Gassem et al., 1991). The serum separation of the experimental samples was determined by determining the serum in ml, which was filtered using coarse filter paper of 25 g yogurt sample at +4°C (Atamer and Sezgin, 1986). In the colour evaluation

of the samples, firstly Hunter device (Colorflex-EZ, Hunterlab, Virginia, USA) was calibrated with black and white ceramic calibration plates. Then, the L*, a* and b* values of the homogenized samples were determined (Cueva and Aryana 2008).

Sensory Analysis

In the study, sensory analyses of the prepared yoghurt samples were performed with a panellist group consisting of 10 people on the 1st, 7th, 14th and 21st days of storage. In sensory analysis, parameters such as appearance, taste, colour, odour, texture (consistency), acidic taste and general acceptability were evaluated according to Bodyfelt et al. (1988).

Statistical Analysis

The results of the research were made using the SPSS program (Version 22.00, SPSS, IBM, NY, USA) with the analysis of variance (factorial trial design) method.

RESULTS AND DISCUSSION

Titratable acidity values in Kavılca flour yoghurt samples varied between 0.81-1.13%. The lowest acidity value was measured on the 1st day of storage in the control group and the highest acidity value was measured in the A2 group on the 21st day of storage (Figure 2). Akın et al. (2019) stated that the titration values increased as the lentil flour concentration increased in the yoghurts they produced using lentil flour.

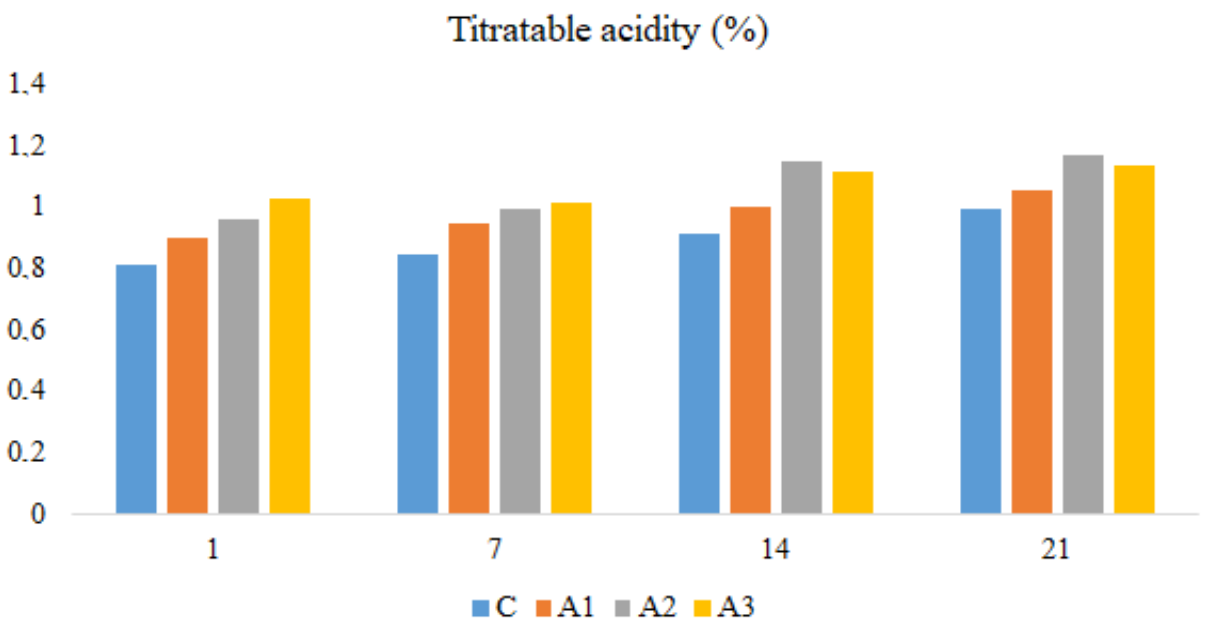

Figure 2. Average titration acidity (%) values of yogurt samples during storage

The pH analysis results of Kavılca flour yoghurt samples during storage are given in Figure 3. During the storage period, the pH values of Kavılca flour yoghurt samples prepared with different concentrations were between 4.06 and 4.57; in the control group samples, it varied between 4.39 and 4.64. Chen et al. (2018) stated that the yoghurt samples produced using chickpea flour contained lower pH values compared to the control group starting from the 1st day of storage, and this result was due to the fact that the use of chickpea flour could increase bacterial activity.

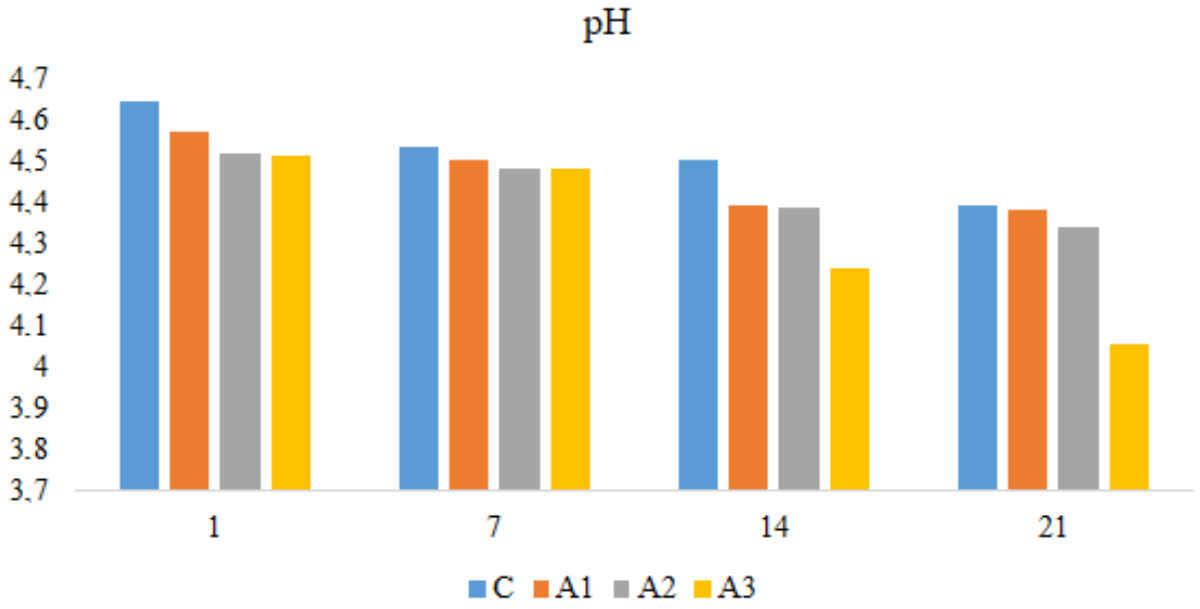

Figure 3. Average pH values of yogurt samples during storage

Viscosity values of yogurt samples are given in Figure 4. In the samples, the lowest viscosity values were observed on the 7th day of storage in the control group (1132.5 cP); the highest was detected on the 21st day of storage (2730 cP) in the A3 coded sample. Küçükçetin et al. (2012) reported that the viscosity value of lentil flour yoghurt samples was higher than that of the control group, and the apparent viscosity values increased significantly as the concentration increased ($p<0.05$). They reported that the highest apparent viscosity value was found in the yoghurt sample containing 3% red lentil flour.

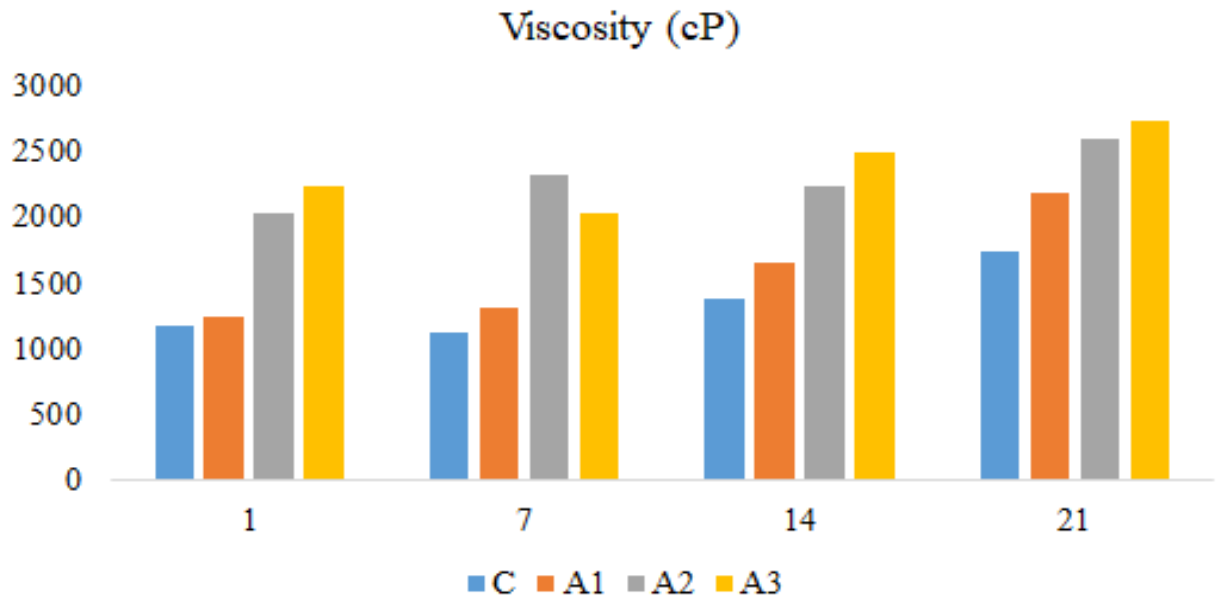

Figure 4. Average viscosity values of yogurt samples during storage

The change in serum separation amounts during the storage period in Kavılca flour yoghurt samples is given in Figure 5. Serum separation values ranged from 4.70 mL/25g to 9.94 mL/25g. When the mean serum separation values were examined, the lowest value was 4.70 mL/25g on the 21st day of storage; the highest value was measured as 9.94 mL/25g on the 1st day of storage. Codină et al. (2016) stated that the addition of quinoa increased serum separation in yoghurts. The researchers explained that

this situation is related to the unstable protein structure and that it has an increasing effect on serum separation as a result of being affected by the weak gel structure.

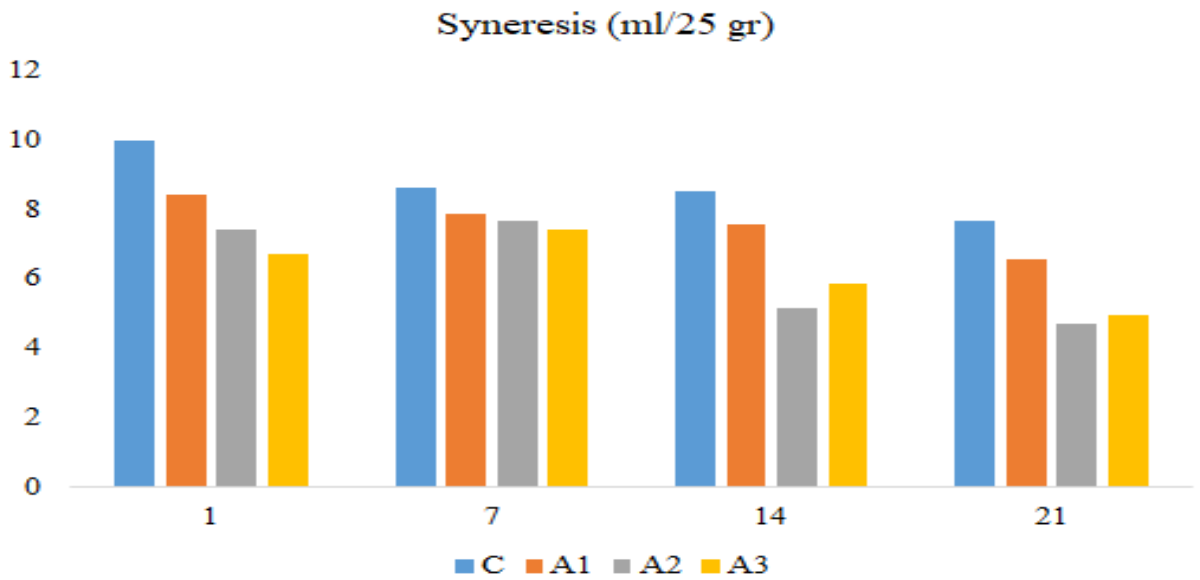

Figure 5. Average serum separation values of yoghurt samples during storage (%)

Kavılca flour caused a decrease in the whiteness value expressed by L* of the samples. However, a* value of the samples prepared with kavılca flour took positive values on the 14th and 21st days. As the amount of kavılca flour added to yoghurts increased, the b* value increased regularly. As a result, it was determined that the effect of the addition of kavılca flour to the yoghurt samples on the colour values (L*, a*, b*) was statistically significant ($p<0.05$) (Table 1). Hafif (2019) reported that the effect of quinoa flour addition on colour values (L*, a*, b*) was statistically significant in probiotic yogurt samples containing quinoa flour ($p<0.01$).

Table 1. Color values of yogurt samples produced using Kavılca flour

Samples	L*	a*	b*
C	90.76 ± 4.86^{b}	1.83 ± 1.31^{a}	9.37 ± 0.38^{ab}
A1	88.36 ± 4.40^{ab}	1.38 ± 0.82^{a}	8.94 ± 0.58^{a}
A2	85.93 ± 4.67^{a}	1.33 ± 0.54^{a}	9.74 ± 0.38^{bc}
A3	85.54 ± 3.12^{a}	1.80 ± 0.33^{a}	10.07 ± 0.89^{c}

$^{a-c}$ Different letters on the same line represent statistical difference ($p<0.05$).

Sensory Analysis Results

The sensory evaluation results of the appearance, colour, taste, odour, consistency, acidic taste and general acceptability parameters of the trial yoghurts are given in Figure 6. The effects of Kavılca flour addition and storage time on the sensory properties of yoghurts were found to be statistically significant ($p<0.01$). The appearance scores of yoghurts were between 6.36 and 8.6; taste scores between 5.4 and 8.7, colour scores between 6.73 and 8.70, odour scores between 6.81 and 8.8, consistency scores between 6.08 and 8.8, acidic taste scores between 5.99 and 8.65 and general acceptability scores were

found between 5.71 and 8.7. Kavılca flour yoghurts were appreciated by the panellists and the A2 sample prepared with 3% concentration received the highest overall acceptability score. Curti et al. (2017) stated that among the yogurt samples with quinoa flour, 1% quinoa flour added yogurt was preferred more by the panellists in the sensory evaluation.

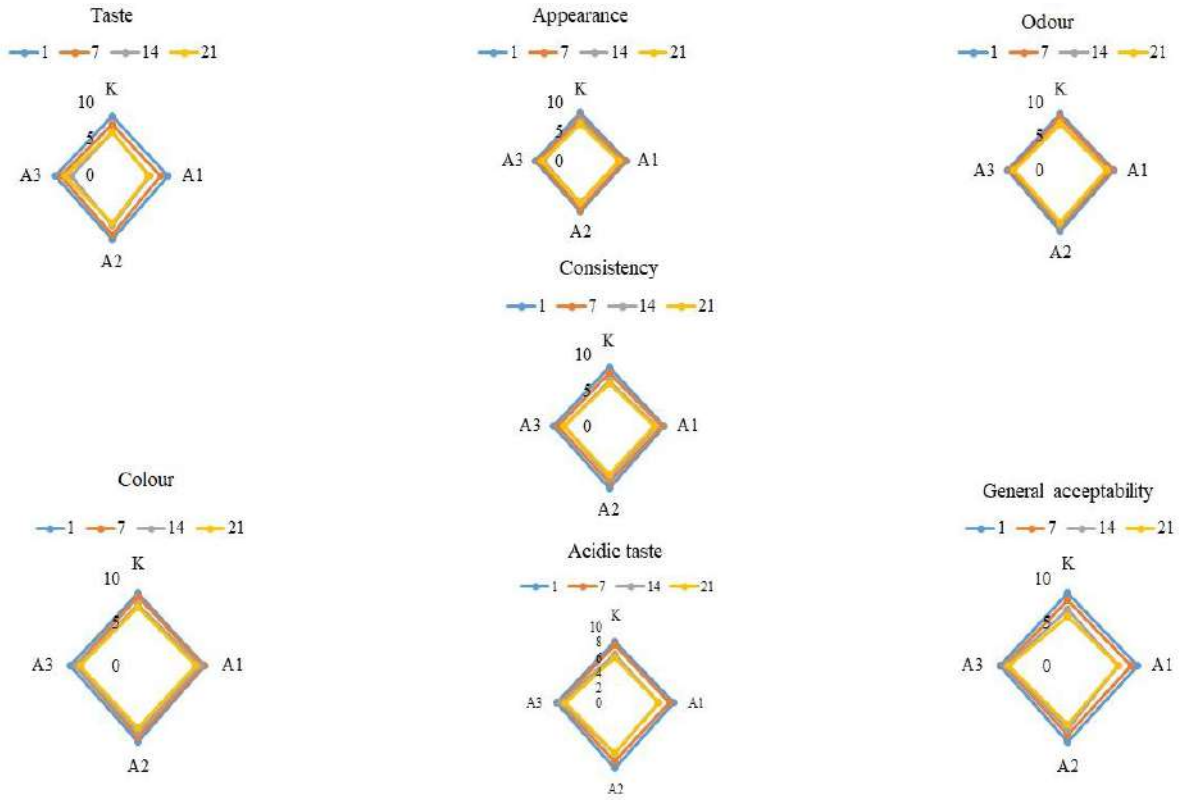

Figure 6. Sensory evaluations of yogurt samples

CONCLUSIONS

In this study, the functional usage possibilities of the addition of kavılca flour in yoghurts were investigated. It was determined that yoghurt samples decreased pH values and increased acidity values with the addition of kavılca flour. It has been determined that the addition of Kavılca flour has an effect on the physical properties of yoghurts such as serum separation and viscosity. According to the results of the sensory analysis, the effect of the addition of kavılca flour at different rates on the appearance, taste, colour, smell, texture, acidic taste and general acceptability scores of the yoghurts was found to be significant ($p<0.01$). The panellists liked the yogurt samples prepared by adding kavılca flour. As a result, in this study, in which the effect of the addition of kavılca flour in different proportions on yoghurts was investigated, it was concluded that it could be used in developing a new and nutritious product.

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EVALUATION OF SOME PHYSICOCHEMICAL PROPERTIES OF SOILS UNDER DIFFERENT PLANTS IN ÇAĞLAYANCERIT

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ABSTRACT

In this study, some physical and chemical properties of agricultural soils where different plants are grown in Çağlayancerit were determined and their distributions were investigated. For this purpose, soil samples from a depth of 0-20 cm were taken from 20 different lands that were farmed according to the grid method with a distance of 2500 m within the district boundaries of Çağlayancerit in Kahramanmaraş province. For the purpose of the research, some physical (texture, saturation) and chemical (pH, EC, CaCO3, organic matter, available P, K, Ca, Mg, Fe, Mn, Cu, Zn and B) properties of the soils were determined. According to the results of the research, the majority of the soils on which different plants are grown have a clay texture and the saturation values are found between 41% and 81%. Most of the soils have neutral reaction (60%), very slightly saline (55%), contain varying amounts of calcareous, and the amount of organic matter has been determined at a low level. It has been determined that 80% of the available phosphorus amount of the soils is insufficient, the potassium level is very high (75%), calcium is good (100%), and magnesium is sufficient (55%). 90% of the iron content of the soils was good, amounts of manganese and copper was sufficient (100%), and 70% of the zinc and boron concentrations were found to be low. As a result, it was determined that the amounts of organic matter, available phosphorus, zinc and boron were insufficient in the soils of the study area.

Keywords: Agricultural soils, Concentration, Çağlayancerit, Distribution, Physicochemical.

INTRODUCTION

The main source of agricultural production is soil. Agricultural soils, which took thousands of years to form, are the only resource that cannot be produced and cannot be renewed. Depending on the climatic characteristics and geographical structure, soils with very different characteristics have been formed from each other, and the plant species cultivated on these soils have also shown differences. Soil fertility is one of the most important factors affecting productivity in agricultural production. Increasing and estimating soil fertility using various methods is one of the current and research priorities. One of the most important factors that increase soil fertility is also plant nutrients. Nutrients are an important part of plant development and the deficiency of one or more of them negatively affects the quality and yield. In some conditions, nutrient excess or nutrient deficiency prevents the uptake of other nutrients by plants, while also negatively affecting yield and quality. On the other hand, determining the physical and chemical properties of the soils and knowing the relationships between these properties and the nutrients in the soil is important for ensuring the highest benefit from fertilization. For the sustainability of our agricultural lands, which are rapidly decreasing and polluted as a result of unplanned urbanization and industrialization, it has become a necessity

to know the physical, chemical and biological properties of the soils well and to take the necessary measures according to these characteristics. The physical and chemical properties of soils and their changes have a significant impact on soil formation processes, productivity and plant growth. Therefore, with this study, it is aimed to determine, evaluate and detect the distribution of some physicochemical characteristics of agricultural soils under different plants in Çağlayancerit.

MATERIAL AND METHOD

Çağlayancerit district is geographically within the borders of the Mediterranean region. It is surrounded by Nurhak in the north, Gölbaşı district of Adıyaman province in the east, Pazarcık in the south and Dulkadiroğlu district in the west. The district center was established in a valley surrounded by Engizek Mountains in the north and Öksüz Mountain in the south. There are 17 affiliated villages and these villages are generally established on mountainous terrain. Arable land is limited in the district, and there are forest areas in the higher parts of the mountainous areas. The altitude of the district center is 1150 m. In the district, the climate type, which has the characteristics of transition between the Mediterranean climate and the continental climate, is dominant. Summer is hot and dry, winter is cold and snowy, and spring is warm and rainy. The area of the district is 642 km². 10,396 hectares (16%) of this area is agricultural land, 23,566 hectares (37%) is forest and heathland, 27,677 hectares (38%) is meadow and pasture, and 5561 hectares (9%) is also non-agricultural land (Anonymous, 2021).

The material of the research consists of 20 soil samples collected from different agricultural locations of Çağlayancerit district. The GPS data of the sampling points are given in Table 1. Soil samples were collected with a stainless steel shovel from a depth of 0-20 cm, in accordance with the general rules (Jackson, 1958), according to the grid method at 2500 m intervals. Soil samples taken were laid on drying benches in laboratory conditions, different particles such as stones and plants were separated and dried. The dried soils were beaten with wooden mallets and passed through a 2 mm steel sieve and made ready for analysis.

In soils ready for analysis; Soil texture was determined according to the modified Bouyoucus hydrometer method (Klute, 1986). Saturation with water (%) was found by adding distilled water to the soil until it is saturated with water, as reported by Richards (1954). Soil reaction (pH) was measured with a Mettler Toledo Seven Compact brand pH meter with glass electrodes after one day in soil saturated with water as reported by Richards (1954). The electrical conductivity (EC) (dS m^{-1}) of the soils was measured in the saturated sludge with the Ezdo PL-700 AL brand electrical conductivity device one day later (Richards, 1954). Lime ($CaCO_3$) (%) was determined volumetrically in Scheibler calcimeter (Klute, 1986). Organic matter (%) was determined by the modified Walkley-Black method specified by Richards (1954). The amount of available phosphorus (mg kg^{-1}) was determined in the Hitachi U-1900 brand Spectrophotometer device according to the method of Olsen et al. (1954). Available K, Ca and Mg contents were determined by measuring with ICP-OES according to ammonium acetate method (Richards, 1954). As explained by Lindsay and Norvell (1978), the extractable microelement (Fe, Mn, Cu and Zn) amounts (mg kg^{-1}) were determined by measuring in the ICP-OES device of the filtrate obtained from the soils shaken with DTPA solution (Klute, 1986). The available boron concentration was found by measuring the filtrates obtained according to the hot water method reported by Klute (1986) in the ICP-OES device.

Statistical Analysis

Descriptive statistics of soil analysis results were calculated in IBM SPSS 25.0 package program.

Table 1. Coordinates and characteristics of the places where soil samples were taken.

Sample No	Coordinates		Altitude	Unirriged/Irrıgated	Cultivated/Planted Crops
	X	Y	m		
1	37.17100	37.75646	1509	Irrigated	Garlic
2	37.20010	37.75729	1484	Unirrigated	Wheat
3	37.22938	37.75429	1534	Irrigated	Orchard
4	37.25527	37.77783	1474	Irrigated	Onion
5	37.19876	37.73304	1427	Irrigated	Sweet cherry
6	37.22911	37.73323	1593	Unirrigated	Walnut
7	37.19836	37.71052	1362	Irrigated	Walnut
8	37.19904	37.68841	1579	Irrigated	Onion
9	37.22891	37.68836	1416	Irrigated	Onion
10	37.28062	37.68761	1189	Irrigated	Walnut
11	37.28261	37.66460	1120	Irrigated	Sour cherry
12	37.33967	37.66394	1066	Irrigated	Corn stubble
13	37.36832	37.68668	1089	Unirrigated	Barley
14	37.45158	37.66247	875	Unirrigated	Wheat
15	37.45242	37.68501	888	Unirrigated	Walnut
16	37.53764	37.81916	993	Unirrigated	Vineyard
17	37.45487	37.77514	978	Irrigated	Sweet cherry
18	37.42466	37.77812	1048	Unirrigated	Wheat
19	37.39876	37.77411	1098	Unirrigated	Wheat
20	37.36957	37.77502	1096	Unirrigated	Vineyard

RESULTS AND DISCUSSION

Descriptive statistics of some physical properties of soils collected from 20 different agricultural areas of Çağlayancerit district are given in Table 2.

Table 2. Some descriptive statistics of some physical properties of soils (n=20).

Parameter	Minimum	Maximum	Average	σ	CV (%)	Skewness
Sand (%)	30.2	76.6	50.9	15.23	29.95	0.40
Silt (%)	5.6	22.8	11.0	5.22	47.48	0.98
Clay (%)	13.2	55.4	38.1	13.06	34.25	-0.41
Saturation (%)	41	81	66	12.80	19.28	-0.70

σ: Standard deviation; CV: Coefficient of variation.

As shown from Table 2, the amount of sand in Çağlayancerit agricultural lands under different plants varies between 30.2-76.6%, and its average value is 50.9%. The standard deviation is 15.23, the coefficient of variation is 29.95%, and the skewness coefficient is 0.40. The silt content of the soils varies between 5.6%-22.8%, with an average value of 11.0%. Its

standard deviation, coefficient of variation, and skewness coefficient are 5.22, 47.48 and 0.98, respectively. While the clay content of the soils varies between 13.2% and 55.4%, the average is 38.1%. Saturation capacity of the soils varies between 41% and 81%, and the average is found to be 66%. Its standard deviation was 12.80, the coefficient of variation was 19.28% and the skewness was -0.70. As can be seen, descriptive statistical data are within the validity limits.

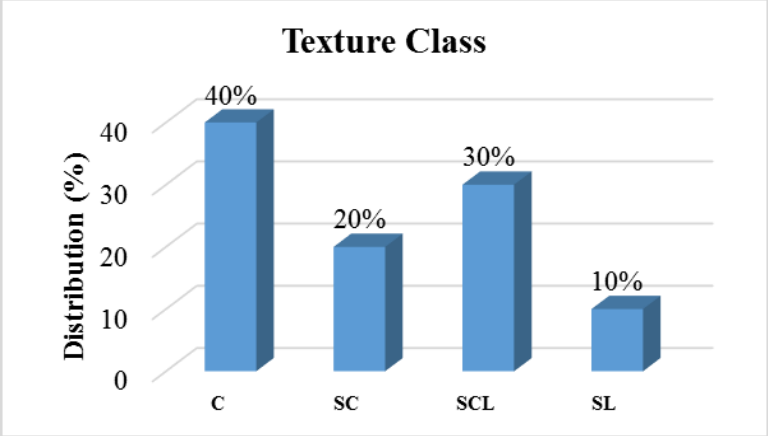


Figure 1. Texture distribution of soils (n= 20).

According to Bouyoucos (1951), Çağlayancerit agricultural soils were determined as 40% clay (C), 20% sandy clay (SC), 30% sandy clay loam (SCL) and 10% sandy loam (SL) in terms of texture (Figure 1). Although the amount of clay textured soils is higher (40%) than the others, when the average amount is evaluated, sandy clay (SC) textured soils are obtained.

Descriptive statistics of some chemical properties of soils grown different plants are given in Table 3.

Table 3. Some descriptive statistics of some chemical properties of soils (n= 20).

Parameter	Minimum	Maximum	Average	σ	CV (%)	Skewness
pH	6.48	7.80	7.25	0.33	4.59	-1.06
EC (dS m^{-1})	0.53	1.21	0.82	0.17	21.44	0.70
$CaCO_3$ (%)	1.86	35.02	13.33	9.92	74.44	0.84
OM (%)	0.78	4.14	1.95	0.96	49.09	0.94
P (mg kg^{-1})	3.00	39.25	9.39	8.64	92.05	2.51
K (mg kg^{-1})	26.39	393.72	238.05	97.56	40.98	-0.07
Ca (mg kg^{-1})	3847.95	12043.74	7436.94	2070.82	27.85	0.40
Mg (mg kg^{-1})	91.60	1019.47	349.59	245.34	70.18	1.43
Fe (mg kg^{-1})	2.32	23.33	8.33	5.25	63.11	1.78
Mn (mg kg^{-1})	3.33	71.94	11.95	14.98	125.36	3.74
Cu (mg kg^{-1})	0.34	3.06	1.29	0.64	49.99	1.35
Zn (mg kg^{-1})	0.13	1.57	0.51	0.45	88.38	1.70
B (mg kg^{-1})	0.09	1.21	0.49	0.27	56.37	1.68

pH: Soil reaction, EC: Electrical conductivity, CaCO3: Lime, OM: Organic matter, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium, Fe: Iron, Mn: Manganese, Cu: Copper, Zn: Zinc and B: Boron.

As it is understood from Table 3, soils with different plants on them have a slightly acid, neutral and slightly alkaline reaction, with an average pH value of 7.25. Standard deviation,

coefficient of variation and skewness were determined as 0.33, 4.59%, -1.06, respectively. The average EC amount of the soils is 0.82 dS m^{-1} and the soils are in non-saline and slightly saline class. The standard deviation is 0.17, the coefficient of variation is 21.44%, and the skewness coefficient is 0.70. Soils where different plants are grown vary between calcareous and too much calcareous in terms of lime content, and the average amount of lime is 13.33%. Statistical parameters were also determined as 9.92, 74.44% and 0.84, respectively. The organic matter content of the soils is mostly low, with an average of 1.95%. Standard deviation, coefficient of variation, and skewness criteria were determined as 0.96, 49.09% and 0.94, respectively. Soils are generally low in phosphorus, with an average phosphorus amount of 9.39 mg kg^{-1}. Standard deviation, coefficient of variation and skewness data were found to be 8.64, 92.05% and 2.51, respectively. Available potassium content of the soils is very high and the average potassium amount is 238.05 mg kg^{-1}. Statistical parameters were determined as 97.56, 40.98% and -0.07, respectively. Ca amounts varied between 3847.95-12043.74 mg kg^{-1}. Its average was 7436.94 mg kg^{-1} and the statistical data were 2070.82, 27.85% and 0.40, respectively. The lowest amount of magnesium in the soil is 91.60 mg kg^{-1} and the highest is 1019.47 mg kg^{-1}. Average amount of Mg is 349.59 mg kg^{-1}, and the magnesium content of the soils is mostly at a sufficient level. Its standard deviation was as 245.34, the coefficient of variation was 70.18%, and the skewness coefficient was also 1.43 determined. The iron content of the soils is generally at a good level, with an average iron content of 8.33 mg kg^{-1}. Statistical parameters were found to be 2.25, 63.11% and 1.78, respectively. The manganese concentrations of the soils are sufficient and the average manganese value is 11.95 mg kg^{-1}. Standard deviation, coefficient of variation, and skewness coefficient were determined as 14.98, 125.36% and 3.74, respectively. Soils are sufficient in terms of copper, and the average copper amount is 1.29 mg kg^{-1}. The standard deviation was 0.64, the coefficient of variation was 49.99%, and the skewness coefficient was 1.35. The zinc content of Çağlayancerit soils is generally low, and the average zinc amount was determined as 0.51 mg kg^{-1}. Descriptive statistical data were found as 0.45, 88.38 and 1.70%, respectively. The available boron level in soils is generally very low, with an average B value of 0.49 mg kg^{-1}. The standard deviation, coefficient of variation and skewness were calculated as 0.27, 56.37% and 1.68, respectively. In general, statistical data are within the validity limits. The fact that some agricultural processes such as tillage, fertilization and irrigation are not carried out regularly, as well as natural conditions such as the topography of the land and the climate of the region, may cause some soil parameters ($CaCO_3$, P, Mg, Fe, Mn, Zn) to not be homogeneously distributed, thus may cause statistical data to be outside the limits of reliability.

The distributions of pH, EC, $CaCO_3$, OM, P, K, Ca, Mg, Fe, Mn, Cu, Zn and B concentrations of the soils under various plants grown in Çağlayancerit district are shown in Figure 2-14.

When the pH of the soils of Çağlayancerit research area is evaluated according to Ülgen and Yurtsever (1995), 10% is slightly acid (6.1-6.5), 60% is neutral (6.6-7.3) and 30% is moderately alkaline (7.4. -8.4) (Figure 2). In a study conducted by Ülgen and Yurtsever (1988), in a total of 64,591 soil samples taken from eight regions of Turkey, the pH distribution of saturation sludge was determined as 0.9% strongly acid, 4.5% moderately acid, 13.4% very slightly acid, 76.5% neutral and slightly alkaline, and 4.7% moderately and strongly alkaline. According to this study, Çağlayancerit agricultural soils were found to have a neutral reaction with a ratio close to Turkey's general. Precipitation, which is one of the climatic factors, plays a role in determining various soil properties, especially pH. Soil pH is high and alkaline in places where annual precipitation is low, and soil pH is low and acidic in places where annual precipitation is high. There are various agricultural basins with different climate and soil characteristics in our country. As a result of the prevailing climatic conditions, most of our country's soils are calcareous and have alkaline reaction (Eyüpoğlu, 1999).

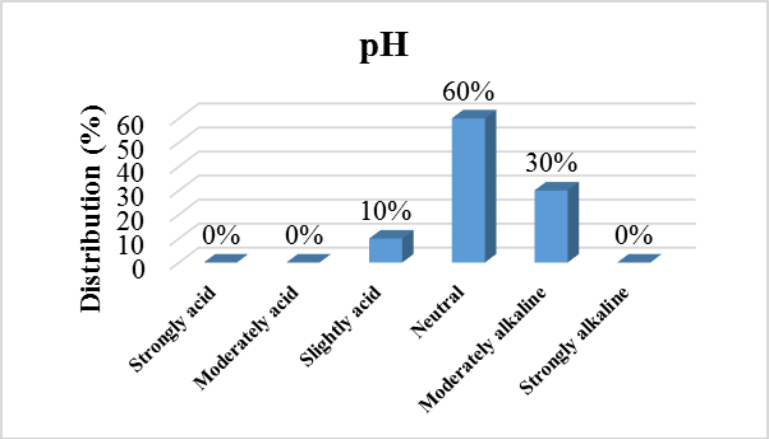


Figure 2. pH distributions of soils (n= 20).

In the EC evaluation of the research soils on which different plants are grown, according to Anonymous (2018), it was determined that 45% of them were non-saline (< 0.75 dS m^{-1}) and 55% of them were also very slightly saline (0.75-2.0 dS m^{-1}) (Figure 3). As seen in the figure also, there is no salinity problem in the study area soils in terms of salt concentrations. Karagöktaş (2012) reported that in a his study conducted in Kahramanmaraş, the total amount of saline varies between 0.07-0.25%, the average is 0.15%, and it is in the class of non-saline and slightly saline. This also shows that our study is compatible with the literature data.

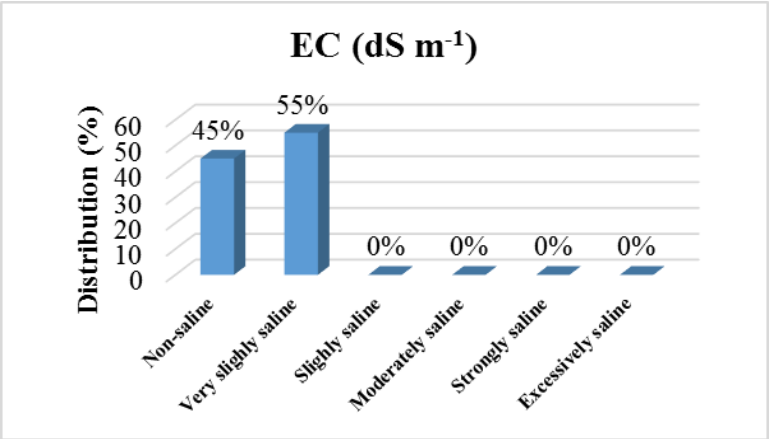


Figure 3. EC distributions of soils (n= 20).

According to the classification reported by Ülgen and Yurtsever (1995), the lime contents of the soils in 25% calcareous (1-5%), in 40% moderately calcareous (5-15%), in 20% much calcareous (15-25%), and in 15% also too much calcareous (> 25%) were determined as (Figure 4). As can be seen, Çağlayancerit agricultural soils vary from calcareous to too much calcareous. It is thought that the reason for this is as well as the parent material, inability to leach lime due to insufficient precipitation, and accumulating in certain layers of the soil profile. In terms of lime content, it has been reported that 26.60% of Turkey's soils are low calcareous, 23.60% moderately calcareous, 18.50% calcareous, 15.70% much calcareous and 15.60% also too much calcareous (TAGEM, 2006). There is a positive correlation between the given literature and our results.

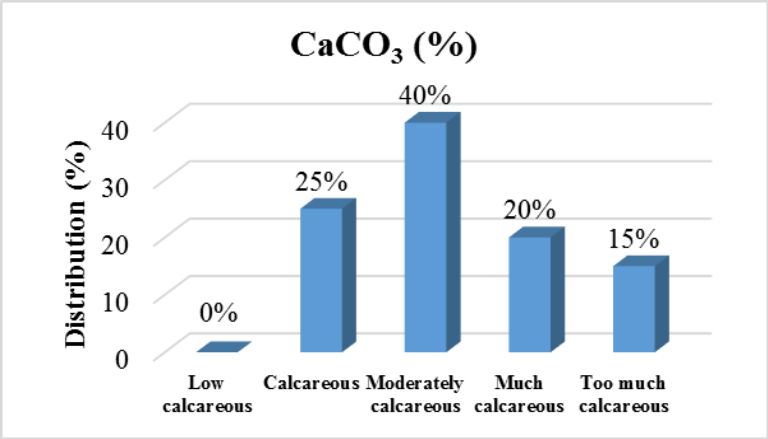


Figure 4. $CaCO_3$ distribution of soils (n= 20).

The amount of organic matter of Çağlayancerit soils, according to the classification of Ülgen and Yurtsever (1995), were found very low in 15% (<1%), low in 45% (1-2%), moderately in 20% (2-3%), high in 15% (3-4%), and very high also in 5% (> 4%) (Figure 5). According to these results, it is understood that the organic matter content of the soils of the study area is mostly low. The amount of organic matter in the soil is closely related to uncontrollable climatic factors such as temperature and precipitation, as well as factors such as tillage status, tillage period, condition of vegetation in the soil, fertilization method, burning of stubble or mixing with the soil. Ülgen (1988) determined organic matter amounts as very low in 19.2%, low in 49.8%, moderately in 22.4%, good in 5.6%, and high also in 3% in a total of 63,613 soil samples colled from eight regions of Turkey. There is a similarity between the data in the literature and our findings.

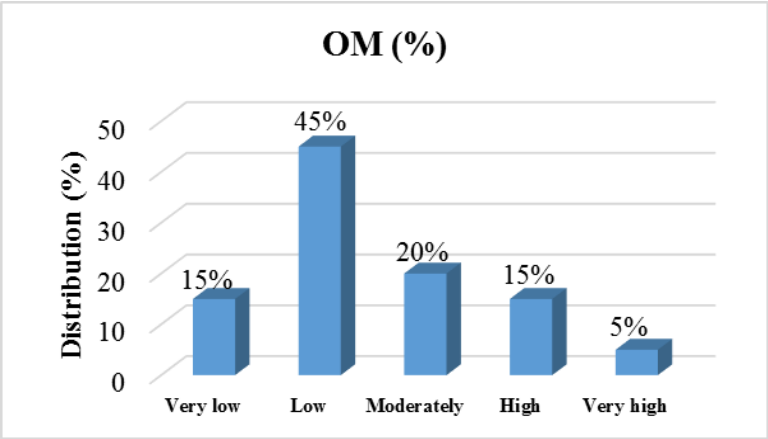


Figure 5. OM distributions of soils (n= 20).

The available phosphorus levels of the research soils were made according to the classification proposed by Rehm et al. (1996). According to this, phosphorus amounts of soils are very low in 25% (0-3 mg kg^{-1}), low in 35% (4-7 mg kg^{-1}), moderately in 25% (8-11 mg kg^{-1}), and very high levels also in 15% (> 16 mg kg^{-1}) (Figure 5). According to these results, it is understood that 85% of Çağlayancerit soils have phosphorus deficiency. In terms of phosphorus content of the soils of Turkey, 28.5% is very low, 26.8% is low, 17.2% is moderately, 9.7% is high and 17.8% is very high (TAGEM, 2006). There is a similarity between the data in the literature and our findings.

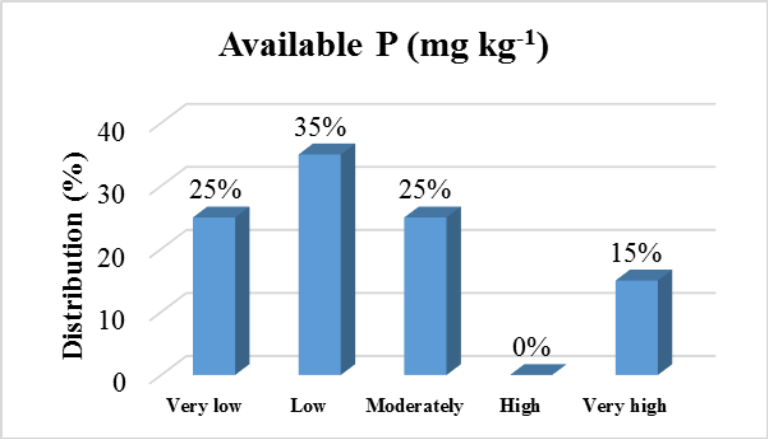


Figure 6. Available P distributions of soils (n= 20).

The available potassium levels of the research soils were made according to the classification proposed by Rehm et al. (1996). Accordingly, the potassium distributions of the soils are very low in 5% (0-40 mg kg^{-1}), high in 20% (121-160 mg kg^{-1}), and very high in 75% (> 161 mg kg^{-1}) (Figure 7). According to these data, 95% of Çağlayancerit agricultural soils do not have a problem of available potassium. It has been reported that climatic factors such as temperature and insufficient precipitation cause potassium minerals to disintegrate into the soil and cause potassium to accumulate in the soil (TAGEM, 2006). Karagöktaş (2012) reported in a study in Afşin-Elbistan that available potassium for the plant ranged from 119-1256 mg kg^{-1} and averaged 643.62 mg kg^{-1}. There is a positive correlation between the given literature and our findings.

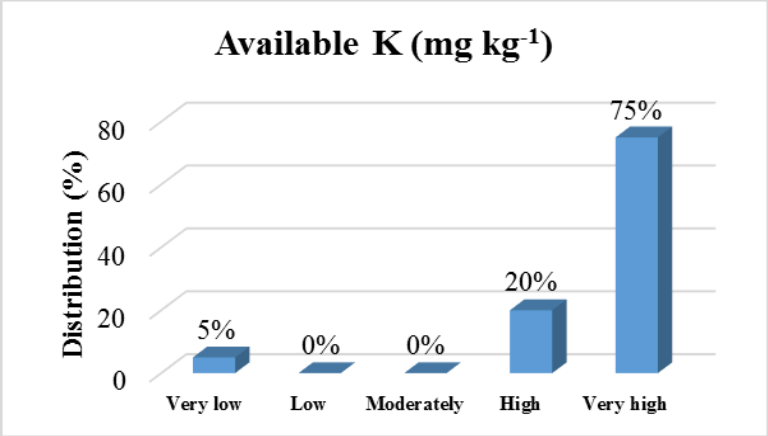
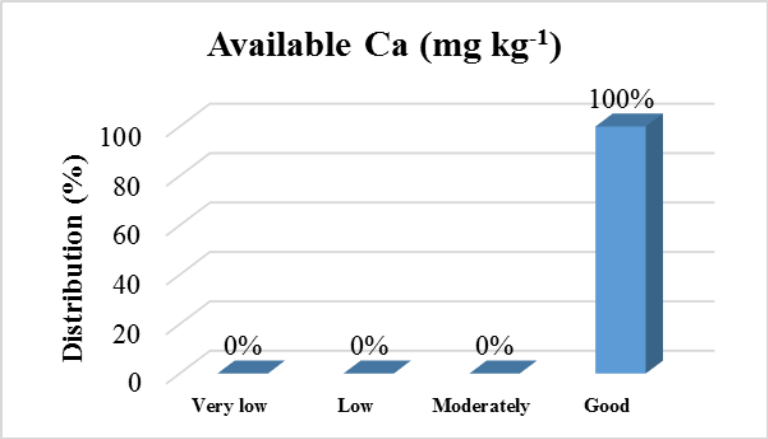


Figure 7. Available K distributions of soils (n= 20).

According to Loue (1968), all of the soils of the research area evaluated are at a good (> 2860 mg kg^{-1}) level in terms of Ca content (Figure 8). From this result, it is understood that there is no problem in terms of available Ca in Çağlayancerit agricultural soils. The lime contents of the soils of our country are generally high. Therefore, calcium concentrations are also high. Alpaslan et al. (2001), the amounts of available calcium of the agricultural lands of the Mediterranean Region ranged from 1742 to 9220 mg kg^{-1}, and the available calcium content of the soils; They reported that 16% were adequate and 84% were also high. The results of the researchers are in agreement with our findings.



Şekil 8. Available Ca distributions of soils (n= 20).

As can be understood from Figure 9, according to FAO (1990) contents of Mg of the studying soils on which different plants are grown are 30% very low (50-160 mg kg^{-1}), 55% sufficient (160-480 mg kg^{-1}), and 15% were also determined to be high (480-1500 mg kg^{-1}). These results show that there is no problem in terms of Mg in 70% of the soils, but there is a problem in 30%.

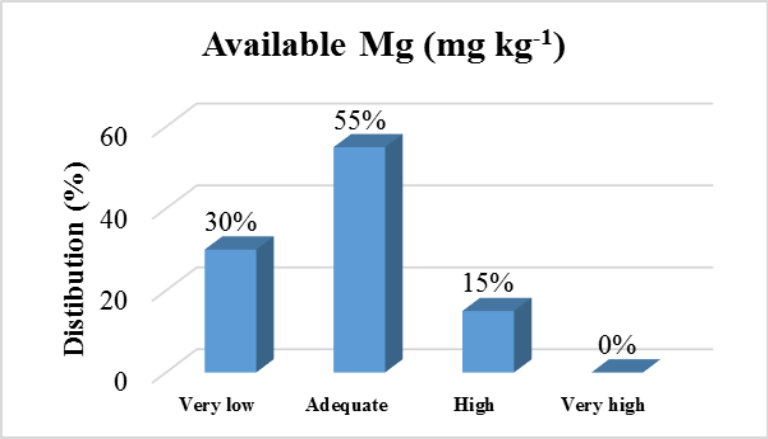


Figure 9. Available Mg distributions of soils (n= 20).

Sufficient and excess Mg nutrient element in the soil is due to the heavy or clay textured soils formed on the magnesium-rich parent material (dolomite and serpentine). Alpaslan et al. (2001) stated that the amount of available Mg in the agricultural soils of the Mediterranean Region varies between 102-2955 mg kg^{-1} and that 1% of the available Mg content is low, 26% is sufficient, 73% is also much and too much. There is a similarity between these results obtained by the researchers and our findings.

The available Fe contents of soils under different plants in Çağlayancerit were evaluated according to Lindsay and Norvell (1978). Accordingly, 5% is inadequent (< 2.5 mg kg^{-1}), 5% is moderately (2.5-4.5 mg kg^{-1}), and 90% is also good (> 4.5 mg kg^{-1}) (Figure 10). The results show that there is no Fe problem in Çağlayancerit agricultural soils. Eyüpoğlu et al. (1996) stated that the available iron concentrations of agricultural soils in Turkey are moderate in 27% and high in 73%. These results, given in the literature, are similar to those we obtained.

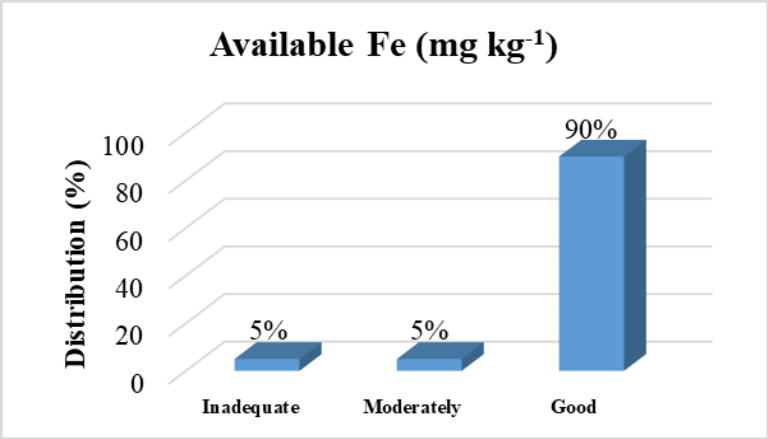


Figure 10. Available Fe distributions of soils (n= 20).

According to Lindsay and Norvell (1978), all of the soils of the study area evaluated are at a adequate (> 1.4 mg kg^{-1}) level in terms of Mn concentration (Figure 11). From this results, it is understood that there is no problem in terms of Mn available in Çağlayancerit agricultural lands. From this results, it is understood that there is no problem in terms of Mn available in Çağlayancerit agricultural lands. Eyüpoğlu et al. (1996) reported that 4% of Turkey's soils are inadequate, 18% are moderately, 44% are adequate and 34% are also high in terms of Mn content. It is understood that our findings are similar to the values given in the literature.

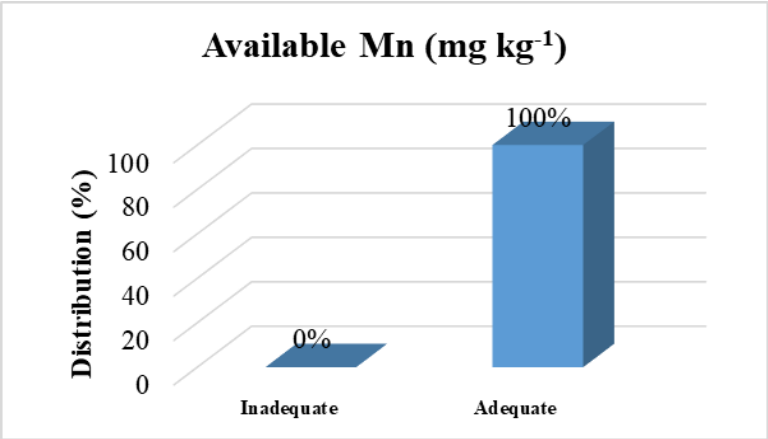


Figure 11. Available Mn distributions of soils (n= 20).

When the Cu concentrations of the soils were compared with the limit values specified by Lindsay and Norvell (1978), 100% were classified as sufficient (> 0.2 mg kg^{-1}) (Figure 12). According to these results, there is no problem in terms of available Cu content of Çağlayancerit agricultural soils. Karaduman and Çimrin (2016) stated that the available Cu amounts of agricultural soils in Gaziantep region vary between 0.14-3.15 mg kg^{-1}, the average is 1.04 mg kg^{-1}, and that 2.84% of the soils are insufficient and 97.16% are sufficient in terms of available Cu content level have been reported. It is seen that there is a positive correlation between the data in the literature and our findings.

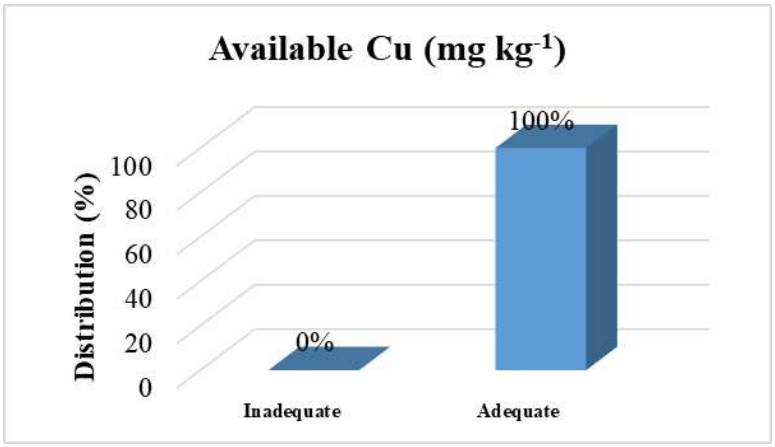


Figure 12. Available Cu distributions of soils (n= 20).

The available zinc amounts were evaluated according to Lindsay and Norvell (1978). Accordingly, it is understood from Figure 13 that 15% of Çağlayancerit agricultural land is very low (< 0.2 mg kg^{-1}), 70% low (0.2-0.7 mg kg^{-1}) and 15% also adequate levels (0.7-2.4 mg kg^{-1}). According to these results, there is a problem in terms of available Zn in 85% of Çağlayancerit agricultural lands. It is thought that the reason is due to soil lime. Eyüpoğlu et al. (1996) reported that 49.83% of Turkey's soils had insufficient available zinc content.

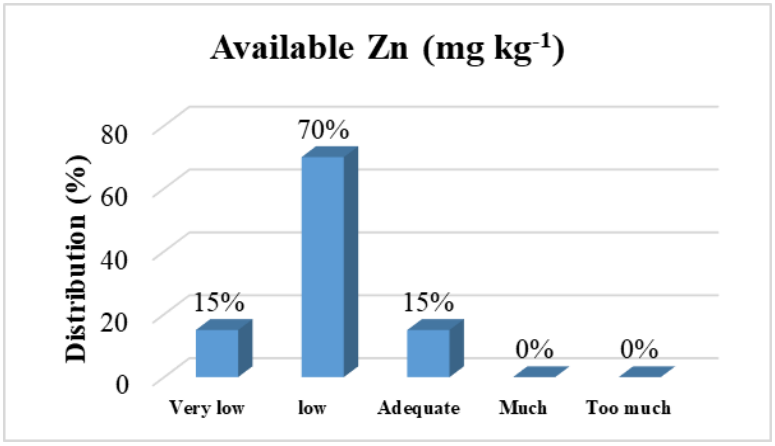


Figure 13. Available Zn distributions of soils (n= 20).

The available B contents of soils under different plants in Çağlayancerit were evaluated according to the limit values reported by Wolf (1971). The available B levels of soils distributed in the form of very low in 70% (< 0.50 mg kg^{-1}), low in 20% (0.50-0.99 mg kg^{-1}) and sufficient in 10% (1.00-2.49 mg kg^{-1}) (Figure 14). Accordingly, 90% of Çağlayancerit agricultural lands are insufficient in terms of available boron. The reason is that the soils are calcareous and the amount of organic matter is low. Alpaslan et al. (2001) reported in a study they conducted on agricultural soils in the Mediterranean Region that the available B concentrations of the soils varied between 0.01-7.14 mg kg^{-1} and that 90% of the soils contained very low and low available B. It is understood that our findings are similar to the values given in the literature.

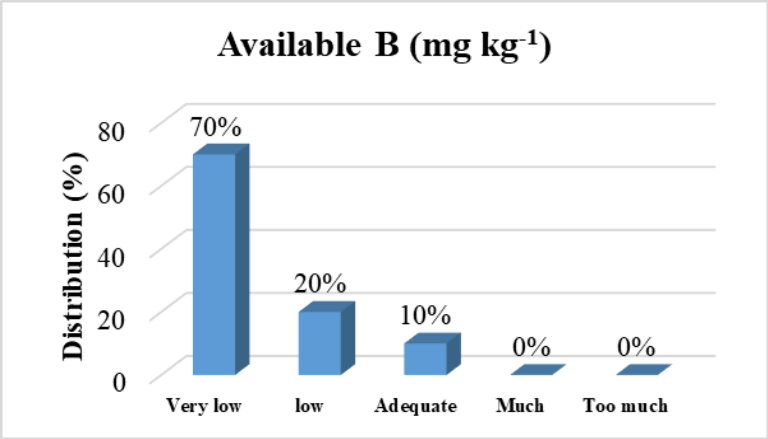


Figure 14. Available B distributions of soils (n= 20).

CONCLUSIONS

It has been determined that the soils under different plants in Çağlayancerit are mostly clay textured, neutral reaction, without salinity problem, calcareous, organic matter, available phosphorus, zinc and boron are insufficient. It was determined that the available potassium, calcium, magnesium, iron, manganese and copper contents were sufficient. It is appropriate to use acidic fertilizers such as ammonium sulfate to increase the uptake of phosphorus, zinc and boron nutrients and to reduce the effect of lime. In addition, sulfur should be applied on much and too much calcareous soils. Fertilizers containing phosphorus, zinc and boron should also be applied in appropriate amounts. In these regions, instead of stubble burning, it should be mixed with the soil, barn manure, organic fertilizers and organomineral fertilizers should be used. Although the potassium and magnesium amounts of Çağlayancerit agricultural soils are found to be sufficient in general, it will be useful to fertilize with these elements depending on the soil analysis in the areas where they are deficient.

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METABOLIC SYNDROME STUDY: AN ASSOCIATION BETWEEN DIABETES MELLITUS AND HTA DISEASE IN SHIJAK POPULATION. GLYCEMI, TRIGLYCERIDES AND HDL- CHOLESTEROL LEVELS STUDY

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ABSTRACT

The aim of the study was to determine the association between Diabetes Mellitus and HTA disease, and the risk for patients with diabetes mellitus to increase the probability for hypertension and coronary artery disease. In this study are evaluate the levels of triglycerides, glycemi and HDL- Cholesterol, the correlation between them, to explain the relation of these components and the affect that they have together for the metabolic syndrome risk, among 245 of the patients (108 male and 137 female), separated in I, II, III, IV, V, VI,VII age group, with 0 - 15 years for I age group, and 10 years difference from each other in II, III, IV, V, VI, VII age group. The metabolic syndrome was defined by the presence of these components: abdominal obesity, hight blood pressure, hypertriglyceridemia, low HDL- Cholesterol and hight fasting glucose. In the study population, patients with diabetes mellitus have 13 % prevalence for metabolic syndrome and the patients with HTA have 12 % prevalence for metabolic syndrome. Hight levels of glycemi and triglycerides increases with the age. Individs number with diabetes and HTA increases with the age. The highest value of glycemi in patients with diabetes mellitus is in the V- age group, with average value of 214 mg/ dl. For triglycerides the highest value is in the VI- age group, with average value of 165 mg/ dl. Increasing of triglycerides and glycemic levels have positive correlation with each other, for each disease - diabetes mellitus (is clearly seen in V, VI, VII age group) and HTA (is clearly seen in IV, V, VI, VII age group). The knowledge for metabolic syndrome can prevent these metabolic abnormalities in early stages and help the patients for an exact diagnosis and to follow a regular lifestyle: a balanced diet, physical activity, avoiding sedentary living and a routine medical examination.

Keywords: Metabolic Syndrome, Diabetes Mellitus, HTA, Triglycerides, Glycemi

INTRODUCTION

The metabolic syndrome (MetS) has been defined as a constellation of risk factors, including obesity, high levels of triglycerides, low levels of high-density lipoprotein cholesterol, elevated serum levels of fasting plasma insulin, and hypertension. These factors tend to cluster together, suggesting a common etiology, and places the individuals at an increased risk for type 2 diabetes mellitus (T2DM) and cardiovascular disease (Patricia Khashayar et al., 2013). Associated with a 3 fold and 2 fold increase in type 2 diabetes and cardiovascular disease, respectively it is thought to be a driver of the modern day epidemics of diabetes and cardiovascular disease and has become a major public health challenge around the world (Paul Zimmet et al., 2005). It has been estimated that 190 million people worldwide have diabetes and it is very likely that this will increase to 324 million by 2025 (Paul Zimmet et al., 2005 ; Zimmet P. et al., 2001).

MetS has gained significant importance recently due to the exponential increase in obesity worldwide. Early diagnosis is important in order to employ lifestyle and risk factor modification (Yogita Rochlani et al, 2017). The term ‘’MetS’’ dates back to at least the late 1950s but came into common usage in the late 1970s to describe various risk factors associated with diabetes, something that had been noted as early as the 1920s (Abhisheck Gupta et al., 2010). An association between hypertriglyceridemia, obesity, insulin resistance, glucose intolerance, hypertension, and coronary artery disease has been documented since the 1960s (Fereideoun Azizi et al., 2003). The prevalence of MetS varies around the world and often corresponds with the prevalence of obesity. There is a wide variation in prevalence based on age, gender, race, ethnicity, and the criteria used for diagnosis. MetS affects a fifth or more of the population of the USA and about a quarter of the population of Europe. South-east Asia has a lower prevalence of MetS but is rapidly moving towards rates similar to the western world (Yogita Rochlani et al., 2017). In 1998, a World Health Organization (WHO) consultation group outlined a provisional classification of diabetes that included a working definition of the metabolic syndrome. The guideline group also recognized CVD (cardiovascular disease) as the primary outcome of the metabolic syndrome. However, it viewed insulin resistance as a required component for diagnosis (Scott M. Grundy et al., 2004). Individuals with metabolic syndrome are at increased risk for CVD (Lakka HM et al., 2002). Hypertriglyceridemia is typically associated with reductions in HDL-C. The relationship between insulin resistance and hypertension has been established and relates to several potentially different mechanisms. (Marc-Andre Cornier et al., 2008). Metabolic syndrome (MS) has become one of the major challenges to public health worldwide due to its significant association with an increased risk of developing type 2 diabetes and cardiovascular disease among children, adolescents, and adults (DeBoer, M.D, 2019). Rapid urbanization, unhealthy diets, and increasingly sedentary lifestyles of populations globally have made obesity an emerging pandemic. This serious public health problem increases the incidence of MS accompanied by a variety of conditions, such as hypertension, hypertriglyceridemia, hypercholesterolemia, and high glucose level (Areej Alowfi et al., 2021). The power of individual components to predict the MetS is subject to tremendous variation, indicating that not all components have equal power in identifying future CVD risk (Patricia Khashayar et al., 2013). In a multivariate analysis of data from the Third National Health and Nutrition Examination Survey data, low HDL-Cholesterol, high blood pressure, and diabetes were considered the most significant predictors of CVD (C. M. Alexander et al., 2003). Metabolic syndrome increased with age but increased even more dramatically as BMI increased. The prevalence of metabolic syndrome varied by the race and ethnicity but the patterns was different for males and females (R. Bethene Ervin, Ph.D et al., 2009).

MATERIAL AND METHOD

Analysis data were obtained from the laboratory of Shijak State Hospital, Durrës, Albania, in the time period January – December 2020.

Data on the geographical position of Shijak:

Shijak municipality is located in the part of central Albania and is part of administrative district of Durres and is bordered by Xhafzotaj, Gjepalaj and Maminas municipalities. In its composition there are respectively 3 neighborhoods: 1. Popular neighborhood; 2. Erzen neighborhood; 3. Kodra neighborhood. (Wikipedia)

Figure 1. Map of Shijak municipality

For the study of metabolic syndrome, is used statistical processing with Excel program. Individuals are divided into age groups. I age group (0 – 14 years old), II age group (15- 24 years old), III age group (25- 34 years old), IV age group (35- 44 years old), V age group (45- 54 years old), VI age group (55- 64 years old) VII age group (over 65 years old). By means of calculations is found the average value of each analytical component. For the description of statistical analyzes is used graphic construction. For changing analytical values between components and for the study of dependence between them is realized the correlation method. In this study are evaluated the levels of triglycerides, glycemi and HDL- Cholesterol, the correlation between them, to explain the relation and the affect of these components, that they have together for the metabolic syndrome risk among 245 of the patients with diabetes mellitus and HTA (hypertension) disease (138 male and 137 female), for the year 2020 (January – December).
Biochemical analysis, from laboratory manuals:
Triglycerides measurements are used in the diagnosis and treatment of hyper- lipidemia. Glucose (glycemia) measurements are used in the diagnosis and treatments of disorders of carbohydrate metabolism such as diabetes mellitus, hypoglycemia and hyperglycemia. (Gasan Production s. r. l ; Human Gesellschaft fur Biochemica und mbH) .

Table 1. Normal values for triglycerides, glucose (glycemia), HDL- Cholesterol

Components	Normal Value
Triglycerides	Men (60- 165 mg/ dl) Women (40- 140 mg/ dl)
Glucose (glycemia)	70- 105 mg/ dl
HDL- Cholesterol	>35 mg/ dl

Table 2. Monoreagent Procedure ‘’ sample starter ‘’

Glucose Monoreagent

	Blank	STD (standard)	Sample
Reagent	1000_l	1000_l	1000_l
Distilled Water	10_l	-	-
Sample	-	-	10_l
Standard	-	10_l	-

For this procedure (glucose monoreagent): Mix, then incubate 10’ at 37º C. Measure the absorbance of sample and standard against the reagent blank.

Triglycerides (liquid reagent)
For this procedure (triglycerides liquid reagent) : Mix, then incubate for 5’ at 37º C. Measure the absorbance of sample and standard against the reagent blank.

Table 3. Bireagent Procedure ‘’ substrate starter ’’

	Blank	STD (standard)	Sample
Reagent R1	800 µL	800 µL	800 µl
Distilled water	10 µl	-	-
Sample	-	-	10 µl
Standard	-	10 µl	-
Mix, incubate at 37º C for 1’ and then add:			
	Blank	STD (standard)	Sample
Reagent R2	200 µl	200 µl	200 µl

RESULTS AND DISCUSSION

Graphich presentation for an individuals group with Diabetes Mellitus disease and HTA disease, to evaluate the level of glycemi, triglycerides, HDL- Cholesterol for I, II, III, IV, V, VI, VII age group.

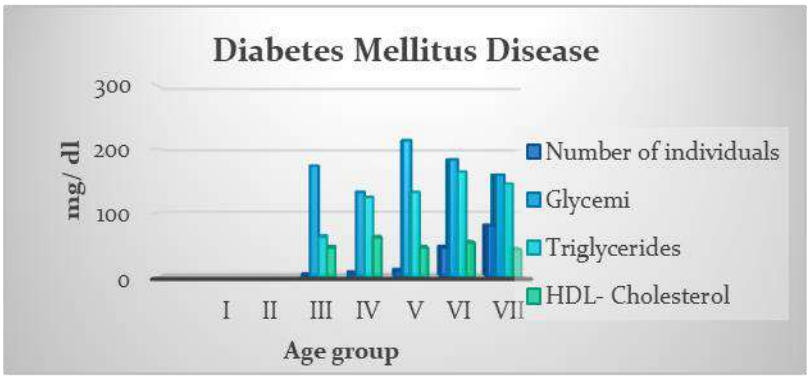

Figure 2. Individuals group with Diabetes Mellitus disease.

Table 4. Glycemi, Triglycerides, HDL- Cholesterol levels for patients with Diabetes Mellitus disease.

Age group	I	II	III	IV	V	VI	VII
Number of individuals			2	5	9	46	80
Glycemi			174	133	214	184	160
Triglycerides			63	125	133	165	146
HDL- Cholesterol			46	62	46	54	43

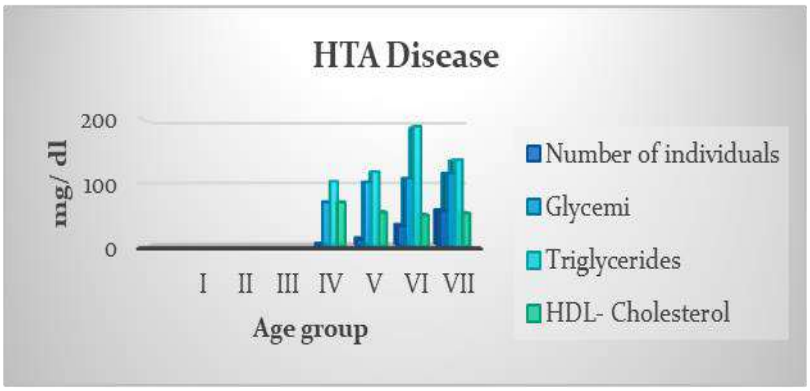

Figure 3. Individuals group with HTA disease.

Table 5. Glycemi, triglycerides, HDL- Cholesterol levels for patients with HTA Disease

Age group	I	II	III	IV	V	VI	VII
Number of individuals				2	11	33	57
Glycemi				70	102	108	116
Triglycerides				103	118	189	137
HDL- Cholesterol				70	54	50	53

Number of individuals for two graphics (for the patients with Diabetes Mellitus and for the patients with HTA Disease), increases with age. In the first and the second age group there are no individuals with Diabetes Mellitus. In the first, second and third age group there are no individuals with HTA disease. In the study population, from observations and calculations of the number of individuals with 245 patients with diabetes mellitus and HTA disease, the patients with diabetes mellitus have 13 % prevalence for metabolic syndrome and the patients with HTA have 12 % prevalence for metabolic syndrome (are patients who also have diabetes mellitus and HTA disease).

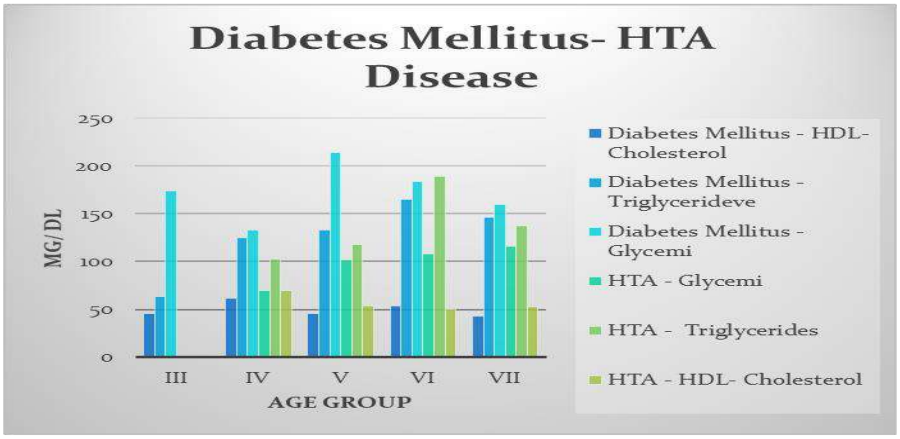

Figure 4. Individuals group analysis for glycemi, triglycerides, HDL- Cholesterol level for each age group.

The graph showes that an increase in glycemic level is associated with an increase in triglycerides level and a decrease in HDL- Cholesterol values. Higher levels of Glycemi for the patients with diabetes mellitus are in V age group, for the patients with HTA are in VII age

group. Higher levels of Triglycerides for the patient with diabetes mellitus are in VI age group, for the patient with HTA are in VI age group. The lowest levels of HDL- Cholesterol for the patient with diabetes mellitus are in VII age group, for the patients with HTA are in VI age group.

CONCLUSIONS

In the table are evaluated the correlation between analytical components.
For all components.
There is a correlation between them.

Table 6. Correlation between analylic components that are factors for Metabolic Syndrome prevalence.

Components	Correlation value
Triglycerides- Glycemi (Diabetes Mellitus Disease)	0.135
Glycemi- HDL- Cholesterol (Diabetes Mellitus Disease)	-0.03
Triglycerides- HDL-Cholesterol (Diabetes Mellitus Disease)	-0.22
Components	Correlation value
Triglycerides- Glycemi (HTA Disease)	0.01
Glycemi- HDL- Cholesterol (HTA Disease)	-0.32
Triglycerides- HDL-Cholesterol (HTA Disease)	-0.4

Glycemic and triglycerides levels, increase with age, while HDL-Cholesterol levels decrease with age.
Number of individuals with Diabetes Mellitus and HTA disease increase with age. There is a positive correlation between triglyceride and glycemic values (in the patient with Diabetes Mellitus and HTA disease).There is a negative correlation between HDL- Cholesterol and glycemic (in the patients with Diabetes Mellitus and HTA disease). There is a negative correlation between HDL- Cholesterol and triglycerides (in the patients with Diabetes Mellitus and HTA disease). Increase in glycemic values is associated with increase in triglyceride value (in the patient with Diabetes Mellitus), leading to the onest of Metabolic Syndrome. Increase in triglyceride values is associated with increase in glycemic value (in the patients with HTA disease), leading to the onest Metabolic Syndrome. Increase in triglyceride and glycemic values is associated with lower HDL- Cholesterol values. Between increasing triglyceride, glycemic values, and decrease in HDL- Cholesterol values, there is correlation. This indicates that the values increased of glycemic and triglycerides, and reduced HDL- Cholesterol values, in the patients with Diabetes Mellitus and HTA disease, are factors of Metabolic Syndrome. The importance of this study is to recognise the risk of the action of three factors together (glycemi, triglyleride, HDL- Cholesterol levels) for Metabolic Syndrome. Timely prevention and acurate diagnosis of Metabolic Syndrome. Medical examination and a regular lifestyle.

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ASSESSMENT OF RICE WASTE FOR THE PRODUCTION OF SUSTAINABLE NATURAL TEXTILE DYE AND CHEMICALS

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ABSTRACT

Wheat, sunflower (*Helianthus annuus* L.) and rice (*Oryza sativa*) are the leading products cultivated in Thrace. The use of agricultural wastes as natural dyestuff and finishing chemicals in textile production can be a good alternative for environmental protection and human health due to their high environmental compatibility, biodegradability and non-toxic nature. In this study, it was aimed to use the extracts obtained from rice husk, one of the agricultural wastes, in textile production by investigating the antimicrobial and dyeing capacities of wool yarns, and thus to bring the rice waste into economy economically. As a mordant material, instead of metal mordants, dyeing was done by using natural mordants such as chitosan, acorn (*Quercus hartwissiana*), thuja (*Thuja orientalis*) cone that will not cause environmental pollution. When the colors and K/S yield values obtained on the wool yarns were examined, it was concluded that the paddy husk extracts could color the wool yarns with a good color yield. The extracts were resistant to 6 different microorganisms (Gram (-) *Escherichia coli* ATCC 25922, Gram (+) *Staphylococcus aureus* ATCC 25923, Gram (+) *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 19115, *Salmonella typhi* ATCC 14028 bacteria and its antimicrobial activity against the yeast *Candida albicans* ATCC 10231. It has been observed that it has antimicrobial effect against *S. aureus, B. cereus* bacteria and yeast fungus *C. albicans*. It has been determined that the antimicrobial effect varies with the concentration and duration of action.

Keywords: Rice Husk, wool yarn, antimicrobial, biomaterial.

INTRODUCTION

The textile industry, which produces a wide variety of different from each other, it is a sector where many chemicals and auxiliary materials are used. Many chemicals that may pose a risk to the environment and human health are used in the coloring and functionalization of textile products. Due to the wide variety of products, a large amount of chemicals with variable properties are used in textile production. As a result, waste water containing a large amount of chemical pollution is formed (Vajnhandl ve Valh, 2014; Verma, Dash, ve Bhunia, 2012). The chemicals used in textile production do not only cause water pollution, but also spread over a wide area globally as soil and air pollution. For this reason, the size of the negative effects on the environment and human health is also large. In addition, textile waste water containing chemicals increases the heavy metal load of agricultural lands and mixes with groundwater. These heavy metals enter the body of all living things through the food chain and cause serious damage to their health in excessive amounts. Some are carcinogenic and can harm children even before birth, while others can trigger allergic reactions (Kant, 2012; Lacasse ve Baumann,

2012). Today, due to increasing consumer demands and environmental protection measures, the textile industry seeks solutions for dyeing and finishing chemicals that have chemical pollution potential. It has become imperative for fabric dyeing and finishing processes to turn into ecological production with non-toxic and sustainable methods. Accordingly, the importance of biotechnology for environmentally friendly production is increasing day by day (Roy Choudhury, 2014; Hasan, Nabi ve Mahmud, 2015).

Most natural dyes are produced from a plant source such as roots, fruit, leaves, bark and wood. Environmentally friendly and sustainable vegetable agricultural wastes can be used as an eco-solution for dyeing and finshing processes. The advantages of colorants obtained from agricultural product wastes can be listed as follows (Gilbert ve Cooke, 2001; Samanta ve Agarwal, 2009);

Does not pose a health and environmental pollution hazard

Paint extracts can be obtained by easy methods

Very high sustainability as a biomaterial for textile production

The dyeing methods of textile products are easy and applicable.

Color measurement, known as the numerical identification of the colors of textile materials or dye solutions, is an important process in the production of colored textiles. Color measurement with computer support with spectrophotometers is used to define the color of a material as depth, yield and tone, to determine dyeing recipes, and to determine color differences in dyed products (Öner, 2001). When a color is expressed in CIELAB, L defines the lightness, a indicates the red/green value, and b indicates the yellow/blue value (Pantone, 2018). Chemicals with antibacterial effect, it is used to eliminate bacterial problems. In antibacterial finishing processes, textile surfaces are treated with antibacterial chemicals.

In this study, it is aimed to use the extracts obtained from rice husk, which is one of the agricultural wastes, in textile production by investigating the antimicrobial properties and dyeing capacity of wool yarns, and thus to bring the rice waste into the economy as a biomaterial. For this purpose, the antimicrobial effects and dyeing potentials of the extracts obtained from the rice waste, which is an important agricultural product in the Thrace Region, were determined by measurements and tests.

MATERIAL AND METHOD

Rice husk (Oryza sativa): After the rice is separated from the rice plant, the rice husk remains as waste material. The husks used in the study were taken from the husk separating machine in Edirne after the rice harvest and dried and used to obtain the extract (Figure 1).

M**ordants:** Natural mordants were used. Acorn, Thuja cone, Chitosan

Acorn (Quercus hartwissiana) (Strandja acorn): It is the seed of the acorn tree. It is rich in tannins and has a hemispherical head called goblet. The goblet parts of the collected acorns (Figure 2) were separated and divided into small pieces in a muller. It is prepared for use as a mordant.

Thuja cone (Thuja orientalis): After the green colored thuja fruits are collected and dried, they are cut into small pieces in a grinder and prepared for extracting.

Chitosan: Chitosan obtained from Sigma Aldrich company was used in the study (medium molecular weight chitosan from shrimp shell, Code: 448877) (Figure 4).

Wool yarn: 4/3 Nm warp 100% wool carpet yarn is used in dyeing.

HT dyeing machine: Ataç lab-dye ht is a brand-model, steel tube, programmable dyeing machine. Since it has equivalent properties with the dyeing machines used in the industry, the dyeing processes of textile materials with plant extract were made in this machine (Figure 5).

The husks taken right after the rice harvest and the natural mordants collected from the acorns and thuja cones were dried at 21ºC in a closed environment, on the cloth, without losing their color. In the preparation of extract from husks, water and hot extraction method were used as solvents. According to the weight of the wool yarn to be dyed, 300% paddy husk, 1/40 liquor ratio in water at 100 ºC for 60 minutes. The extraction process was carried out in a thermostatic heater by magnetically stirring. It was aimed to determine the antimicrobial properties of plant extracts by testing the antimicrobial activity of extracts obtained from rice husks.

The prepared husk extracts are resistant to 6 different microorganisms (Gram (-) *E. coli* ATCC 25922, Gram (+) *S. aureus* ATCC 25923, Gram (+) *B. cereus* ATCC 11778, *L. monocytogenes* ATCC 19115, *S. typhi* ATCC 14028 bacteria and antimicrobial activity against yeast fungus *Candida albicans* ATCC 10231 was investigated by CLSI (Clinical Laboratory Standards Institute) microbroth distillation method. *Ampicillin* and *Gentamicin* in bacterial cultures and *Amphotericin* B in yeast cultures were used as antibiotic control. Antibiotics and solute stock solutions were filtered through a 0.45 µm sterile filter for sterilization. Distilled water and 1% acetic acid mixture was used as solvent.. Bacteria and yeast cultures were inoculated on each plate and incubated at 37 ºC for 24 and 48 hours. % vitality values were determined (Orlab, 2019). Antimicrobial performances were tested in the TÜTAGEM Laboratory of Trakya University.

In the pre-mordanting process with oak or thuja mordants, a solution of 1/25 liquor was prepared by using 200% mordant according to the weight of the textile material to be dyed, and this solution was added to the textile materials and mordanted at 80°C for 60 minutes. When the solution cooled down, the textile materials were removed, rinsed with distilled water and dried at 60 °C. Before dyeing, it was slightly dampened.

In the pre-mordanting process with chitosan; 10g/L chitosan solution by weight was prepared using commercial chitosan in powder form, and 1% acetic acid was added and mixed at 100°C for 30 minutes in a magnetic stirrer. After the chitosan is dissolved, the textile materials are padded with this chitosan solution, which is prepared, in a laboratory type scarf machine with 60% squeezing pressure, dried at 100°C, and dried at 110°C for 1 minute fixed and pre-mordanting was performed.

Dyeing was done in order to determine the wool yarn dyeing potential of the obtained rice husk extract. Weighed, moistened wool yarns were dyed with extracts prepared before in a steel tube dyeing machine at 80°C for 60 minutes. After dyeing, the samples were subjected to cold rinsing - warm rinsing - warm soaping (at 40 °C with non-ionic soap) - cold rinsing and dried in a tumble dryer at 60°C. Color measurements of the dyed samples were made by determining the K/S and L, a and b values using a computer color matching system. Color measurement was measured with X-RITE brand color spectrophotometer in accordance with TS EN ISO 105-J03, 2010 standard, at D65 daylight source setting, with a 10 degree viewing angle. The (K/S) values used as an indicator of color yield were calculated with the spectrophotometer software.

Figure 1. Rice husk

Figure 2. Dried acorns

Figure 3. Dried thuja cone

Figure 4. Commercial chitosan

Figure 5. HT dyeing machine

RESULTS AND DISCUSSION

Antimicrobial effect

Antimicrobial effects of rice husk extract against *E. coli*, *S. typhi*, *S. aureus*, *L. monocytogenes*, *B. cereus* bacteria and yeast fungus *C. albicans* were determined as % vitality for different concentrations at 24 and 48 hour contact times. In Figure 6 and Figure 7, it is seen that the antimicrobial effect of rice husk extracts against *S. aures*, *C. albicans* and *B. cereus* bacteria varies with the concentration and duration of action in the analyzes performed at different concentrations at 24 and 48 hours of action time. It was determined that it did not show any antimicrobial effect on bacteria other than these.

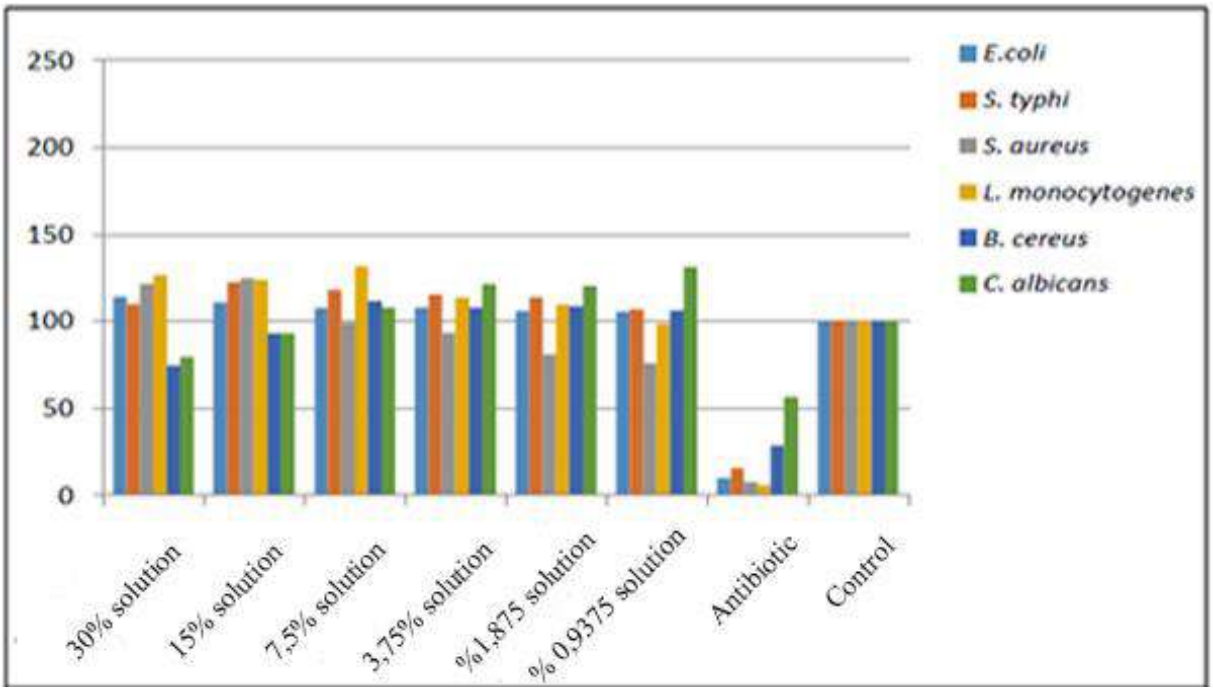

Figure 6. 24 hour % vitality values of rice husk extract

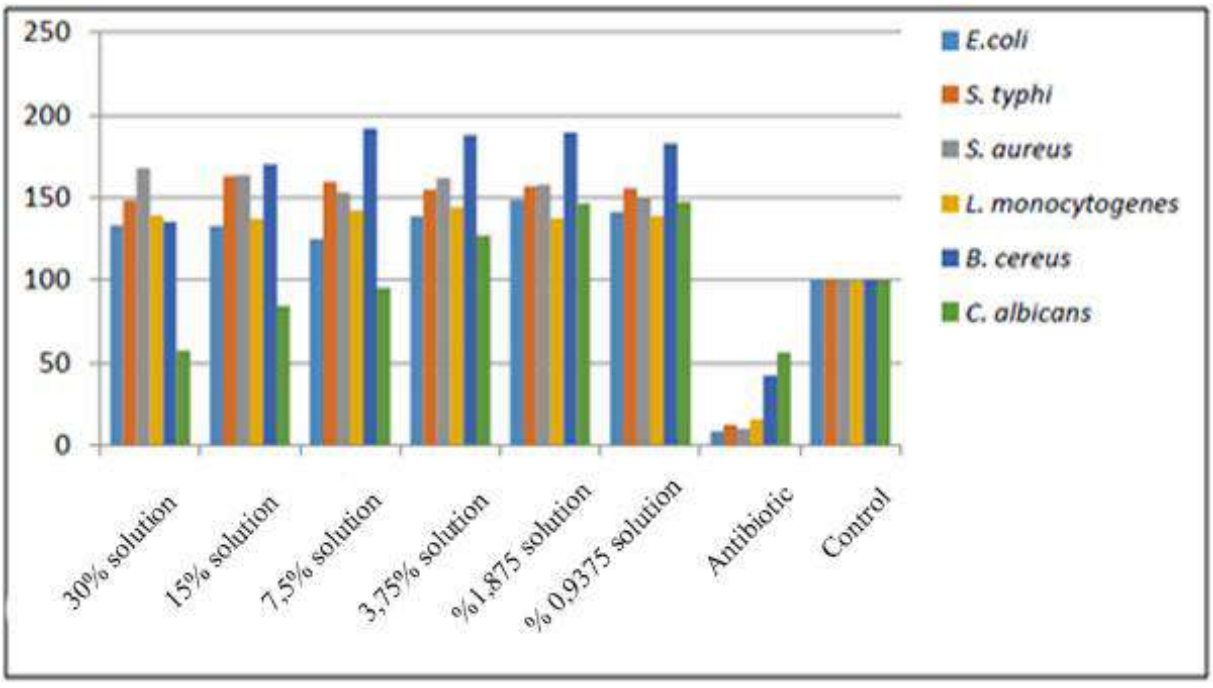

Figure 7. 48 hour % vitality values of rice husk extract

Color measurements

The colors obtained on the wool yarn dyed with the extracts were examined and compared in terms of color tone and darkness, the effect of the mordant variety on the obtained colors was determined, and the color efficiency was interpreted with the K/S values in the 400 wavelength

range. Photographs of the dyed samples were taken in daylight (Table 1) and their colors were visually evaluated and compared with the L a b values (Table 2).

Table 1. Colors obtained in textile materials with extracts

Dyestuff	Mordant	Wool yarn
Rice Husk	Without mordant	
	With chitosan mordant	
	With acorns mordant	
	With thuja cone mordant	

Table 2. L a, b results of Wool Yarn Dyeing with Extract

Dyeing Method	**L (0-100: lightest-darkest)**	**a (+red /- green) value**	**b (+ yellow/ -blue) value**
Without Mordant	68.61	2.12	16.98
With Chitosan Mordant	66.71	2.08	17.32
With Acorns Mordant	59.06	4.11	16.42
With Thuja Cone Mordant	64.00	2.95	17.58

When the a values of the wool yarns dyed with the extracts were compared in terms of green-red tones (Table 2), the green indicator -a value was not seen in the colors obtained in the dyeings, and the +a values, which is the redness indicator, were determined. It was determined that the highest red tone was with acorn mordant, and the least red tone was in dyeing with chitosan mordant.

When the b values of the wool yarns were compared in terms of yellow – blue tones, the blue indicator –b value was not found, and the yellow indicator +b values were determined in all dyeings. It was observed that the highest yellow tone was observed with the thuja mordant, and the least yellow tone was in the dyeings made with the acorn mordant.

When evaluated in terms of L value, the lightest color was obtained in dyeing without mordant, and the darkest color was obtained in dyeing with acorn mordant.

K/S values of wool yarns dyed with extracts measured at 400 nm wavelength

The K/S values of the wool yarns measured with a 400 nm wavelength spectrophotometer were examined according to the D65/10 lighting and angle mode and are shown in Table 3.

Table 3. K/S values at 400 nm wavelength of Wool Yarn Dyeing with Extract

Dyeing Method	**K/S**	**Color Efficiency**
Without Mordant	2,22	well
With Chitosan Mordant	2,25	well
With Acorns Mordant	4,01	well
With Thuja Cone Mordant	3,72	well

The highest K/S value in wool yarn dyeing with rice husk extracts was obtained with acorn mordant. It has been determined that with the rice husk extracts, wool yarns without mordant and with mordant can be colored with good color yield (Karabulut, 2015).

CONCLUSIONS

Textiles and apparel will continue to be basic necessities, so it has become important to use sustainable solutions and non-toxic dyestuffs for chemical pollution caused by textile industry production. The results clearly show that the use of traditional agricultural wastes in textile production will create an important natural resource in order for environmentally friendly textile production to be sustainable. The data obtained in the study show that rice husks, which is one of the agricultural product wastes, can be evaluated as an antimicrobial agent and a natural dyestuff in textile production.

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APPLICATION OF NUTRIENT POLLUTION INDEX AND WATER POLLUTION INDEX TO EVALUATE THE DRINKING WATER QUALITY OF THE VILLAGES LOCATED IN EDIRNE, TURKEY

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ABSTRACT

This study was carried out to evaluate the drinking water qualities of 51 villages located in Edirne Province of Turkey by using Nutrient Pollution Index (NPI) and Water Quality Index (WQI). For ths purpose, drinking water samples were collected from villages of İpsala, Keşan, Uzunköprü and Meriç Districts in autumn season of 2017. Some physical and chemical water quality parameters including electrical conductivity (EC), total dissolved solids (TDS), turbidity, nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and cyanide (CN) were determined and NPI and WQI were applied to experimental data in order to evaluate the drinking water qualities. Geographic Information System (GIS) was also used to make a visual explanation by presenting distribution maps of recorded NPI and WQI scores. According to the results of applied ecological indicators, although the intense agricultural activities carried out in the basin, it has been determined that the drinking water of the region has quite high quality and all the investigated locations have been found as having "Excellent – A Grade" water quality in terms of WQI and "No pollution" water quality in terms of NPI, in general.

Keywords: Edirne villages, Drinking water quality, Nutrient Pollution Index, Water Quality Index, Geographic Information System

INTRODUCTION

Freshwater quality has a vital importance and they are among the most significant and adversely affected components of the environment. Contamination caused by anthropogenic applications decreases the water quality day by day and decreases the usage potential of limited water resources. Therefore, monitoring water quality in especially rural lands, which are potentially under effect of an agricultural pressure, has a great importance (Çiçek et al., 2013; Köse et al., 2015; Tokatlı and Baştatlı, 2016; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021; Tokatlı, 2021).

İpsala District, which shows a natural structure consisting of wavy plains with low hills, is located in Edirne City. İpsala District with a surface area of 753 km², has an economic structure based on especially agriculture. The total population of İpsala District is about 30,000 with 10,000 in the center and 20,000 in the villages (Anonymous, 2016; http://www.edirnekulturturizm.gov.tr; http://www.ipsala.gov.tr; http://www.turkstat.gov.tr/). Keşan District with a surface area of 1,087 km² is located in the southern half of Edirne City. The county economy is predominantly agriculture and animal husbandry and the total area of the province is 108,699 hectares. The total population of Keşan District is about 80,000 with 60,000 in the center and 20,000 in the villages (Anonymous, 2016; http://www.edirnekulturturizm.gov.tr; http://www.kesan.gov.tr; http://www.turkstat.gov.tr/). Uzunköprü District is the first place among the districts of Edirne Province in terms of its water basin. The Ergene River flows through the middle of the Uzunköprü District and flows into the Meriç River. 60% of the people live in the countryside and provide their subsistence from

agriculture. The total population of Uzunköprü District is about 60,000 with 40,000 in the center and 20,000 in the villages (Anonymous, 2016; http://www.edirnekulturturizm.gov.tr; http://www.uzunkopru.gov.tr; http://www.turkstat.gov.tr/). Meriç District, which has a surface area of 448 km², is located on the south – western edge of Lalapaşa Plateau in the middle part of Edirne City. Subsistence in Meriç District is agriculture and animal husbandry in general. The total population of Meriç District is about 15,000 with 3,000 in the center and 12,000 in the villages (Anonymous, 2016; http://www.edirnekulturturizm.gov.tr; http://www.meric.gov.tr; http://www.turkstat.gov.tr/).

The aim of this research was to evaluate the drinking water quality of İpsala, Meriç, Keşan and Uzunköprü Districts by using Nutrient Pollution Index (NPI) and Water Quality Index (WQI) and present the detected data visually by using GIS based distribution maps.

MATERIAL AND METHOD

Study Area and Collection of Drinking Water Samples

Drinking water samples were collected in autumn season of 2017 from 51 villages from the drill fountains in the İpsala, Keşan, Uzunköprü and Meriç Districts. Groundwater with a volume of three wells was purged before sampling. Drinking water samples were then collected at the outflow of drill pump in polyethylene bottles. Coordinate information and locations were given in Table 1.

Table 1. Location properties of villages

Station Number	District	Locality	Coordinates		Station Number	District	Locality	Coordinates	
			North	South				North	South
S1	Keşan District	Dişbudak	40.72012	26.57460	S28	Meriç District	Adasaranlı	41.08403	26.35795
S2		Yayla	40.62822	26.38887	S29		Subaşı	41.14356	26.37485
S3		Borağı	40.70750	26.43023	S30		Meriç	41.19035	26.41944
S4		Mercan	40.74779	26.60413	S31		Umurca	41.20001	26.35920
S5		Danışment	40.61973	26.42850	S32		Alibey	41.24518	26.35476
S6		Beyköy	40.66763	26.51088	S33		Küpdere	41.22291	26.46626
S7		Orhaniye	40.73180	26.42855	S34		Akçadam	41.30541	26.53097
S8		Büyükdoğanca	40.77319	26.58404	S35		Kavaklı	41.23313	26.52256
S9		İzzetiye	40.81222	26.64535	S36		Yakupbey	41.23989	26.55337
S10		Erikli	40.64211	26.45612	S37		Paşayenice	41.19908	26.55366
S11		Karahisar	40.76458	26.50405	S38		Akıncılar	41.19265	26.51727
S12		Çeltikköy	40.68376	26.55570	S39		Küplü	41.10457	26.34976
S13		Şabanmera	40.67849	26.40786	S40	Uzunköprü District	Kırçasalih	41.39338	26.80224
S14		Akhoca	40.71820	26.40434	S41		Yeniköy	41.33851	26.76877
S15		Kılıçköy	40.78402	26.55357	S42		Değirmenci	41.31121	26.70025
S16	İpsala District	Turpçular	40.94092	26.43437	S43		Uzunköprü	41.26563	26.68482
S17		Hacıköy	40.97936	26.54988	S44		Çöpköy	41.21776	26.82452
S18		İbriktepe	41.00769	26.50721	S45		Ömerbey	41.26768	26.83598
S19		Sultanköy	41.02544	26.45104	S46		Sipahi	41.22890	26.89057
S20		Sarıcaali	40.98506	26.38257	S47		Kavacık	41.18497	26.66849
S21		İpsala	40.92147	26.38144	S48		Kurtbey	41.14359	26.57965
S22		Paşaköy	40.85033	26.32039	S49		Hamidiye	41.15265	26.66731
S23		Karpuzlu	40.83215	26.29482	S50		Türkobası	41.09383	26.60723
S24		Kocahıdır	40.81130	26.40674	S51		Altınyazı	41.07232	26.57499
S25		Aliço	40.84104	26.43971					
S26		Esetçe	40.87074	26.44392					
S27		Karaağaç	41.06235	26.53022					

Physicochemical Analysis

Measurements of electrical conductivity (EC) and total dissolved solid (TDS) parameters were performed by using Hach Portable Multi – Parameter Measurement Device (HQ40D) and turbidity parameter was performed by using Hach Portable Turbidimeter Device (2100Q) during the field studies. Nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and cyanide (CN) parameters were performed by using Hach Colorimeter Device (DR890) during the laboratory studies.

Nutrient Pollution Index (NPI)

NPI is an important technique to evaluate the drinking water quality in terms of nutrient contamination (Isiuku and Enyoh, 2020). The formula of NPI is given in the Equation (6).

$$NPI = (CN/MAC_N) + (CP/MAC_P) \quad (1)$$

$C_{N/P}$ are the levels of NO_3 and PO_4 detected in the water samples. $MAC_{N/P}$ are the maximum permissible levels of NO_3 and PO_4 specified by WHO (2011). The scale of NPI is given in Table 2.

Water Quality Index (WQI)

WQI is a widely used method to assess the drinking water quality (Wang et al., 2017; Varol, 2020; Ustaoğlu et al., 2020). The formula of WQI is given in the Equation (2) and (3).

$$WQI = \sum \left[W_I \times \left(\frac{C_i}{S_i} \right) \times 100 \right] \quad (2)$$

$$W\imath = \frac{W_i}{\Sigma W_i} \quad (3)$$

W_I is relative weight. W_i coefficients are assigned as 5 (maximum) – 1 (minimum), according to the effects of toxicants on health. C_i is the parameter level determined in water. S_i is the standard value for drinking water specified by WHO (2011) and TS266 (2005). The scale of WQI is given in Table 2 (Xiao et al., 2019).

Table 2. Water quality classes in terms of applied ecologic indices

WQI		NPI	
Value	**Water Quality Classes**	**Value**	**Water Quality Classes**
< 50	Excellent quality	< 1	No pollution
50 – 100	Good quality	1 – 3	Moderate polluted
100 – 200	Poor quality	3 – 6	Considerable polluted
200 – 300	Very Poor quality	> 6	Very high polluted
> 300	Unsuitable for drinking purpose		

RESULT AND DISCUSSION

Results of the applies Nutrient Pollution Index (NPI) and Water Quality Index (WQI) in the drinking water of İpsala, Meriç, Uzunköprü and Keşan Districts are given in Figure 1 and Figure 2.

According to the calculated NPI scores by using the parameters of nitrate (NO_3) and phosphate (PO_4) detected in the drinking waters of İpsala, Keşan, Meriç and Uzunköprü Districts, all the investigated villages have been recorded as having "No pollution" water quality.

According to the calculated WQI scores by using the parameters of electrical conductivity (EC), total dissolved solids (TDS), turbidity, nitrate (NO_3), nitrite (NO_2), phosphate (PO_4) and cyanide (CN) detected in the drinking waters of İpsala, Keşan, Meriç and Uzunköprü Districts, all the investigated villages have been recorded as having "Excellent – A Grade" water quality.

The risk order of the parameters used in the applied NPI index was determined as PO_4 > NO_3, in general, while the the risk order of the parameters used in the applied WQI index was determined as TDS > EC > CN > Turbidity > PO_4 > NO_3 > NO_2, in general.

EC is a measure of ability of water to pass an electrical current and it is affected by the presence of dissolved solids. TDS, which depends mainly on the solubility of rocks and soils, is defined as the quantity of dissolved material in water. EC and TDS variables in water are closely related and these parameters may indicate the general water characteristic. Discharges to groundwater may change the EC and TDS levels and sewage water and irrigation practices are known as significantly effective factors on these parameters (Wetzel, 2001; Manahan, 2011). The main reason of the recorded quite high risks of EC and TDS in WQI application may be irrigation practices and the filtration from septic tanks in villages.

Fertilizers used in agricultural applications increase the concentrations of nitrogen and phosphorus compounds in water and soil (Wetzel, 2001; Manahan, 2011). The main sources of nitrogen and phosphorus compounds in groundwater are known as anthropogenic activities including mainly; nitrogen – phosphate rich fertilizers, animal feedlots and municipal wastewater, sludge and septic tanks (Tokatlı, 2014). Although the research area of the present study includes a rural area and agricultural activities are carried out intensively in the region, the main reason for recorded quite low NPI scores in drinking water resources is thought to be the clayey soil structure of the region, which prevents the passage of organic pollutants from the surface to the groundwater.

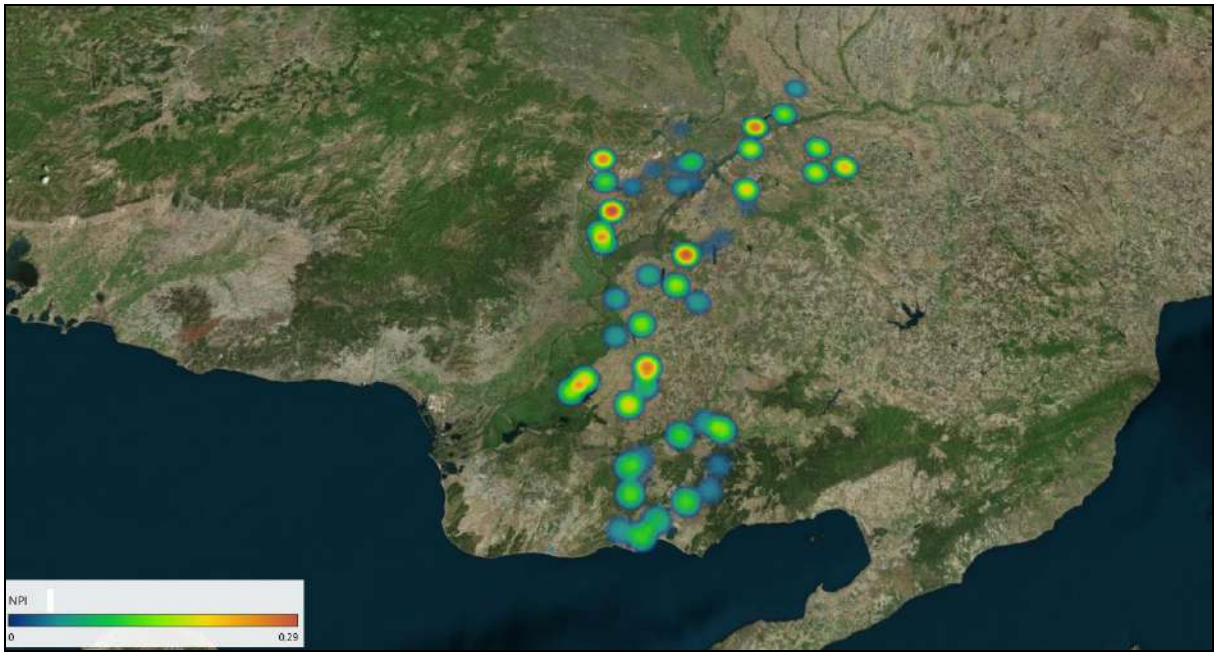

Figure 1. Distributions of recorded NPI scores

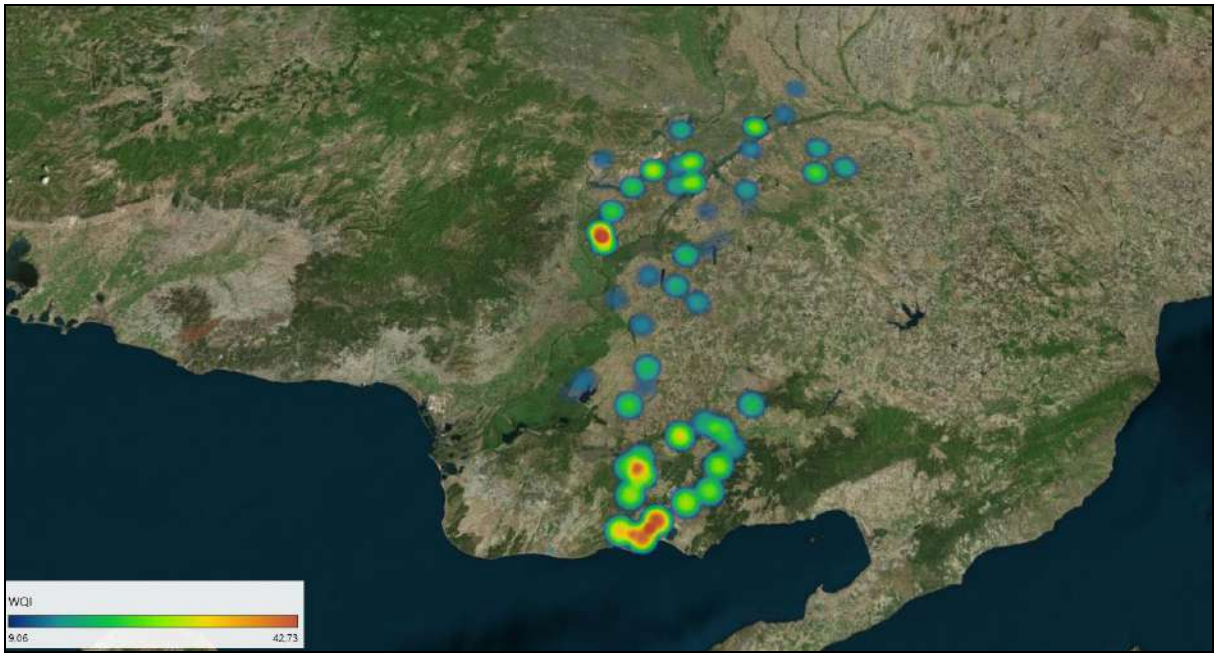

Figure 2. Distributions of recorded WQI scores

CONCLUSIONS

In this study, drinking water quality of İpsala, Meriç, Keşan and Uzunköprü Districts located in the Edirne Province of Turkey were evaluated by using 2 widely used drinking water quality assessment indices including Nutrient Pollution Index (NPI) and Water Quality Index (WQI). Also Geographic Information System (GIS) was applied to detected data in order to evaluate the detected index scores by providing distribution maps. According to the results of applied ecological indicators, drinking waters of the villages located in the İpsala, Keşan, Meriç and Uzunköprü Districts have "Excellent – A Grade" water quality in terms of applied WQI, while they have "No pollution" water quality in terms of applied NPI. The data of the current investigation has also demonstrated the benefits and usability of ecological indicators such as WQI and NPI in drinking water quality assessment studies.

ACKNOWLEDGEMENT

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GROUNDWATER QUALITY ASSESSMENT FOR IRRIGATION PURPOSES IN THE VILLAGES OF A SIGNIFICANT AGRICULTURAL AND INDUSTRIAL REGION IN TURKEY

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ABSTRACT

Meriç – Ergene River Basin is the most significant lotic habitat and the main freshwater resource of Thrace Region. Despite its grate importance for the region in terms of especially irrigation purposes, it is also known as one of the most polluted watersheds in Turkey. The aim of this study was to assess the groundwater quality of Meriç – Ergene River Basin in terms of irrigation water supply. For this purpose, groundwater samples were collected from 30 selected villages located in the watershed in summer season of 2018 and Na, K, Mg and Ca levels were determined by using an ICP-MS. Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI) were applied to detected data in order to evaluate the groundwater quality in terms of irrigation. According to the results of this research, groundwater quality of Meriç – Ergene River Basin was found as in quite low level in terms of use in irrigation purposes, in general. As a result of applied irrigation water quality assessment indices, 6.67% of total locations were recorded as "Not applicable" in terms of SAR; 36.67% of total locations were recorded as "Not applicable" in terms of Na%; 10% of total locations were recorded as "Not suitable" in terms of MR; and 60% of total locations were recorded as "Not suitable" in terms of KI.

Keywords: Meriç – Ergene River Basin, Groundwater, Irrigation Water Quality

INTRODUCTION

It is known that only about 3% of water is fresh and suitable for human consumption and about 30% of this freshwater is located in underground. Groundwater resources are the most important sources of drinking and irrigation water supply for many villages. But numbers of organic and inorganic pollutants sourced from anthropogenic activities have been identified as strong contaminants found in both surface and groundwater (Gupta, 1997; Hudak, 1999; Çiçek et al., 2013; Özer and Köklü, 2019; Ustaoğlu and Tepe; 2019; Köse et al., 2020; Varol and Balcı, 2020; Tokatlı et al., 2021). Therefore, monitoring and assessment of groundwater quality have a critical importance both for human and ecosystem health in especially industrial and agricultural zones like Thrace Region.

Meriç – Ergene River Basin is one of the most important river basins of Turkey and known to be exposed to serious pollution due to rapid urbanization and industrialization and intensive agricultural applications. Ergene River is the main branch of Meriç River and domestic wastes, pollution caused by organized industrial sites, especially sodium and salt containing agricultural derange water pollution and slaughterhouse waste were reported as the major sources of pollution in the Ergene River (Tokatlı and Başlatlı, 2016; Tokatlı and Ustaoğlu, 2020; Tokatlı, 2021; Tokatlı and Varol, 2021).

The aim of this research was to determine the sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca) accumulations in groundwater of Meriç – Ergene River Basin and evaluate the water quality in terms of irrigation water supply by using Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI).

MATERIALS AND METHODS

Study Area and Collection of Samples

Groundwater samples were collected in summer season of 2018 from 30 stations from the drill fountains of the villages located in the Meriç – Ergene River Basin. Groundwater samples were then collected at the outflow of drill pump in polyethylene bottles. Station codes and localities of selected stations are given in Table 1. The map of Meriç – Ergene River Basin and the selected stations are given in Figure 1.

Table 1. Location properties of selected stations

Station Code	Location	Station Code	Location	Station Code	Location
S1	Muratlı	**S11**	Müsellim	**S21**	Danişment
S2	Sarılar	**S12**	Düğüncübaşı	**S22**	Çöpköy
S3	Çorlu	**S13**	Lüleburgaz	**S23**	Bayramlı
S4	Velimeşe	**S14**	Babaeski	**S24**	Uzunköprü
S5	Çerkezköy	**S15**	Alpullu	**S25**	Salarlı
S6	Saray	**S16**	Karakavak	**S26**	Kurtbey
S7	Karlı	**S17**	Kadriye	**S27**	Yenicegörece
S8	Marmaracık	**S18**	Çerkezmüsellim	**S28**	Meriç
S9	Vakıflar	**S19**	Hayrabolu	**S29**	Adasarhanlı
S10	Karamusul	**S20**	Pehlivanköy	**S30**	İpsala

Element Analysis

For determination of Na, K, Mg and Ca concentrations in groundwater, water samples of one liter were adjusted to pH 2 by adding 2 ml of HNO_3 into each. Afterwards, all the samples were filtered (cellulose nitrate, 0.45 µm) in such a way as to make their volumes to 50 ml with ultra-pure water. The element levels in water samples were determined by using the "Agilent 7700 xx" branded Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) device in Trakya University Technology Research and Development Application and Research Center (TÜTAGEM). The center has an international accreditation certificate within the scope of TS EN / ISO IEC 17025 issued by TÜRKAK (representative of the World Accreditation Authority in Turkey). The element analyses were recorded as means triplicate measurements (APHA, 1992; EPA, 2001).

Irrigation Water Quality Assessment Indices

The formulas of the applied irrigation water quality evaluation indices re given below and the evaluation scales of the applied indices are given in Table 2.

$$SAR = \frac{Na}{\sqrt{\frac{(Ca+Mg)}{2}}} \quad (1)$$

$$\% = \left(\frac{Na+K}{Na+K+Mg+Ca}\right) * 100 \quad (2)$$

$$MR = \left(\frac{Mg}{Mg+Ca}\right) * 100 \quad (3)$$

$$KI = \frac{Na}{Mg + Ca} \quad (4)$$

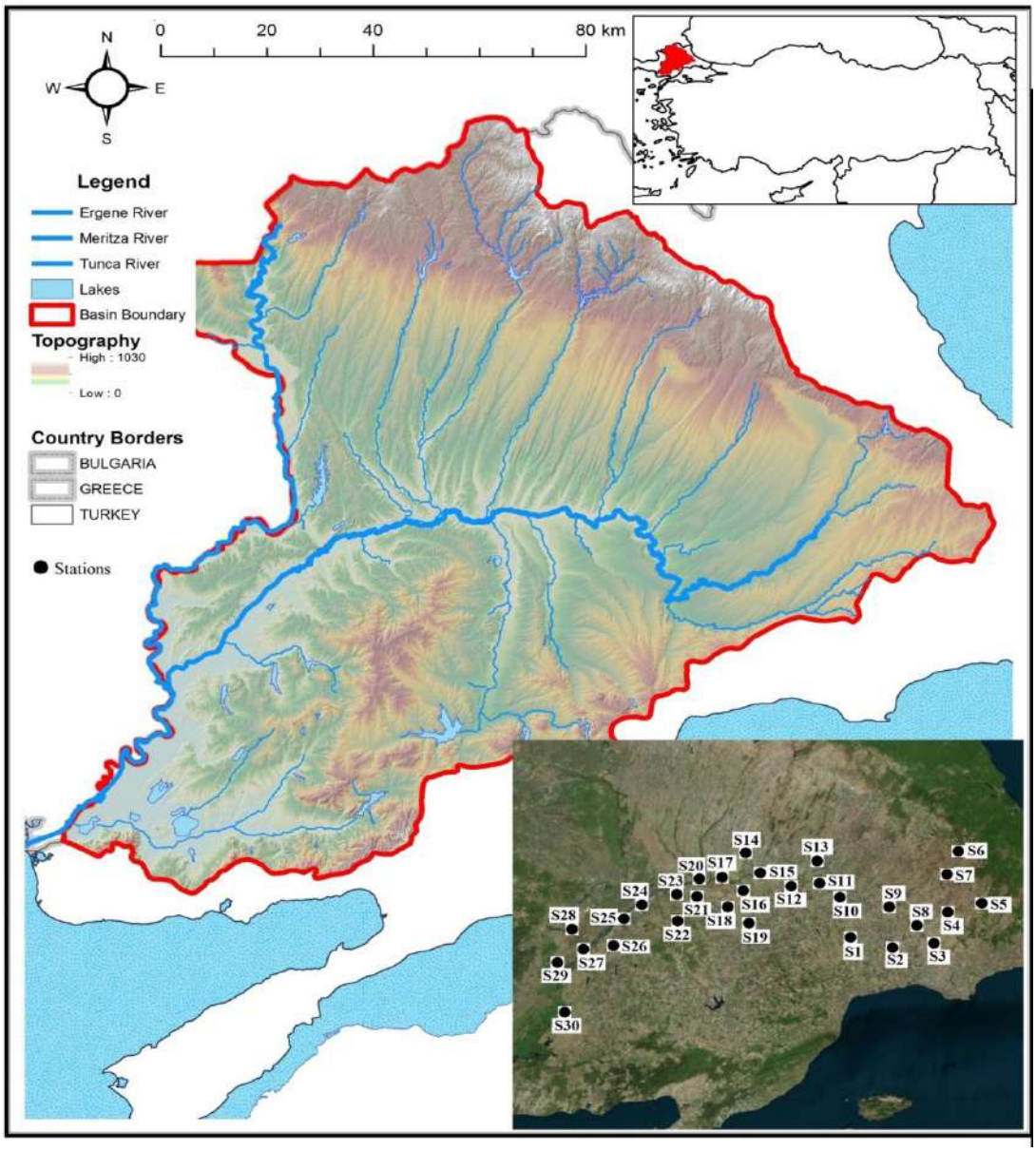

Figure 1. Meriç – Ergene River Basin and selected stations

Table 2. Irrigation water evaluation indices

Name of Index	Evaluation Scale	Name of Index	Evaluation Scale
Sodium Adsorption Rate (Richards, 1954)	< 20 Excellent 20 – 40 Good 40 – 60 Permissible 60 – 80 Doubtful > 80 Not applicable	Magnesium Rate (Raghunath, 1987)	< 50 Suitable > 50 Not suitable
Sodium Percentage (Wilcox, 1955)	< 20 Excellent 20 – 40 Good 40 – 60 Permissible 60 – 80 Doubtful > 80 Not applicable	Kelly Index (Kelly, 1963)	< 1 Suitable > 1 Not suitable

RESULTS AND DISCUSSION

In this research, Sodium Adsorption Rate (SAR), Sodium Percentage (Na%), Magnesium Rate (MR) and Kelly Index (KI), which are among of the most widely used irrigation water quality assessment tools, are applied to detected data in order to evaluate the groundwater quality of Meriç – Ergene River Basin in terms of irrigation water supply. The results of applied irrigation water quality assessment indices are given in Figure 2.

As a result of applied SAR, 60% of total locations were found as "Excellent (<20)", 16.67% of total locations were found as "Good (20-40)", 13.33% of total locations were found as "Permissible (40-60)", 3.33% of total locations were found as "Doubtful (60-80)" and 6.67% of total locations were found as "Not applicable (>80)". As a result of applied Na%, 0% of total locations were found as "Excellent (<20)", 23.33% of total locations were found as "Good (20-40)", 26.67% of total locations were found as "Permissible (40-60)", 13.33% of total locations were found as "Doubtful (60-80)" and 36.67% of total locations were found as "Not applicable (>80)". As a result of applied MR, 90% of total locations were found as "Suitable (<50)", while 10% of total locations were found as "Not suitable (>50)". As a result of applied KI, 40% of total locations were found as "Suitable (<1)", while 60% of total locations were found as "Not suitable (>1)".

In addition to the intensive agricultural applications, Thrace Region is also known as a significant industry zone. There are numbers of industrial enterprises on the watershed mainly in the Çorlu, Çerkezköy, Muratlı and Lüleburgaz districts. Therefore, Meriç – Ergene River Basin is being exposed to an intensive agricultural and industrial pressure (Tokatlı and Baştatlı, 2016; Tokatlı and Ustaoğlu, 2020; Tokatlı, 2021; Tokatlı and Varol, 2021). As a result of applied macro elements based indices, groundwater quality of Meriç – Ergene River Basin in terms of irrigation water supply was found as quite risky, in general.

CONCLUSIONS

In the present investigation, groundwater quality of Meriç – Ergene River Basin was evaluated in terms of irrigation water supply by using SAR, Na%, MR and KI. As a result of applied macro elements based indices, groundwater of Meriç – Ergene River Basin was found as in quite low quality and use of groundwater resources of the villages in terms of irrigation water supply was found as quite risky, in general. The data of this investigation also reflects the importance, applicability and necessity of the use of different irrigation water quality assessment indices together on evaluation of groundwater resources.

ACKNOWLEDGEMENT

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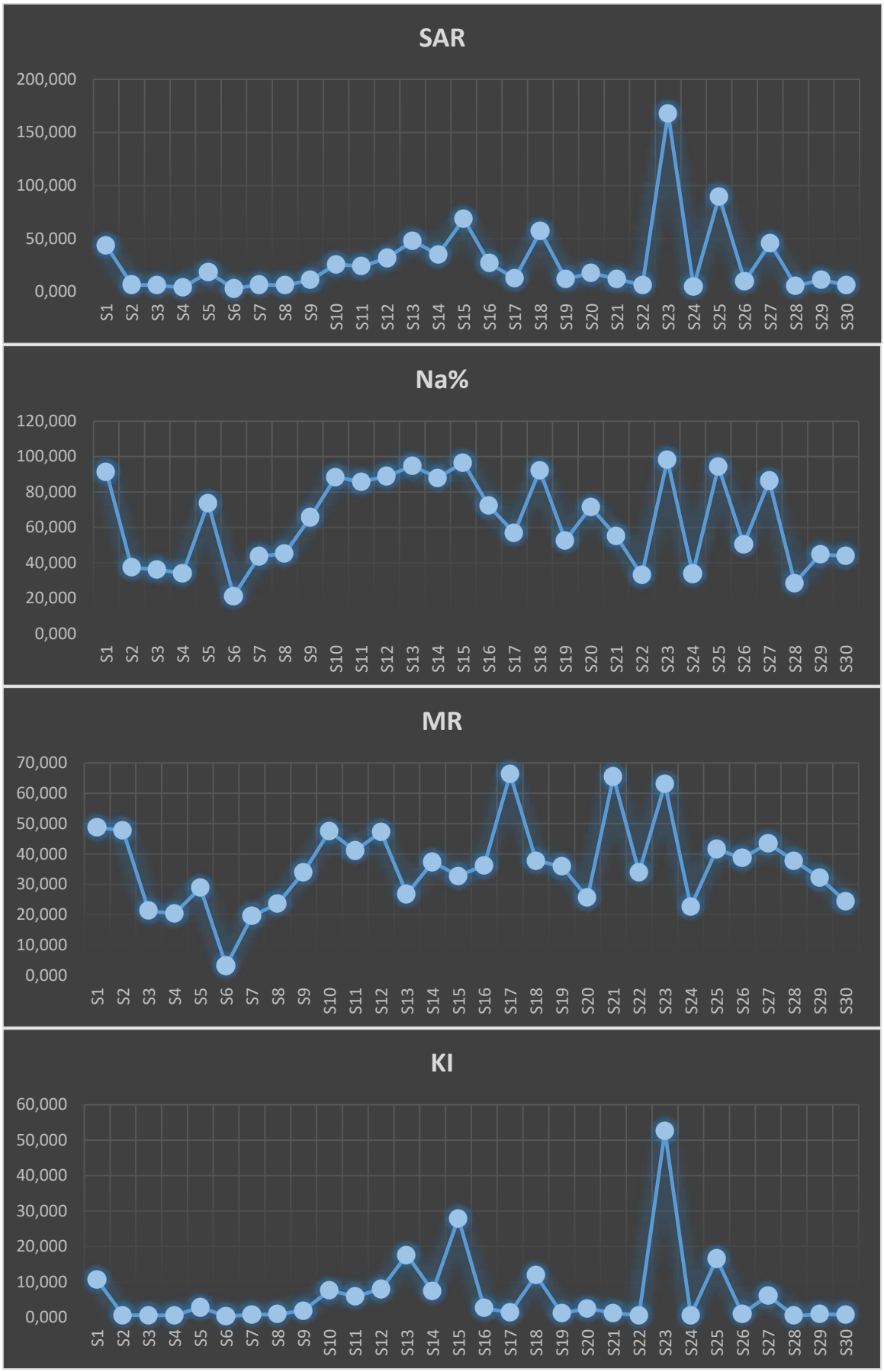

Figure 2. SAR, Na%, MR and KI scores of investigated villages

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A FORTHCOMING THREAT FOR WINTER WHEAT: FERAL RYE (Secale cereale L.)

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ABSTRACT

Feral rye (*Secale cereale* L.) is an annual grassy weed species which is a native weed to Anatolia and is commonly found in wheat fields. It has a growth ability faster than wheat because it is more tolerant to adverse climatic and environmental conditions. Therefore, feral rye competes for nutrient, water, and space with wheat from the beginning of growth to the harvest. In addition to its direct impact, feral rye seed is generally harvested during wheat harvest, reduces value of wheat seed, and may carry the ergot which is a fungus cause an important human disease, ergotism, by its ergotoxine and alkaloids. A roadside survey was conducted along Ankara-Bilecik state road to determine the presence and abundance of feral rye in wheat fields adjoining to the road in 2020 and 2021. As a result of the survey, feral rye was found nearly in one-third of Eskişehir and Ankara's wheat field, and quarter of Bilecik's. It was also found that feral rye infestations in wheat were two common kinds; scattered and patch. The abundance of feral rye in Ankara, Eskişehir, and Bilecik were changed in 0.23-8.79, 0.32-10.35 and, 0,12-14.63 plant m^{-2}, respectively. A preliminary study was also conducted in Gölbaşı, Ankara and Bozüyük, Bilecik to determine yield losses in wheat due to natural feral rye infestation. Grain yield loss in scattered field was 18.47% in Gölbaşı whereas it was changed 4.23 to 13.72% in Bozüyük. The grain loss in patches reached to 62.81%, depending on the feral rye number in the patch. These results indicated that feral rye is a significant weed species in Ankara, Eskişehir and Bilecik Provinces, and its impact on the wheat yield will increase in the near future.

Keywords: Sunflower, Sustainable production, Drought tolerance, Hybrid, Yield traits, Yield performance,

INTRODUCTION

Feral rye is, as native weed species, commonly found in many crop fields of Turkey (Zohary, 1971). Since Anatolia is one of its gene centres, soil bank is heavily contaminated with its seeds in Turkey. Feral rye has an extraordinary survive ability when exposure to hard climatic conditions, even if the winter temperature decline very low level (White et al., 2006). At the early growth stages of winter wheat, its competition may aggressively than other weed species because it has a similar growth habit to the crop and complete its root growth before the crop (White et al., 2006).

Wheat is the most important grain crop in terms of the production area and yield because it is grown nearly all the regions of Turkey (TUİK, 2021). Even if wheat may adopt the adverse environmental conditions of Turkey, some pests, diseases and weeds result in grain yield loss.

Feral rye is the main host of ergot (*Claviceps purpurea* (Fr.) Tul.). Weeds compete with the wheat plants because of water, nutrient, space, and sunlight, especially at the early stages of the growth. The grain loss may reach 92% (Cobel and Fay, 1985; Westra and D'Amato, 1989; GRDC, 2021). depending on the weed species. Economical loss in winter wheat is not only limited with weed-crop competition. Actually, seed contamination during harvest of winter wheat may result in more economic loss than competition since the seeds of feral rye are harvested at the same time (White et al., 2006). Previous studies indicated that the seeds of feral rye have contaminated the wheat grain during the harvest (Özkil and Kara, 2006; Tepe, 1998; Karaca and Güncan, 2009).

The study was aimed to determine the presence and abundance of feral rye in wheat fields adjoining to the road in 2020 and 2021, and to determine its economic impact on the winter wheat yield in Ankara and Bilecik Provinces.

MATERIAL AND METHOD

A roadside survey was planned along the Ankara-Bilecik state road (285 km) to determine the presence and abundance of feral rye in wheat fields adjoining to the road (Figure 1). The road is one of the important transportation routes connecting the Central Anatolia Region to the South Marmara Region. The fields near the road have mainly been covered by winter wheat, sunflower, and maize. The route has the Continental or the Mediterranean climate conditions with 11-12°C average temperature and 372-569 mm rainfall. The surveys were conducted in 58 sampling points in April and August 2020 and 2021. The interval between the sampling points over the road was at least 5 km. The records of presence or absence and density per square metre belonging to feral rye were taken from 8 points in each visited field.

Figure 1. Survey fields were indicated by black-red line

Economic impact of feral rye on winter wheat was investigated with a preliminary field trial conducted in Gölbaşı, Ankara and Bozüyük, Bilecik. Two winter wheat fields naturally infested by feral rye were selected for the observations, and their edges were determined by red plastic strips. Additionally, the plots in the same field that had not contained feral rye were also included in the trials. At the harvest, the 3 quadrants, each of them was one square meter, were manually harvested from 15 cm above the soil surface, and the wheat samples were put in the paper bags. The samples were transported to the laboratory and waited in the shade for 2 days. Then the grain was manually separated from the hay. The wheat grain was weighted after the cleaning stage and the data were recorded.

RESULTS AND DISCUSSION

Feral rye distribution

The road consisted of the three main parts: Ankara (90 km), Eskişehir (150) and Bilecik (45 km). The winter wheat field surveyed during the journey was 18, 29, and 11, respectively. Feral rye was found in 7 winter fields in Ankara, and the least density was 0.23 plant m^{-2} while the highest was 8.79 plant m^{-2}. The weed density varied 0.32 plant m^{-2} to 10.35 plant m^{-2} in 10 winter wheat fields of Eskişehir. In Bilecik, three winter field was only infested by feral rye, but a field in Poyra village had the highest feral rye abundance in the survey.

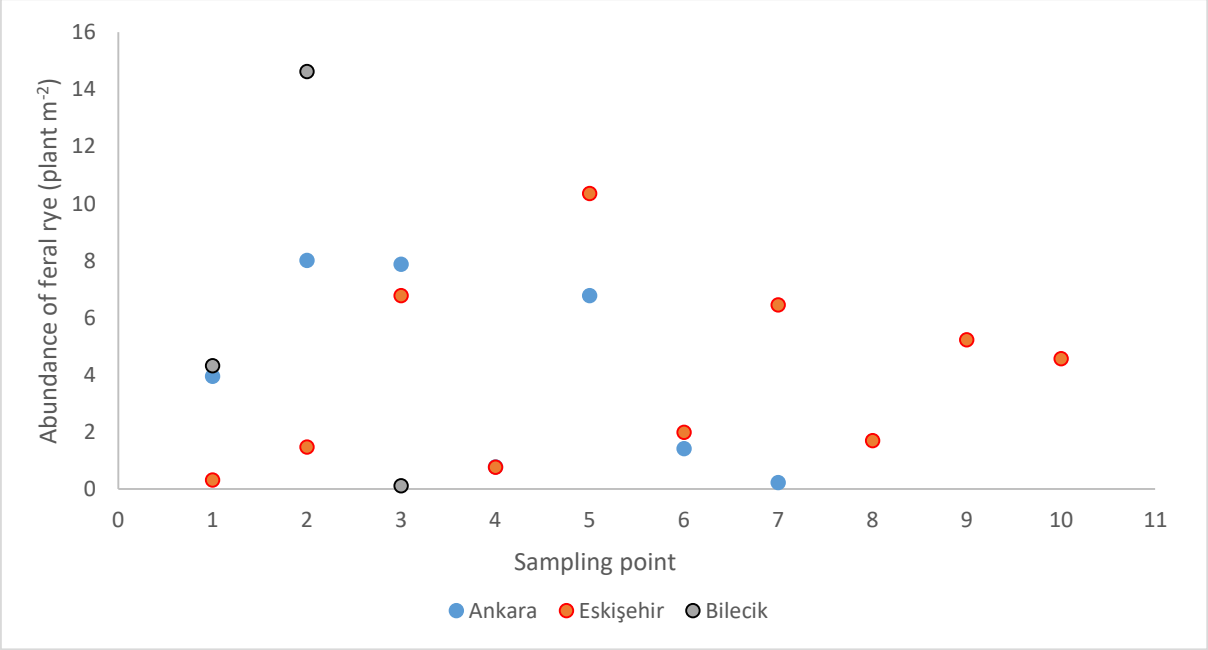

Figure 2. Presence and abundance of feral rye in winter wheat fields in Ankara, Eskişehir, and Bilecik.

Feral rye has completed its growth stage before winter wheat in all fields visited (Figure 3). The spikes of feral rye turned down while the spikes of winter wheat stood upright. Some of the seeds in the spikes of feral rye easily separated from the spike and included in the soil seed bank via wind or human activities.

Figure 3. Feral rye in winter wheat

Economic losses

A preliminary study was conducted in three winter wheat fields were naturally infested by feral rye, Gölbaşı, Ankara and Bozüyük, Bilecik (2 fields) to determine yield losses caused by the weed. It was observed that feral rye scattered in nearly all parts of the winter wheat field in Gölbaşı, Ankara (Figure 4). The average yield declined in the quadrants was 18.47% compared to the quadrants non-infested by feral rye. In the second field, Bozüyük, Bilecik (Kandilli village), two kinds of feral rye distribution were observed in winter wheat. The first was scattered distribution which was found in nearly all parts of the winter wheat field similar to Gölbaşı, Ankara. The average yield loss was 13.72% in the quadrants. The second distribution was the patch (Figure 5). The yield loss in the patches reached to 62.81%, depending on the feral rye number in the patch.

Figure 4. Scattered distribution of feral rye in winter wheat

Figure 5. Scattered distribution of feral rye in winter wheat

These results indicated that feral rye is a significant weed species in Ankara, Eskişehir and Bilecik Provinces similar to other countries in the world. There has not been registered herbicide to control feral rye in Turkey; therefore, the wheat-growers have no solution for it, except cultural precautions. Additionally, no official cultural precaution has been declared for

Turkish growers, yet. The impact of feral rye on the wheat yield will increase day by day because global climate change has changed the climatic conditions in favour of feral rye.

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SANITARY DIAGNOSIS OF ALEPPO PINE TREES USING THE ARCHI METHOD IN CHETTABA FOREST (ALGERIA)

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ABSTRACT

The study presented aims to establish the state of the forest of Chettaba by a diagnosis on 4 forest plots. A first assessment of the health of the crowns is carried out by the ARCHI method, this method is based on a morphological analysis of all the aerial part from the observation. The main objective of this work is to know the health status of the Chettaba forest massif (located in the North-East of Algeria). Knowledge of the architecture of trees of a large number of species has enabled the transposition of these notions to forestry technicians and has led to the implementation of observation protocols to establish a diagnosis of the architecture giving indications on the level of stress suffered by the tree, to establish its reactivity and therefore to propose a prediction of the evolution of its architecture. The keys integrate three series of observations: the sequential structure established during growth, which provides information on the development stage of the tree, the symptoms of degradation, the architecture of the tree crown (mortality, depletion of the branching) and finally the processes of restoration of the crown resulting essentially from the development of epicormic twigs. The results obtained indicate that the two dominant ARCHI types are ARCHI I and ARCHI S. The nominal values were observed in the ARCHI I and ARCHI S types, then the minimum values are recorded in the ARCHI R and ARCHI D types. The highest value is recorded to ARCHI I and ARCHI S with percentages of 42.12 and 41.04% respectively while the healthy ARCHI type is low with the value of 23.76%. These results contribute to the improvement of knowledge on current condition indicators of natural *Pinus halpensis* stands that can be used as a basis in the management of Chettaba forest. The diagnostic results show that the majority of trees are subject to climatic, pedological and anthropogenic stresses and their health status is quite low.

Keywords: Tree diagnosis, Aleppo pine, ARCHI, decline.

INTRODUCTION

The pines of the "*halepensis*" group represent a major forestry capital on the Mediterranean rim. According to Le Houërou (1980), they occupy about 6.8 million hectares. In Algeria, Aleppo pine is very frequent in all the mountainous massifs, from the coastal Tell to the Saharan Atlas (Kadik 1983), and although it has often been badly treated by man, there are still vast stands in Oranie (Bel Abbes, Saida, Ouarsenis regions), in Algérois (Medea-Boghar, Monts de Bibans, Monts des Ouled Nail), and in Constantinois (Aurès, Tébessa region especially). The wood of the pine is used in construction, industry, carpentry, wood and paper pulp, for hand shoring, shipbuilding and carpentry (Maestre and Cortina, 2004). Pine is also used in cosmetics due to its richness in fatty acids, vitamin E, polyphenols and natural antioxidants. Pine seeds are used in the food industry (pastry) (Cheikh - Rouhou et al., 2006).

To account for this dynamic, the Institute for Forestry Development has developed a diagnostic tool called: the ARCHI method. "ARCHI" is a diminutive of "Architecture", because the method is based on a reading of the architecture of trees. The principle is to carry out two series of observations: the first concerns the symptoms of degradation of the crown (leaf deficit, abnormal coloration, mortality...). The second concerns the processes of restoration of the crown (development of gourmands, covering of wounds, and resumption of growth...). The study of the balance of power between these agonistic processes of degradation and restoration allows us to make a diagnosis of the tree. For simplicity, the number of possible outcomes was limited to five: the healthy tree, the stressed tree, the resilient tree, the tree in crown descent and the tree in irreversible decline (Drénou, 2013). The objective of this study is to assess the health status of Aleppo pine stands in the forest of Chettaba (Algeria).

MATERIAL AND METHOD

Presentation of the study area

Forest of Chettaba is located southwest of Constantine (Algeria). The estimate terrain elevation above sea level is 865 meters. The study area is located on the map topographic Constantine Scale 1/200 000 sheet N° 17 and located between the coordinates 36°19'4" north latitude and 6°28'36" East longitude. The forest of Chettaba spreads over an area of 2398 ha and 94a, and is perfectly limited and divided into six districts. Extreme altitudes of the forest is about 1104 m (maximum altitude) and 652 m (minimum altitude), corresponding to each of them respectively following map coordinates: (x = 839, y = 344), (x '= 839.9, y' = 340.3). Its bioclimatic is semi-arid to sub-humid. The average annual rainfall is estimated between 670 and 800 mm and the mean annual temperature of the region is 18°C, with an average of the warmest month above 35°C and the coldest month varies between 1.25 and 3.05°C. A large plant grouping as the forest of Chettaba can be studied in its entirety, especially when it concerns hundreds of acres to be treated in the detail (Fig. 1).

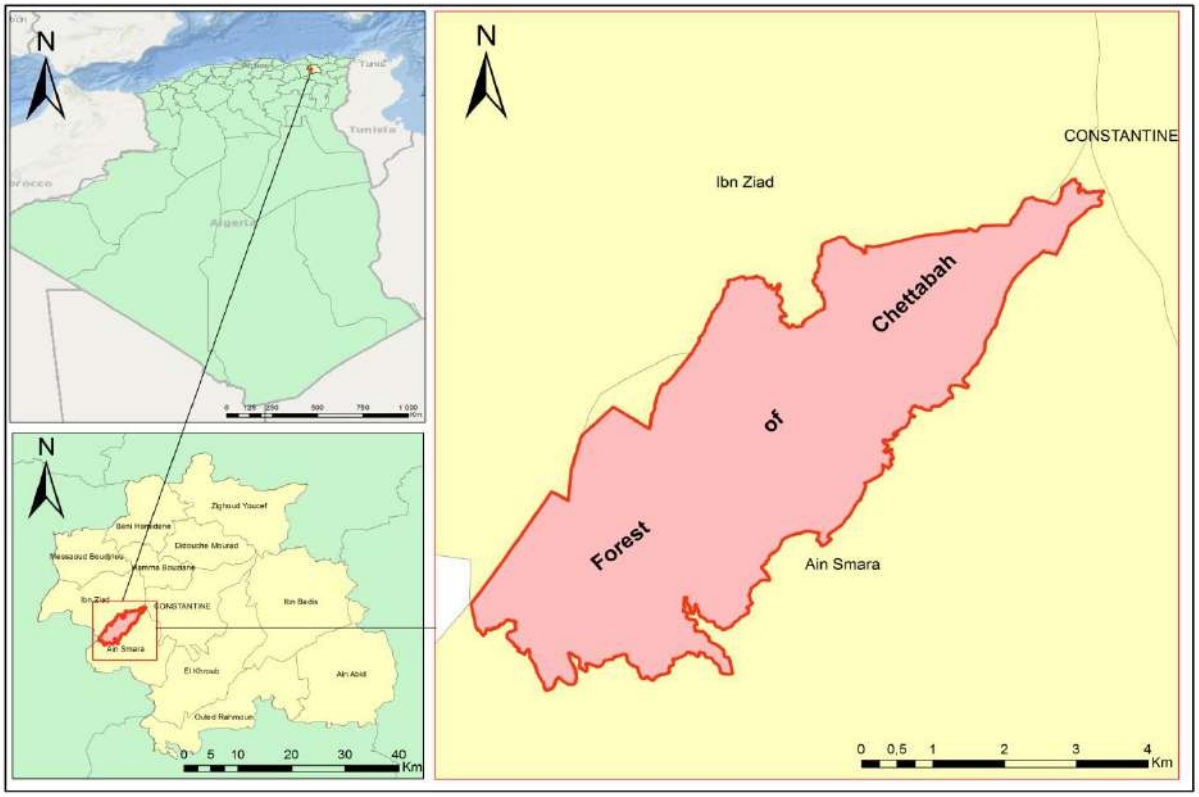

Figure 1. The map of study area.

Tree Architecture Method (ARCHI)

To characterize the health status of our stands, the institute for forest development has developed a diagnostic tool called the ARCHI method, which is based on a reading of tree architecture (Drénou et al., 2013; Eichhorn et al., 2016). The principle is to carry out two series of observations: the first concerns the symptoms of crown degradation (leaf deficit, abnormal coloring, mortality, etc.), and the second concerns crown restoration processes (development of greedy, wound recovery, growth recovery, etc.). The study of the balance of power between these antagonistic degradation-restoration processes enables a diagnosis to be made on the tree.

RESULTS AND DISCUSSION

Knowledge of the architecture of trees of a large number of species has enabled the transposition of these notions to forestry technicians and has led to the implementation of observation protocols to establish a diagnosis of the architecture giving indications on the level of stress suffered by the tree, to establish its reactivity and therefore to propose a prediction of the evolution of its architecture (Drénou, 2014; Drénou and Caraglio, 2019). The ARCHI diagnostic method was developed in order to provide managers with the simplest possible decision-making tool enabling them to carry out a health analysis of the stands and to extract information on the interventions to be recommended (Drénou et al., 2011; 2012).

The study presented aims to establish the vitality status of the Sidi R'Ghies forest massif using a diagnosis on 8 plots. A first assessment of the crown health status is carried out using the ARCHI method, which is based on a morphological analysis of the entire aerial part based on observation. The keys integrate three series of observations: the sequential structure set up during growth, which provides information on the tree's development stage, symptoms of

degradation, tree crown architecture (mortality, branching impoverishment), and finally the crown restoration processes resulting mainly from the development of epicormic twigs (Eichhorn et al., 2010; Drénou et al., 2013; Rached-Kanouni et al., 2020).

The results obtained from the field surveys were limited to 4 plots and are shown in Table 1. Through the results of table 1 concerning the Aleppo pine, the nominal values are observed in the observed in the ARCHI S type (42.12%), the ARCHI I type (41.04) and the ARCHI H type (23.76). For ARCHI I type, the highest value is recorded in plots 1 and 2 (24 and 10 in order). The maximum value of ARCHI S type is obtained in plot 1 and 2 as well. The maximum value of type ARCHI H is obtained in plot 3 and then the minimum values are around zero trees in plot 2 and 4. The smallest values are noticed for the ARCHI D, ARCHI R and ARCHI M types; they are null for almost all the plots (Fig. 2).

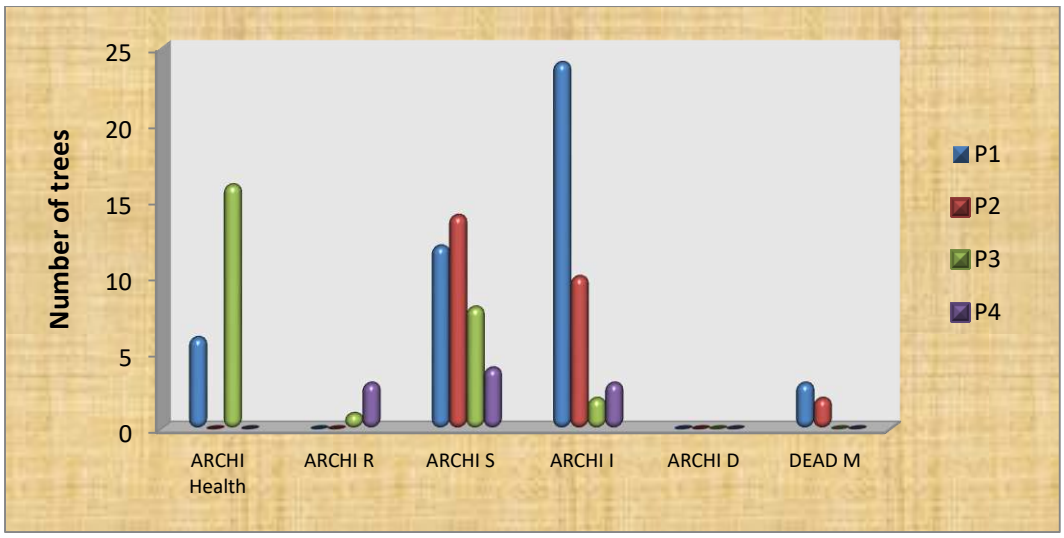

Figure 2. The different states of Aleppo pine According to the ARCHI type.

ARCHI R, ARCHI D and ARCHI H type's trees with its characteristics are: In the absence of additional stress, these types will revert to the healthy type. In terms of the width of the rings formed, after a period of decay, the radial progression recovers. A resilient Aleppo pin is potentially a tree of the future, provided that the log is of good quality and the plant is capable of producing timber. As with ARCHI S type trees (A stress condition), it is not possible to make a statement on the tree's future. Either the stress is too recent, or the five architectural descriptors used are insufficient to make prognosis. Thus, starting from a normal state, under the influence of various factors, there may be deviations from normal followed by returns to normal. This cyclical behavior is not uncommon. In our study, the results obtained indicate that the majority of trees are of the ARCHI I and ARCHI S type in the Aleppo pine in the 4 plots, which can be explained by the influence of various ecological factors in the study area characterized by rocky soil and a semi-arid climate.

CONCLUSION

In conclusion, the massif forest of chettaba is considere as a very rich forest area in terms of biological diversity. The ARCHI diagnostic method was developed to provide managers with the simplest possible decision-making tool for analyzing the health of stands and drawing conclusions about the interventions to be recommended. Monitoring the evolution of this health situation and identifying the agents directly or indirectly involved is a complex

task and requires regular follow-up over several years. This forest is mainly composed of Aleppo pine which are currently in a degraded state due to various natural and anthropic factors. The objective of this work is to know the health status of these two species using the ARCHI method. The diagnostic results show that the Aleppo pine is subject to climatic, pedological, and anthropogenic stresses and their health status is quite low. It is important to take into account the results obtained to establish a management plan to protect this forest massif against various degradation factors.

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EFFECT OF CHROMIUM ON PARENTAL STRAIN ALTERNARIA ALTERNATA AND ITS THREE COLOR MUTANTS STRAINS

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ABSTRACT

In this study, minimal inhibitory concentrations (MICs) of sodium chromate were determined for wild type (w.t) AA_1 strain of *Alternaria alternata* with its three derivatives strains are SW_1, SW_2 and SW_3 which have color mutation with lacking melanin genes. MIC of AA1 was reached to 10 mM that is less than MICs of other color mutants strains. However, SW_1, SW_2 their MICs were reached to 20 mM unlike MIC of SW_3 strain that was 14 mM. Stability of growth also was occurred on certain different concentrations of chromate for each strain. The cause of variation in chromate MICs for these strains is still unknown. Probably, because of loss melanin genes that bind or control other physiological properties that related on trace element resistance. Furthermore, 14 resistant strains were distributed between UV induced and spontaneous mutations of chromate were isolated from AA_1 and SW_2. Reverse mutation also was tested, 10 reverse mutation are reverse whereas 4 strain are forward mutants. Morphological changes also were recorded in these mutants.

Keywords: *Alternaria alternate*, Color mutations, Chromate, Resistant mutants.

INTRODUCTION

Alternaria alternata is an opportunistic plant pathogen that tops of the list plant pathogens which affect various species of plants and causing different economic damages (Hadi, 2019; Rathod, 2012); therefore controlling process is difficult except using fungicides that are a more widespread and cheaper means. However, adding some heavy metals to these chemicals may make their effectiveness more efficiency (Shoaib et al., 2015).

On the other hand, the release of these heavy metals to the environment, whether from these fungicides or various other industries, is a frightening matter; therefore many articles were looking for organisms those capable of removing these pollutant metals either by resistance or tolerance of their high concentrations (Ezzouhri et al., 2009). Therefore, it became necessary to measure the pathogen tolerance levels for these heavy metals, so the *A. alternata* fungus was used in the current study which have the black pigment known as melanin (Shoaib et al., 2015). It is known by high resistance and protection properties, especially when it is exposure to external chemical agents (Fernandes et al., 2016).

Consequently, the current research aims to measure the tolerance and resistance of the wild strain *A. alternata* fungus (have black-colored conidia) and their derivatives color mutants SW_1, SW_2 and SW_3 strains (lacking melanin) of the chromium element. As well as the genetic effect of chromium with or without of the ultraviolet radiation in its laboratory form, with studying the effect of presence or lack melanin on the tolerance these strains to the heavy metal, which is chromium.

MATERIAL AND METHODS

Test organism: The study was carried out on w.t strain of *A. alternata* (named AA_1) with black color conidia. With three color mutant strains are SW1, SW2 and SW3 which it's colonies color were white to pale pink their source is from (Hadi and Dhahi, 2012) article.

1. Media and chemicals

A. Minimal Medium (MM): attended this medium as (Caten, 1979).

B. Potato Dextrose Agar (PDA) and Potato Sucrose Agar (PSA) media: They were used for obtaining intensive and sustaining growth (Pitt and Hocking, 2009) . Whereas PSA medium in which, the dextrose replaced by equivalent sucrose (Hadi and Dhahi, 2012).

C. Sodium deoxycholate solution (D): a final concentration 400mg/ml of this solution was used to obtain single colonies which is suitable for this fungus as in (Hadi and Dhahi, 2012).

D. Sodium chromate stock solution: The stock solution was prepared from the Sodium chromate powder with a concentration of 1 Molar from the Department of Chemistry / College of Science / University of Mosul, which was supplied from Sigma-Aldrich Company.

2. Preparation of the Spore Suspension

It was prepared by growing the test strain for 5-7 days on the PDA media, then the conidia were washed by sterile lope under sterile conditions; dilutions (10^{-6}-10^{-7}) were prepared that were give an appropriate conidia that can be counted in control plates.

3. Culture and Incubation Conditions

The strains under study were cultured and incubated in the aforementioned media under sterile conditions and at 28 ± 2° C temperature.

4. Minimal inhibitory concentrations (MICs) Measurement

MICs of sodium chromate was measured by adding ascending concentrations to MM media for reaching concentrations at which a 100% inhibition rate occurred and these concentrations were considered the MICs.

5. Isolation of Chromate Resistant Mutants

The induced resistant mutants to chromate were isolated by exposing the conidia to UV rays with lethal intensity according to (Hadi and Dhahi, 2012), and then it was cultured on MM media containing MIC concentrations according to each strain, and at the same time, control media were cultured for finding the living counts. Spontaneous resistant mutants were obtained by culturing the conidia on media containing MIC of chromate without exposing it to any mutagen and leaving it until the well-developing chromate-resistant colonies.

6. Reverse Mutants Test

Chromate resistant mutants were cultured on the MM media plus MIC of chromate at the same time they were inoculated on the control media. To track the mutant's growth and their resistance to chromium, we compare the growth of the resistant mutants on the media with or without MIC of chromate. The inoculation process was carried out by making a master plate of resistant mutants and inoculating the aforementioned media using a pin replica method.

RESULTS AND DISCUSSION

1. MICs of Chromate

It is noticed from (Table 1) that the MIC of the SW_1 strain is 20 mM, which is the same MIC for the SW_2 strain. The inhibition percentage 40% for SW_1 strain was stable at different concentrations of chromate were the 2, 4, 6 mM. This is what happened with the inhibition percentage of the SW_2 strain, which was stable for two times, once at the 6,8,10 Mm concentrations at 66.6% inhibition percentage and at the 14, 16 mM concentrations, when the inhibition percentage reached 83.3% (Table 2).

Although both SW_1 and SW_2 strains have the same final MICs 20mM but inhibition percentage was constant at different concentrations (Tables 1, 2). The MICs stability of the SW_1 strain occurred at low concentrations 2, 4 and 6 mM, while the MIC of SW_2 strain was stable at higher concentrations and reached at the first time to the 10 mM then to 16mM. This indicates that the genes or gene which responsible of chromate resistance or tolerance in these two lacking melanin strains (that carry the color mutation) may be found in two different locations. The 50% inhibition percentage of two above-mentioned strains on the at 4-8 mM was higher than the inhibitory effect of chromium at 47%, that reached to 1 mM on wild type (w.t) strain of the same fungus in another study (Ezzouhri et al.,2009).

Table 1: MIC of chromate on SW_1 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
SW_1	0	1	0
	2	0.6	40
	4	0.6	40
	6	0.6	40
	8	0.5	50
	10	0.4	60
	14	0.3	70
	20	0.0	100

Table 2: MIC of chromate on SW_2 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
SW_2	0	1.2	0
	2	0.7	41.6
	4	0.6	50
	6	0.4	66.6
	8	0.4	66.6
	10	0.4	66.6
	14	0.2	83.3
	16	0.2	83.3
	20	0.0	100

The chromate MIC of SW_3 strain was 14 mM (Table 3), which is lower than the MIC of the previous two strains SW_1 and SW_2 by the 6 mM. This strain did not show a stability in the inhibition percentage as happened with the SW_1 and SW_2 strains because the inhibition

percentage continued to increase exponentially until it reached 100% at a 14 mM concentration. This also indicates genetic variations in the chromium resistance among the three strains that lacking melanin genes.

Table 3: MIC of chromate on SW_3 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
SW_3	0	1.3	0
	2	0.5	61.5
	4	0.4	69.2
	6	0..3	76.9
	8	0.2	84.6
	10	0.1	92.3
	14	0.0	100

The inhibition percentage of AA_1 (w.t strain) was 100% at 10 mM concentration, which in all cases is less than reached MICs by other studies such as (Zafar et al.,2007), but it is similar to another study that found 50% inhibition percentage was occurred at 1 mM of chromate (Ezzouhri et al.,2009).

The MIC of the w.t strain less than the MICs of three white strains, this means chromium resistance isn't related with melanin presence or lacking in this fungus (Tables 1, 2, 3 and 4). All of the melanin lacking strains has higher MICs that reached to 20 mM in the SW_1 and SW_2 strains, whereas 14 mM in SW_3 strain when they compared with MIC of AA_1 strain that reached to 10 mM.

Table 4: MIC of chromate on AA_1 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
AA_1	0	1.5	0
	2	0.5	66.6
	4	0.2	86.6
	8	0.2	86.6
	10	0.0	100

The variation of chromium resistance between MICs of the lacking melanin strains (have a color mutation) and w.t strains can only be explained by one reason, which is the genes or gene controlling the melanin pathway are able to impair or change other functions (to the better or worse) such as resistance to heavy metals (Fetzner et al., 2014), which is actually happened in our current study.

However, the targeting of the responsible genes for melanin not only affects the physical characteristics, but the morphological characteristics are also affected in the fungus *Sclerotinia sclerotium* (Liang et al.,2018) in addition to the increased production of exonzymes such as cellulase, protease, amylase and pectinase to compensate the deficiency of melanin mutants in *Bipolaris sorkiniana* (Chand et al.,2014).

We conclude from the foregoing that it is possible to take advantage of the chromate resistance characteristic of the three strains with color mutation SW_1, SW_2 and SW_3 which were reached to 20 mM (Table 1 and 2) and 14 mM (Table 3). To benefit from them it in ridding the environment from heavy metals more than using the same wild strain. However,this happened when isolating a wild strain of *Aspergillus flavus*, which has a high chromat-tolerance (Kumar and Dwivedi, 2019). Anywhere, it is less tolerant than the strains with the color mutation in *A. alternata* in our current study. The mutants may be similar to the parental strain in terms of pathogenicity but they are different in other traits, as X-ray resistant strains of *Alternaria sp.* (Babalola, 2009).

In the current study, the chromates MICs were not measured at different environmental conditions, as in (Kumar and Dwivedi, 2019).Furthermore, despite the stability of the environmental conditions for conducting the experiments, the white mutants (lacking melanin) strains were showed high resistance to chromium, this may belong to the included excess of heavy metal in their subcellular parts (Cornejo et al., 2013), these subcellular parts are melanin-free. This confirms the possibility of using these color mutants to withdraw chromium pollution from the environment, which is researchers are trying to obtain it as well as mention in (Chen et al., 2019). The current study also enhances another aspect which is the possibility of using chromium by adding it to some natural or industrial pesticides as a fatal component for controlling the wild strain *A. alternata* fungus due to its sensitivity to it (the present study) this need extensive prospective studies.

2. Isolation of Chromate Resistant Mutants

The total number of chromate resistant mutants from the two strains w.t and SW_2 was 14 (Table 5), which were distributed between 8 resistant mutants from w.t black isolates of *A. alternata* and six resistant mutants from the SW_2 strain, while no resistant mutants were obtained from the remaining strains such as SW1 and SW3.

Table 5: resistant mutants of chromate with reverse and forward mutants count

strain	Treatment type	Mutants count	Reverse mutants	Forward remaining mutantss
W.t	0	2	1	1
	U.V	6	4	2
SW_2	0	0	-	-
	U.V	6	5	1

Indeed, many experiments failed, which we didn't mention in Table 5, because no chromate-resistant mutant was obtained, which were 10 experiments distributed between UV or spontaneous treatment (0) which were performed on all strains .The reason was not known, this may be due to the fungal spores influencing by the chemicals toxicity (Bhajbhuje, 2014) that coming from the chromate with the killing intensity of UV rays with the (Hadi and Dhahi, 2012). Thus, spores of treated strains did not germinate therefore; I sometimes reduced the MIC to obtain more mutants. Two spontaneous mutants (Table 5) were also obtained from w.t strain.

The continuous growth of resistant mutants on chromate media with lethal concentration for preserving mutants, as well as control media without adding chromate in order to study them intensively. We noticed there are mutants that could not grow and resist on the chromate medium, but rather became growing on the control media only. Thus these mutants were

considered are reverse or recurrent mutations, and this may be due to one of the following reasons: either because of the duplications or their changes in specific locations of the chromatids and along the chromosome, leading to an opposite direction of the mutation or due to certain replications of the DNA (Holliday, 1964).

Or, the reversal of the mutation may occur at a finer level at the gene level, by substituting a specific gene as an alternative for the defective gene or within the gene itself by replacing the healthy allele for the damaged allele. In both cases shows phenotype to the examiner, it is in the fact not the phenotype because its genotype remains mutations containing that are not visible in its phenotype (Clark. and Pazdernik 2013).

However, it may be due to the phenomenon of multinucleate conidium in in this fungus, which is the reason that I see more likely because of the conidium contains more than one nucleus (Knox-Davies, 1979). So that the mutation effect of one nucleus that present in the conidium is covered by effect of other perfect nuclei that are not mutated and thus the wild phenotype returns to the mutant strain.

3. Morphological Changes of Resistant Mutants

There are morphological changes that occurred to chromate-resistant mutants (fig 1), which corresponds many studies in it the fungi changed morphologically when exposed to certain chemical pressures (Fernandes et al., 2016; Kumar and Dwivedi, 2019). They were characterized by being very dense, coherent and not widespread even if the color of the mycelium has become lemon colored (whether the resistant mutants are isolated from the w.t black strain or the mutant lacking melanin (Table 5), possibly due to the taking of morphological changes of the resistant strains from as a defense means to preserve their cells.

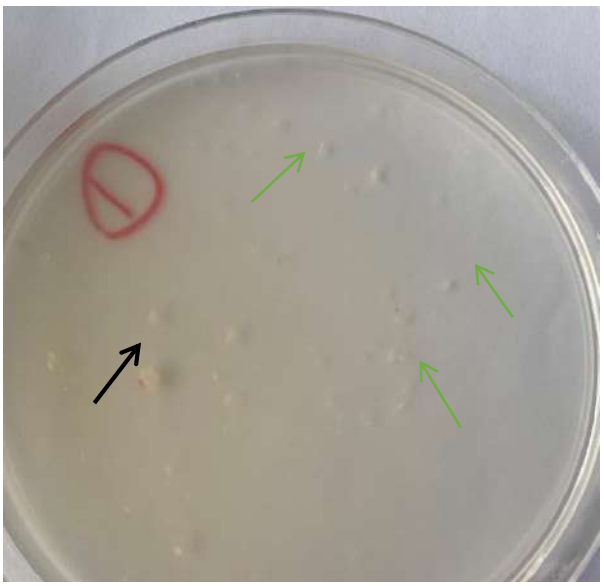

Figure 1: morphological changes of chromate resistant mutants. Green arrows indicate poor resistant that ignored while black arrow refer to strong resistant that was completed tests on it.

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GASTROINTESTINAL HELMINTHS OF SHEEP BREED IN SPREAD BELGRADE AREA IN PERIOD 2018-2019

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Abstract

During 2018-2019. we were examined parasitic fauna of sheep in spread Belgrade area (Serbia). Coprological, and post-mortem examination revealed the following helminth species: *Teladorsagia* (*Ostertagia*) *circumcincta* in 75,23%, *Ostertagia trifurcata* 71,53%, *O.ostertagi* 21.99%, *Trichostrongylus axei* 62,23%, *T.colubriformis* 69,57%, *T.vitrinus* 62,85%, *Nematodirus spathiger* 77,43%, *N,filicolis* 33,31%, *Haemonchus contortus* 58,95%, *Marshallagia marshalli* 27,77%, *Skrjabinema ovis* 11,31%, *Bunostomum trigonocephalum* 13,28%, *Chabertia ovina* 63.85%, *Oesophagostomum venulosum* 27.91%,*Cooperia curticei* 60.52%,*C.oncophora* 28,39% and *C.punctata* 13,28%. The obtain results was compares with the results of research from 2009-2010 and the impact of changes in microlimatic and environmental conditions on the biodiversity of GI heminate sheep in this area.

Key words*:* sheep, gastrointestinal helminths, Belgrade

INTRODUCTION

Belgrade is the capital and largest city of Serbia. It is located at the confluence of the Sava and Danube rivers and the crossroads of the Pannonian Plain and the Balkan Peninsula. The city has an urban area of 360 km^2 while together with its metropolitan area it covers 3,223 km^2. The spread area of Belgrade has extremely favorable conditions for modern

agricultural production (climate, agricultural land, watercourses, developed processing industry). This economic branch is of strategic importance for supplying Belgrade with food products, along with the resources that abound in the wider environment (Vojvodina and Šumadija). The Belgrade region has a significant land potential of about 322,292 hectares of agricultural land, which makes up 70% of the total territory of the City of Belgrade.

During last decade small ruminant breeding is constantly growing and start to play an important role in providing animal protein for food, especially for those living in a village near Belgrade. Sheep are milked and they produce the bulk milk supply, together with a proportion of the meat that is consumed.

The first serious studies of parasitic fauna of sheep and goats in the area of Belgrade were done in the period 2009-2010 (Pavlović et al., 2009b,2012). In the meantime, there has been an increase in the number of herds, changes in microlimatic conditions and environmental conditions (Pavlović and Ivanović,2015b). Due to the sudden urbanization, which is inevitably accompanied by pollution of land and water, an increase in the number of non-owner dogs, the approach of wild animals to human settlements (foxes, etc.). This has affected the quality of the environment, the grazing areas where sheep are kept, as well as the global epidemiological and hygienic condition of the city. All this affects, together with parasitic infections, the production results (milk yield, growth, quantity and quality of wool) in sheep (Pavlović et al.2009a).

For these reasons, after ten years, we returned to examining the parasitic fauna of small ruminants in the area of Belgrade in order to see the current situation causing these changes.

MATERIAL AND METHODS

The study of endoparasites infection performed during 2018-2019. we were carried out in 152 flocks of sheep originated from from 6 Belgrade districs Mladenovac, Lazarevac, Obrenovac, Grocka, and Vozdovac (from the village Mladenovac, Vlaska, Mala Krsna, Velika Krsna,Medjuluzje, Senjak, Velika Ivanca, Orašac, Mala Vrbica, Rajkovac, Dubona, Šepšin, Resnik, Ritopek, Vrčin, Vinča, Leštane, Pinosava, Grocka, Velike Granice, Granice, Koracica, Jagnjilo, Markovac, Lazarevac, Arapovac, Junkovac, Leskovac, Sokolovo, Rabrovac, Vrbovno, Zvecka, Krtinska and Stepojevac). During our examination we were examined total of 631 fecal samples. Examination we performed using standard coprological technique (Euzeby,1981).

Geographical and climate data about examined area was next: Belgrade is situated in South-Eastern Europe**,** on the Balkan Peninsula**.** It lies at the point where the river Sava merges into the Danube**,** on the slope between two alluvial planes. The river waters surround it from three sides, and that is why since ancient times it has been the guardian of river passages. Belgrade's climate exhibits influences of oceanic, humid continental and humid subtropical zones, with four seasons and uniformly spread precipitation.Monthly averages range from 0.4 °C in January to 21 .8 °C in July, with an annual mean of 12.2 °C. There are, on average, 31 days a y ear when the temperature is above 30 °C, and 95 days when the temperature is ab ove 25 °C. Belgrade receives about 680 millimeters of precipitation a year, with late spring being wettest (http://www.hidmet.gov.rs/).

Total of 73 sheep and lambs we were examined by post-mortem examination. Total differential worm counts were done on all the alimentary tract using the standard paristology necropsy technique described by Pavlović and Anđelić-Buzadţić (2010b). Determination of adult and eggs of parasites were done by keys given by Euzeby (1981) and Anderson (2000).

RESULTS AND DISCUSION

The faecal samples were obtained from a different source all together as they were collected from flocks in the field, and the results support the other findings. These counts were also of value in providing some information's on the peripartuirent egg rise. The number of guts and lungs examined in this survey thought small in number, but in combination with results of coprological examination, samples appeared to represent the population adequately.

In period 2018-2019 we found next helminth species: *Teladorsagia* (*Ostertagia*) *circumcincta* in 75,23%, *Ostertagia trifurcata* 71,53%, *O.ostertagi* 21.99%, *Trichostrongylus axei* 62,23%, *T.colubriformis* 69,57%, *T.vitrinus* 62,85%, *Nematodirus spathiger* 77,43%, *N,filicolis* 33,31%, *Haemonchus contortus* 58,95%, *Marshallagia marshalli* 27,77%, *Skrjabinema ovis* 11,31%, *Bunostomum trigonocephalum* 13,28%, *Chabertia ovina* 63.85%, *Oesophagostomum venulosum* 27.91%, *Cooperia curticei* 60.52%,*C.oncophora* 28,39% and *C.punctata* 13,28%.

Most prevalence species of nematode are *Trichostrongylus* and *Nematodirus* species. The distribution of species within the established genera also varied. Within the genus *Ostertagia* most abundant were dominated by *Ostertagia circumcincta* and *O.trifurcata*. Prevalence of infection with Ostertagia ostertagi and *Ostertagia occidentalis* was higher during the colder periods of the year.Among the species of the genus *Trichostrongylus* was the most prevalent *Trichostrongylus colubriformis*. Extensity of infection with *Trichostrongylus axei* and *T.vitrinus* varied, without any regularity. Extensity of infection with *Nematodirus filicollis* and *N.spathiiger* demonstrated a tendency to increase and leveled off at the highest level of the whole study period.

If we make a comparison with the results we had during the research done in the period 2009-2010, it can be seen that the prevalence of certain types of parasites has decreased, but the number of parasite species has increased. There are many reasons for that, and the main one is that in the past period there has been a significant increase in the number of herds in the villages around the city. these were mainly animals that were procured from other parts of Serbia where these types of parasites are present (table 1, figure 1).

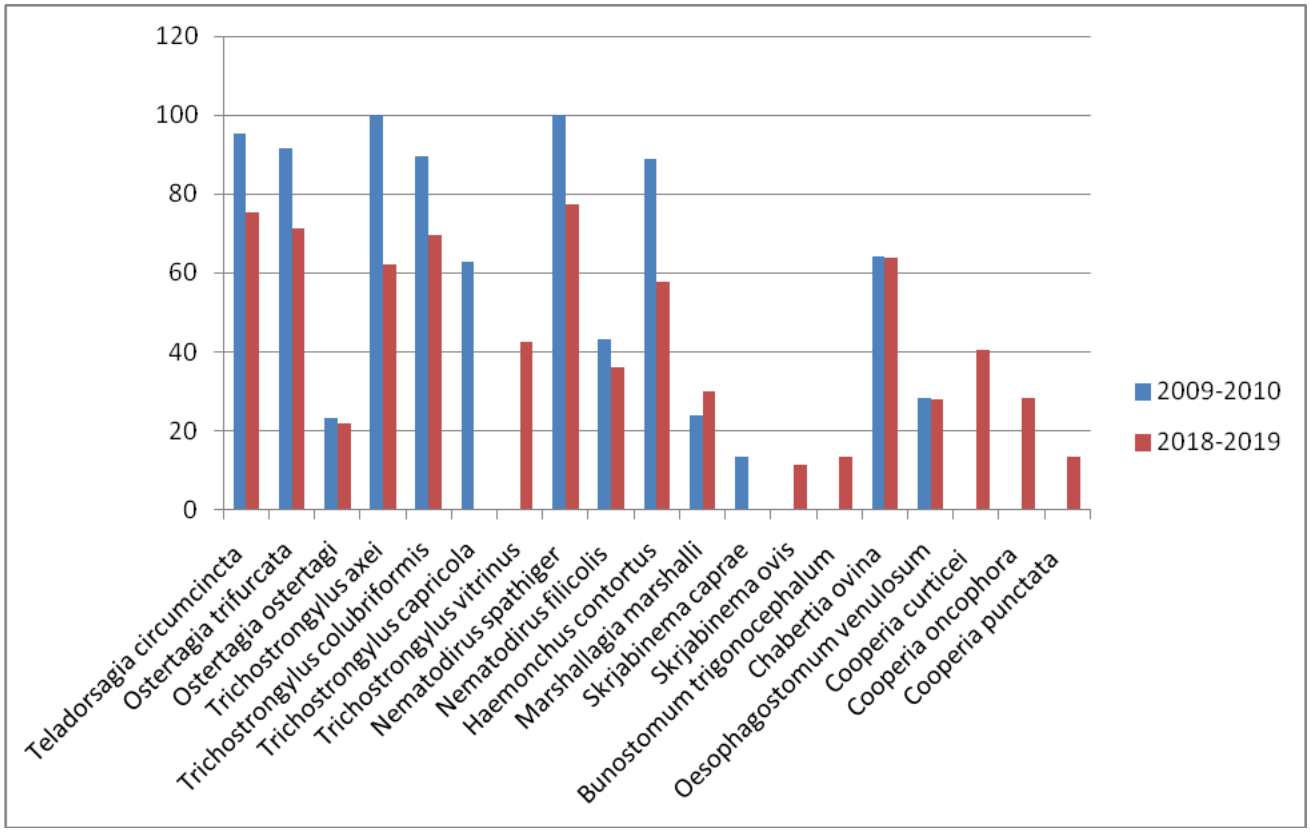
Table 1. Comparative prevalence of GI helminths og sheep in perid 2009-210 and 2018-2019

YEARS			
2009-2010		**2018-2019**	
parasites species	**%**	**parasites species**	**%**
Teladorsagia (*Ostertagia*) *circumcincta*	95.23	*Teladorsagia* (*Ostertagia*) *circumcincta*	75.23
Ostertagia trifurcata	91.53	*Ostertagia trifurcata*	71.33
Ostertagia ostertagi	23.33	*Ostertagia ostertagi*	21.99
Trchostrongylus axei	100.00	*Trichostrongylus axei*	62.23
Trichostrongylus colubriformis	89.57	*Trichostrongylus colubriformis*	69.57
Trichostrongylus capricola	62.85	*Trichostrongylus vitrinus*	42.45
Nematodirus spathiger	100.00	*Nematodirus spathiger*	77.43
Nematodirus filicolis	43.31	*Nematodirus filicolis*	35.91
Hameonchus contortus	88.95	*Haemonchus contortus*	57.65
Marshallagia marshalli	23.77	*Marshallagia marshalli*	29.89
Skrjabinema caprae	13.28	*Skrjabinema ovis*	11.31
Chabertia ovina	64.14	*Bunostomum trigonocephalum*	13.28
Oesophagostomum venulosum	28.39	*Chabertia ovina*	63.85
		Oesophagostomum venulosum	27.91
		Cooperia curticei	40.52
		Cooperia oncophora	28.39
		Cooperia punctata	13.28

During our research done in the period 2018-2019, a difference in the biodiversity of GI helminths is noticed in comparison with the research done in the period 2009-2010 (Pavlović et al.2012). This research has for the first time identified parasites of the genus *Cooperia* that were not previously present in the Belgrade area. They are usually found in sheep flocks in southern and south-east Serbia (Pavlović et al.2013a,b). Simultaneously with the increase in the number of GI helminth species, the prevalence of previously established species decreased. This is most noticeable in the three bridge prevalence genera of the GI nematode *Ostertagia, Trichostrongylus* and *Nematodirus* species.

Found parasites species were present at small ruminats in other parts of Serbia. This was confirmed by during examination performed in the hilly areas of Serbia (Šar Planina, Stara Planina) (Vujić et al.,1911,2015a), south, south-east and south-west part of Serbia, (Pavlović et al.2013a,b, 2018a), at Timok District (Jovanović et al.1991), Belgrade area (Pavlović et al.2009,2012) Vojvodina (Pavlović et al.2017b) and Kosovo (Pavlović et al.1995,Milanović et al.2018). Same parasitic species were occurred at other Balkan countries like Macedonia or Bulgaria (Georgievski,1989, Zurliiski and Rusev,1990).

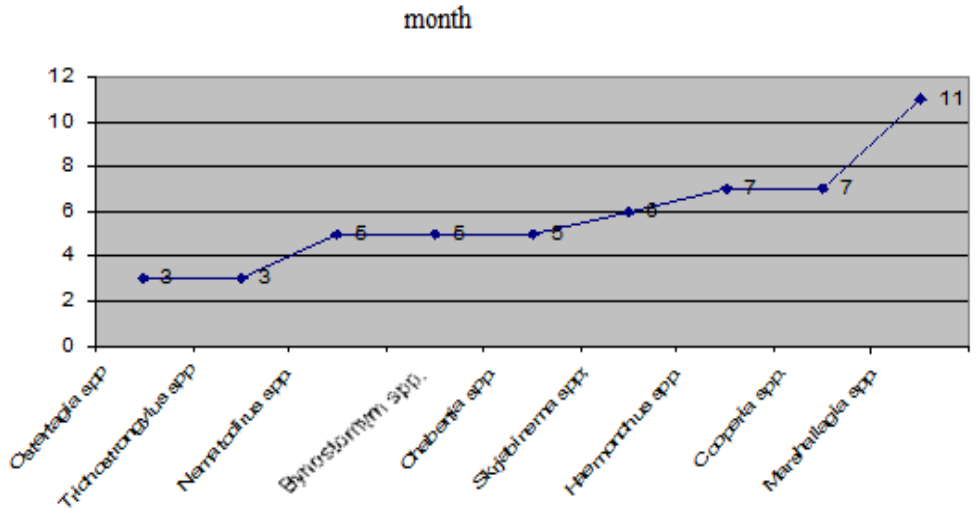
Figure 2. Comparative prevalence of GI helminths og sheep in perid 2009-210 and 2018-2019



Although most of the gastro-intestinal species appear to follow this general pattern of seasonal distribution, some variations in intensively and duration of these characteristics with different worm species occurred (Pavlović and Ivanović,2018). Thus with *Trichostrongylus* and *Nematodirus* species infection at mature sheep the spring peak was more pronounced that the autumn infection.

The season dynamics of the established parasites species was as follows (figure2):
- In March have occurred *Ostertagia spp.* and *Trichostrongylus spp.*
- In May, the observed infection with *Nematodirus spp*, *Bunostomum spp.* and *Chabertia spp.* (*ovina*)
- In June was the first record of *Skrjabinema spp*;
- In July were established eggs of *Haemonchus spp.* (*contortus*) and *Cooperia spp.*
- In November showed the presence of *Marshallagia spp*

Figure 2. The season dynamics of GI parasitesx in period 2018-2019



Climate variations are a significant factor of seasonal distribution of certain species of sheep helminates (Ardeleanu et al.2007**).** There are discrepancies in the seasonal distribution between certain regions in Serbia. Thus, certain species within the genera *Ostertagia*, *Trichostrongylus* and *Nematodirus* occur earlier in the plains (north Serbia Vojvodina) and the area of Belgrade than in hilly and mountainous areas (Pavlović et al.2015b, 2017b, Pavlović and Ivanović,2018).

Generally speaking the occured parasites represent a global problem. Way of breeding usualy at shepeng had prerequisite to a lot of infections including parasitoses. Pasture breeding make possible contact within sheep and eggs, larvar stages and intermediate host of parasites. Those induce that there are no one sheep without parasites. The countries of Magreb, Middle East and Northern Africa are also in permanent probleme with parasitic infections and losses ensued by them. Negative influence of parasitic infection reflected througt lost of weight and decrement quantum of lactation (Bahgat et al.1988, Dogana, et al.1989, Ashraf and Nepote,1989, Fakae,1990, Smith,1990, Quesadaet al.1990).

CONCLUSION

However, since the parasitic infections are in majority sub clinical this problem is not played due attention by a sheep breeder from the village in the Belgrade area. The prophylactic treatment is not conducted in the majority of flocks or it is only partially performed what can be seen by the records from the slaughter line and from production results. In aim of introducing parasites fauna of sheep and prepare measure to its control we must to continue our examination. This was the only way to obtain better product results, characteristics and quality of sheep and lambs meat.

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AESTHETIC PROPERTIES OF SOME WOODY PLANT TAXONS THAT CAN BE USED IN URBAN ROAD AFFORESTATIONS

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ABSTRACT

One of the most important elements of urban landscape is urban roads. These roads, which constitute the most important transportation networks of cities, have also seen as light green locations that enrich value to the city. The afforestation for urban roads and streets should be designed to provide beautiful views to the travelers and pedestrians along the road. Woody plant taxa to be used in road afforestation have an effective appearance with their form, color and sizes. In this research, the some woody plant taxa used in urban afforestation of urban roads which have become the focal points of the city due to diversification of transportation networks have been studied. The aesthetic properties of the woody plant taxa were evaluated and recommendations were developed.

Keywords: Urban road afforestatıons, Woody landscape plants, Planting desıgn

INTRODUCTION

The roads in the transportation networks of cities are one of the most important elements that give the city an aesthetic feature. Urban roads, medians, pedestrian roads, etc. are located in urban open green spaces. Along with determining the development direction of the city, roads also interconnect urban areas (Aslanboğa, 1997; Sağlık et.al., 2012; Akdeniz et.al., 2019). It is necessary to carry out effective planning and designs to provide the opportunity to pass through a beautiful landscape for those traveling along urban roads (Önder and Polat 2007; Şengül, 2011). Roads, which are places for drivers to pass through, also have business, shopping, and recreational purposes for pedestrians, apart from transportation. The designs to be made on the roads will not only support the image of the city but also have positive effects on the health of the city (Aslanboğa, 1986; Dirik, 2014; Yazıcı, 2017; Akdeniz et.al., 2019).

The most important planning initiative that affects the relations between the road and the environment is planting. Plantings not only ensure that the driver's perception and attention are always open (Ertekin and Çorbacı, 2010; Sağlık et.al., 2020) but also give an identity to the city and create an aesthetic appearance. The aesthetic features of plants are an important issue in design, and directing the designs in a way that provides aesthetic satisfaction with the views of the landscape, their smell, the senses of the plants, the sounds created by the wind and rain on the leaves and branches is a factor that directly affects the preferability and usability of the landscape and the positive perception it creates in the user. Therefore, although aesthetic satisfaction is an important goal of planting design, an effective plant design should complement, revitalize and unite the area with the selection of suitable species that are

aesthetically pleasing (Erbaş, 2003; Basıç, 2016; Richard and Gobster, 1990; Yılmaz et. al., 2019).The aesthetic value of plants is provided by their dendrological and visual characteristics such as form, color, and texture (Yılmaz et.al., 2019). The visual effect created by the color, texture, form, and size of the trees used on urban roads is undeniable (Aklıbaşında and Erdoğan, 2016). The characteristics of the plant material to be used such as changing color according to the seasons, leaf shapes, light and shadow plays, autumn coloring, flower and fruit color, and leafless appearance in winter are important. (Çorbacı and Ertekin, 2010; Aklıbaşında and Erdoğan, 2016).

In this study, some woody plant taxa that can be used on urban roads were determined by using different sources, and taxa were evaluated in terms of their aesthetic properties by examining form, texture, and color.

Some plant taxa that can be used on urban roads

A total of 60 coniferous and leafy taxa that can be used in urban road planting were determined and evaluated (Table 1, Table 2) (Aslanboğa, 1997; Akdeniz et.al., 2019; Aklıbaşında and Erdoğan, 2016). An evaluation of the coniferous taxa reveals that the coniferous taxa used on urban roads are generally from the Cupressaceae and Pinaceae families. Among the coniferous taxa, *xCupressoscyparis leylandii* is one of the most frequently used taxa, especially with its branches that do not break easily and its characteristic to be resistant to abiotic and biotic pests. Cedrus sp., Picea sp., and Cupressus sp., etc. can be used. Leafy taxa are mostly from Aceraceae, Betulaceae, Fabaceae, and Oleaceae families. Since taxa such as Salix sp. and Morus sp, have strong root growth, attention should be paid not to use them where sewer and infrastructure pipes are located but taxa such as Acer sp., which is resistant to the mechanical pressures brought by the urban environment, and in regions where there is the effect of the sea, Elaeagnus sp. and Fraxinus sp. can be preferred (Aslanboğa, 1986; Seçkin, 1986; Küçük and Gül 2005; Yılmaz, 2019).

Table 1. Some coniferous taxa that can be used in urban road planting

Families	**Taxa**
Cupressaceae	*Cupressus arizonica*
	Cupressus sempervirens
	Cupressus macrocarpa 'Goldcrest'
	xCupressoscyparis leylandii
	Juniperus horizontalis
	Juniperus sabina
Pinaceae	*Abies nordmanniana*
	Cedrus atlantica
	Pinus nigra
	Picea abies
	Picea orientalis
	Picea pungens
	Pinus pinea

Table 2. Some leafy taxa that can be used in urban road planting

Families	**Taxa**
Aceraceae	*Acer platanoides*
	Acer pseudoplatanus
	Acer saccharinum
	Acer negundo
	Acer campestre
Anacardiaceae	*Rhus typhina*
Apocynaceae	*Nerium oleander*
Betulaceae	*Alnus glutinosa*
	Betula pendula
	Betula papyrifera
	Carpinus betulus
Berberidaceae	*Berberis thunbergii 'Atropurpurea*
Cannabaceae	*Celtis australis*
Caprifoliaceae	*Viburnum tinus*
Fabaceae	*Albizia julibrissin*
	Cercis siliquastrum
	Gleditsia triacanthos
	Robinia pseudoacacia "Umbraculifera"
	Robinia pseudoacacia
	Sophora japonica
Fagaceae	*Quercus robur*
	Ouercus cerris
	Quercus palustris
Lamiaceae	*Rosmarinus officinalis*
Lythraceae	*Lagerstromia indica*
Lauraceae	*Laurus nobilis*
Magnoliaceae	*Liriodendron tulipifera*
	Magnolia grandiflora
Malvaceae	*Tilia tomentosa*
	Hibiscus syriacus
Meliaceae	*Melia azaderach*
Moraceae	*Morus nigra*
Oleaceae	*Ligustrum ovalifolium*
	Ligustrum ionandrum
	Fraxinus angustifolia
	Fraxinus ornus
	Syringa vulgaris
Paulowniaceae	*Paulownia tomentosa*
Platanaceae	*Platanus orientalis*
Rosaceae	*Crataegus monogyna*
	Malus floribunda
	Prunus cerasifera
	Prunus serrulata "Kanzan"
Salicaceae	*Salix alba*
Sapindaceae	*Koelreuteria paniculata*
Simaroubaceae	*Ailanthus altissima*
Ulmaceae	*Ulmus carpinifolia*

Aesthetic properties of plant taxa that can be used on urban roads

Some plant taxa to be used on urban roads were evaluated in terms of aesthetic properties by examining form, texture, and color.

The Form Property

Form emerges as an aesthetic feature that has continuity and can be perceived in all seasons. The form is a design element that is firstly perceived by people as the outer outline and silhouette of the plant and stands out. The plant form provides the coordination of plants with other elements. Pyramidal-column-shaped plants create a focal point by increasing the height in the design, while diffuse forms create width in the design area. Round forms, on the other hand, need to be used more in design than other forms as neutral forms that do not have the feature of orientation. Round forms create a visual link between pyramidal and emanative forms. However, pendent forms also direct our gaze from top to bottom, since the branches are a structure that hangs down (Ayaşlıgil, 2004; Korkut and Kiper, 2021). Taxa to be used in road planting were examined in six groups in terms of form characteristics as pyramidal (conical), round (spherical), oval, creeping, weeping, and scattered (Table 3). Among the taxa evaluated, the majority of them were observed to be round-spherical-shaped taxa with 32 of them.

Table 3. Form characteristics of some taxa that can be used on urban roads

Round (Spherical Form		
Acer negundo	*Pinus pinea*	*Morus nigra*
Acer platanoides	*Platanus orientalis*	*Nerium oleander*
Acer campestre	*Prunus cerasifera*	*Paulownia tomentosa*
Acer pseudoplatanus	*Robinia pseudoacacia*	*Fraxinus angustifolia*
Acer saccharinum	*Robinia pseudoacacia*	*Fraxinus ornus*
Alnus glutinosa	*"Umbraculifera"*	*Koelreuteria paniculata*
Celtis australis	*Sophora japonica*	*Ulmus carpinifolia*
Cercis siliquastrum	*Viburnum tinus*	*Hibiscus syriacus*
Laurus nobilis	*Quercus robur*	*Tilia tomentosa*
Melia azaderach	*Ouercus cerris*	*Gleditsia triacanthos*
Crataegus monogyna	*Liriodendron tulipifera*	*Prunus cerasifera*
Malus floribunda		
Pyramidal (Conical) Form		**Oval Form**
Cupressus sempervirens	*Picea pungens*	*Berberis thunbergii*
Magnolia grandiflora	*Cupressus arizonica*	*'Atropurpurea*
Picea orientalis	*xCupressoscyparis leylandii*	*Lagerstromia indica*
Abies nordmanniana	*Pinus nigra*	*Rosmarinus officinalis*
Betula papyrifera	*Picea abies*	*Ligustrum ovalifolium*
Cupressus macrocarpa	*Carpinus betulus*	*Ligustrum ionandrum*
'Goldcrest'	*Quercus palustris*	*Prunus serrulata "Kanzan"*
Creeping Form	**Weeping Form**	**Scattered Form**
Juniperus horizontalis	*Betula pendula*	*Rhus typhina*
Juniperus sabina	*Salix alba*	*Albizia jülibrissin*
		Ailanthus altissima
		Syringa vulgaris

While 13 taxa were in column-pyramidal form, 7 taxa were oval, 4 taxa were in scattered form, and 2 taxa each were in creeping and weeping forms. Round-shaped taxa such as *Acer negundo* and *Gleditsia triacanthos* etc. and taxa with scattered forms such as *Ailanthus altissima* etc. are used for shading as street trees. While pyramidal forms such as Magnolia grandiflora etc. can be used at intersections, taxa such as *xCupressoscyparis leylandii, Pinus pinea,* etc. can be used along the central refuge. Also, the taxon *xCupressoscyparis leylandii* creates a landscape effect.

The Texture Property

The texture is a visual design component that can be felt with the eye and the sense of touch. The texture of a plant is the holistic appearance of the plant with its general structure, branching feature, leaf shape, and twigs. As an element that adds dimension and movement to design, texture allows the plant to be perceived with its general habitus characteristics when viewed from afar.

Table 4. Texture characteristics of some taxa that can be used on urban roads

Fine Texture	
Betula papyrifera	*Melia azaderach*
Betula pendula	*Robinia pseudoacacia*
Fraxinus angustifolia	*Robinia pseudoacacia "Umbraculifera"*
Fraxinus ornus	*Salix alba*
Gleditsia triacanthos	*Sophora japonica*
Hibiscus syriacus	*Syringa vulgaris*
Lagerstroemia indica	Pinus pinea
Pinus nigra	
Medium Texture	
Acer saccharinum	*Prunus cerasifera*
Albizia julibrissin	*Quercus palustris*
Berberis thunbergii 'Atropurpurea'	*Rosmarinus officinalis*
Carpinus betulus	*Malus floribunda*
Cercis siliquastrum	*xCupressoscyparis leylandii*
Coarse Texture	
Abies nordmanniana	*Liriodendron tulipifera*
Acer campestre	*Magnolia grandiflora*
Acer negundo	*Morus nigra*
Acer platanoides	*Nerium oleander*
Acer pseudoplatanus	*Ouercus cerris*
Ailanthus altissima	*Paulownia tomentosa*
Alnus glutinosa	*Picea abies*
Cedrus atlantica	*Picea orientalis*
Celtis australis	*Picea pungens*
Crataegus monogyna	*Platanus orientalis*
Cupressus arizonica	*Prunus serrulata "Kanzan"*
Cupressus macrocarpa 'Goldcrest'	*Quercus robur*
Cupressus sempervirens	*Rhus typhina*
Juniperus horizontalis	*Tilia tomentosa*
Juniperus sabina	*Ulmus carpinifolia*
Koelreuteria paniculata	*Viburnum tinus*
Ligustrum ionandrum	*Laurus nobilis*
Ligustrum ovalifolium	

Course-textured plants should be used in the background in the design, and when used in large numbers, they cause the area to appear smaller. Most of the coniferous taxa are coarse-textured taxa. Medium texture, which is perceived in the case of medium-sized leaves, branches, etc., plays a unifying role by providing a transition between coarse and fine textures. On the other hand, the fine texture is suitable for creating a calm and soft surface character, creating a background for textures and being used in the front of buildings, and as a texture that is the last to be noticed in the design, looks brighter and allows tricks of the light by enabling light to pass through (Ayaşlıgil, 2004; Korkut and Kiper, 2021). Taxa that can be used in road planting were examined in terms of texture in three groups as fine, medium, and coarse-textured (Table 4). The taxa selected with the maximum number 37 within the scope of the study were the coarse-textured ones. While there are 13 taxa with fine texture, there are 10 taxa with a medium texture. While coarse and medium-textured taxa such as *Laurus nobilis* and *Cercis siliquastrum* can be used on pedestrianized roads, fine-textured taxa such as *Pinus nigra* and *Pinus pinea* may be preferred for use in central refuges. (Ürgenç, 1998; Akdeniz et.al., 2019).

The Color Property

Color is an important design element for plant material, emphasizing a line or route, drawing attention to the center of the plan, and giving it personality. Plants, leaves, flowers, fruits, etc. help define spaces by creating a visual effect with their many color features. Colors also affect human psychology. Warm colors make the space seem small by making the objects appear close as converging colors, while cold colors cause the space to seem farther away. Moreover, green color provides a sense of calmness, peace, and security, yellow makes one feel a sense of energy and white color appears as a color that has a calming effect. Red and orange colors, on the other hand, are colors that create tension if watched for a long time, so it is important to use them carefully. While using color in design, different colors and color tones are utilized and colors are used together with textures (Altınçekiç, 2000; Whitehouse et.al. 2001; Eroğlu et.al., 2005; Ender et.al., 2016; Akdeniz, 2020; Korkut and Kiper, 2021). The taxa to be used in road planting were evaluated in four parts as flower, leaf, and autumn coloration and also flowering times in terms of color characteristics. Among the taxa considered in the study, green/light green/yellowish-green flowers have the maximum number 12. 10 taxa have White/Cream/Creamy white flowers, 6 taxa have yellow/yellow-orange flowers, while 3 taxa have red flowers. There are 4 taxa with flowers in different colors *(Nerium oleander, Syringa vulgaris, Hibiscus syriacus, Lagerstromia indica),* (Table 5). However, it is seen that taxa often flower in spring and summer and stay in flower for 1-6 months. While taxa such as *Acer sp., Berberis thunbergii 'Atropurpurea', Betula pendula* and *Malus floribunda,* etc. taxa remain flowering for 1 month, taxa such as *Viburnum tinus, Albizia julibrissin, Magnolia grandiflora,* and *Laurus nobilis,* etc. remain in flower for 3 months. The longest flowering taxon is *Rosmarinus officinalis* for 6 months (Table 6). *Nerium oleander and Lagerstroemeria indica* taxa with different colored flowers are preferred as highly decorative plants with their aesthetic appearance. Taxa such as *Laurus nobilis* and *Ligustrum ovalifolium* with white and yellow flowers can also be preferred as street trees in areas with pedestrian traffic. It is also possible for these taxa to be used by being crowned from high. On the other hand, in the central refuge and areas with heavy traffic, flowerless coniferous *Pinus sp., Cedrus sp., and Abies sp.,* etc. are commonly preferred species (Ürgenç, 1998; Aklıbaşında and Erdoğan; 2016).

Table 5. Flower colors of some taxa that can be used on urban roads

White/Cream/Creamy White		**Yellow / Yellow-Orange**
Crataegus monogyna *Fraxinus ornus* *Fraxinus angustifolia* *Magnolia grandiflora* *Robinia pseudoacacia* *Ligustrum ionandrum*	*Robinia pseudoacacia "Umbraculifera"* *Sophora japonica* *Viburnum tinus* *Ligustrum ovalifolium*	*Acer platanoides* *Berberis thunbergii 'Atropurpurea'* *Laurus nobilis* *Liriodendron tulipifera* *Rhus typhina* *Salix alba* *Tilia tomentosa*
Pink	**Green/ Light Green/ Yellow-Green**	
Albizia jülibrissin *Cercis siliquastrum* *Malus floribunda* *Prunus cerasifera* *Prunus serrulata "Kanzan"*	*Acer campestre* *Acer negundo* *Acer pseudoplatanus* *Ailanthus altissima* *Carpinus betulus* *Celtis australis*	*Gleditsia triacanthos* *Koelreuteria paniculata* *Morus nigra* *Quercus cerris* *Quercus palustris* *Quercus robur*
Red	**Lilac-Purple**	**Brown/ Brown-Yellow**
Acer saccharinum *Ulmus carpinifolia* *Alnus glutinosa*	*Paulownia tomentosa* *Rosmarinus officinalis* *Melia azaderach*	*Platanus orientalis* *Betula pendula* *Betula papyrifera*

Table 6. Flowering times of some taxa that can be used on urban roads

Taxa	January	February	March	April	May	June	July	August	September	October	November	December
Acer platanoides				■								
Acer pseudoplatanus					■							
Acer saccharinum L.			■									
Acer negundo				■								
Acer campestre					■							
Albizia jülibrissin						■	■	■				
Alnus glutinosa			■	■								
Ailanthus altissima					■	■						
Berberis thunbergii 'Atropurpurea'				■								
Betula pendula				■								
Betula papyrifera			■	■								
Carpinus betulus							■	■				
Cercis siliquastrum				■	■							
Celtis australis			■	■	■							
Crataegus monogyna				■								
Fraxinus angustifolia				■	■							
Fraxinus ornus					■	■						
Gleditsia triacanthos					■							
Hibiscus syriacus						■						
Koelreuteria paniculata					■	■						
Lagerstromia indica								■	■			
Laurus nobilis			■	■	■							
Ligustrum ovalifolium							■					
Ligustrum ionandrum							■	■				
Liriodendron tulipifera						■	■					
Malus folibunda				■								
Melia azaderach				■	■							
Magnolia grandiflora						■	■	■				
Morus nigra					■	■						
Nerium oelander						■						
Paulownia tomentosa		■	■									
Platanus orientalis				■								
Prunus cerasifera			■	■								
Prunus serrulata "Kanzan"				■	■							
Robinia pseudoacacia'Umbraculifera'				■	■	■						
Robinia pseudoacacia				■	■	■						
Rosmarinus officinalis			■	■	■	■	■	■				
Rhus typhina					■	■	■					
Salix alba				■	■							
Sophora japonica							■	■				
Syringa vulgaris				■	■							
Quercus robur								■	■			
Ouercus cerris								■	■			
Quercus palustris								■	■			
Tilia tomentosa						■	■					
Ulmus carpinifolia			■									
Viburnum tinus			■	■	■							

An evaluation of the taxa that can be used in road planting according to their leaf colors and autumn coloration reveals that the majority of them have Green/Light Green tones with 29 taxa. While 20 taxa, mainly coniferous species, are dark green, 5 taxa have Blue / Bluish-green leaves and 2 taxa have red leaves (Table 7). It is possible to observe that 34 taxa create an autumn color effect with the leaves turning yellow, red, and orange in autumn (Table 8). The use of coniferous species, which contrast with other plants with their dark green colors, helps to highlight the road and provides guidance. Taxa with dark green leaves such as *Pinus nigra* and *x Cupressoscyparis leylandii* can be used as buffer strips around car parks and in central refuges. Taxa such as *Acer saccharinum*, a medium-sized tree with beautiful red coloration in autumn, can be used on narrow avenues and streets, and on medium-wide avenues, taxa such as *Fraxinus excelsior and Celtis australis, etc.,* which exhibit a decorative appearance with their yellow color and of which leaves do not fall for a long time, can be used. On the other hand, the *Tilia tomentosa* taxon is advantageous for road planting with its silvery underside of leaves and yellow leaf coloration in autumn. Again, the *Quercus palustiris* taxon is effective in autumn with its red color, but it is also preferred for its rapid growth in the first years (Ürgenç, 1998; Aklıbaşında and Erdoğan, 2016).

Table 7. Leaf colors of some taxa that can be used on urban roads

Green/Light Green		
Acer negundo *Acer platanoides* *Acer pseudoplatanus* *Acer saccharinum* *Albizia jülibrissin* *Betula pendula* *Cercis siliquastrum* *Gleditsia triacanthos* *Malus floribunda* *Paulownia tomentosa* *Platanus orientalis*	*Cupressus sempervirens* *Ligustrum ionandrum* *Ligustrum ovalifolium* *Morus nigra* *Prunus serrulata "Kanzan"* *Quercus robur* *Rhus typhina* *Robinia pseudoacacia* *"Umbraculifera"* *Hibiscus syriacus*	*Sophora japonica* *Syringa vulgaris* *Tilia tomentosa* *Ulmus carpinifolia* *Betula papyrifera* *Carpinus betulus* *Koelreuteria paniculata* *Lagerstromia indica* *Robinia pseudoacacia* *Liriodendron tulipifera*
Dark Green		**Blue/Blue-Green**
Abies nordmanniana *Acer campestre* *Alnus glutinosa* *Ailanthus altissima* *xCupressoscyparis leylandii* *Crataegus monogyna* *Laurus nobilis* *Melia azaderach* *Pinus pinea* *Viburnum tinus*	*Fraxinus ornus* *Pinus nigra* *Fraxinus angustifolia* *Picea abies* *Ouercus cerris* *Quercus palustris* *Magnolia grandiflora* *Nerium oleander* *Picea orientalis* *Acer campestre*	*Cedrus atlantica* *Cupressus arizonica* *Juniperus horizantalis* *Juniperus sabina* *Picea pungens*
Red	**Green/Grey-Green**	**Green/Yellow-Green**
Berberis thunbergii 'Atropurpurea' *Prunus cerasifera*	*Celtis australis* *Salix alba*	*Cupressus macrocarpa 'Goldcrest'*

Table 8. Taxa with effective autumn coloration that can be used on urban roads

Autumn Coloration Effective Taxa		
Acer platanoides	*Berberis thunbergii*	*Gleditsia triacanthos*
Acer pseudoplatanus	*'Atropurpurea'*	*Fraxinus ornus*
Acer saccharinum	*Betula pendula*	*Catalpa bignonioides*
Acer negundo	*Betula papyrifera*	*Koelreuteria paniculata*
Acer campestre	*Carpinus betulus*	*Lagerstromia indica*
Alnus glutinosa	*Fraxinus angustifolia*	*Liriodendron tulipifera*
Celtis australis	*Malus folibunda*	*Malus folibunda*
Melia azaderach	*Prunus cerasifera*	*Paulownia tomentosa*
Rhus typhina	*Sophora japonica*	*Tilia tomentosa*
Morus nigra	*Quercus robur*	*Ulmus carpinifolia*
Salix alba	*Ouercus cerris*	*Quercus palustris*
Ailanthus altissima	*Robinia pseudoacacia*	*binia pseudoacacia "Umbraculifera"*

CONCLUSION

Roads, which are one of the transportation networks of cities, also constitute an important part of open and green areas, and it is important to design them in a way that offers aesthetic beauty. The fact that the designs to be made on the roads will make both the people of the city and the visitors feel positive emotions by presenting effective views and contribute to the image of the city is due to the effective planting of the roads. Road planting should be such that it does not obstruct the view of pedestrians and drivers and do not distract them. While form, texture, color, etc. of plants in design add an aesthetic value to the roads with their features and appearance during the transitions of the seasons, they also provide vitality and movement by allowing drivers and pedestrians to get closer to nature. Whether the plant material to be used on the roads is coniferous or leafy taxa, care should be taken to ensure that it is in a suitable harmony in terms of form, texture, and color. While taxa with round and scattered forms provide shadow effect on the roadsides, taxa that are effective with their form and color features are preferred to emphasize in refuges and intersections. Coniferous taxa with dark green leaves and coarse texture are used to create a background. Moreover, aesthetically effective views will be created with the designs to be made by taking into account the flowering and autumn coloration of coniferous and leafy taxa in different periods along the road.

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IMPACT OF THE CLIMATIC CONDITIONS OF MERSIN PROVINCE ON THE CULTIVATION OF MUNG BEAN (Vigna radiata (L.) WILCZEK)

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ABSTRACT

Mung bean *Vigna radiata* (L.) Wilczek is an edible legume plant used as a grain in human nutrition and its green parts and straw in animal nutrition. Mung bean belongs to the family of Fabaceae and it is a tropical legume species. It is widely cultivated in Asian countries, and its seeds are quite extensive in lysine and protein. In addition to its nutritional importance, this plant is also important with its drought resistance. This drought-resistant plant is not cultivated in cities with a hot climate like Mersin. In this study, mung beans were successfully grown in Mersin. The total yield of the plant, the number of pods per plant, the number of seeds per pod, the grain yield per plant were analyzed. The data obtained were compared with mung beans grown in other cities and countries which have warm climates. The research was carried out in the climatic conditions of Mersin province in 2021. In case of possible drought, this plant may need to be planted, and it is thought that the data obtained from this study will be necessary for this reason.

Keywords: Cultivation, Mersin, mung bean, yield

INTRODUCTION

Legumes are frequently preferred by a large part of the population and producers due to their high protein content and high energy consumption (IITA, 2016). Mung bean, which is among the legumes, is a valuable plant with high protein content (20-25%), rich in various vitamins (A, B1, B2, C), oil, and minerals, and has a very important place in human nutrition (Prabhavat, 1987). Mung bean is a warm climate plant, small-seeded, hairy, erect or semi-erect, creeping, herbaceous, annual (Şehirali, 1988). About 80% of the amount produced is of South Asian origin (Tomooka et al., 1992). To date, mung beans have been grown in many countries by adapting to different climatic conditions since the production cost is low. Although the crop growing period is short, it can be easily adapted to a wide variety of climatic conditions and

different soils. In many regions, conditions throughout the seasons are generally suitable for growing mung beans (Pratap et al., 2013). It can be grown successfully even in hot summer months and dry conditions (Sekhon, 2008). In addition, the low phytic acid concentration has an extra advantage over other legume-focused diets (Kataria et al., 1989). They can bind the free nitrogen of the air to the soil through the nodules formed by the rhizobium bacteria in their roots (Ahmed et al., 2006). Although it is an important food source, the interest of Turkish farmers in agriculture in Turkey has decreased due to the high costs of inputs such as fertilizer, energy, and seeds. According to TÜIK (2013), there is a significant decrease in pulses cultivation areas. Although mung bean production cannot be carried out very effectively in our country, the need rate may increase with the population growth rate in the following periods. In this case, it is important to effectively cultivate mung beans in our country to meet the sufficient level of product and it has the potential to be a good option for the growers. Various studies have been carried out in Turkey to determine the various morphological characters of mung beans (Toker et al., 2002; Çancı and Toker, 2005; Çancı and Toker, 2014). In this study, it was aimed to evaluate mung beans in terms of yield characteristics in Mersin, the Mediterranean Region of Turkey.

MATERIAL AND METHOD

The seeds of the mung bean were provided from the Sta Quality Control Food Laboratory (http://stalab.com.tr/), Mersin, Turkey (Figure 1). The field experiment was conducted in the field of Mersin, Turkey in 2021. The fresh and healthy seeds of mungbean (*Vigna radiata* L. Wilczek) were used in the present study.

Figure 1. The seeds of mungbean were provided from Sta Lab. Mersin, Turkey

The seeds were cleaned manually to remove all foreign matter such as dust, dirt, stones, and chaff as well as immature, broken grains. The field experiment was arranged in a randomized complete block design with three replications. Seeds were sown manually keeping a distance of 45 cm between rows. The growing plants were fertilized weekly with various fertilizers. Days to emergence (E), days to first flowering (FF), pods number per plant (PP), seeds per pod (SP), were determined in the study. Plants were harvested at maturity and 5 plants were randomly selected. The number of pods per plant and seeds per pod was counted.

RESULTS AND DISCUSSION

The field experiments were conducted one season Mersin in Turkey to identify for climatical conditions of Mersin on mungbean production. It was observed that the seeds planted in the soil started to germinate in the first week. The plant showed a stunted type of development (Figure 2).

Figure 2. The view of the plants and flower structure from the field

Mung bean is self-fertile and its fruits are long, brown, bronze, or black in maturity. Pods are oblique or drooping, long, hairy, grains are long cylindrical. Seeds are green, brown, black stained, drought-resistant (Bozoğlu and Topal, 2005). It has been reported that there is a high variation in mung bean in terms of growth, phenology, yield characteristics, and grain yield (Yimram et al., 2009). When the morphological appearances of the pods obtained as a result of the study were examined, it was seen that the pod color was green (Figure 3-A) when fresh and close to black when dried (Figure 3-B).

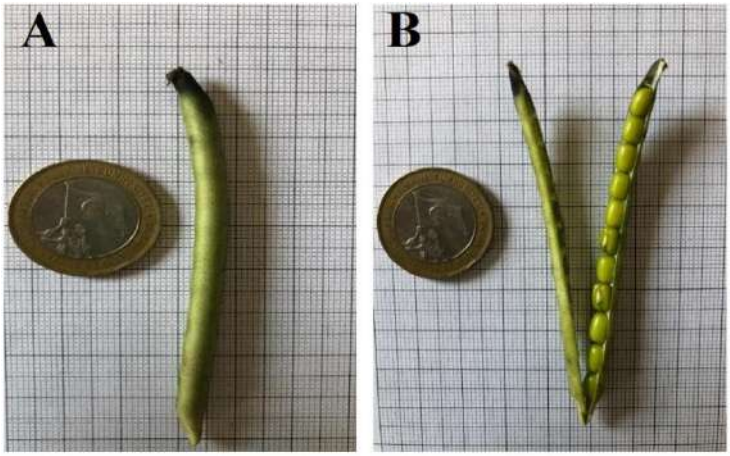

Figure 3. The view of the developed mung bean pod (A) closed and (B) open

The experiment was carried out during March-May 2021. This preliminary experiment indicated in terms of days to emergence (15 days), days to first flowering (57.3 days) as shown in Table 1. The yield data were obtained was using 5 randomly selected plants in each replicate for the number of pods per plant and, the number of seeds per pod.

Table 1. The days to emergence, first flowering, number of pods per plant, and seeds per pod of mungbean

Days to emergence (E)	15 (day)	Pods per plant(PP)	12.2 (pod/plant)
Days to first flowering (FF)	57.3 (days)	Seeds per plant(SP)	9.17 (seed/pod)

The yield of mung beans measured in the study was the number of pods per plant and the number of seeds per pod. The seed yield is governed by many genetic factors as well as environmental factors that are interdependent (Huseyin and Cengiz, 2014). In addition, plant density affects plant development and grain yield in mung beans (Jahan and Abdul, 2004). The grain yield per unit area is a function of the yield of individual plants and the population density. Both the yield and yield attributes are markedly influenced by the population density (Jahan and Abdul, 2004). The increase of biological yield has beneficial for grain yield at a certain limit. In this study, the average number of pods was 12.2 per plant, and the seeds per plant were 9.17. The data obtained were compared with mung beans grown in other cities and countries which have warm climates. Gebeloglu et al. (1992) carried out a study for the adaptation of mung beans in Tokat conditions. The researchers repeated the study in 3 different periods as spring, summer, and autumn, but in that study, although all of the sown seeds germinated, the seedlings died when the temperature dropped. On the other hand, they received successful

results from the study conducted in June. They found that the number of pods in the plant was 11.93-35.20 and the number of seeds in the pod was 9.13-13.53. As the average of the varieties, they found that the time to flowering occurs between 54.33 and 64.33 days. In a study conducted for 2 years to determine the phenological, morphological, and agronomic characters of 19 mung bean cultivars in Antalya ecological conditions, the researcher reported that the starting time of flowering is 46-71 days, the number of seeds is 6-11 pods, and the number of pods is 12-42 per plant (Toker et al. 2002). In other studies on the same subject, it was determined that the number of pods varied between 10.0-103.0 units/plant (Paroda and Thomas 1988, Toker et al. 2002). In a similar study, it was determined that the flowering period of mung bean genotypes varied between 46-71 days (Toker et al. 2002). These results are in parallel with the results presented in this study.

CONCLUSIONS

Turkey has rich ecological and biological diversity due to its location. This rich ecology, in which almost every climate type is observed, allows many crops to be grown. From this point of view, it is necessary to emphasize the importance of mung beans, which are grown in different parts of the world and locally grown in small areas in our country, in addition to known edible legumes. In the present study, four quantitative characters were evaluated. Despite the increasing temperature conditions in Mersin, high yields were obtained from mung bean and its cultivation may increase in Mersin. In case of possible drought, this plant may need to be planted, and it is thought that the data obtained from this study will be necessary for this reason. However, mung bean is a resource for research in many fields, not only in terms of crop yield but also in terms of nitrogen fixation.

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EFFECT OF HIGH TEMPERATURES ON THE ADAPTATION OF CEDRUS ATLANTICA

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ABSTRACT

Climate change in the Mediterranean region is the cause of physiognomic variations in forest ecosystems. This pathology manifests itself in the form of the thermal constraints that trees undergo in their natural range, affecting their growth and productivity. The most disastrous consequences are the risk of displacement of their biogeographical ranges. This work focuses on the adaptation capacities of Atlas cedar (*Cedrus atlantica*) to thermal variability using biochemical markers (proline, soluble sugars and chlorophyll). Seedlings, raised in a nursery, were subjected to a short-term stress of 3 hours at a temperature range between 35°C and 50°C. Soluble sugars are closely related, with a decrease in their content in stressed seedlings. Proline accumulation is positively correlated with warm temperatures and reaches its maximum at 50°C. High temperatures induce an increase in chlorophyll (a) at 40°C (21.66%), while a decrease from 45°C onwards of 25% is obtained. The most accumulative organs are the roots for proline and the leaves for sugars. The accumulated quantities could be linked to the level of tolerance to thermal stress, contributing to the maintenance of cell turgidity, created by the osmotic adjustment for which proline and sugars are responsible.

Keywords: *Cedrus atlantica*, high temperatures, proline, soluble sugars, chlorophyll.

INTRODUCTION

Current climate change is a complex phenomenon in the Mediterranean region (Rossello et al., 2016): summer is warming up faster than winter (+0.5°C vs. +0.2°C per decade), and for each of these seasons, daily minimum temperatures (+0.7 and +0.3°C) are increasing faster than maximum temperatures (+0.25 and +0.15°C). These differences accentuate the negative effects on vegetation. The already high summer stress gradually becomes unbearable for many species. But the faster increase in minimum temperatures is not good news either. In summer, the very hot nights and the lack of dew do not allow the vegetation to rehydrate between two hot days. In late autumn and winter, less cold leads to a late halt in plant growth, and an early recovery at the end of winter, or even no halt at all for some species (Vennetier et al., 2011).

As a result, there is already an increase in the risk of frost damage during the rare episodes of intense cold that are unavoidable, even if they are very short: on the one hand at altitude], on the other hand in the lower Mediterranean zone (Vennetier et al., 2018). A warmer winter also means less snow on the Mediterranean mountains (Spandre et al., 2019), which means more runoff and less water reserves in the soil, thus counteracting the positive effect of an advanced growth start (Zhang et al., 2019). The earlier use of the soil water reserve, and a high water requirement due to higher temperatures in spring, lead to a rapid depletion of this reserve. Both

phenomena increase the duration and intensity of the summer drought, especially as summer rainfall tends to decrease.

Future climate projections indicate that the Mediterranean climate is likely to gain the equivalent of 25-50% of its current area northwards under the RCP4.5 and RCP8.5 scenarios respectively (Barredo et al., 2018), in areas where vegetation is not adapted to summer drought, but also to lose up to 16% of this area on the south side to arid and desertified areas. In practice, climate change is already having effects, mostly negative, on most of the physical and biological components of Mediterranean forests.

From a thermal point of view, the ranges given are between a rise of 0 and 2 to 2.5°C over about 30 years. Mediterranean forest ecosystems are composed of sclerophyllous and deciduous trees as well as conifers, including endemic and emblematic species such as the Atlas cedar "*Cedrus atlantica*". The distribution areas of these species have varied significantly over the last 20 millennia in relation to global climate change. Thus, the cedar is confronted with heat stress and drought which may affect the behaviour of the species, as changes in morphology, physiology and metabolism are observed at the level of the whole plant during heat stress.

MATERIAL AND METHODS

Our study was carried out on Atlas cedar (*Cedrus atlantica*) seedlings raised in a nursery. The one-year-old seedlings were subjected to high temperatures (35°C, 40°C, 45°C and 50°C) for a period of 3 hours. Biochemical analyses (soluble sugars, proline and chlorophylls) were performed on Atlas cedar seedling organs.

Extraction and estimation of total soluble sugars

The sugar content in the extracts normally was analyzed by a phenol-sulfuric acid method (Dubois et al., 1956). This colorimetric method determines only the amount of total sugars. This is the most widely used colorimetric method to date for determination of carbohydrate concentration in aqueous solutions. The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible color. The standard procedure of this method is as follows. A 2 ml aliquot of a carbohydrate solution is mixed with 1 ml of 5% aqueous solution of phenol in a test tube. Subsequently, 5 ml of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 2 ml aliquot of carbohydrate is replaced by DDI water. The phenol used in this procedure was redistilled and 5% phenol in water (v/v) was prepared immediately before the measurements.

Proline method

The method used is that of Troll and Lindsley (1955). Each sample collected (100 mg of the vegetal substance), is immediately weighed, and then put in a test tube. A 2 ml volume of methanol at 40% is added to the sample, and then the whole is heated at 85°C in a double boiler for 1h at 85°C. After cooling 1 ml of the extraction solution is added to 2 ml of acetic acid, 25 mg of ninhydrine and 1 ml of mixture distilled water- acetic acid- orthophosphoric acid of density 1.7 (120, 300, 80,: v/v/v). The whole is heated up to boiling point during 30mn in a double boiler at 100°C, then let to cool down and added 5 ml of toluene. After agitation in

Vortex, a pinch of sodium sulphates (Na_2 SO_4) is added in each tube. The reading of the optic density is done at 528 nm.

Determination of photosynthetic pigments

The determination of chlorophylls is based on the method of Millerioux (1975). 0.1 g of fresh plant material taken at the heading stage was ground dry and then in 10 ml of 80% acetone. The grindings were centrifuged at 3000 rpm for 15 min. The supernatant containing the pigments was recovered. The optical densities are read at wavelengths 663, 647 nm for chlorophylls a and b respectively. The chlorophyll content is determined according to the equations of Lichtenthaler (1987):

- Chl a = 12.25 DO (663) – 2.7 DO (647) (µg/ml).
- Chl b = 21.5 DO (647) – 4.7 DO (663) (µg/ml).
- Chl (a+b) = 7.15 DO (663) + 18.71 DO (647) (µg/ml).

RESULTS AND DISCUSSION

The results obtained indicate that there is an evolution of the content of soluble sugars in the different organs subjected to high temperatures during a 3h period. This evolution is more important from 45°C onwards in the needles than in the stems and roots. The highest content is obtained in the needles at 40°C and the lowest in the roots at 35°C. The analysis of variance with 2 classification criteria shows that there is a significant difference between organs and between temperatures (p=0.001). The results obtained show that the highest content of soluble sugars is found at 40°C for the temperatures and at the level of the needles; whereas the lowest content is recorded at the level of the roots (Tab. 1).

Table1. Newman-Keuls test at 5% test.

Temperature	Groups	Organs	Groups
40°C	986.03	Needles	857.96a
45°C	888.11	Stems	681.70b
35°C	828.09	Roots	577.89c
50°C	729.62		

The behavior of Atlas cedar seedlings stressed at high temperatures shows a very significant increase in the amounts of soluble sugars in the different organs depending on the intensity of the stress with a relatively higher accumulation in the needles. It has long been known that the level of sugars increases considerably in plants subjected to different types of stress; indeed, this was verified by Rached-Kanouni et al. (2012) in adult *Eucalyptus microtheca* under different water stresses. The presence of these soluble sugars in periods of heat and drought would protect thylakoids from irreversible membrane damage and would exert a favorable action on protoplasmic resistance to drought. Gibson et al. (1994) state that temperature, associated with insolation, could be a determining factor for the accumulation of sugars.

Concerning chlorophyll, the results obtained show that the levels of chlorophyll (a) and (b) vary according to temperature. The measurements show that the highest values are obtained at 35°C and 40°C for both chlorophylls. It can be seen that the levels of chlorophyll (b) are lower than those of chlorophyll (a). As a result, the lowest levels are recorded at 50°C. This is confirmed by the 2-criteria analysis of variance, which shows a highly significant difference according to temperature ($p<0.001$) (Tab. 2). The comparison of the chlorophyll means (a) and (b) by the Newman and Keuls test at 5% reveals two groups, where we note the assignment of 35°C and 40°C treatments to group (A) and 45°C and 50°C temperatures to group (B).

Table 2. Newman-Keuls test at 5% test.

Temperature	Groups (Ch.a)	Temperature	Groups (Ch.b)
35°C	13.007	35°C	1.534
40°C	12.637	40°C	1.490
45°C	10.084	45°C	1.189
50°C	6.555	50°C	0.773

Chlorophyll is very important in the process of photosynthesis. Indeed, it is a catalyst for reactions. Temperature affects photosynthesis in the short and long term, and plants can, to varying degrees, acclimatize to changes, and this acclimatization can lead to a decrease in photosynthesis (Cornic, 2007). The modification of the composition and content of pigments would therefore be a character of adaptation to the environment (Foyer et al., 2002). Faced with sub-optimal temperatures, plants reduce their chlorophyll a and b content and accumulate zeaxanthin and antheraxanthin (Haldimann, 1999).

Proline levels show fluctuations at the organ level and between temperatures. The temperature of 50°C shows the highest levels in the different organs, while the lowest values are recorded at 35°C. These results are statistically confirmed (Tab. 3).

Table 3. Newman-Keuls test at 5% test.

Temperature	Groups	Organs	Groups
35°C	1.19b	Roots	4.86a
40°C	1.23b	Needles	3.61b
45°C	2.35a	Stems	2.90c
50°C	3.67a		

The behaviour of Atlas cedar seedlings stressed at different levels of high temperature shows a very significant increase in the amounts of free proline in the different organs with the intensity and duration of stress. Indeed, they are low under normal conditions; whereas they are higher in the different organs of seedlings transferred to high temperatures, with a relatively higher accumulation in the roots. Many studies report that proline accumulates in the plant under

unfavourable conditions (Sivaramakrishnan et al., 1988), which reflects the character of stress resistance (Greenway and Munns, 1980). Zerrad (2006), suggests that proline at high concentrations acts as a solute for osmotic adjustment, and also to serve as a reservoir of nitrogenous compounds and carbon for subsequent use in growth.

CONCLUSION

The effect of high temperatures) is very highly significant for the biochemical parameters. Our results show that Atlas cedar seedlings are able to accumulate different osmolytes like proline, soluble sugars in different organs in response to heat stress. The accumulation of proline was well marked in the roots at temperatures of 45°C and 50°C indicating the resilience of the cedar to thermal stress. This accumulation is preferential in the roots. Osmoregulators such as proline and soluble sugars play an important role in osmotic adjustment and stabilisation of plant cells.

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MEANS OF ACQUIRING NATURAL COLORING AGENT FROM Vitis labrusca L.

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ABSTRACT

Color, which is among the appearance features of food, comes forth as a substantial factor determining the preferences of the consumers. Artificial and natural coloring are utilized to ensure the stability of the color of the food. Anthocyanins refer to natural coloring agents commonly used in foods. Apart from adding color, anthocyanins increase the oxidative stability of the foods containing them to due to their antioxidant properties. In this study, anthocyanin-based extracts of *Vitis labrusca* L. fruit have been prepared, commonly named as the fox grape growing naturally in the Black Sea Region of Turkey by utilizing two different solutions (distilled water and acidized ethanol) and two different extraction method (conventional and ultrasonic). Certain physical and chemical properties, chemical compound and mineral substance profile of the prepared extracts have been determined. As a result of the analysis, vitamin C revealed an increase in extracts compared to fruit, while total phenolic content increased in other extracts except for the extract acquired by using the water solution with the conventional extraction. While total monomeric anthocyanin indicates a decrease in the water extracts, an increase has been revealed in the acetic acid extracts. As a result of the statistical evaluation performed, it was identified that the effect of extraction method and solvent type on the amount of vitamin C, total phenolic content and total monomeric anthocyanin were very significant ($p<0.01$). The method had a significant effect ($p<0.01$) on the antioxidant properties. A total of 45 compounds have been identified in the fruit and the extracts there of. 1-isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3α-ol, the compound found with the highest rate (20.97%) has been identified in the extract acquired by using the water solution with the conventional extraction. A total of 16 elements have been identified in the mineral composition analysis. Potassium mineral with the highest amount has been identified in both fruit and extracts thereof. While a decrease has been observed in the extracts in comparison with the fruit in the entire minerals identified, the amount of potassium mineral revealed an increase only in the extract acquired with water applying the conventional method.

Keywords: *Vitis labrusca* L., Anthocyanin-based extract, Ultrasonic extraction, Conventional extraction,

INTRODUCTION

Fragrant grape (Isabella grape, *Vitis labrusca* L.) is a grape variety resistant to fungal diseases that grows naturally in humid and cold climates of Turkey, America, England, Russia, Brazil, Canada, Germany and Japan. In Turkey, it is consumed both fresh and used in the production of a local dessert called molasses and pepeçura (Çelik 2011; Ertaş and Karadağ, 2013; Çelik et al. 2013). B.C. IV. In the XIX century, there were large and fertile fragrant vineyards in Trabzon and even wine was sent to Italy, but in the 19th century. It is reported that these vineyards were uprooted in the 19th century in order to increase tobacco cultivation areas (Çelik 2011). *V. labrusca* L. izabella is known as fragrant black grape, strawberry grape, black grape or American grape (Ekbiç et al. 2015). Its varieties are called Bordeaux, Izabella and Concord (Lima et al. 2014). Bordeaux and Izabella varieties are widely used in wine and fruit juice production in the United States (Scola et al. 2011). It has a unique aroma with a thick crust and seeds, and it is easily separated from the interior as the crust part is slippery (Yılmaz and Çelik 2005). It blooms in May-June (Çelik 2011) and fruit is harvested in September-October (Kurt 2015).

The skin constitutes 50% of the weight of the Isabella grape. The content of the waxy layer covering the fruit peel consists of 50-70% oleanolic acid. It has been determined that the majority of polyphenols and especially anthocyanins in the fruit composition are in the residue after fruit juice production and wine production (Toaldo et al., 2013). Anthocyanidin group compounds (delphinidin, petunidin, malvidin, cyanidin and peonidin) form the red and black colors of the fruit (Gülcü et al. 2008). *V. labrusca* anthocyanins consist of diglucosides formed by a molecule of glucose attached to the third and fifth carbons (Söylemezoğlu 2003). It has been reported that the dominant anthocyanin of the fruit is malvidin 3,5-diglucoside (Granato et al., 2015).

It has been stated that the polyphenolic compounds in red grape juice, by showing antioxidant properties, block free radicals and prevent the accumulation of fats in the body by oxidation (Toaldo et al. 2015). Resveratrol, which is a polyphenolic compound, is found in high amounts in grape skin and this compound is a substance that protects plants with its antifungal properties and has antioxidant activity. Black grape skin contains more resveratrol than white grape skin. It is stated that resveratrol has properties such as reducing bad cholesterol in the body, enhancing memory, anti-inflammatory, relieving Alzheimer's disease and antioxidant (Güder 2012; Çelik 2014; Kuck and Noreña 2016).

Studies have shown that V. labrusca significantly reduces the progression of atherosclerosis, reduces oxidative stress and has neuroprotective effects in the brain, and fragrant grape concentrate is a product recommended to increase blood values and strengthen the immune system of cancer patients receiving chemotherapy, it has been reported that when healthy individuals consume 1 g/kg black grapes, the plasma antioxidant potential increases in the body at the 4th hour and it is a natural antioxidant source due to the resveratrol and anthocyanin content in its composition (Burak and Çimen 1999; Hort et al., 2012; Cardozo et al., 2013; Çelik, 2014; Silva et al., 2017).

In the literature review, there are studies in which the composition analyzes of the naturally grown V. labrusca fruit in Turkey were carried out (Aktaş, 2012; Eroğlu, 2012; Güder, 2012; Saltoğlu, 2014; Yüksel, 2014; Üneş, 2016; Demirkol, 2016) and it has been seen that the resources on the evaluation possibilities of this fruit are limited (Güder, 2012; Güder et al., 2014; Ateş, 2017). The fruit peel is used to make cookies by turning into flour (Abreu et al. 2019), the extract powder of the Concord grape variety is added to bread and extruded products to obtain functional products (Caba, 2015), and the fruit is added to yogurt made with goat milk due to its high anthocyanin and resveratrol content. (Silva et al. 2017). The above-mentioned studies have been carried out on the determination and evaluation possibilities of the *V.*

labrusca fruit. However, no study has been found regarding the evaluation of anthocyanins of *V. labrusca* fruit as a natural colorant.

MATERIALS AND METHODS

Material

Fragrant grape fruit was obtained from Turkey Trabzon Province Dernekpazarı in October. Figure 1 shows the Fragrant grape tree and its fruit. The fruits were kept at -20 ºC until used in the analysis. The characteristics of the fruit to be used as material are given in Table 1.

Table 1. Characteristics of fragrant grape fruit

	Fruit weight (g)	Sugar (%)	Dry matter (%)	Ash (%)	Protein (%)	Titratable acidity (%MA)
Fragrant grape	2.48±0.08	3.66±0.10	16.75±2.12	0.50±0.01	0.89±0.08	0.44±0.00

*MA: Malic acid

Method

Extraction

After the fruit is taken as a whole and homogenized with a blender, 50 g is weighed and ethanol acidified with 7% acetic acid: pure water (1:1) and pure water solvent and homogenized with ultraturrax (Heidolph Silent Crusher M, Germany) and ultrasonically extracted. For this purpose, it was extracted in an ultrasonic water bath (JSSB-30T, JSR, Korea) at 35°C for 2 hours at a frequency of 30 Khz. In order to prevent the temperature increase caused by vibration, the temperature was kept constant by constantly measuring and adding cold water. For classical extraction, 50 g is again weighed and 200 ml of ethanol acidified with 7% acetic acid:pure water (1:1) and 200 ml of distilled water solvent are separately homogenized with ultraturrax (Heidolph Silent Crusher M, Germany) in a shaking water bath. (JSSB-30T, JSR, Korea) were incubated at 35°C for 2 hours in a dark environment.

In both extraction methods, after these steps, the extract was filtered through 4-layer cheesecloth at the end of the period and then filtered through whatman No:1 filter paper. The obtained extract was evaporated with a rotary evaporator (Heidolph Laborota 4000 Efficient, Germany) at 60°C until the solvent was completely removed. The residue in the rotary evaporator balloon was washed with distilled water and taken into glass jars and made up to 60 ml with distilled water. The extracts were stored at -20°C until analysis and production.

Figure 1. The fragrant grape tree and its fruit (Trabzon in Turkey)

Meyve püresinin %7 asetik asit ile asitlendirilmiş etanol:saf su (1:1) ile ultrasonik ekstraksiyonla elde edilen ekstraktı ÜUA, meyve püresinin saf su ile ultrasonik ekstraksiyonla elde edilen ekstraktı ÜUS, olarak adlandırılmıştır. Meyve püresinin %7 asetik asit ile asitlendirilmiş etanol:saf su (1:1) ile klasik ekstraksiyonla elde edilen ekstraktı ÜKA, meyve püresinin saf su ile ultrasonik ekstraksiyonla elde edilen ekstraktı ÜKS, olarak adlandırılmıştır. Şekil 2'de klasik ve ultrasonik yöntemle meyve ve çekirdekten %7 asetik asit ile asitlendirilmiş etanol:saf su (1:1) ve saf su çözgenleriyle elde edilmiş olan ekstraktların fotoğrafları verilmiştir.

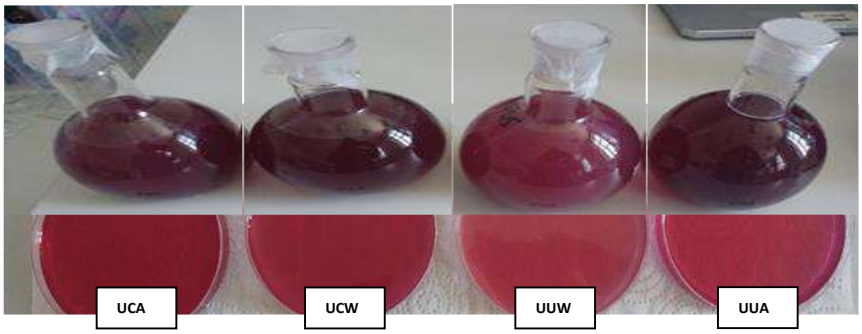

Figure 2. Extracts from fragrant grape fruit; UCA: ethanol acidified with 7% acetic acid of fruit puree : extract obtained by classical extraction with distilled water (1:1), UCW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUA: ethanol of fruit puree acidified with 7% acetic acid: extract obtained by ultrasonic extraction with purified water (1:1).

Physical and chemical analyzes

It was determined with a OHAUS Starter 3100 USA model pH-meter calibrated with buffer solutions (pH 4.00 and 7.00) (Cemeroğlu 1992).

Color density was measured with a minolta colorimeter (Chroma Meter, CR- 200, Japan) device based on three-dimensional color measurement in fruits and extracts. Color measurements of the samples were made on a white background. According to the CIELAB color scale, L* values are brightness (0=black, 100=white) (darkness/lightness), (Y) axis, a* value (X) axis is red in the range of +60 and 0, and green- (+a red, -a green), b* value (Z) are values showing the color intensities between +60 and 0, yellow in the range of +60 and -60, blue (+b yellow, -b blue) color intensities (Luo, 2006; Polatoğlu, 2013). Hue angle (Hº) and chroma values (C) were calculated using a* and b* color values. Hue value indicates 0º=bluish red, 90°=yellow, 180°=green, 270°=blue and 360°=red. The chroma value indicates the hue angle and the saturation or intensity of the central color (Wrolstad et al., 2005). C, H° and ΔE values were calculated using the following equations (Rosso and Mercadante, 2007). The ΔE value is defined as the difference between the initial color values and the final color values to measure color changes and is important in stored foods. If ΔE=1 there is no difference, ΔE>3 the color difference is very significant, 1.5<ΔE<3 different and 1.5<ΔE small difference (Pathare et al. 2013). ΔE>5 is observed as two different colors (Matsuo et al., 2019).

$$C=(a^2+b^2)^{1/2} \quad (1)$$

$$H°=\arctan(b/a) \quad (2)$$

$$\Delta E=\pm(\Delta L^2+\Delta a^2+\Delta b^2)^{1/2} \quad (3)$$

The amount of vitamin C was determined photometrically by using special test kits and RQ Flex Plus 10 (Merck, Germany) device developed to determine the amount of vitamin C in fruits and extracts (Ercisli and Orhan 2008).

For total phenolic content analysis and antioxidant analyses, 25 g of fragrant grapes and its extracts, which were turned into pulp, were directly weighed and mixed with 75 ml of 90% ethanol in a magnetic stirrer, in the dark and with the mouth closed for 6 hours. Then it was filtered through Whatman No.1 filter paper and the filtrate was evaporated at 50°C. The inside of the balloon was rinsed with distilled water and placed in 25 ml tubes and stored at -20°C until analysis.

The basis of the determination of total phenolic content is the conversion of phenolic compounds to the oxidized form by reducing the Folin-Ciocalteu reagent in basic medium (Singleton et al., 1999; Cemeroğlu, 2013). For the determination of total phenolic substance, 1 ml of the extracts was taken and 46 ml of distilled water and 1 ml of FCR (Folin-Ciocalteau-Reagent) were added. After waiting for 3 minutes, 3 ml of 2% saturated sodium carbonate (Na2CO3) solution was added and mixed with a magnetic stirrer for 2 hours in the dark. At the end of this period, the absorbance of the sample was measured in a spectrophotometer (T60V Spectrometer, PG Instruments) at a wavelength of 760 nm. The phenolic content of the samples was calculated as gallic acid equivalent (µg GAE/mg sample) by using the equation obtained from the graph prepared with the gallic acid standard (Gulcin et al. 2002).

Total monomeric anthocyanin determination was made spectrophotometrically using the pH differential method developed by Giusti and Wrolstad (2005). Absorbances were read at 510 nm and 700 nm wavelengths in pH 1.0 and pH 4.5 buffer solutions. The total amount of anthocyanin was calculated to be equivalent to milligram cyanidin-3-glycoside per gram extract.

TMA (mg/L)= A(MW)(DF)*1000/(ε)(L) (1)

TMA: Total monomeric anthocyanin amount
A: Absorbance difference (measured absorbance difference at pH 1.0 and 4.5)
A= (A510-A700)pH1 - (A510-A700)pH4.5
MA: 449.2 (molecular weight of cyanidin 3-glycoside)
DF: Dilution factor
ε: Molar absorbtivity
L: Layer thickness of the absorbance measuring cuvette (1 cm)

The prepared sample extracts were transferred to test tubes with a concentration of 10, 20, 30 µg/µl in 3 parallels, and the final volume was completed with ethanol as 3 ml. Finally, 1 ml of DPPH• radical removal solution prepared in 1 Mm ethanol was added and mixed with vortex (WiseMix, VM-10) and after incubation for 30 minutes in the dark, reduction/lightening was measured in a spectrophotometer (T60V Spectrometer, PG Instruments) at a wavelength of 517 nm. (Sonboli 2015). The control sample was prepared by adding 3 ml of ethanol and 1 ml of DPPH• solution. Trolox were used as standard antioxidants. The percent inhibition of the DPPH• radical scavenging activity reaction was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_{DPPH} - A_{SAMPLE})/A_{DPPH}] \times 100$$

A_{DPPH}: Absorbance of the DPPH blank sample
A_{SAMPLE}: Absorbance of the sample

The sample extracts were transferred to test tubes with a concentration of 10, 20, 30 µg/µl in 3 parallels, and ethanol was added so that the final volume was 1.5 ml. Finally, 0.5 ml ABTS•+ radical solution was added and mixed with vortex and kept in the dark for 30 minutes. After incubation, reduction was measured spectrophotometrically at a wavelength of 734 nm. The control was prepared by adding 1.5 ml of ethanol and 0.5 ml of ABTS•+ solution. The ABTS•+

radical scavenging activity reaction inhibition percentage was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_{ABTS} - A_{SAMPLE}) / A_{ABTS}] \times 100$$

A_{ABTS}: Absorbance of the ABTS blank sample
A_{SAMPLE}: Absorbance of the sample

The concentration that caused 50% inhibition of the radical was determined by linear regression from the graph drawn with the % inhibition values calculated against different concentrations of antioxidants, and the results were expressed as IC50 (µg/ml) (Yılmaz 2011; Bektas et al. 2016; Bardakçı 2017). There is an inverse correlation between IC_{50} value and antioxidant activity. A low calculated IC_{50} value indicates high antioxidant activity, and a high IC_{50} value indicates low antioxidant activity (Karataş, 2014).

Chemical compounds analyzes

The chemical compounds were detected using an Agilent 7820A gas chromatography device, a 5977 mass spectroscopy detector, a 7673 series autosampler and ChemStation (Agilent Technologies, Palo Alto, CA) software. The fragrant grape fruit and its extracts were weighed 5 g and vortexed (Heidolph Reax Top, D-91126 Schwabach, Germany) with 50 mL of methanol to obtain a homogeneous mixture and dissolved in a magnetic stirrer (Orbital Shaker SSL1, UK) for 12 hours. After mixing and thawing, samples were filtered with Whatman filter paper and put into 1.5 mL glass vials through a 0.22 µm micro filter through disposable syringes. The compounds were separated on an HP-5 MS column with a film thickness of 0.25 µm (30 m x 0.25 mm inner diameter, USA) using 1 µl splitless injection mode with 1 ml / minute flow rate and 70 eV ionization energy using helium as the carrier gas. The identification of chemical compounds was carried out using the National Institute of Standards and Technology (NIST) gas chromatography–mass spectrometry (GC-MS) library and reference standard substances.

Mineral substance composition analyzes

The mineral profiles of the fragrant grape fruit and its extracts were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using an Agilent 7800. Initially, the samples were subjected to wet burning. In a microwave-based system (Milestone connect ETHOS UP microwave) in a wet burning process, in a total of 30 minutes for 15 minutes in 2 steps; It has been done with the condition of applying 200 °C and 1800 W at each step. As a result of the wet burning process, teflon tube contents were completed to 50 ml with ultrapure water and the tube contents were filtered using a 0.45 µm membrane filter. The samples were taken from 50 ml of 100 µl and completed to 10 ml with a mixture of 2% HNO_3 and 0.5% HCl and analyzed with ICP-MS. Using the calibration curve against the standards obtained with ICP-MS, the amounts of the sodium (Na), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), potassium (K), zinc (Zn), selenium (Se), copper (Cu) and aluminum (Al) elements in the samples were determined (NMKL NordVal International, 2007)**.**

Statistical analysis

In the research, the analyzes were carried out on samples with 3 replications. All data were evaluated statistically with IBM SPSS Statistics Version 20.0 package program.

RESULTS AND DISCUSSION

As a result of the analysis, pH, L*, a*, b*, C, Hº and ΔE values of fragrant grapes and their extracts were determined and given in Table 2. As a result of the statistical analysis, it was determined that the effect of the method applied on the pH, L*, a*, b*, C, Hº and ΔE values was insignificant ($p > 0.05$). It was determined that the effect of the solvent used on pH, a*, C, Hº and ΔE values was very important ($p< 0.01$) (Table 2). The pH of fragrant grape fruit and extracts differed significantly according to the solvent used ($p < 0.01$). The pH was found to be lower in the extracts compared to the fruit (Table 2). When the a* value, which is the color value that determines redness, is examined, it was determined that the values of the UCW and UUW extracts were the highest. Again, when the C values are examined, it is seen that these two extracts have more vivid colors and when the Hº value is considered, the sample closest to the red axis is UUW (Table 2). It was determined that the ΔE value calculated according to the fruit was very different from the fruit color of the UUW extract and there was a significant color difference in the other extracts (Table 2).

Table 2. Some physical and chemical properties of fragrant grapes and its extracts

	Fragrant grape	UCA	UCW	UUA	UUW	Solvent	Method
pH	3.20±0.00^{a}	2.92±0.01^{c}	2.93±0.02^{c}	2.87±0.00^{d}	3.01±0.00^{b}	**	ns
L*	23.67±1.40^{a}	22.88±0.81^{a}	23.01±0.60^{a}	22.13±0.57^{a}	23.79±0.66^{a}	ns	ns
a*	2.80±0.14^{d}	5.49±0.28^{b}	7.17±0.34^{a}	4.88±0.28^{c}	7.71±0.40^{a}	**	ns
b*	1.40±0.17^{b}	2.21±0.14^{a}	2.28±0.17^{a}	1.99±0.23^{ab}	2.00±0.67^{ab}	ns	ns
C	3.13±0.20^{d}	5.92±0.30^{b}	7.53±0.27^{a}	5.27±0.31^{c}	7.98±0,44^{a}	**	ns
Hº	26.42±1.78^{a}	21.91±0.60^{ab}	17.66±2.04^{bc}	22.18±2.01^{ab}	14.51±4.68^{c}	**	ns
ΔE	-	3.31±0.43^{b}	4.79±0.10^{a}	3.15±0.45^{b}	5.16±0.44^{a}	**	ns

UCA: ethanol acidified with 7% acetic acid of fruit puree: extract obtained by classical extraction with distilled water (1:1), UCW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUA: ethanol of fruit puree acidified with 7% acetic acid: extract obtained by ultrasonic extraction with purified water (1:1).

a-e: means with different letters in the same line are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$

The total phenolic content in the fruit was determined as 56.22 µg GAE/g. When the phenolic content of the extracts was examined, it was determined that the total phenolic content increased in the extracts except for the UCW extract. It was determined that the total amount of monomeric anthocyanin was 4.40 mg/L in the fruit and TMA increased in the extracts except for the UCW and UUW extracts (Table 3). When the amount of vitamin C, total phenolic substance and total anthocyanin amounts were examined, it was determined that UUA extract had the highest amounts (Table 2-3). When the antioxidant properties of fragrant grape fruit and its extracts were examined, it was determined that the UCA extract showed the highest antioxidant property in both DPPH and ABTS analyzes (Table 3). When UCA was evaluated according to trolox, it showed a low activity. As a result of the statistical analyzes, it was determined that the Solvent variable had a very significant effect ($p < 0.01$) on the total phenolic content and total monomeric anthocyanin amounts, while the effect was insignificant for DPPH and had a significant effect for ABTS ($p < 0.05$). It was determined that the treatment had a very significant effect ($p < 0.01$) on the total phenolic substance, total monomeric anthocyanin amounts, and IC_{50} values for DPPH and ABTS ($p < 0.01$).

Table 3. Some chemical properties of fragrant grapes and its extracts

	Fragrant grape	UCA	UCW	UUA	UUW	Troloks	Solvent	Method
Vitamin C	47.00±0.00^{e}	228.00±0.00^{b}	95.00±0.00^{c}	266.00±0.00^{a}	94.00±0.00^{d}	-	**	**
Total phenolic content (µg GAE/g)	56.22±1.24^{c}	112.87±4.57^{b}	55.25±5.65^{c}	152.00±5.44^{a}	60.26±0.22^{c}	-	**	**
Total monomeric anthocyanin (mg/L)	4.40±0.17^{c}	14.06±0.05^{b}	3.88±0.08^{d}	14.79±0.31^{a}	4.31±0.15^{c}	-	**	**
DPPH ($IC_{50,}$ mg/ml)	248.62±29.13^{ab}	85.78±1.33^{c}	193.60±66.24^{b}	273.72±80.64^{a}	231.44±1.87^{ab}	9.48±0.41^{d}	ns	**
ABTS ($IC_{50,}$ mg/ml)	99.37±12.75^{b}	74.67±15.57^{b}	74.97±39.08^{b}	82.76±35.47^{b}	147.22±20.43^{a}	8.68±1.03^{c}	*	**

UCA: ethanol acidified with 7% acetic acid of fruit puree: extract obtained by classical extraction with distilled water (1:1), UCW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUA: ethanol of fruit puree acidified with 7% acetic acid: extract obtained by ultrasonic extraction with purified water (1:1).

a-e: means with different letters in the same line are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$

As a result of GC analysis, a total of 45 compounds were detected in fragrant grape fruit and its extracts. While 33 chemical compounds were not detected in fragrant grape fruit, they were detected in various proportions in their extracts (Table 4). The lowest rate of pyranone (8.47%) was detected in the fruit. In the extracts, the most compounds (20 units) were detected in the UUW extract, while the least amount of compounds (14 units) were detected in the UCA extract. 1-isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3α-ol compound was found to be the highest (20.97%) compound in fruits and extracts (Table 4).

As a result of the statistical analysis, when the effect of the solvent on the chemical compounds detected in the samples was examined, it was determined that it had a very significant effect on 6 compounds ($p < 0.01$) and a significant effect on 31 compounds ($p < 0.05$). On the other hand, it was determined that the method had a very significant ($p < 0.01$) effect on the chemical compounds of the samples in 7 compounds, and a significant effect ($p < 0.05$) on 30 compounds (Table 4).

Table 4. Chemical compound profile of fragrant grape fruit and its extracts

Peak Number	Compound	Retention time (min)	Relative peak area (%)						
			Fragrant grape	UCW	UUW	UCA	UUA	Solvent	Method
1	Acetic acid	3.20	1.49+0.00^{a}	ND	ND	ND	ND	ns	ns
2	2,3-bütanediol	3.40	ND	0.37+0.01^{a}	ND	ND	ND	*	*
3	Furfural	3.70	5.28+0.00^{d}	4.56+0.00^{e}	6.30+0.00^{b}	5.67+0.00^{c}	7.59+0.00^{a}	**	**
4	2-furanmethanol	3.90	ND	0.19+0.00^{b}	0.33+0.00^{a}	ND	ND	**	*
5	5-methyl furfural	4.90	ND	ND	0.28+0.00^{b}	ND	0.33+0.00^{a}	*	**
6	2,4-dihydroxy-2,5-dimethyl(-3(2H)-furanone	5.20	1.22+0.00^{a}	ND	0.60+0.00^{b}	ND	0.34+0.00^{c}	*	**
7	Heptose	6.10	ND	0.66+0.00^{a}	ND	ND	ND	*	*
8	pyranone	7.80	8.47+0.00^{e}	10.56+0.00^{a}	8.81+0.01^{c}	10.30+0.00^{b}	8.73+0.00^{d}	**	*
9	Maltose	10.60	ND	1.94+0.00^{a}	ND	ND	ND	*	*
10	6-Acetyl-β-d-mannose	8.20	3.69+0.00^{a}	0.20+0.00^{e}	0.34+0.00^{d}	0.40+0.00^{b}	0.36+0.00^{c}	*	ns
11	Melezitose	13.60	3.59+0.00^{c}	5.26+0.00^{b}	9.89+0.00^{a}	0.69+0.00^{e}	2.73+0.00^{d}	**	**
12	palmitic acid	19.10	ND	0.64+0.00^{a}	ND	ND	ND	*	*
13	palmitic acid β monoglyceride	30.90	4.44+0.00^{a}	ND	ND	ND	1.23+0.00^{b}	*	*
14	Ethyl iso-allocholate	34.70	ND	2.31+0.00^{b}	ND	2.83+0.28^{a}	0.53+0.00^{c}	ns	ns
15	5,8,11-heptadecatriynoicacid methyl ester	20.10	2.23+0.00^{a}	ND	ND	ND	ND	ns	ns
16	Stearic acid diglycerin ester	33.30	ND	ND	0.67+0.00^{b}	ND	1.20+0.00^{a}	*	**
17	5-methyl-2-furfural	4.90	ND	ND	ND	0.23+0.00^{a}	ND	*	*
18	4,5-diamino-2-pyrimidinol	6.80	1.35+0.00^{a}	ND	ND	ND	ND	ns	ns
19	Methyl 4-nitrohexanote	10.40	3.86+0.00^{a}	ND	ND	ND	ND	ns	ns
20	Dodecanoic acid, 1-methyl ethyl ester	13.80	1.01+0.00^{a}	ND	ND	ND	ND	ns	ns
21	Stearic acid β-monoglycerid	33.30	2.53+0.00^{a}	ND	ND	0.73+0.00^{b}	ND	*	*
22	(2-hydroxy-1-methoxy) ethyl furan	4.80	ND	2.02+0.00^{a}	0.16+0.00^{d}	0.85+0.00^{b}	0.18+0.00^{c}	ns	**
23	Furyl hydroxy methyl ketone	6.60	ND	1.21+0.00^{a}	0.90+0.00^{c}	0.82+0.00^{d}	0.93+0.00^{b}	ns	ns
24	Hepta-2,4-dienoic acid methyl ester	7.50	ND	0.44+0.00^{a}	0.20+0.00b	ND	ND	**	*
25	1-isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3α-ol	16.10	ND	20.97+0.00^{a}	ND	ND	ND	*	*
26	1-(1-butyny) cyclopentanol	20.50	ND	2.45+0.00^{a}	ND	ND	ND	*	*
27	linoleic acid ethyl ester	30.80	ND	8.80+0.01^{a}	ND	ND	ND	*	*
28	α-bromo-γ-valerolactone	3.20	ND	ND	0.14+0.00^{a}	ND	ND	*	*
29	Carbono cyanidic acid ethyl ester	3.50	ND	ND	0.29+0.00^{a}	ND	ND	*	*
30	Melibiose	6.20	ND	ND	0.53+0.00^{a}	ND	ND	*	*
31	Orcinol	6.50	ND	ND	0.20+0.00^{a}	ND	0.15+0.00^{b}	*	**
32	N-nitrosoazocyclononane	6.90	ND	ND	0.45+0.00^{a}	ND	ND	*	*
33	Estra 1,3,5(10)-trien-17β-ol	19.80	ND	ND	0.26+0.00^{a}	ND	ND	*	*
34	17-chloro-7-heptadecyne	20.50	ND	ND	1.87+0.00^{a}	ND	ND	*	*
35	2-hydroxy-1-[(palmitoyloxy)methyl)] ethyl palmitate	30.70	ND	ND	2.35+0.00^{a}	ND	ND	*	*
36	Trilinolein	38.30	ND	ND	0.38+0.00^{a}	ND	ND	*	*
37	5-tetrahydro-2H-pyran-3-yl-ethanethioate	3.50	ND	ND	ND	0.22+0.00^{a}	ND	*	*
38	3-Thiazolidine carboxamidine,2-imino	6.10	ND	ND	ND	0.54+0.00^{a}	ND	*	*
39	Methyl (2E,4E)-2,4-heptadienoate	7.50	ND	ND	ND	0.36+0.00^{a}	0.28+0.00^{b}	**	*
40	9-oxabicyclo[6,1,0]non-6-en-2-one	20.50	ND	ND	ND	4.45+0.00^{a}	ND	*	*
41	2-hexadecanoylglycerol	30.90	ND	ND	ND	0.95+0.00^{a}	ND	*	*
42	Ethyl cyano formate	3.50	ND	ND	ND	ND	0.28+0.00^{a}	*	*
43	Methyl 6-oxoheptanoate	6.10	ND	ND	ND	ND	0.41+0.00^{a}	*	*
44	5-Acetoxymethyl-2,4-heptadienoate	8.70	ND	ND	ND	ND	0.39+0.00^{a}	*	*
45	17-octadecynoicacid	20.50	ND	ND	ND	ND	3.07+0.00^{a}	*	*

UCA: ethanol acidified with 7% acetic acid of fruit puree: extract obtained by classical extraction with distilled water (1:1), UCW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUA: ethanol of fruit puree acidified with 7% acetic acid: extract obtained by ultrasonic extraction with purified water (1:1).

a-e: means with different letters in the same line are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$

As a result of the mineral composition analyzes made in fragrant grape fruit and its extracts, it was determined that calcium was the highest detected mineral in both fruit and extracts (Table 5). Considering the results of the analysis, the calcium and phosphorus amounts of the UCW extract increased compared to the fruit, while the amounts of all other minerals showed a decrease in the extracts compared to the fruit. As a result of the statistical analysis, it was concluded that the effect of the solvent on the mineral amounts was very significant ($p < 0.01$) on the amounts of calcium, phosphorus and lead minerals, while the effect for the other minerals was insignificant ($p > 0.05$) (Table 5). On the other hand, it was determined that the method had a significant effect ($p < 0.01$) on the amounts of potassium, calcium, zinc, phosphorus, lead and barium in the samples, while it had a significant effect on the amounts of manganese and copper ($p < 0.05$) (Table 5).

Table 5. Mineral substance amounts of fragrant grapes and its extracts

	Fragrant grape	UCW	UCA	UUW	UUA	Sign Solvent	Sign Method
Li	ND	ND	ND	ND	ND	-	-
Na	90.47±0.00^{a}	9.89±0.00^{b}	5.22±0.00^{e}	9.15±0.00^{d}	9.23±0.00^{c}	ns	ns
Mg	206.85±0.00^{a}	188.24±0.00^{b}	114.47±0.00^{e}	142.41±0.00^{d}	151.23±0.00^{c}	ns	ns
K	2258.56±0.00^{c}	3471.71±0.00^{a}	1555.42±0.00^{d}	2801.32±0.00^{b}	1265.02±0.00^{e}	**	**
Ca	906.85±0.00^{a}	51.46±0.00^{b}	20.94±0.00^{e}	44.16±0.00^{c}	29.34±0.00^{d}	ns	**
Cr	0.83±0.00^{a}	ND	ND	ND	ND	ns	ns
Mn	6.38±0.00^{a}	2.44±0.00^{b}	1.47±0.00^{d}	1.97±0.00^{c}	1.96±0.00^{c}	ns	*
Fe	104.79±0.00^{a}	1.36±0.00^{c}	1.04±0.00^{e}	1.30±0.00^{d}	1.93±0.00^{b}	ns	ns
Cu	5.12±0.00^{a}	0.99±0.00^{e}	2.98±0.00^{b}	1.51±0.00^{d}	1.59±0.00^{c}	ns	*
Zn	120.20±0.00^{a}	4.02±0.00^{b}	1.41±0.00^{d}	2.50±0.00^{c}	0.34±0.00^{e}	**	**
Al	61.54±0.00^{a}	ND	ND	ND	ND	ns	ns
P	242.55±0.00^{b}	278.12±0.00^{a}	134.98±0.00^{e}	209.86±0.00^{c}	173.63±0.00^{d}	ns	**
As	0.03±0.01^{a}	ND	ND	ND	ND	ns	ns
Se	0.25±0.00^{a}	ND	ND	ND	ND	ns	ns
Cd	0.02±0.00^{a}	ND	ND	ND	ND	ns	ns
Sn	ND	ND	ND	ND	ND	-	-
Hg	ND	ND	ND	ND	ND	-	-
Pb	14.33±0.00^{a}	0.14±0.00^{c}	0.05±0.00^{e}	0.21±0.00^{b}	0.10±0.00^{d}	**	**
Ba	75.23±0.00^{a}	2.24±0.00^{b}	0.75±0.00^{e}	1.93±0.00^{c}	0.78±0.00^{d}	ns	**

UCA: ethanol acidified with 7% acetic acid of fruit puree: extract obtained by classical extraction with distilled water (1:1), UCW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUW: extract obtained by ultrasonic extraction of fruit puree with distilled water, UUA: ethanol of fruit puree acidified with 7% acetic acid: extract obtained by ultrasonic extraction with purified water (1:1).

a-e: means with different letters in the same line are significantly different ($p < 0.05$); Sign: Significance; ns: not significant ($p > 0.05$); *$p < 0.05$; **$p < 0.01$

CONCLUSIONS

Trabzon İlinde doğal olarak yetişen kokulu üzüm meyvesinin fiziksel ve kimyasal özellikleri belirlenmiştir. Kokulu üzüm meyvesinin doğal renklendirici olarak kullanımı

noktasında toplam antosiyanin miktarı bakımından zengin bir kaynak olduğu saptanmıştır. Bu çalışmanın verileri ışığında hazırlanan kokulu üzüm meyvesi ekstraktlarının gıdalarda doğal renklendirici olarak kullanım olanaklarının saptanması için gıda çalışmaları gerekmektedir.

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DETERMINATION OF THE BEST SEEDING DATE FOR SEED PRODUCTION IN ANNUAL RYEGRASS

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ABSTRACT

This research was carried out in Samsun ecological conditions in the vegetation period of 2020-2021 in order to determine the most suitable seeding date in terms of seed yield in two newly registered annual ryegrass cultivars. Six different planting times (15 October, 30 October, 15 November, 30 November, 15 January, 1 February) were applied in the study, and Ilkadım and Koca Yaşar varieties were used as plant material in the experiment. The experiment was set up according to split plot design in randomized blocks with four replications. While there was no difference between the cultivars in terms of the characteristics examined, it was determined that the differences between seeding dates were significant. The highest plant height was obtained from the plantings made between October 15 and November 30, and it was determined that the plant height shortened as the planting date was delayed. Both the spike length and the spikelet number decreased as the planting date was delayed. The highest seed yield was obtained from the planting done on November 15, as 220 kg/da. While the autumn sowings were in the same statistical group, the seed yield decreased significantly in the sowings made in January and February, and fell to 130.7 kg/da in the 1 February sowing. While there was no difference between autumn sowings in terms of thousand-grain weight, it was determined that there was a significant decrease in January and February sowings. According to the one-year results obtained from this study, it can be recommended to plant Ilkadım and Koca Yaşar annual ryegrass cultivars in the coastal areas of Samsun between 15 October and 15 November in terms of seed yield.

Key Words: Annual ryegrass, seeding date, cultivar, seed yield

INTRODUCTION

It is very important for the ever-increasing population in the world and in our country to sustain a healthy life and to have an adequate and balanced diet. In order to reach animal products in a sustainable and inexpensive way, animal husbandry activities in the agricultural sector should be given due importance. One of the most important problems of animal husbandry in our country is the inability to meet the needs of adequate and high quality roughage. Feed expenses constitute a large part of the inputs in the livestock sector. This rate is known to be about 70%. Roughage constitutes 78% of feed expenses and concentrate feeds constitute 22% (Harmanşah, 2018). The cheapest feed source is meadows and pastures. In our country, high and quality yields cannot be obtained from our meadows and pastures because of deterioration. For this reason, the first source of reference in providing economic and sustainable feed in livestock enterprises should be the production of forage crops. One of the most important reasons why forage crops agriculture could not be expanded is the seed problem (Acar et al., 2000).

Annual ryegrass (*Lolium multiflorum* L.), which is from the wheat family, is an annual, cultivated, Southern European origin species in the grass genus. Annual ryegrass spreads naturally in Turkey and takes names such as Italian grass, milkweed, ryegrass (Özköse and

Acar, 2018). Annual ryegrass is a tall fodder plant that develops rapidly in terms of agricultural characteristics, provides high grass yield, and can give a large number of forms. It is a grassy forage plant with the potential to increase its production even more with its features such as being grown as a mixture with one-year grasses and legumes forage crops in regions with mild winters, hay production and grazing, as well as being able to enter crop rotation with one-year crops in field agriculture. Annual ryegrass, which is grown as a winter intermediate product in temperate regions, can also be planted as a main product to produce forage and seeds (Kuşvuran and Tansı, 2005). Due to the high and quality forage yield, the need for seeds of this plant is increasing due to the increase in annual ryegrass cultivation areas in our country.

The seed forms the basis of biological and cultural diversity and the first link of the food chain. In order to increase production and productivity, it is necessary to use the seeds of varieties with high genetic potential and whose genetic capacity can be maximized in the environmental conditions of the region where they will be grown (Acar, 1994). With the use of high quality seeds in agricultural production, yield increases can be achieved at rates exceeding 20% to 100% (Kara et al., 2014). Our country consists of different climatic zones and each climatic zone consists of a different number of sub-climates called "microclimate". The bred varieties are very sensitive to changes in environmental conditions (Manga, 1991). For this reason, in order to increase the cultivation areas of forage crops, varieties of forage plant species are developed in different climatic regions of our country, which can reveal their genetic potential at the highest level. It is important to ensure sufficient production of quality seeds of these developed varieties and to distribute them to the relevant producers in a timely manner and at reasonable prices. Considering such features, researches to increase seed yield in annual ryegrass gain great importance.

As with all plants, one of the cultural practices that affect the seed yield increase in annual ryegrass is to determine the most appropriate planting date. Sowing date varies according to varieties, as well as according to ecological regions. With this study, it was aimed to determine the most suitable planting date in terms of seed production in annual ryegrass in our region.

MATERIAL AND METHOD

This research was carried out at the Ambarköprü trial station of the Black Sea Agricultural Research Institute in the vegetation period of 2020-2021. The coordinates of the trial area are between 41° 13' 11" N - 36° 40' 02" E latitude and longitude and its height is 17 meters above sea level.

Soil samples taken from 0-20 cm depth of the experimental area were analysed in the Black Sea Agricultural Research Institute Soil Department laboratory. According to soil analysis results; the soils of the experimental area were clayey loam and slightly alkaline (7.35), unsalted (0.027%), lime (6.8%) and organic matter content medium (1.23%), phosphorus content very low (2.00 kg da-1) and potassium content insufficient (55 kg da-1).

According to the climate data of Samsun for many years (1991-2020), the average annual temperature is 15.0 ^{o}C, the hottest month is August (24.5 ^{o}C), and the coldest month is February (7.3 ^{o}C). While the annual total precipitation amount is 729.7 mm on average for many years, it has been recorded that the highest precipitation falls in December (82.5 mm) and the least precipitation falls in July (38.9 mm) (Anon, 2021).

Annual ryegrass cultivars named İlkadım and Koca Yaşar, which were used as plant material in the experiment, were obtained from the Field Crops Department of the Black Sea Agricultural Research Institute. Six different sowing dates (15 October, 30 October, 15 November, 30 November, 15 January, 1 February) were investigated. The experiment was set up split plot design in a randomized block with four replications. The cultivars were in the main plot and the planting dates were in the subplot. In the experiment, each plot consisted of 5 rows

with 20 cm row spacing. The row length is 4 m and the plot area is 4 m^2. A gap of 1.5 m was left between the plots and 3 m between the blocks, and the total trial area is 1069.5 m^2. In planting, 3 kg of seeds were used per decare, and fertilization was made with 5 kg of N (nitrogen) and 8 kg of P (phosphorus). During the shooting period of the plants, 5 kg of nitrogen was supplemented, thus, in total 10 kg of nitrogen was used per decare.

Harvest, in the period when the ears turn yellow; Autumn sowings (October 15, October 30, November 15, November 30) was done on June 22, and winter plantings were done on July 15 (January 15, February 1) with a parcel harvester. Before harvest, 10 plant samples were taken randomly from the plots and plant height, spike length and number of spikelets were determined. After the seeds were dried in the open air for two days and passed through the selector, the seed yields and thousand kernel weights were found. Statistical analysis was applied to the obtained data using SPSS 22.0 statistical package program.

RESULTS AND DISCUSSION

Plant Height

While there was no difference between cultivars in terms of plant height, it was determined that the difference between planting dates was significant. While the average plant height values were determined to be between 93.1-125.4 cm, the highest plant height was 122.7 cm on October 15, and the lowest plant height value was 95.1 cm on February 1, according to the average of the varieties. In terms of plant height, all autumn plantings were in the highest group and winter plantings were in the lowest group (Table 1).

Table 1. Average plant height values according to varieties and planting dates (cm)*

Cultivars	**Seeding Dates**						
	October 15	October 30	November 15	November 30	January 15	February 1	Mean
İlkadım	120.0	122.3	123.6	117.2	101.1	93.1	112.9
Koca Yaşar	125.4	120.3	118.2	120.0	100.1	97.1	113.5
Mean	122.7 [a]	121.3[a]	120.9[a]	118.6[a]	100.6[b]	95.1[b]	

(*) There is no statistical difference at the $p \leq 0.05$ level between the means shown with the same letter on the same line.

In studies on annual ryegrass in different regions of our country, different values were obtained in plant height. These values are; Dinç (1995) reported 113.27-129.30 cm in Edirne, 86.17-96.17 cm in Şanlıurfa, İnce (2000), and 89.36-95.42 cm in Eskişehir, Yaman (2019) and these results are partially consistent with our findings. In the study conducted by Özel (1989) in Çukurova, the plant height was 123.4-231.00 cm in seed production plots, 110.07-176.20 cm in forage production plots, Pişkin (2007) was 40.56-47.45 cm in Aksaray, Çolak (2016) was 59.5- 61.3 cm, and Kuşvuran and Tansı (2005) reported 60.35-85.99 cm in Çukurova. The differences between the findings may be caused by factors such as the varieties used, soil characteristics, ecological factors of the regions, and cultural methods applied.

Spike Length

While it was determined that the difference between the varieties in terms of spike length was insignificant, it was determined that there was a significant difference between the planting

dates. According to the data obtained from this study, the length of the spike varies between 24.1-31.3 cm. According to the average of the cultivars, the highest spike length was observed on October 15 with 30.4 cm, and the lowest spike length was observed on February 1 with 24.5 cm. However, all autumn plantings were in the highest group (Table 2).

Table 2. Average spike length values according to cultivars and sowing dates (cm)*

Cultivars	**Seeding Dates**						
	October 15	October 30	November 15	November 30	January 15	February 1	Mean
İlkadım	30.3	31.3	31.3	30.4	27.9	24.9	29.3
Koca Yaşar	30.5	29.1	28.2	29.4	26.2	24.1	27.9
Mean	30.4[a]	30.2[a]	29.7[a]	29.9[a]	27.1[b]	24.5[c]	

(*) There is no statistical difference at the p≤0.05 level between the means shown with the same letter on the same line.

Although the early sowing date caused an increase in the spike length, there was no statistical difference between autumn sowings. Uygun (1994) stated that the spike length of annual ryegrass varied between 27.46 and 29.01 cm, which are similar to our findings. Kuşvuran and Tansı (2005) reported that the mean spike length ranged from 17.90-18.98 cm, Gültekin (2008) applied different fertilizer forms and reported that the mean spike length values varied between 31.52-35.33 cm, and Yaman (2019) said it was varied between 17.48 and 20.65 cm, which differed from our findings. Different sowing dates, applied methods, ecological conditions of the regions and varieties can be shown as factors in the formation of different results between studies.

Number of spikelets

In terms of the average number of spikelets in a spike, it was determined that the difference between cultivars was insignificant, but the differences between sowing dates were significant. According to the data obtained from the research, it is seen that the number of spikelets varies between 22.9-33.8 pieces/spike. According to the average of the cultivars, the highest number of spikelets was determined as 33.0 and 32.8 pieces/spike from the plantings made on 15 and 30 October. As the sowing date delayed, the number decreased regularly and decreased to 23.5 on 1 February, the last sowing date (Table 3).

Table 3. Average number of spikelets (number/head)*

Cultivars	**Seeding Dates**						
	October 15	October 30	November 15	November 30	January 15	February 1	Mean
İlkadım	32.2	33.1	31.2	29.0	26.1	24.2	29.3
Koca Yaşar	33.8	32.6	30.8	28.9	25.0	22.9	29.0
Mean	33.0[a]	32.8[a]	31.0[b]	28.9[c]	25.5[d]	23.5[e]	

(*) There is no statistical difference at the p≤0.05 level between the means shown with the same letter on the same line.

In our country, many studies have been carried out on the number of spikelets on annual ryegrass yield and yield components. Serin and Gökkuş (1993) reported that there are 38

spikelets per spike. Uygun (1994) stated that the number of spikelets of Italian ryegrass is between 22.51 and 23.64 in Thrace conditions. Pişkin (2007) determined the number of spikelets as 16.53-18.06 pieces/spike in his study named the effects of different seed amounts on yield components. Yaman (2019) reported that the number of spikelets is between 17.00 and 20.25 pieces/spike according to different nitrogen doses. The differences between studies; It can be caused by the climatic conditions of the regions, sowing dates, soil characteristics, planting and maintenance methods and genotype differences.

Seed Yield

When Table 4 is examined, it is understood that there is no significant difference between varieties, but there is a significant differences between planting dates. According to the data obtained, the seed yield varied between 129.0 and 225.8 kg/da. As the average of the varieties, the highest seed yield of 220.0 kg/da was obtained from the 15 November sowing, while all autumn sowings were included in the same statistical group. The seed yield decreased regularly in sowing after the 15th of November and decreased to 130.7 kg/da in 1 February sowing (Table 4).

Table 4. Average seed yield values according to varieties and sowing times (kg/da)*

Cultivars	**Seeding Dates**						
	October 15	October 30	November 15	November 30	January 15	February 1	Mean
İlkadım	200.7	166.9	225.8	177.9	143.2	132.4	174.5
Koca Yaşar	172.8	205.6	214.3	177.4	143.0	129.0	173.7
Mean	186.7^{ab}	186.2^{ab}	220.0^{a}	177.6^{ab}	143.1^{bc}	130.7^{c}	

(*) There is no statistical difference at the p≤0.05 level between the means shown with the same letter on the same line.

İnce (2000) 39.00-61.70 kg/da in Şanlıurfa, Kuşvuran and Tansı (2005) 17.53-34.13 kg/da in Çukurova, Pişkin (2007) 68.32-88.47 kg/da in Aksaray, Gültekin (2008) 22.09-64.85 kg/da, in Çukurova, Yaman (2019) 84.0-132.0 kg/da in Eskişehir were obtained. The reported values are considerably lower than the yields determined in this study. Özel (1989) determined the seed yield as 98.14-164.29 kg/da in his study in Çukurova, which is partially similar to the values obtained from this study. It can be said that the variety, planting date, climatic and environmental conditions of the regions and cultural practices are effective in obtaining different results in the studies.

Thousand Seed Weight

While there was no significant difference between varieties in terms of thousand-seed weight of seeds, it was determined that sowing dates significantly affected thousand-seed weight. Although the highest thousand grain weight value was determined on the average of the cultivars on October 15 (2.66 g), all autumn treatments were in the same group. On the other hand, winter plantings formed a second low group (Table 5).

Table 5. Average thousand-grain weight values according to cultivars and sowing times (g)*

Cultivars	**Seeding Dates**						
	October 15	October 30	November 15	November 30	January 15	February 1	Mean
İlkadım	2.60	2.54	2.42	2.47	2.25	2.09	2.39
Koca Yaşar	2.72	2.37	2.37	2.54	2.09	2.09	2.36
Mean	2.66^{a}	2.45^{a}	2.39^{ab}	2.50^{a}	2.17^{bc}	2.09^{c}	

(*) There is no statistical difference at the p≤0.05 level between the means shown with the same letter on the same line.

In some studies on the weight of a thousand grains in annual ryegrass; Avcıoğlu and Geren (1996) reported that the weight of one thousand grains of annual ryegrass is between 1.8 and 2.4 g. Pişkin (2007) found a thousand grain weight as 2.1-2.7 g in his study with different seed sowing rates. Yaman (2019), in his study at different nitrogen doses, stated that the thousand grain weight of annual ryegrass is between 2.49 and 2.67 g. Our findings are generally in agreement with previous studies.

CONCLUSION

In the light of the data obtained from this study, which used two annual ryegrass cultivars and six different sowing dates and carried out for one year, it was concluded that it would be appropriate to plant annual ryegrass between October 15 and November 15 in Samsun coastal areas for high and quality seed production. However, longer-term results are needed to increase the reliability of the results.

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EFFECT OF SOIL STRUCTURE DETERIORATION ON WATER RETENTION OF CAMBISOLS

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ABSTRACT

Soil structure deterioration is the most widespread consequence of long-term cultivation of soil. The soil susceptibility to this form of soil physical degradation depends on genetic features of soil. The aim of this study was to characterize the soil structure and its effect on water retention of Haplic Cambisols under long-term potato cultivation and adjacent grassland in the region of the Samokov hollow, southwestern Bulgaria. The top soil layers were analyzed for determination of soil texture, soil organic carbon, pH, soil bulk density, total porosity, aggregate size distribution, water stability of soil aggregates, and soil water retention curve (SWRC). The water retentions at low suctions (pF 0.4-2.5) were determined on undisturbed samples in the process of draining by suction type apparatus and on disturbed soil samples by TDR/MUX/mpts device during air drying. It was found that deterioration of soil structure as result of long-term potato cultivation was well expressed by the lower water stability of soil aggregates, field capacity and plant available water capacity in comparison to grassland. Another indictor for structureless of the cultivated soil was the coincidence of SWRC obtained in case of the intact soil cores and of the disturbed soil. The obtained results showed that long-term use of Haplic Cambisols for potato cultivation lead to significant soil structure deterioration in this relatively cool and humid region.

Keywords: soil aggregation, water stability of soil aggregates, soil water retention curve, suction plate apparatus, TDR/MUX/mpts device

INTRODUCTION

Soil structure deterioration is the most widespread consequence of long-term cultivation of soil (Dilkova, 2014). The soil quality indicators used for assessing land change effects are total porosity, aeration capacity, plant available water capacity, water stability of soil aggregates, soil organic carbon content, pore size distribution parameters, infiltration rate (Dilkova et al., 1998; Dilkova, 2014; Gajić, 2013; Reynolds et al., 2009; Kercheva et al., 2017). Soil susceptibility to physical degradation depends on genetic features of soil and was assessed for some main agricultural soil varieties in low lands of the country (Dilkova et al., 1998; Dilkova, 2014). Changes of soil physical properties of Cambisols (Brown forest soils) under different vegetation cover in mountainous regions were discussed by Kercheva et al. (2019). There are no comparative studies on the long-term effects of cultivation on soil physical properties and organic matter of this type of soil in relative high-land territories, such as the Samokov hollow (950 m a.s.l.). The region is known as suitable for potato growing in Bulgaria.

The aim of this study was to characterize soil structure and its effect on water retention of Haplic Cambisols under long-term potato cultivation and adjacent grassland in the region of the Samokov hollow, southwestern Bulgaria.

MATERIAL AND METHODS

The study was conducted on Brown forest soil (Haplc Cambisols according to WRB 2015) under grassland and adjacent ploughed area used for potato growing in the Samokov hollow (23.535E; 42.336N; altitude 945 m a.s.l.), southwestern Bulgaria. The region is relatively cool with mean air temperatures of the coldest month (January) -3.4ºC and of the hottest one (July) 17.4ºC. The annual sum of precipitation is 670 mm with summer maximum of 202 mm.

The laboratory analyses of the soil samples taken from the top soil layers included determination of soil particle size distribution, soil organic carbon content, pH in H_2O, soil bulk density, total porosity, aggregate size distribution, water stability of soil aggregates, and soil water retention curve.

The particle-size distribution was determined by sieving and the pipette method (ISO 11277, 2009). Fractions of sand (2-0.063 mm), silt (0.063-0.002 mm) and clay (<0.002 mm) were determined according to ISO 11277 (2009) for applying the textural classification of IUSS Working Group WRB (2015). Total soil organic carbon content (SOC, %) was determined by the modified Tjurin's method (Filcheva and Tsadilas, 2002, Kononova, 1963). Based on these data the resilience of soil structure was assessed by the structural stability index (SI) proposed by Pieri (1992):

$$SI=1.724 \times SOC/(silt+clay) \times 100\% \quad (1)$$

The acidity of soil was measured by pH meter (ISO 10390: 2011).

Vertically oriented intact soil cores were sampled in 4 replicates in 100 cm^3 metal cylinders for determination of bulk density (Db) (ISO 11272:1998) and water retention by the suction type apparatus till pF 2.5 (ISO 11274:1998). Particle density (Ds) analysis was carried out in water with 100 cm^3 pycnometers. Total porosity (Pt) was calculated using the measured bulk density (Db) and particle density (Ds):

$$Pt=(1-Db/Ds)\times 100\% \quad (2)$$

The distribution of dry-sieved aggregates between aggregate size classes (>10, 10-5, 5-3, 3-1, 1-0.25, and <0.25 mm) was determined by manual dry sieving of air dried soil using set of sieves arranged from top to bottom with decreasing size of the openings. The proportion of each aggregates class (DSA) was calculated relative to the summed total weights of all the aggregate size classes. Mean weight diameter (MWD, mm) of the fractions less than 10 mm was calculated.

The water stable aggregates were determined by the method of Savinov, modification of Vershinin and Revut (Revut, 1969). Four soil samples (20 g each) were prepared for wet sieving: one composite sample ($F_{0.25\text{-}10}$) by taking equal quantity (5 g) of air dried aggregates from four fractions: 10–5, 5–3, 3–1, and 1-0.25 mm; three replicate samples (20 g each) with air dried aggregates from a single fraction 3–1 mm ($F_{1\text{-}3}$). The wet sieving was done by the device of Savinov an hour after direct immersion of the air dried soil aggregates sample into water (slaked pretreatment). The correction for aggregate-sized sand content (skeleton) was done according to Six et al. (2000):

$$WSAi=Pi-Si \quad (3)$$

where WSAi – is the proportion of water stable aggregate of size class i (i=10-5, 5-3, 3-1, 1-0.25 mm), Pi is the proportion of the aggregates remaining on the sieve (5, 3, 1, and 0.25 mm) after wet sieving to the weight of the sample prior to the wet sieving, Si is the proportion of

sand with size i in aggregates of size i after disruption. The proportion of water unstable aggregates (P<0.25) was calculated as 100%-the total sum of Pi. Mean weight diameter of water stable aggregates (MWDwet) was also calculated.

The water stability of aggregates is expressed by the ratio (MWDR) of mean weight diameters of aggregates after (MWDwet, mm) and before wet sieving and by the percent of water stable macro aggregates >0.25 mm (WSA>0.25, %) (Dilkova, 2014).

Soil water retention at suctions less than pF 2.5 (-33 kPa) was determined using two laboratory methods. The first one (M1) was a suction plate method similar to ISO11274:1998 using Shot filters G5 connected to hanging column for measuring soil water content at pF 1, 1.7 and 2, and to a vacuum chamber for pF 2.5 as described by Kercheva et al. (2017, 2019). The second method (M2) used TDR/MUX/mpts measurement device (Time Domain Reflectometry meter with a multiplexer for measurement of moisture, matric pressure, temperature and salinity of soils) for measuring soil volumetric water content and soil matric potential.

Soil water retention at suction 1500 kPa (pF 4.2) was determined using fine (<2 mm) earth samples by pressure membrane apparatus (Soilmoisture equipment Corp.). The hygroscopic water content at pF 5.6 at water adsorption part of the soil water retention curve (SWRC) was determined using vapor pressure method with controlled relative humidity 75% in desiccators containing saturated solution of NaCl.

The field capacity was estimated by the water content retained at suction 10 kPa (pF 2.0). The plant available water capacity (PAWC) was determined as the difference between water retained at pF2.0 and pF4.2:

$$PAWC=W_{pF2.0}-W_{pF4.2} \quad (4)$$

Air capacity, AC was calculated as difference between total porosity (Pt) and volumetric water content corresponding to FC ($\theta_{pF2.0}$):

$$AC=Pt-\theta_{pF2.0} \quad (5)$$

Relative FC was calculated as $\theta_{pF2.0}$/Pt.

More details on these and other soil physical indicators and their optimal limits can be found in Reynolds et al (2009).

RESULTS AND DISCUSSION

Soil texture was classified as loam in the top 0-10 cm soil layers under both arable and grassland (Table 1). Soil organic carbon content (SOC) was nearly two times lower in the arable layer than under grassland. It was classified as high (1.8-3%) under grassland and low (0.6-1.2%) under the arable according to the proposed criteria of Filcheva (2014). According to Greenland (1981) tillage-induced loss of soil structure may occur when SOC<2.3%wt. These results corresponded to the values of the structural index (SI), calculated by the silt and clay fractions and SOC (eq. 1). SI showed high risk of degradation under grassland as 5<SI≤7% and highly degraded soil under cultivation (SI ≤5%).

The soil reaction was very strongly acidic as pH in H_2O was less than 4 (Table 1).

The higher SOC and finer texture under grassland leaded to lower particle density (Ds) and higher hygroscopic water content ($W_{pF5.6}$) (Table 1) and wilting point ($W_{pF4.2}$) (Table 2). The coarser texture of the arable soil was accompanied with the higher content of skeleton as it was determined after wet sieving (Fig. 2). The higher bulk density (Db) of the arable soil than under grass confirmed the instability of soil structure and it proneness to compaction.

Table 1. Soil texture fractions and class, soil organic carbon content (SOC), pH, soil bulk (Db) and particle (Ds) density and hygroscopic soil water content at pF 5.6 ($W_{pF5.6}$) of the studied topsoil layers of the arable and grassland Cambisols.

Land use	Sand %	Silt %	Clay %	Texture class	SOC, %	SI, %	pH in H_2O	Db, g cm^{-3}	Ds, g cm^{-3}	$W_{pF5.6}$, %wt
Arable	46	32	22	Loam	1.10	3.5	3.8	1.15	2.65	2.8
Grassland	39	36	25	Loam	1.97	5.6	3.8	1.04	2.59	3.8

Soil air capacity showed no aeration deficit as AC≥10%vol in both studied cases (Table 2). The tillage leaded to two times higher AC of the arable soil. This was on the account of less retention capacity of the arable soil at pF 2.0 (used in this study as the measure of FC) and at pF 2.5 (also used in other studies as a measure of FC). The relative field capacity ($\theta_{pF2.0}$/Pt) was below the optimal range (0.6-0.7) (Reynolds et al., 2009) for microbial production of nitrate in the arable soil and above it in grassland. PAWC was optimal (≥20%vol) under grassland and good (15≤PAWC<20) in the arable soil. The air capacity and the retention properties of the till soil suggested high water permeability and no risk for waterlogging which is beneficial for potato growing.

The formation of clods (>10 mm aggregates) was better expressed in the arable soil (Fig. 1). The mean weight diameter was almost the same – around 3 mm, but the agronomical valuable aggregates between 0.25-10 mm prevailed under grassland (79%), while they were 56% in the arable soil (Fig. 1). The water stable aggregates >0.25 mm of fraction F_{1-3} was more than two times higher under grassland than in the arable soil.

Table 2 Soil quality indicators. Water content (W) by mass retained at potentials pF 2.0, 2.5 and 4.2. PAWC plant available water content, AC air capacity, WSA – waster stable aggregates in fraction F1-3 mm. $\theta_{pF2.0}$/Pt – relative field capacity

Land use	Pt, %vol	$W_{2.0}$, % wt	$W_{2.5}$, % wt	$W_{4.2}$, % wt	PAWC, % vol	$\theta_{pF2.0}$/Pt	AC, %vol	WSA_{F1-3} >0.25 mm, %
Arable	56.5	25.1	21.4	9.0	18.5	0.51	27.6	31.9±4.5
Grassland	59.8	46.7	39.0	13.2	32.9	0.78	13.1	84.7±0.6

The composite sample $F_{0.25-10}$ showed instability of aggregates greater than 3 mm in the arable soil. When expressed as MWDR (ratio of mean weight diameter after and before wet sieving) the water stability of the arable soil was assessed as medium (MWDR=0.25) while the water stability of the aggregates under grassland was high (MWDR=0.84) (Fig. 2).

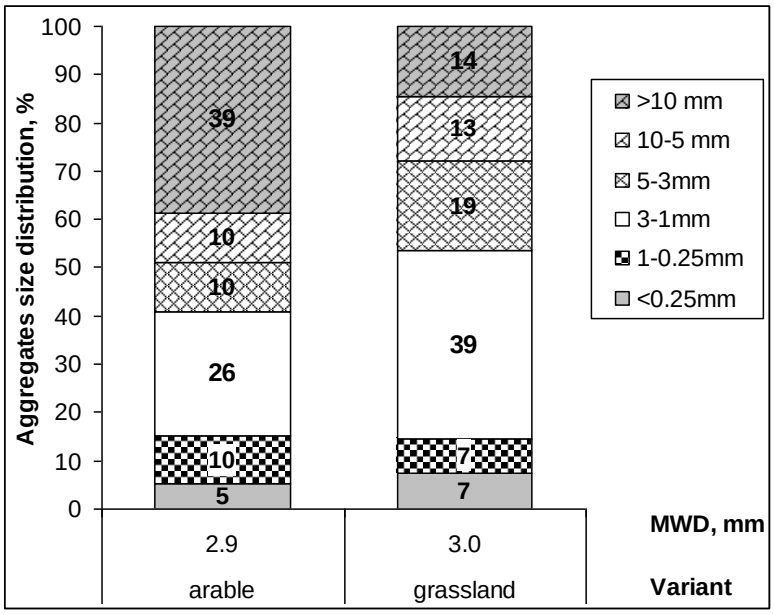

Fig. 1. Aggregates size distribution and mean weight diameter of dry aggregates (MWD).

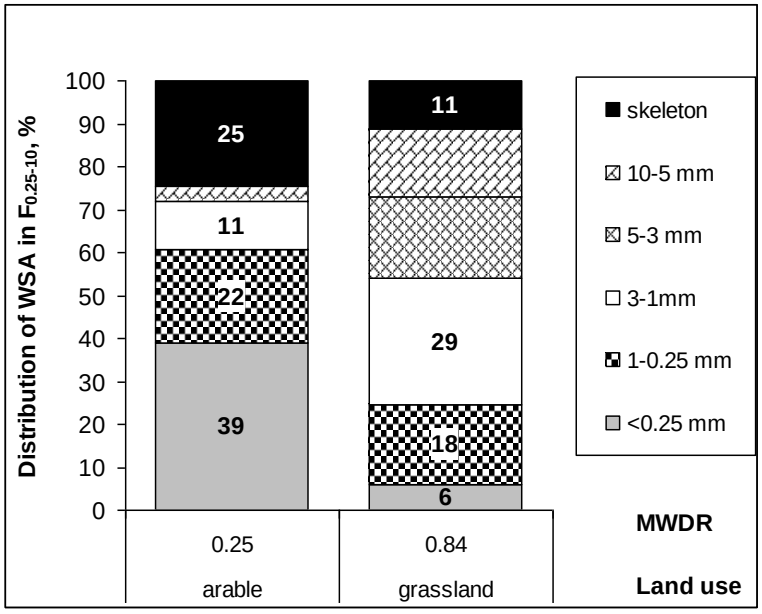

Fig. 2 Size distribution of water-stable aggregates in the composite sample ($F_{0.25-10}$) and mean weight diameter ratio after and before wet sieving (MWDR).

The soil water retention curves were presented in Fig. 3. The similarity between SWRC obtained on the intact soil cores and on the disturbed soil at lower suctions (less than pF 2.5) is another indication for structureless of the cultivated soil. Under grassland the SWRC of the intact cores was higher suggesting presence of macro pores. Even disturbed soil under grass showed higher water retention capacity than cultivated soil. This can be explained with presence of greater amount of structure pores in water stable soil macro-aggregates.

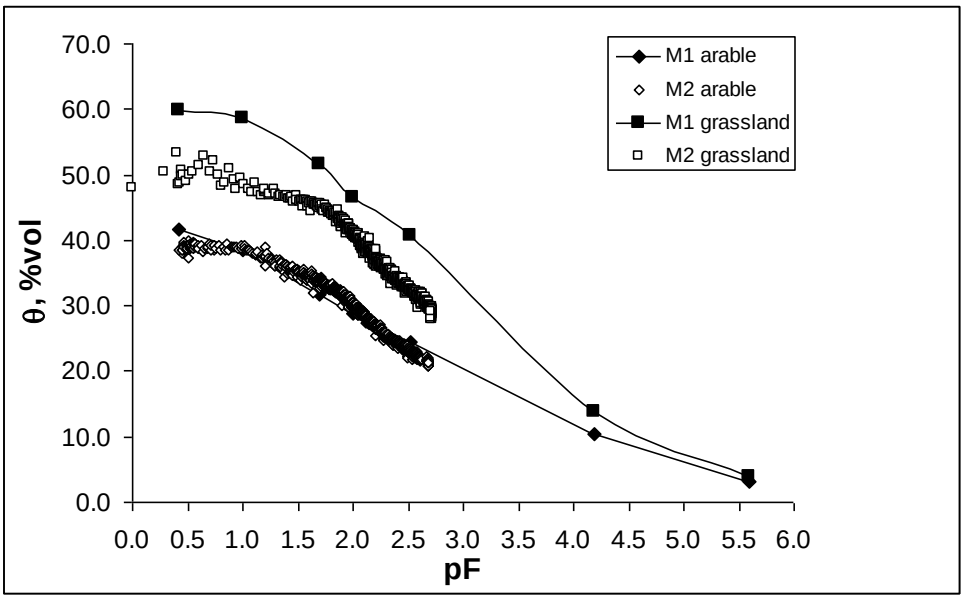

Fig. 3. Water retention measured on undisturbed soil cores (M1) and disturbed sample (M2)

CONCLUSIONS

Several indicators of soil structure related to solid phase and soil porous system of Haplic Cambisols under grassland and cultivation were determined. The use of TDR/MUX/mpts device during air drying provided detailed information of soil water retention curve of the studied soil in the range of pF 0 to 2.7. The obtained characteristics of soil structure proved the significance of water stability of soil aggregates for improving the water retention capacity of Haplic Cambisols. The long-term use of Haplic Cambisols for potato cultivation leaded to significant soil structure deterioration in this relatively cool and humid region. The high air capacity and low retention properties of the till soil suggested high water permeability and no risk for waterlogging which is beneficial for potato growing.

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EFFECT OF BIOCHAR AND MANURE ON SOIL QUALITY INDICATORS OF FLUVISOL

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The effect of separate and combined application of manure (4 t ha^{-1}) and biochar produced by maize cobs (500 and 750 kg ha^{-1}) on soil quality indicators was studied within the field experiment on sandy loam Fluvisol under broad bean cultivation (*Vicia Faba, L.*). The studied soil characteristics were physicochemical properties, soil organic carbon content, water retention and adsorption characteristics, bulk density, total porosity, aeration capacity, water stability of soil aggregates and soil thermal conductivity. The applied amendments did not influence the physicochemical characteristics of the topsoil. Increase of the soil organic carbon content was observed only in the variants with the combined application of manure and biochar. In these variants it was observed higher content of water retained at matric potential -1500 kPa (wilting point) than in the control. As the rate of increase of water retained at potential -33 kPa (field capacity) was lower, there was no or even negative effect on plant available water. The soil bulk density at 0-5 cm was higher and respectively the total porosity was lower in treated variants than in the control, but at depth 10-15 cm the tendency was the opposite. A decrease of aeration capacity at 0-10 cm depth was observed in all amended variants and it was critically low (7.2%vol.) when only manure was applied. Water stability of aggregates in all variants was low, but in the amended variants the water stability of aggregates fraction of 1-3 mm size slightly increased. The relative field capacity increased in all treated variants which can be considered as a positive effect of the applied manure and biochar. The soil thermal conductivity measured at 10-15 cm depth in the field with KD2pro device was the highest 1.506 W $m^{-1}K^{-1}$ in the control variant in comparison with the variant with manure 1.268 W $m^{-1}K^{-1}$, biochar 1.489 W $m^{-1}K^{-1}$ and combined manure + biochar 1.372 W $m^{-1}K^{-1}$.

Keywords: soil hydraulic properties, soil thermal conductivity, water stability of soil aggregates, biochar, manure, broad bean

INTRODUCTION

Numerous studies were conducted for evaluating the effect of biochar (BC) properties on soil physical characteristics (soil water holding capacity, density, filtration, specific surface area, thermal properties etc.) (Piccolo et al., 1996; Sohi et al., 2010; Ojeda et al., 2015; Usowicz et al., 2016). Zhang et al. (2016) pointed out the paucity of investigations based on field experiments, highly degraded soils and use of biochar produced by crop residues and commercial products as soil amendments. The combined application of BC with manure is considered as beneficial for increasing of soil fertility because the BC is inert material (Arthur et al., 2015).

The aim of this study was to assess the effect of separate and combined application of manure and biochar produced by maize cobs on soil quality indicators within the field experiment on sandy loam Fluvisol under broad bean (*Vicia Faba*, L.) growing.

MATERIAL AND METHODS

The field experiment with separate and combined application of manure (4 t ha^{-1}) and biochar produced by maize cobs (500 and 750 kg ha^{-1}) on Fluvisol was carried out in 2018 under growing of broad bean (*Vicia Faba, L.*) in the experimental field of the University of Forestry in Vrazhdebna, Sofia field (23.436E,42.708N, elevation 532 m a.s.l). The region is relatively cool with mean air temperatures of the coldest month January -1.3ºC and of the hottest one July 20.3ºC. The long-term mean annual sum of precipitation is 573 mm.

The experimental design included the following variants: V1) control - no biochar and manure; V2) manure - 4 t ha^{-1}; V3) biochar – 500 kg ha^{-1}; V4) manure 4 t ha^{-1}+ biochar 500 kg ha^{-1}; V5) manure 4 t ha^{-1} + biochar 750 kg ha^{-1} (Petrova et al., 2019).

The laboratory analyses of the undisturbed and disturbed soil samples taken from the top soil layers included determination of physicochemical properties, soil organic carbon content, water retention and adsorption characteristics, bulk density, total porosity, aeration capacity, water stability of soil aggregates and soil thermal conductivity.

Particle-size distribution was determined by sieving and pipette method (ISO 11277, 2009). Fractions of sand (2-0.063 mm), silt (0.063-0.002 mm) and clay (<0.002 mm) were determined according to ISO 11277 (2009) for applying the textural classification of IUSS Working Group WRB (2015). Total soil organic carbon content (SOC, %) was determined by the modified Tjurin's method (Filcheva and Tsadilas, 2002, Kononova, 1963). Based on these data the resilience of soil structure was assessed by the structural stability index (SI) proposed by Pieri (1992) (Reynolds et al., 2009):

$$SI=1.724 \times SOC/(silt+clay) \times 100\% \quad (1)$$

The acidity of soil was measured by pH meter (ISO 10390: 2011).

Vertically oriented intact soil cores were sampled in 4 replicates in 100 cm^3 metal cylinders for determination of bulk density (Db) (ISO 11272:1998) and water retention till pF 2.5 by the suction type apparatus (ISO 11274:1998). Particle density (Ds) analysis was carried out in water with 100 cm^3 pycnometers. Total porosity (Pt) was calculated using the measured bulk density (Db) and particle density (Ds):

$$Pt=(1-Db/Ds)\times 100\% \quad (2)$$

Distribution of dry-sieved aggregates between size classes (>10, 10-5, 5-3, 3-1, 1-0.25, and <0.25 mm) was determined by manual dry sieving of air dried soil using set of sieves arranged from top to bottom with decreasing size of the openings. The proportion of each aggregates class (DSA) was calculated relative to the summed total weights of all the aggregate size classes. Mean weight diameter (MWD, mm) of the fraction less than 10 mm was calculated.

The water stable aggregates were determined by the method of Savinov, modification of Vershinin and Revut (Revut, 1969). Four soil samples (20 g each) were prepared for wet sieving: one composite sample ($F_{0.25-10}$) by taking equal quantity (5 g) of air dried aggregates from four fractions: 10–5, 5–3, 3–1, and 1-0.25 mm; three replicate samples (20 g each) with air dried aggregates from a single fraction 3–1 mm (F_{1-3}). The wet sieving was done by the device of Savinov an hour after direct immersion of the air dried soil aggregates sample into

water (slaked pretreatment). The correction for aggregate-sized sand content (skeleton) was done according to Six et al. (2000):

$$WSAi=Pi-Si \quad (3)$$

where WSAi – is the proportion of water stable aggregate of size class i (i=10-5, 5-3, 3-1, 1-0.25 mm), Pi is the proportion of the aggregates remaining on the sieve (5, 3, 1, and 0.25 mm) after wet sieving to the weight of the sample placed on the set of sieves prior to the wet sieving, Si is the proportion of sand with size i in aggregates of size i after disruption. The proportion of water unstable aggregates (P<0.25) was calculated as 100%-the total sum of Pi. Mean weight diameter of water stable aggregates (MWDwet) was also calculated.

The water stability of aggregates is expressed by the ratio (MWDR) of mean weight diameters of aggregates after (MWDwet, mm) and before wet sieving and by the percent of water stable macro aggregates >0.25 mm (WSA>0.25, %) (Dilkova, 2014).

Soil water retention at suctions less than pF 2.5 (-33 kPa) was determined on the undisturbed soil samples from the control variant by the suction plate method similar to ISO11274:1998 using Shot filters G5 connected to hanging column for measuring soil water content at pF 1, 1.7 and 2, and to a vacuum chamber for pF 2.5 as described by Kercheva et al. (2017, 2019). Soil water retention at pF 2.5 was measured also by ceramic pressure plate extractor (Soilmoisture equipment Corp.) using 3-5 mm soil aggregates from all variants. Soil water retention at suction 1500 kPa (pF 4.2) was determined on fine earth (<2 mm) samples by pressure membrane apparatus (Soilmoisture equipment Corp.). The hygroscopic water content at pF 5.6 at water adsorption part of the curves was determined using vapor pressure method with controlled relative humidity 75% in desiccators containing saturated solution of NaCl.

The plant available water capacity (PAWC) was determined as the difference between water retained at pF2.5 (field capacity) and pF4.2 (wilting point):

$$PAWC=W_{pF2.5}-W_{pF4.2} \quad (4)$$

Air capacity, AC was calculated as difference between total porosity (Pt) and volumetric water content $\theta_{pF2.5}$ corresponding to pF2.5:

$$AC=Pt-\theta_{pF2.5} \quad (5)$$

Relative FC was calculated as $\theta_{pF2.5}/Pt$.

In the current study direct measurements of thermal conductivity of the studied variants were conducted in the field with KD2Pro reader-logger device and its single needle TR-1 sensor.

RESULTS AND DISCUSSION

Soil texture of the studied soil was sandy loam in surface 0-10 cm and finer (sandy clay loam) at 25-30 cm depth (Table 1). The soil organic carbon content decreased from medium in the surface 0-10 cm layer to low at the bottom of the arable soil layer (Table 1). The structural stability index (SI, Eq.1) indicated degradation (SI≤5.0%) of the control variant and of V2 (single manure application). In the variants with biochar application the values of SI increased, but still the risk of soil degradation according to this indicator was assessed as high (5.0<SI≤7.0%) (Table 3). An increase of the soil organic carbon content was observed only in the variants with the combined application of manure and biochar. In these variants it was observed higher content of water retained at matric potential -1500 kPa (pF 4.2 wilting point) and at pF 5.6 (Wh hygroscopic water content) than in the control. Our results stay in line with

reported positive effect of high sorption properties of BC on soils with low clay and SOC content (Arthur et al., 2015; Amoakwah et al., 2017).

The physiochemical properties did not change significantly in the studied variants as can be seen by the small standard deviation between the variants (Table 2). The soil reaction was neutral pH in H_2O was 6.6 in average (Table 2).

Table 1. Soil texture fractions and class (ISO11277, 2009), and soil organic carbon content (SOC) of the control variant (V1)

Depth, cm	Sand, %	Silt, %	Clay, %	Texture class	SOC, %
0-10	54	30	16	SL	1.31
25-30	53	22	25	SCL	0.63

Table 2. Average and standard deviation of physicochemical properties of surface 0-10 cm soil layer of the studied variants

pH in	Exchange cations, cmol kg^{-1} soil							Base
H_2O	$T_{8.2}$≡CEC	T_{CA}	T_A	$H_{8.2}$	Al	Ca	Mg	saturation, %
6.60±0.02	22.8±0.2	19.1±0.2	3.7±0.2	2.6±0.1	0	18.3±0.3	1.8±0.1	88.4±0.2

Soil bulk density at 0-5 cm depth was low and increased in depth in all variants except V2 which was more compacted in the whole 0-30 cm layer (Table 3). The soil compactness of this coarse textured soil was provoked by the irrigation of the broad bean (Petrova et al., 2019) due to the low water stability of soil aggregates (Table 3, Fig. 2). A decrease of aeration capacity at 0-10 cm depth was observed in all amended variants and it was critically low (7.2%vol.) when only manure was applied.

The effect of soil amendments on soil aggregation was seen in the distribution of dry aggregates (Fig. 1). The control variant (V1) was characterized with significant presence of clods (>10 mm), while it can be suggested that the amended material prevented clod formation. There was no significant difference between studied variants regarding WSA_{F1-3} >0.25 mm of the top 0-10 cm layer. Water stability of aggregates in all variants was low, but in the amended variants the water stability of aggregates fraction F_{1-3} slightly increased. This can be explained by the water stability of the amended material and not by the formation of soil aggregates as it did not influence the water retention properties (Table 3) and the water stability of macroaggregates, which was very low (MWDR=0.12-0.17) (Fig. 2). The stability of aggregates increased in depth 25-30 cm due to the higher clay content.

Table 3 Soil quality indicators in studied variants: V1) control - no biochar and manure; V2) manure - 4 t ha^{-1}; V3) biochar – 500 kg ha^{-1}; V4) manure 4 t ha^{-1}+ biochar 500 kg ha^{-1}; V5) manure 4 t ha^{-1} + biochar 750 kg ha^{-1}

Variables	Depth, cm	V1	V2	V3	V4	V5
SOC, %	0-10	1.31	1.17	1.36	1.41	1.55
	25-30	0.63				
SI, %	0-10	4.9	4.4	5.1	5.3	5.8
Db, $g.cm^{-3}$	0-5	1.45	1.67	1.49	1.52	1.48
	10-15	1.72	1.68	1.56	1.68	1.54
	25-30	1.72	1.64	1.71	1.70	1.68
Pt, %vol	0-5	45.7	37.6	44.1	42.6	43.7
	10-15	35.5	36.9	41.5	36.6	41.6
	25-30	35.9	38.9	36.1	36.7	37.4
Wh, %wt	0-10	2.67	2.57	2.60	3.00	3.20
W_{1500}, %wt	0-10	7.15	7.21	7.27	8.32	9.00
W_{33}, %wt ([a])	0-10	16.1				
W_{33}, %wt ([b])	0-10	18.1	18.2	17.4	18.8	18.5
θ_{33}, %by vol ([b])	0-10	26.2	30.4	25.9	28.6	27.4
PAWC, % by vol ([b])	0-10	15.9	18.4	15.1	15.9	14.1
θ_{33}/Pt ([b])	0-10	0.57	0.81	0.59	0.67	0.63
AC, % by vol ([b])	0-10	19.5	7.2	18.2	14.0	16.3
WSA_{F1-3} >0.25 mm	0-10	24.7	26.8	28.7	26.6	26.5
	25-30	33.6				
Skeleton $_{F1-3}$ >0.25 mm	0-10	24.1	24.1	23.5	25.2	23.4

([a]) – determined on undisturbed soil cores; ([b]) determined on soil aggregates

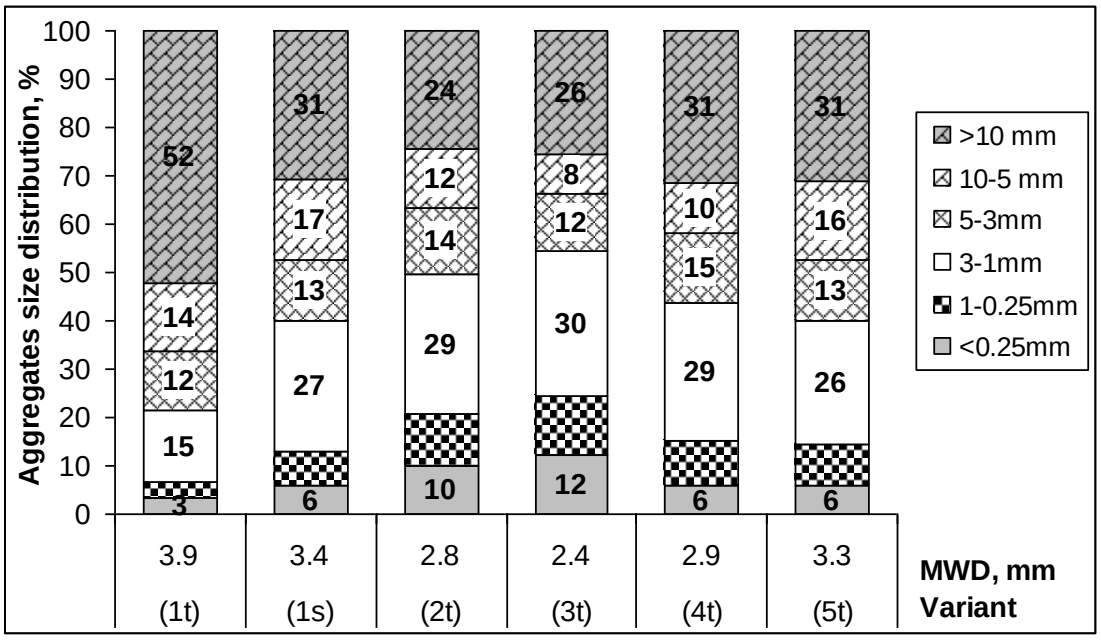

Fig. 1. Aggregates size distribution and mean weight diameter of dry aggregates (MWD) in topsoil (t) 0-5 cm and subsoil (s) 25-30 cm soil layer of the studied variants (1÷5).

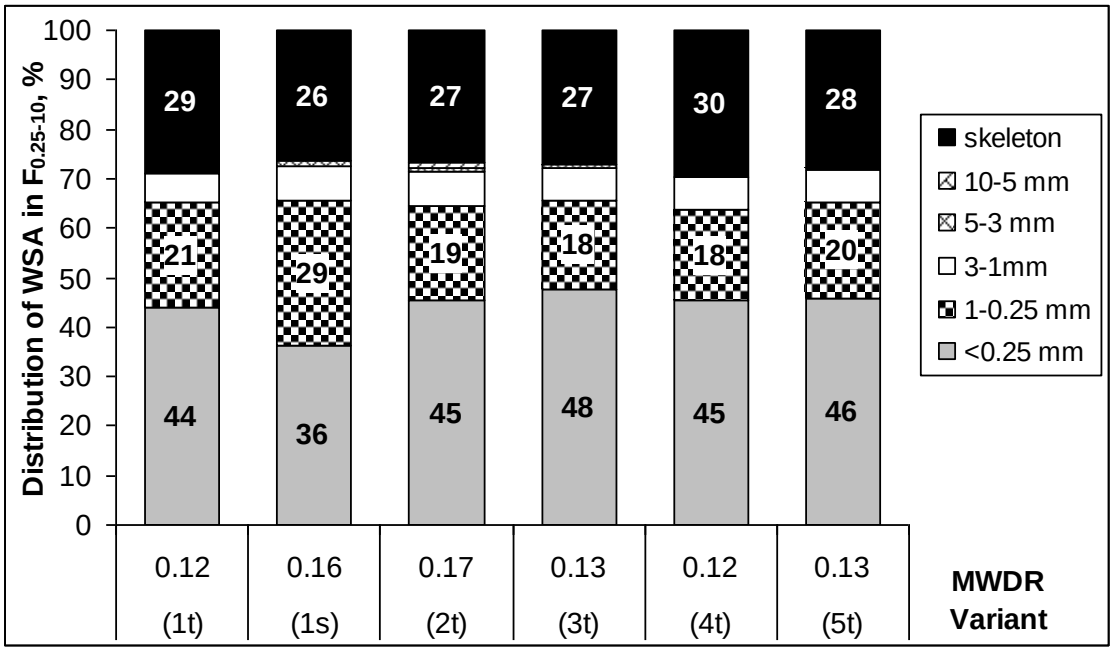

Fig. 2 Size distribution of water-stable aggregates in the composite sample ($F_{0.25-10}$) and mean weight diameter ratio after and before wet sieving (MWDR).

Soil water retention curves in the control variants showed that higher amount of larger pores was observed only in the surface 0-5 cm soil layer. The difference between soil moisture at pF 2.5 and 2.0, both potentials often used as a measure for field capacity, decreased from 2.9%wt in the upper 0-5 cm soil layer to 1.7%wt in the finer textured 25-30 cm layer. The water retained in the intact soil cores at pF 2.5 was 2% lower than that the one measured on aggregates of 3-5 mm size in Richards chamber (Table 3).

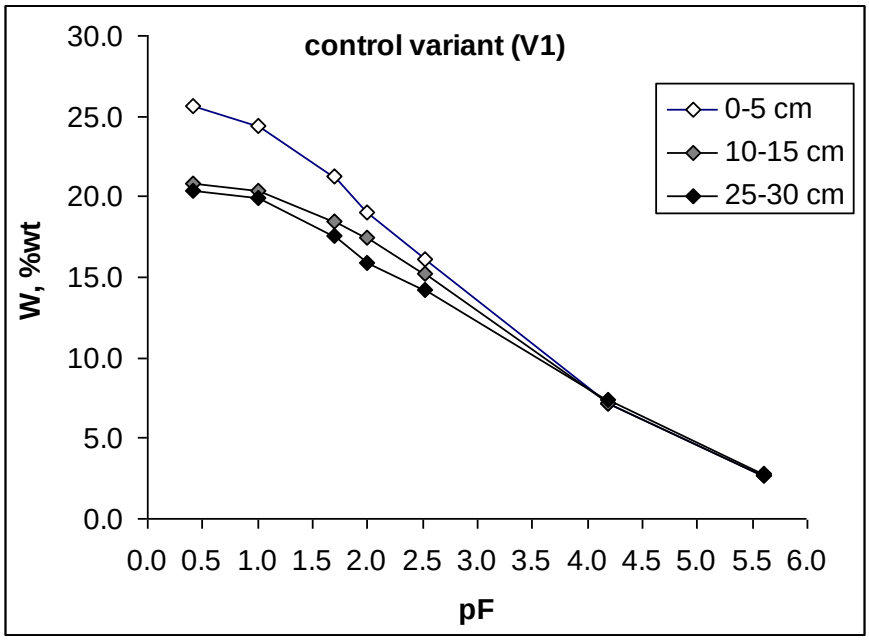

Fig. 3 Soil water retention curves at three depths of the arable soil layer of the control variant (V1).

The relative field capacity increased in all treated variants which can be considered as a positive effect of the applied manure and biochar. As the rate of increase of water retained at

potential -33 kPa (pF 2.5) in the amended variants was lower than the water retained at pF4.2, there was no or even negative effect on plant available water capacity (Table 3).

The soil moisture content at time of sampling was around and above field capacity. The soil thermal conductivity measured at 10-15 cm depth in the field during soil sampling was the highest 1.506 W $m^{-1}K^{-1}$ in the control variant in comparison with the variant with manure 1.268 W $m^{-1}K^{-1}$, biochar 1.489 W $m^{-1}K^{-1}$ and combined manure + biochar 1.372 W $m^{-1}K^{-1}$ (Table. 4). As the single application of biochar did not change soil thermal conductivity, it can be concluded that the applied manure decreased thermal conductivity.

Table 4 Soil thermal conductivity at sampling at 10-15 cm depth.

Variables	V1	V2	V3	V4	V5
θ, %vol.	28.9	30.9	30.1	30.0	32.1
θ/Pt	0.82	0.84	0.72	0.82	0.77
λ, W $m^{-1}K^{-1}$	1.506	1.268	1.489	1.385	1.360

CONCLUSIONS

The obtained data of soil properties did not show significant positive effect of the combined application of manure and biochar produced from maize cobs on sandy loam Fluvisol when growing of broad bean. This can be due to relatively low rates and spatial heterogeneity of the amended material and lack of effect in the first year after its incorporation in this coarse texture soil.

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SANITRY SITUATION OF THE MACHROUHA FOREZT (ALGERIA)

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ABSTRACT

The Machrouha forest (Algeria) is used for forestry and ecotourism. Managed by the Conservation des Forêts de Souk Ahras, this forest is made up of several silvicultural species (cork oak and Zeen oak) spanning an area of 16.448 ha. This work aims to assess the current state of health of this forest. 4 plots were chosen at random for this study. The dendrometric data measured on the sample trees are used to characterize the structure of this forest; thus, information collected on the same samples is analyzed using ARCHI methods which are based on a reading of the architecture of the trees. The diagnostic results show that the majority of the trees in these plots are currently in a healthy state, a minority are stressed or resilient by various natural and anthropogenic factors and an average biodiversity. Therefore, the health of the forest is good and is classified as a viable ecosystem. But these potentialities are insufficient: pressures must be reduced and better conservation of the ecosystem is ensured. To this end, the present study suggests development primarily through silvicultural interventions promoting the regeneration of these two species and including a permanent ecological monitoring system. The latter makes it possible to frame all the interventions.

Keywords: Cork oak, Zeen oak, structure, ARCHI.

INTRODUCTION

The forest is considered an ecosystem with multiple roles that should be conserved or restored. It is an excellent biodiversity conservatory because there are more animal and plant species in this biotope than in open areas (Dajoz, 2007). In recent years, a great problem of sanitary degradation of forest ecosystems (deciduous and coniferous) has arisen since the end of the 19th century. It increased in many countries at the beginning of the last century, especially in Europe, but also in North America (Sinclair, 1967).

The *Quercus* genus is one of the most species-rich forest genera. It includes several hundred woody species from temperate and Mediterranean zones, America, Europe and Asia (Barbero et al., 1991).

Sclerophyllous oaks participate in, or even constitute practically by themselves, various landscape types highly characteristic of the Mediterranean world. It is of course essentially the Mediterranean evergreen forest which represents, when it has not been destroyed, the physiognomic unit most generally assimilated to the Mediterranean climate and vegetation. (Haichour, 2009).

Currently, many natural Mediterranean forests and reforestation are in imbalance. The oaks among the main forest species affected (especially cork oak) in France, Spain, Portugal, Morocco and Algeria (Varela, 2008; Rached-Kanouni, 2013, Rached Rached-Kanouni et al., 2020).

In Algeria, oaks represent a forest capital where they cover nearly 40% of the Algerian forest (Alatou, 1994). The multiple aggressions are overgrazing, repeated fires, attacks of pathogens and insects, aging, poor silvicultural practices, as well as climate change, especially the increasingly prolonged summer droughts, leading to dieback and weakening of oak groves, especially subterranean (Becker and Levy, 1983; Delatour, 1980).

The objective of this work is to evaluate the health and quality of the cork and zeen oak stands of the Machrouha forest. In order to characterize these stands, we conducted dendrometric surveys, and various observations were made within the forest during the winter and spring of the year 2021. These observations mainly concerned the evaluation of the health status of the oak stand through the examination of the crown. The vigor of the stands was assessed by the ARCHI method.

The "ARCHI" method is a diminutive of "Architecture", because it is based on a reading of the trees' architecture. The principle is to carry out two series of observations. The first one concerns the symptoms of degradation of the crown (leaf deficit, abnormal coloration, mortality...); the second one concerns the processes of restoration of the crown (development of suckers, covering of wounds, resumption of growth...).

MATERIAL AND METHOD

Characteristics of the study area

Our work was carried out in the commune of Machrouha, its area is 22623 ha, the forest area is 16448 ha and the afforestation rate 73%. It is the most wooded commune of the wilaya of Souk-Ahras. It has a total population of 17614 inhabitants. The commune of Machrouha is located in the north of the wilaya of Souk-Ahras. It is considered as a tourist region; it represents a place of rest and treatment of respiratory diseases in view of its healthy and clean climate. The forests of the wilaya of Souk-Ahras are divided into two natural sectors: one in the south grouping the forests of Aleppo pine and the other in the north represented by the forests of cork oak and Zeen oak, the latter extend over 24232 hectares including 13080 hectares of cork oak.

Located on the heights of the Atlas tellien, the willaya of Souk-Ahras is exposed to Mediterranean climatic influences in the north on the one hand, and desert in the south on the other hand (Zouaidia, 2006).

Method of study

Horizontal analysis consists of studying the spatial structure in terms of abundance and dominance.

Abundance gives the number of stems of a species in the stand. It is expressed as the number of N per hectare (N/ha). Dominance is expressed as the sum of the land area (G). It is formulated by $G = \Sigma g = \Sigma (\Sigma \pi D^2/4)$ and is expressed in square meters/ha. D is the diameter of the tree at 1.30 m from the ground. The dominance gives an idea of the degree of filling of the forest, i.e. the part of the surface occupied by stems. It is therefore an indicator of the production of the platform. For the number N, the basal area G is also determined by the diameter class.

The stability of a stand is given by the value of the slenderness coefficient (EC), which is expressed by the height/diameter ratio (Robisoa et al., 2008).

The ARCHI method

Method for diagnosing tree decline and resilience capacities, based on architectural analysis of aerial parts (Drénou, 2013). The principle is to carry out two series of observations: the first concerns the symptoms of crown degradation (leaf deficit, abnormal coloration, mortality...); the second concerns the processes of crown restoration (development of suckers, covering of wounds, resumption of growth...) (Sabatier et al., 2014). This tool is used to prognosticate the short-term future of trees showing symptoms of dieback ((Drénou, 2013). For each species, a determination key guides the observer through a sequence of simple questions to one of the following prognoses (Table 1) :

Table 1. Different architectural types of forest trees.

Healthy tree (ARCHI Health)	Tree whose architecture is in conformity with its development stage.
Stressed tree (ARCHI S)	Tree whose architecture deviates from the reference sequence (uncertain future).
Resilient tree (ARCHI R)	Tree showing a dynamic return to normal.
Tree in crown descent (ARCHI D)	Tree building a new crown under the original one.
Tree in irreversible decline (ARCHI I)	Tree blocked in a situation of non-return to the reference sequence.

RESULTS AND DISCUSSION

The dendrometric parameters of the 4 plots of the Machrouha forest are shown in Table 2. The relative density of the stand is too high in plot P1 characterized by *Q. suber* with 256 feet/ha, plot P3 shows a very low abundance with 22 feet/ha and 89 feet/ha respectively for *Q. suber* and *Q. Canarensis* respectively. The minimum average diameter obtained is 32.09 cm for plot P2 (*Quercus suber*) and 41.21 cm for plot 4 (*Quercus canarensis*), which means that these two stands are at the young forest stage. The maximum average diameter observed was 61.94 and 72.51cm respectively for the cork oak and the zeen oak in plot 3, which reached the mature forest stage with more or less large diameters. The highest average height was also found for *Quercus suber* in plot 2 (23.01m) and the lowest in plot 1.

Table 2. Dendrometric characteristics of the species studied.

Plots	Species	D (cm)	H (m)	H/D	N/ha	G (m²/ha)
P1	*Q. suber*	40,28	9,03	22,50	256	0,14
P2	*Q. suber*	32,09	7,41	23,01	178	0,08
P3	*Q. canariensis*	72,51	18,23	25,59	89	0,43
	Q. suber	61,94	16,28	13,64	22	0,30
P4	*Q. canariensis*	41,21	12,62	32,46	144	0,16

The average slenderness coefficients varied between 13.64 and 32.46 in the 4 plots. The variation of H/D is irregular and stable with a complete and dense cover; this result suggests that the slenderness coefficient is a function of the average diameter and thus of the age of the stand.

The study presented aims to establish the state of the forest of EL Machrouha by a simple diagnosis. The evaluation of the health of the crowns is carried out by the ARCHI method. The

study of the architecture of a plant organism is based on a morphological analysis of the entire aerial part. Its principle consists in describing in situ all the main structural forms that the plant follows during its development, in order to deduce by comparison the dynamics of growth linking them over time. The results obtained from the field surveys were limited to 4 plots: P1, P2 cork oak plots, P3 mixed plot (cork and zeen oaks) and P4 zeen oak plot are illustrated in Table 3. There is a large number of ARCHI Health type trees (36.54%); second is the ARCHI R type with a percentage of 32.69%. The trees of type ARCHI S present 30.77%. The ARCHI I type and ARCHI D type are totally absent.

Table 3. The different states of cork oak and zea oak according to the ARCHI type.

Parcelle	Espèce	ARCHI H	ARCHI R	ARCHI S	ARCHI I	ARCHI D
P1	*Q. suber*	10	2	4	0	0
P2	*Q. suber*	0	3	10	0	0
P3	*Q. canariensis*	3	5	0	0	0
	Q. suber	2	0	0	0	0
P4	*Q. canariensis*	4	7	2	0	0

The ARCHI R type is recorded in all 4 plots, while the ARCHI S, Health type is recorded in 3 plots. We notice the absence of ARCHI Health trees in plot 2 and the absence of ARCHI S trees in plot 3 (Figure 2).

The maximum value of ARCHI Health type is obtained in plot 1 and the minimum values are around 5 trees in plot 4. The smallest values are noticed for ARCHI D and ARCHI I types; they are zero for all plots.

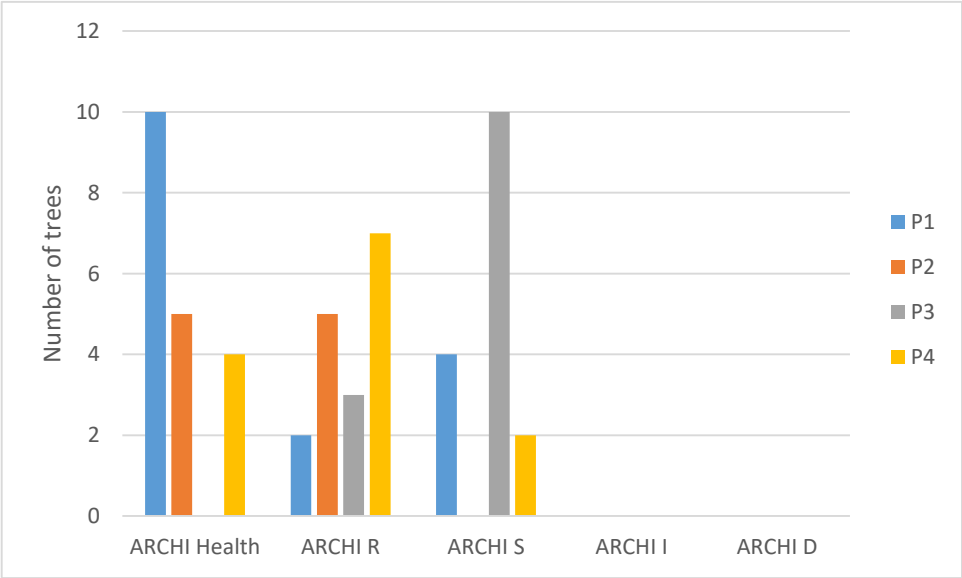

Figure 1. The different types of cork and zeen oaks ARCHI.

Our results indicate that the majority of oak trees in Machrouha forest are ARCHI Health and ARCHI R type (69.23%). The ARCHI Health type characterized by trees with no

significant symptoms of crown degradation and whose architecture is consistent with its stage of development (Drénou et al., 2019).

ARCHI R type trees to its own characteristics are: in the absence of additional stress, this type will revert to healthy type. In terms of ring width formed, after a period of decay, radial progression recovers. A resilient oak is potentially a future tree, provided the log is of good quality and the site is suitable for timber production. As for the ARCHI S type trees (state of Stress), it is not possible to pronounce on the future of the tree. Either the stress is too recent, or the five architectural descriptors used are insufficient to make a prognosis. Sometimes the stress follows a first stress that the oak has overcome and consequently affects the suckers already in place. In all cases, it is necessary to follow the evolution of ARCHI S trees over time before making a prognosis (Drénou et al., 2011).

During our study, the results obtained indicate that the majority of trees are of the ARCHI Health, ARCHI R and ARCHI S types in the cork and zeen oaks plots, this can be explained by the influence of various ecological factors of the study area characterized by more or less favorable soils and climate.

The Archi method offers the forester the possibility of placing his stand, already identified as dying, in the dynamics of dieback: is it an irreversible dieback (leading to death in a variable time interval), in a stressful situation, or in the process of resilience (Rached Rached-Kanouni et al., 2020).

The knowledge of the architecture of trees of a large number of species has allowed the transposition of these concepts to forestry technicians and has resulted in the establishment of observation protocols to establish a diagnosis of the architecture giving indications on the level of stress suffered by the tree, to establish its reactivity and therefore to propose a prediction of the evolution of its architecture. The evaluation of the fragmentation of the crown of trees is thus linked to a capacity of growth and thus of carbon sequestration.

CONCLUSION

Tree architecture reflects the non-linear dynamics of tree response to stress. It takes into account the specificities of each species and the observation of epicormic branches. Its observation gives a short-term prognosis on the future of the trees and prioritizes the series of observations to be carried out in the form of keys for determining the types. This type of analysis, privileging a qualitative rather than quantitative estimation of dieback symptoms. The diagnostic results show that the cork oak and zeen oak of the Mashrouha forest is in good health despite the negligible presence of dead trees, the absence of trees descending from the tops and trees with irreversible decline. The knowledge of the architecture of trees of a large number of species has allowed the transposition of these concepts to forestry technicians and has resulted in the establishment of observation protocols to establish a diagnosis of the architecture giving indications on the level of stress suffered by the tree, to establish its reactivity and therefore to propose a prediction of the evolution of its architecture.

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POSSIBILITIES OF USING WIND TURBINES IN DISINFECTION OF GREENHOUSE SOILS

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ABSTRACT

Our country has a significant potential when it comes to wind and solar energy, two of the renewable energy sources. Wind energy takes its highest value on the coasts of the Marmara and Aegean Seas. Hence, the use of electrical energy obtained from the wind turbines in Tekirdağ located in the north of the Marmara Sea, in terms of agricultural production, has been addressed.

The electrical energy obtained from wind turbines is used for heating the resistance wiring whose electricity consumption is low (25 W m^{-1}) whose temperature increases up to 70-80 ^{o}C. Soil disinfection can be carried out in a greenhouse by increasing the soil temperature through placing them into the soil at a depth of 10-15 cm. The fact that the wind turbine system to be established for this purpose will be 4-5 kWh will fulfill the need. The use of chemical agents in disinfection to be conducted with chemical drugs that are very toxic to living beings will also be eliminated. In the disinfection application, a solarization application by coating the greenhouse soil with a transparent polyethylene cover will also increase the success of the disinfection process. Such works must be performed in the summer months when there is no production in greenhouses.

The electrical energy obtained from the wind turbines can ensure the operation of the fans in the ventilation of the greenhouses, at times when the production is performed in the greenhouses. It may also meet the energy needs of some tools and equipment.

Keywords: *Wind energy, Wind turbine, Greenhouse, Soil disinfection, Solarization*

INTRODUCTION

Today's societies aim to meet their needs without inflicting consequences that may adversely affect future generations. Nevertheless, the possible need for energy resources in the world keeps increasing day by day. In this, especially in developing countries, energy demand will be even more intense in parallel with population growth, industrialization, increase in the welfare level of people, and technological developments. The fact that fossil energy resources pose serious environmental issues in the world, existence of the danger of depletion of its reserves in the near future, and the dependence on the source countries cause various political and economic setbacks. In addition, due to reasons like price instability, the interest in renewable energy resources has increased (Yılmaz, 2012; Özen et al., 2015).

Especially in developed countries, energy resources like hydroelectric, wind, geothermal, solar, biomass, wave, hydrogen etc., which are renewable energy resources, are utilized in various ways, particularly in electricity generation. Turkey is more advantageous concerning renewable energy resources compared to fossil resources (Yılmaz, 2012).

In spite of all this, while various energy resources are used in the world, 85.5% of those resources cover fossil resources like petroleum, natural gas, and coal (Koç et al., 2018).
The fact that maintenance and operating costs of installation facilities connected to renewable energy resources are low and presence of no raw material costs render such resources attractive. In renewable energy, solar and wind energy are dominant in order of importance, compared to others, (Özen et al., 2015).

Wind Energy

Wind is caused by the temperature differences between various parts of earth's surfaces because of the solar radiation. The difference of heat absorbtion at various parts of earth's surface causes the temperature, humidity, and pressure of the air to become different, and air to move between different pressure points. Approximately 2% of the solar energy reaching the world transforms into wind energy (Anonymous, 2021a).
Wind is the air movements that emerge through the impact of high and low pressures as well as forces that occur as a result of uneven heating and cooling of the earth's surface. Wind energy is the movement energy of air flow. It forms the wind by ensuring that the air moves on earth. The major forces in the atmosphere, which affect the speed of the wind, are pressure gradient difference, deflecting impact of the earth's rotation (coriolis force), as well as centrifugal and friction forces (Elibüyük and Üçgül, 2014).
Wind is expressed with two parameters as speed and direction. Wind speed increases with height. Theoretical power is directly proportional to the cube of its speed (Anonymous, 2021a).

Turkey's Wind Energy Potential

The areas where Turkey receives the most wind are coastal areas and high areas. The areas around the Marmara Sea, Northern Aegean region and the areas in the south of the province of Hatay are the places receiving the highest wind speed. When it comes to seasonal average values, the coastlines and the surrounding area of the Marmara Sea reach the times when the wind speed becomes at the most severe levels in December, January, February, and March (Tunus, 2019).
With the advent of autumn, wind speeds start to increase as of September. Strong wind speeds start blowing from December and the strongest wind speeds take place in January and February. Starting from March, the decrease continues in April and May. The wind speed that reaches its lowest level in June continues until September during the summer months (Tunus, 2019).

Wind Turbines

Wind energy is the type of energy that has the most development and is subject to the most investment among renewable energy resources in the world. Electricity generation from wind is provided by a wind turbine. A wind turbine converts wind energy into motion energy and electrical energy with the help of a generator. Basic components of wind energy systems are a wind turbine, power generator, charge regulator, static power converter unit (inverter), energy storage unit (battery), and control mechanism. The electrical output is connected to a load or to the power grid in line with the application (Toprak, 2011). Wind turbines can start generating electricity only at a particular wind speed. A wind turbine performs energy generation at the lowest (cut-in) and the highest (cut-out) wind speed rates. The cut-in speed of modern wind turbines is between 2-4 ms^{-1}, their nominal speed is between 10 and 15 ms^{-1}, and their cut-out speed is between 25 and 35 ms^{-1}. At the wind speed determined for every wind turbine, the

power obtained from the system reaches the biggest value. In order to ensure that the system is not damaged, switching of wind turbines to the stop position after a certain wind speed is ensured automatically. This maximum speed is referred to as the cut-out speed of the system (Anonymous, 2021a).

In Turkey, it has been accepted that wind power plants having the power of 5 MW per square kilometer in areas 50 m above ground level and with above 7.5 ms^{-1} wind speeds can be erected. Accordingly, Turkey's wind energy potential has been identified as 48000 MW (Anonymous, 2021a).

Design of a Wind Energy System

In the system to be installed for soil heating and disinfection in a greenhouse with wind turbines (Figure 1), there may be wind turbine, battery pack, battery charge regulator, inverter, auxiliary electronic circuits, and resistance wiring that will heat the soil (Yüksel and Yüksel-Türkboyları, 2018).

In Figure 1, the inverter, charge regulator, battery group and resistance wires have been purchased in a project supported by the Scientific Research Project Office of T.N.K.U. They have been used in a high tunnel that belongs to Vocational School of Technical Sciences. The Picture of wind turbines have taken at a location close to Çevrimkaya (Tekirdağ).

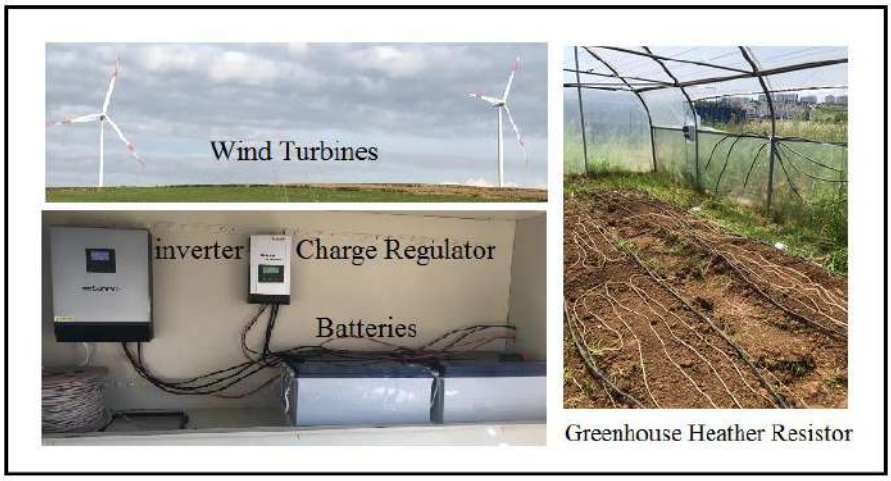

Figure 1. Elements of the wind turbine and greenhouse soil disinfection system

The power of wind turbines that convert the movement of air into electrical energy is determined according to the amount of energy needed. In wind energy systems, the entire conversion output from the power that can be obtained from the wind to the electrical output is between 25-35% (Toprak, 2011; Tunus, 2019). Due to the irregularity of the winds, there appears to be a discontinuous order in electricity generation from wind (Şenel and Koç, 2015). This brings out the energy supply-demand disagreement. As a result, an energy storage issue takes place and the batteries used to store the excess energy also increase the costs (Tunus, 2019). To generate excess energy, it is necessary to use a wind turbine that is a little higher than the calculated power value.

By regulating the current coming from the wind turbine, the battery charge regulator prevents the battery from being overcharged or completely discharged. Hence, damage to the batteries is prevented and their time of use is extended. The charge regulator cuts off the current from

the wind turbine or the current from which the load is drawn, according to the state of the battery.

Alternating or direct current can be generated in a wind turbine. Nevertheless, when the current is high or not needed, it must be stored in batteries. Since alternating current is generally used in systems, an inverter must be added to the output of the batteries in the system (Toprak, 2011; Yüksel and Yüksel-Türkboyları, 2018).

The use of the energy obtained from the wind energy system in the disinfection of greenhouse soils is possible with the conversion of that energy into heat energy. It is, for this purpose, possible to place and use resistance wires in the soil. It is desirable that these wires do not generate a temperature higher than 85-95 ^{o}C. High temperatures cause the loss of aliveness of beneficial soil bacteria along with harmful soil bacteria. To adjust the temperature of the resistance wires, a rheostat or dimmer must be incorporated into the system (Yüksel and Yüksel-Türkboyları, 2018).

In this study, a different use of wind energy from renewable energy resources in greenhouses was addressed. The use of electrical energy obtained from wind turbines in the disinfection of greenhouse soils was studied. The impacts of the obtained electrical energy on the disinfection of greenhouse soils were determined with the help of increasing temperature and solarization method, with the resistance wires placed under the ground.

Solarization and Disinfection in Greenhouses

In our country, greenhouse producers cultivate the same plant every season. This causes the same microorganisms and pests in the soil to increment continuously and the emergence of soil exhaustion. On the other hand, the fact that greenhouse soil air is low and the soil moisture is high due to irrigation causes rapid proliferation and spread of all kinds of diseases and pests in the greenhouse (Altındişli-Atağ et al.,2012). Disinfection is the prevention of bacteria, fungi, and viruses that cause infection in living beings from becoming active (Nar, 2021). To perform healthy, efficient, and high quality production in greenhouses, the impact of soil pests and diseases is reduced by a certain degree of disinfection (Yüksel and Yüksel-Türkboyları, 2018). Many of the disease agents and nematodes that cause injuries in culture plants are present in the soil. The fight against those soil-based factors in greenhouse soil can be carried out through physical and chemical means. In physical disinfection, the greenhouse soil can be heated to a certain temperature by means of using the sun directly or by various methods (Yüksel-Türkboyları et al., 2019a). Chemical disinfection is the application of some extremely toxic chemicals (pesticides) to the soil. This method is extremely dangerous for the environment and human health, and inflicts an adverse impact on the natural balance. Such substances can return to humans and animals through the food chain (Kitiş, 2012). Moreover, they have many undesirable impacts like resistance to diseases, pests, and weeds (Fennimore and Doohan, 2008). This causes an increase in weeds in the soil, a decrease in the yield of cultivated plants, and an increase in hoeing expenses. For today's agricultural production to be sustainable and in order to protect human and environmental health, the conscious and controlled use of chemical drugs must be ensured. The use of chemicals must be restricted as much as possible by taking measures in this regard (Özkan et al., 2003; Delen et al., 2005).

The use and production of MeBr, one of the very toxic pesticides used in soil disinfection, were prohibited worldwide. As a result of this, studies have been conducted on methods that use higher temperatures, which is one of the physical methods, in the fight against soil pests. It was determined that soil solarization applications are effective in the purification from pests in a greenhouse environment (Katan, 1987; Yüksel-Türkboyları et al., 2019a). Solarization ensures

the increase of the temperature of the soil under the cover, with the sun's rays, subsequent to covering the soil surface with polyethylene or organic materials..
The solar radiation permeability of the polyethylene cover used in solarization is more effective on the soil temperature. The surface temperature of the black polyethylene cover is higher than the transparent cover. However, the temperature of the soil under the transparent polyethylene cover is higher than the black polyethylene cover. It is more advantageous to use transparent polyethylene covers in solarization (Yüksel-Türkboyları et al., 2019b).

MATERIALS AND METHODS

General Properties of the Research Site

The province of Tekirdağ where the study was conducted is in the area of Thrace situated in the northwest of Turkey. Thrace is a peninsula located in the southeast of the European continent. The peninsula is surrounded by seas and straits on three sides. Tekirdağ is also located in the north of the Marmara Sea. As a geographical position, it is situated between the 26˚41′-28˚10′ eastern longitudes and 40˚35′-41˚35′ northern latitudes. Due to the absence of high mountains and deep valleys, it can be defined as slightly rough. Its geological structure is quite young and it belongs to the 4th Period. When it comes to climate type, it is semi-humid, dry in summers and rainy in autumn, winter, and spring. Summer and winter seasons are windy (Anonymous, 2021b). According to the wind energy potential atlas, Tekirdağ and the Marmara Sea coasts in our country have high wind speeds (Karık et al., 2017; Tunus, 2019). Thus, Tekirdağ has an important advantage in view of generating power from the wind.

Method

Heating of the soil as a physical disinfection method in greenhouses has been scrutinized. Likewise, it is aimed at solarization to increase the temperature of the soil with the sun rays in the summer months. Nevertheless, it is specified that solarization soil disinfection must be conducted at least four weeks as the soil temperature has not reached the desired height (Kitiş, 2012). Hence, it is aimed in our study to raise the soil temperature up to 70 ºC-80 ºC and carry out the disinfection in a very short time by burying the resistance wires at a 10 to 15 cm depth for increasing soil temperature.
The Thrace Region is one of the regions that receive the most wind energy in our country (Tunus, 2019; Toprak, 2011). That is why the required electrical energy can be generated by the wind turbines that will be installed in the region. Disinfection can be conducted effectively by raising the soil temperature, where the generated electrical energy can bu utilized to in the resistance wires placed into the soil. With the use of such a system, the harmful impacts of the chemicals to be used in disinfection will be prevented and its cost will be eliminated. Expansion of the system, particularly in culture plants that have high economic yield, will be a more economical way of production through reducing chemical inputs for the health of producers and consumers.

Use of Wind Turbines in Soil Disinfection of Greenhouses

A discontinuous order in electricity generation from the wind is observed because of the irregularity of the winds. During periods when the wind does not blow at a sufficient speed, there is not enough energy production (Şenel and Koç, 2015). Hence, the wind energy system must be supported by batteries. The batteries provide energy to the system when electricity is not produced in it (Yüksel and Yüksel-Türkboyları, 2018).

To generate heat from electrical energy, resistance wires or heat bands are used in the system (Anonymous, 2021c). These resistance wires must be resistant to soil and water in order to be used in a greenhouse. The energy consumption of the resistance wires produced for this purpose is 25-30 W m^{-1} or that of the heat band is 17-18 W m^{-1}. During the production seasons, resistance wires may be required to be used at low temperatures in greenhouses. Soil heating can be conducted to increase the root activities of the plants grown in the greenhouse and ensure their rapid development, especially in cold seasons. It ensures the increase of the yield by the intake of more water and nutrients from the soil with the heated root zone and roots of the plants (Korkmaz and Saltalı, 2012). In such cases, a thermostat must be incorporated into the system in order to adjust the intensity of the electric current in the system and to reduce the temperature. Soil disinfection in greenhouses where solarization is applied will be more effective and in a shorter time with the wind turbine system to be installed, because higher soil temperature is obtained through the system. It is stated that soil disinfection in greenhouses with solarization must be carried out for four weeks as the shortest period (Kitiş, 2012). The primary target here is to increase the soil temperature to high degrees in order to reduce the effectiveness of harmful disease factors, nematodes, and microorganisms in the soil. By using heating resistance wires together with wind turbines, the soil temperature in the greenhouse can be increased to 70-80 ^{o}C. This ensures that soil disinfection is performed in less than a week, compared to solarization (Yüksel and Yüksel-Türkboyları, 2018).

Energy Need of the System

A discontinuous situation appears to be present in electricity generation from wind in the wind turbine system to be installed, as the winds are not regular (Şenel and Koç, 2015). A battery group must be incorporated into the system to ensure continuity in energy.

It can be used for heating and disinfection of greenhouse soils where solarization is applied, with the electrical energy obtained from wind turbines. For this purpose, resistance wires can be placed with certain distances like 25-30 cm and at a depth of 10-15 cm into the greenhouse soil.

Although the power of the system to be installed with wind turbines varies depending on the size of the area to be disinfected, it can be around 4-5 kWh (Yüksel and Yüksel-Türkboyları, 2018). Due to the discontinuity of the wind, only a part of this energy can be used. A soil length of 120 to 160 m can be heated in 3-4 kWh, with a resistance wire whose energy consumption is 25 W m^{-1}. By locating the wires in a 25-50 cm distance within the greenhouse soil, an area of 90 to 160 m^2 can be disinfected. Disinfection of a larger area can be ensured by the frequent relocation of the resistance wires laid in the greenhouse soil.

CONCLUSION

Soil disinfection is applied to prevent the reproduction and spread of diseases and pests that cause a decrease in efficiency in greenhouses. Soil disinfection is carried out by chemical and physical means. The toxic substances used in the application performed through the chemical method bring along results that are not suitable for humans and living beings. To prevent it, the use of wind and solar energy systems from renewable energy resources, which is one of the physical methods, can provide significant advantages. The wind turbine system, which provides advantages in terms of both health and economy, can be benefited from in this regard.

The electrical energy obtained through the wind energy system can be used for different purposes in greenhouses. This manner of use differs in the periods of production and the periods of non-production.

With the wind turbine system, soil disinfection can be performed in summer when the weather is hot and no production is present. If soil disinfection is supported by solarization, more positive results can be obtained.

In the production seasons like autumn, winter, and spring, the root zone of the plant can be heated with a wind turbine system. For this, resistance wires and a thermostat must be added to the system. This way, the length of low temperature and the resistance wire to be used increase by providing less energy to the system. A larger area can be heated as well. Thus, the chemical and biological activities of the heated plant roots increase. It is ensured that the yield is increased, by raising the amount of water and nutrients taken from the soil by plant roots with increased activity.

With the excess energy obtained from the wind turbine energy system, mandatory ventilation and cooling can be carried out in greenhouses.

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THE PHOTOCATALYTIC DEGRADATION OF THIAZINE AND ANTHRAQUINONE DYES IN AQUEOUS SUSPENSION OF MgO

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ABSTRACT

The removal of organic dyes from aqueous media is a significant effort for environmental protection. Among various treatment methods, heterogeneous photocatalysis is a promising advanced oxidation process to eliminate the organic effluent dyes. This method is based on the usage of UV-A light and a semiconductor simultaneously. MgO is a favorable photocatalyst due to its advantages such as being environmentally friendly, cost-effective, and having high chemical stability. The present work was focused on the determination of photocatalytic degradation of two different dyes categorized according to their chromophores under UV light. Methylene blue (MB) and Reactive Blue 19 (RB-19) were used as thiazine and anthraquinone model dye compounds, respectively. The structural changes in the functional groups of dyes with the presence of MgO involved in the photocatalytic process have been assessed by using ATR-FTIR. The photocatalytic degradation kinetics of dyes were followed by UV–vis spectroscopy. A higher removal efficiency was obtained for the degradation of RB-19 dye compared to MB. Moreover, the effect of initial dye concentration on the degradation of dyes were studied. The maximum removal efficiencies for both dye solutions were obtained at 2.5 mg/L.

Keywords: Heterogeneous photocatalysis, methylene blue, MgO, Reactive Blue 19.

INTRODUCTION

Dyes are commonly classified according to their chromophore structures such as azo, thiazines, anthraquinone, azo dyes (Rochkind et al., 2015). Most of them are containing aromatic rings in their structures and thus highly toxic, carcinogenic, non-biodegradable and mutagenic for human being and aquatic life. Methylene blue (MB) is a well-known, cationic dye with the thiazine structure. This carcinogenic, heterocyclic aromatic pollutant is used in many industries, mostly in textile industry. Another group constructed on the molecular structure is reactive anthraquinone dyes. Reactive Blue 19 (RB-19) is a commercial textile dye containing anthraquinone based vinyl sulphone. In particular, the anthraquinone structures can cause acute toxicity and mutagenic effect (Fanchiang and Tseng, 2009b). Therefore, the removal of textile wastewater containing thiazine and anthraquinone dyes is a critical issue for human health and environment (Fanchiang and Tseng, 2009b; He et al., 2008).

In recent years, advanced oxidation processes (AOPs) have been proposed for the treatment of dye wastewater containing recalcitrant dyes (Din et al., 2021; Fanchiang and Tseng, 2009a; Fanchiang and Tseng, 2009b). Among AOPs, photocatalysis is commonly employed as an efficient light induced method to degrade organic dyes in aqueous solutions. The system is based on the formation of oxidative hydroxyl radicals after the initiation of catalyst surface. The most widely used photocatalysts are TiO_2 and ZnO in this field (Javaid and Qazi, 2019; Rochkind et al., 2015; Zangeneh et al., 2015). The unique properties of MgO such as being low cost, and nontoxic, having high stability, low dielectric constant and refractive index makes it a promising candidate for photocatalysis. Several studies have been

reported on the photocatalytic degradation of dyes using MgO (Bagheri Gh et al., 2015; Balakrishnan et al., 2020; Fakhri-Mirzanagh et al., 2020; Jorfi et al., 2016; Mageshwari et al., 2013; Ratnam et al., 2020; Zheng et al., 2019).

The objective of this study is to examine the photocatalytic degradation kinetics of thiazine and anthraquinone dyes using MgO under UV light. RB-19 and MB were chosen as the model pollutants used commonly in textile industry. The photocatalytic studies were monitored by UV-vis spectroscopy and FTIR spectroscopy. Additionally, the effect of initial dye concentration effect on the degradation of dyes were investigated.

MATERIAL AND METHOD

MgO (Riedel-de Haën) powder was used as photocatalyst as provided by the supplier. All aqueous solutions were prepared with distilled water. The chemical structure of RB-19 and MB dyes were given in Figure 1. Testing of the photocatalytic activity were achieved in a cylindrical Pyrex reaction vessel. A 125W black light fluorescent lamp (λ_{max} 365 nm) was used as light source. The light intensity reaching the reaction medium was $I_o=1.65 \times 10^{16}$ quanta/sec (Parker, 1997). The photocatalytic experiments were performed without pH adjustment at MgO amount of 0.50 g/L. The irradiated dye solution (50 mL) was filtered immediately through 0.22 μm cellulose acetate filters to remove MgO. The photocatalytic degradation experiments of both dyes were carried out by varying the initial dye concentrations from 2.5 mg/L to 10 mg/L. The absorbance values of the samples were acquired by a Thermo Scientific Genesys 10S double beam spectrophotometer using 1 cm quartz cells. FTIR measurements were achieved using a Thermo Scientific Nicolet 6700 spectrometer equipped with an attenuated total reflection (ATR) accessory. Samples were filtered and then dried in an oven at 105 ^oC until constant weight for FTIR analysis.

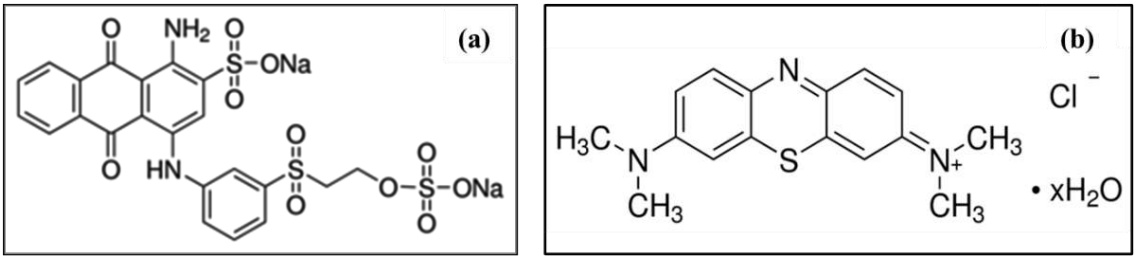

Figure 1. The chemical structure of (a) RB-19 (λ_{max}= 594 nm, MW= 626.54 g/mol) and (b) MB (λ_{max}= 664 nm, MW= 319.85 g/mol).

RESULTS AND DISCUSSION

Effect of Initial Dye Concentration

In order to investigate the initial dye concentration effect on photocatalysis, three different initial RB-19 and MB dye concentrations (2.5 mg/L, 5 mg/L, 10 mg/L) were used.

The removal percentage values of RB-19 and MB were calculated from Equation (1), where C_0 is the initial concentration of the dye and C is the dye concentration at time t. The results were presented in Figure 2 and the removal efficiencies of RB-19 (30 min), and MB (180 min) with respect to initial dye concentrations were given in Table 1.

$$Removal\% = \frac{C_o - C}{C_o} x100 \quad (1)$$

Table 1. The removal efficiencies of RB-19 (30 min) and MB (180 min).

	2.5 mg/L	**5 mg/L**	**10 mg/L**
RB-19	92.3	90.5	88.6
MB	61.4	61.0	56.9

The maximum degree of photocatalytic degradation for both dye solutions was obtained at 2.5 mg/L. It was observed that the removal percentage value was decreased gradually with the increase in the initial concentration of RB-19 and MB. The more dye molecules adsorbed on the photocatalyst surface with increasing concentration resulting in a decrease in the active sites of the photocatalysts may be given as the possible explanation for this behavior (Anju Chanu et al., 2019; Sohrabnezhad, 2011).

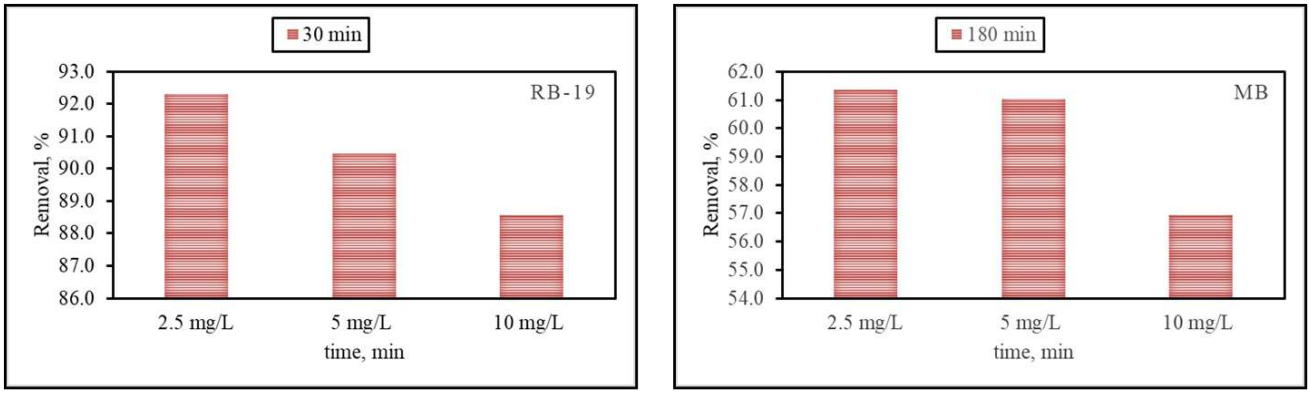

Figure 2. The effect of initial dye concentration on the degradation of RB-19 and MB.

Kinetic Study

The results revealed that the photocatalytic degradation of RB-19 and MB followed pseudo first order of kinetics expressed by Equation (2).

$$\ln[A] = -kt + \ln[A_o] \quad (2)$$

where,

A_o: initial absorbance of dye expressed as $A_{,o,}$
A: absorbance of dye expressed as A at time t,
t: irradiation time, min,
k: pseudo first order reaction rate constant, min^{-1}.

The kinetic degradation rate constants were calculated to be $k=54.5x10^{-3}$ min^{-1}, and $k=4.59x10^{-3}$ min^{-1} for RB-19 and MB, respectively.

FTIR spectroscopy

FTIR spectroscopy was used to identify functional group changes of the adsorbed RB-19 and MB dye molecules during photocatalysis. The spectrum of RB-19 was represented in Figure 3(a). The peaks at 676 cm^{-1} and 891 cm^{-1} were related to aromatic rings, while 1182 cm^{-1} was corresponding to C-N aromatic groups. The observed peak at 1619 cm^{-1} was attributed to the combination of stretching vibration of C=O conjugated with C=C. The peak at 1404 cm^{-1} was assigned to the vibration of C–H. The peaks observed at 1037 cm^{-1} and 1113 cm^{-1} were associated with C-NH_2. The spectrum of adsorbed RB-19 (10 mg/L) on MgO was monitored with respect to the pure RB-19. A new peak appeared at 1063 cm^{-1} was related to

the presence of O-H. The peaks assigned to aromatic groups and the vibration of C–H were shifted to 885 cm^{-1} and 1407 cm^{-1}, respectively. After 30 min irradiation, these peaks were significantly decreased. Moreover, the disappearance of peak at 1619 cm^{-1} indicated the cleavage of anthraquinone rings during photocatalysis (Fanchiang and Tseng, 2009a).

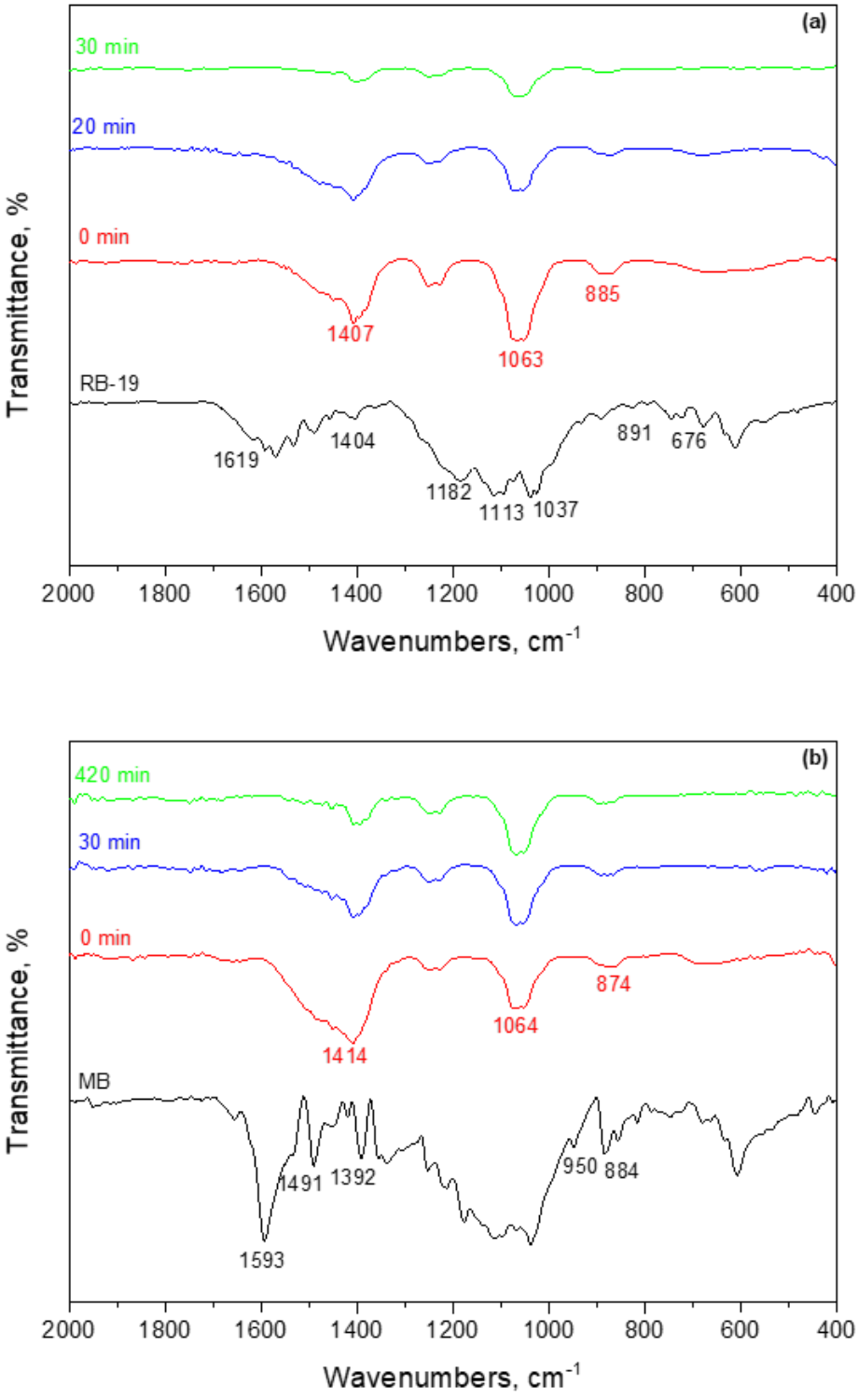

Figure 3. Stacked FTIR spectra of (a) RB-19 (10 mg/L), and (b) MB (10 mg/L),

The FTIR spectrum obtained from MB dye powder was displayed in Figure 3(b). The peaks at 1392 cm^{-1} and 950 cm^{-1} were assigned to the vibrations of C–H and =C–H aromatic

structures of MB, respectively. The intense peak observed at the region 1593 cm^{-1} related to the stretching vibrations of the C=C and C=N double bonds in the heterocycle. The peak located at 1491 cm^{-1} were assigned to a $C=S^{+}$ vibration. The peak at 884 cm^{-1} was related to aromatic ring (Fanchiang and Tseng, 2009a; Hasnat et al., 2015; Kumar et al., 2015; Ovchinnikov et al., 2016). The spectrum of MB (10 mg/L) adsorbed on MgO was monitored with respect to the pure MB. In particular, the peaks at 1392 cm^{-1} and 884 cm^{-1} were shifted to 1414 cm^{-1} and 874 cm^{-1}, respectively. A new peak at 1064 cm^{-1} corresponding to O-H was appeared. The peak at 1593 cm^{-1} attributed to the heterocycle structure of MB was almost disappeared after 420 min irradiation. The observed changes in the frequency and intensity of MB confirmed the interaction between MgO and MB. (Kiran et al., 2017; Selvam et al., 2011). The most important decrease in the intensities of the peaks were observed after 420 min irradiation.

CONCLUSIONS

The photocatalytic degradation kinetics of MgO nanoparticles on MB (thiazine dye) and RB-19 (anthraquinone) dyes were investigated. The kinetics of dyes followed pseudo first-order kinetics. Based on the results, a higher kinetic degradation rate constant was achieved for RB-19 ($k=54.5x10^{-3}$ min^{-1}) compared to MB ($k=4.59x10^{-3}$ min^{-1}). The photocatalytic studies revealed that MgO nanoparticles could be considered as a more efficient photocatalyst for the degradation of RB-19 rather than MB. The observed changes in the frequency and intensity of both dyes in the FTIR spectra confirmed the interaction between MgO and dyes.

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INHIBITORY EFFICACY OF LACTIC ACID BACTERIA AGAINST BIOFILM PRODUCTION OF DELETION MUTANTS OF Salmonella TYPHIMURIUM 14028 STRAIN IN TERMS OF dam AND seqA GENES

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ABSTRACT

In this study, the effects of culture filtrates of lactic acid bacteria inhibit the growth of planktonic forms of *S.* Typhimurium 1408 wild-type strain on biofilm formation and eradication in mutants with deletion of a *dam,* and *seqA* genes of the said bacterium were investigated. As a result of the trials carried out on polystyrene microtitre plates, it was determined that 6 lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus sake, Pediococcus acidilactici, Bifidobacterium longum, Bifidobacterium bifidum, and Pediococcus pentosaceous)* used in the trials completely inhibited biofilm formation in these mutants at optimum biofilm production times. In these experiments, culture filtrates were inoculated into biofilm-producing media together with the biofilm producer strain. In the eradication studies of biofilm structures, culture filtrates were added to the medium after the biofilm structures matured. In eradication studies carried out in this way, it was determined that the culture filtrates of lactic acid bacteria did not show any biofilm eradication efficiency.

Keywords: *S.* Typhimurium, *dam, seqA,* biofilm, lactic acid bacteria

INTRODUCTION

Bacterial biofilms are multicellular-like life forms that result from the proliferation of bacterial cells by adherence to different organic or inorganic surfaces and their coordinated genetic differentiation. The genetic differentiation that occurs during the maturation process of the forms, as mentioned above, leads to the physiological differentiation of the cells in the biofilm community and ultimately to take on different roles in the biofilm (Donlan and Costerton 2002). As a result of being surrounded by a fluid polymeric matrix containing carbohydrates, proteins, lipids, and extracellular DNA, they become resistant to environmental stress conditions, so it is very difficult to remove them from their environment. For this reason,

biofilm forms of pathogenic bacteria, especially in the food industry and medical environments, are seen as the main causes of both economic losses and serious health problems (Hall-Stoodley et al., 2006; Hoiby et al., 2010).

Salmonella serovariates are in the first place among food-borne pathogenic microorganisms worldwide when the economic losses and health problems (salmonellosis) they cause are taken into account. As mentioned above, about 85% of *Salmonella* infections are caused by the biofilm forms, not the planktonic forms of the bacteria in question. For these reasons, these sources of infection become permanent in the environments in which they occur. Elimination of these persistent Salmonella infections is one of the main sanitation problems in the food industry and the medical field (Davey and O'toole 2000). Since almost all of the different sanitation practices that are effective against planktonic forms of bacteria show very low activity against biofilm forms of the same bacteria, it has become a necessity to develop new antibiofilm strategies (Sutherland et al., 1997; Shi and Zhu 2009; Simões et al., 2010; İpek and Zorba 2018).

In this study, in the light of the information obtained from the genetic regulation of biofilm formation, the behavior of genetic variants against sanitation agents was determined, and it was aimed to obtain basic information for the development of new generation antibiofilm agent combinations. For this purpose; The efficacy of food-grade antibiofilm agents was determined in deletion-silenced mutants of a *dam* and s*eqA* genes (Uğur et al., 2018).

MATERIALS AND METHODS

Biomaterial

S. Typhimurium 14028 wild-type strain and mutants of this strain with deletion of a *dam* and *seqA* genes (Uğur et al., 2018) were grown in LB broth or agar media. Culture stocks were prepared in LB broth media containing 60% glycerol and stored at -80 0C. Lactic acid bacteria were grown in M17 broth media, and stock cultures were prepared in M17 broth containing 20% glycerol.

Determination of Antimicrobial Activities of Lactic Acid Bacteria Against Salmonella

Antimicrobial activity of lactic bacteria against *Salmonella* Typhimurium 14028 strain and its mutants with deletion of a *dam* and *seqA* genes were determined using the method suggested by van Belkum et al. (1989). Culture filtrates of lactic acid bacteria used in well

diffusion experiments were prepared by precipitating broth cultures by centrifugation and passing the supernatant liquids from these media through sterile membrane filters (0.45 μm pore diameter).

Biofilm Assays

Biofilm production trials in *Salmonella* strains were carried out in 96-well polystyrene microtiter plates. M17 broth without NaCl was used as the medium, and strains were incubated at 20 ^{0}C for 72 hours under static conditions for biofilm formation.

In order to determine the effectiveness of lactic acid bacteria on the inhibition of biofilm production of Salmonella strains or the eradication of produced biofilms; Sterile culture filtrates prepared from lactic acid bacteria used in the experiments were transferred as 30 μL to microtiter plate wells containing 30 μL *Salmonella* strain cultures prepared for biofilm formation. Antibiofilm activities were checked for 72 hours. (Stepanovic et al., 2000). In the quantitative determination of biofilm, Vestby et al. (2009) proposed method was used.

RESULTS AND DISCUSSION

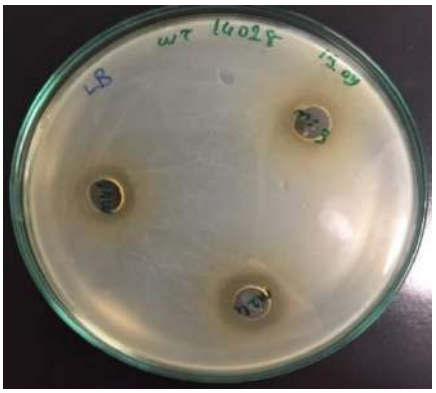
Inhibitory Effect of Lactic Acid Bacteria Against Planktonic Forms of *Salmonella* Strains

Inhibition activities of lactic acid bacteria used in the experiments against *Salmonella* Typhimurium 14028 wild type strain and its mutants in a *dam* and *seqA* genes were confirmed by the well diffusion method after the agar spot test was performed (Figure 1).

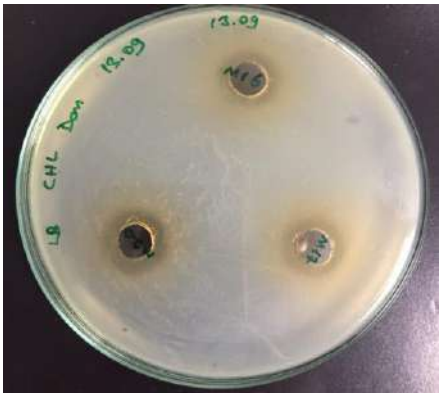
The fact that LAB strains showing strong antimicrobial activity were also defined as bacteriocin producers, in addition to other probiotic properties, in previous studies by our research group (M16: *Lactobacillus plantarum*, plantarisin producer, M17: *Lactobacillus sake*, sakacin producer, M20: *Pediococcus acidilactici*, pediocin producer, M23: *Bifidobacterium lognum*, bifidosin A producer, M24: *Bifidobacterium bifidum*, bifidosin B producer, and M46: *Pediococcus pentosaceous*, bacteriocin-like antimicrobial compound producer) (Tezel 2019) explain these powerful antimicrobial activities. Although it is accepted that bacteriocins produced by bacteria are not effective against Gram-negative microorganisms, it has been determined that when the cell wall structure is weakened with different antimicrobial agents other than bacteriocins, these bacteriocins can also be effective against Gram-negative pathogens (Klaenhammer 1993; Hernandez et al., 2005). The literature data in this direction explains the findings that we obtained in our experiments. The bacteriocin producer LAB strains

also show effective inhibitory activity against Gram-negative bacteria. It is highly probable that other chemical compounds, especially the acids formed by these strains due to fermentation, have effects that destabilize the cell wall and membrane structure and thus increase the bacteriocin activity.

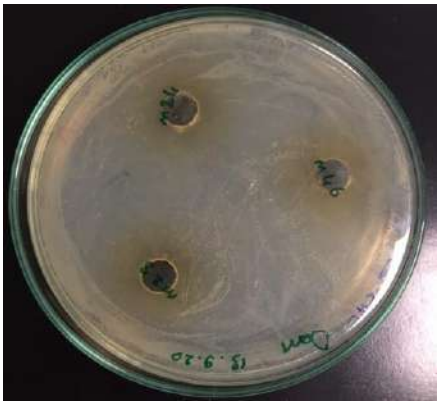
WT 14028 (M16, M17, M20)



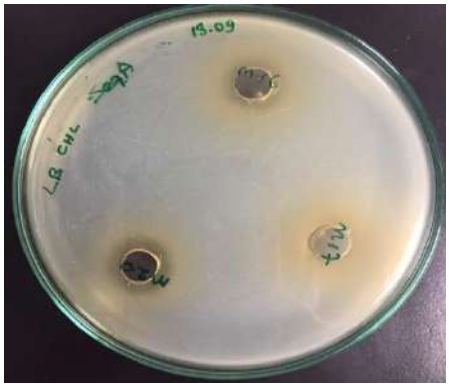
WT 14028 (M23, M24, M46)



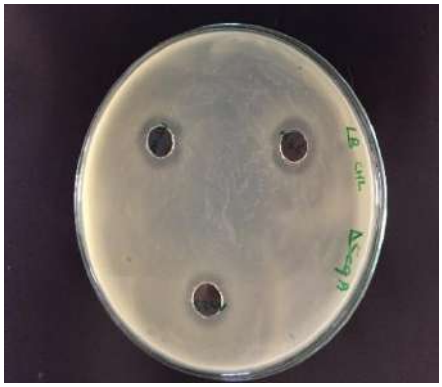
Δ*dam* (M16, M17, M20)



Δ*dam* (M23, M24, M46)



Δ*seqA* (M16, M17, M20)

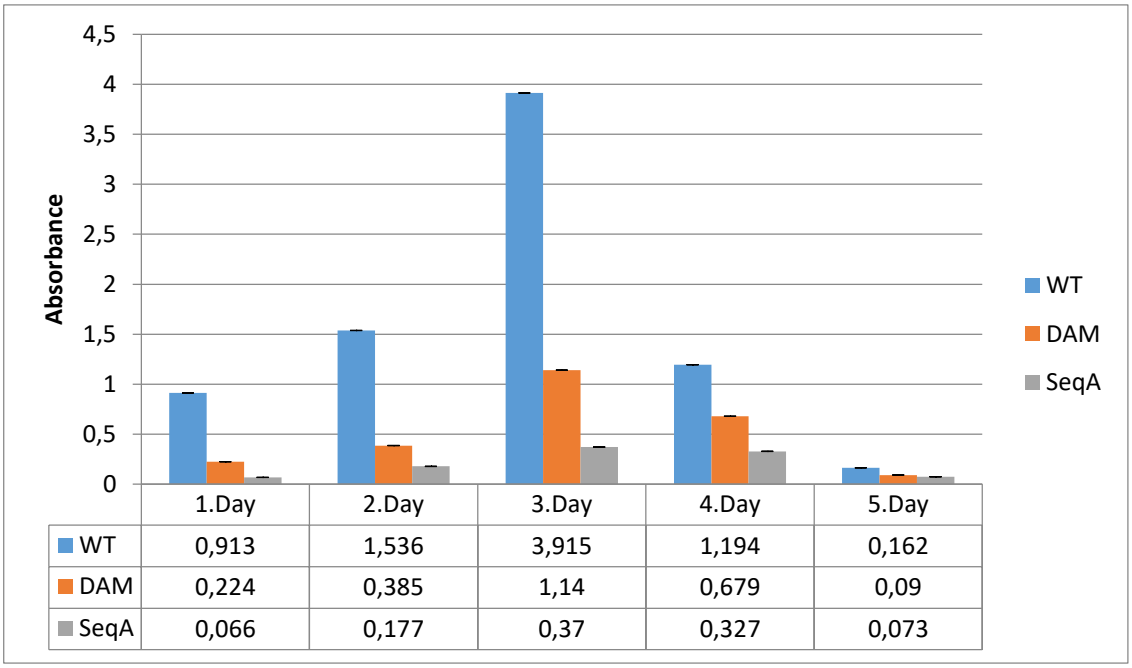


Δ*seqA* (M16, M17, M20)

Figure 1 Antimicrobial activities of lactic acid bacteria against *Salmonella*

Inhibition of Formation of *Salmonella* Biofilms by Lactic Acid Bacteria and Effect on Eradication of Mature Biofilms

Before determining the effect of lactic acid bacteria on the biofilm-forming abilities of *Salmonella* strains, optimum biofilm production times were determined for wild-type strain and mutants at 20 ℃. Accordingly, this period was determined as 72 hours in the wild-type strain and mutants used in the experiment (Figure 2).



	1.Day	2.Day	3.Day	4.Day	5.Day
WT	0,913	1,536	3,915	1,194	0,162
DAM	0,224	0,385	1,14	0,679	0,09
SeqA	0,066	0,177	0,37	0,327	0,073

Figure .2. Biofilm production on polystyrene surfaces of *S.* Typhimurium 14028 wild type strain and its mutants in terms of *dam* and *seqA* gene

After this determination, the experiments were continued by adding sterile culture filtrates of lactic acid bacteria to the biofilm production media of *Salmonella* strains, and it was determined that all of the lactic acid bacteria used completely inhibited biofilm formation in mutant strains in terms of *dam* and *seqA* gene (Figure 3).

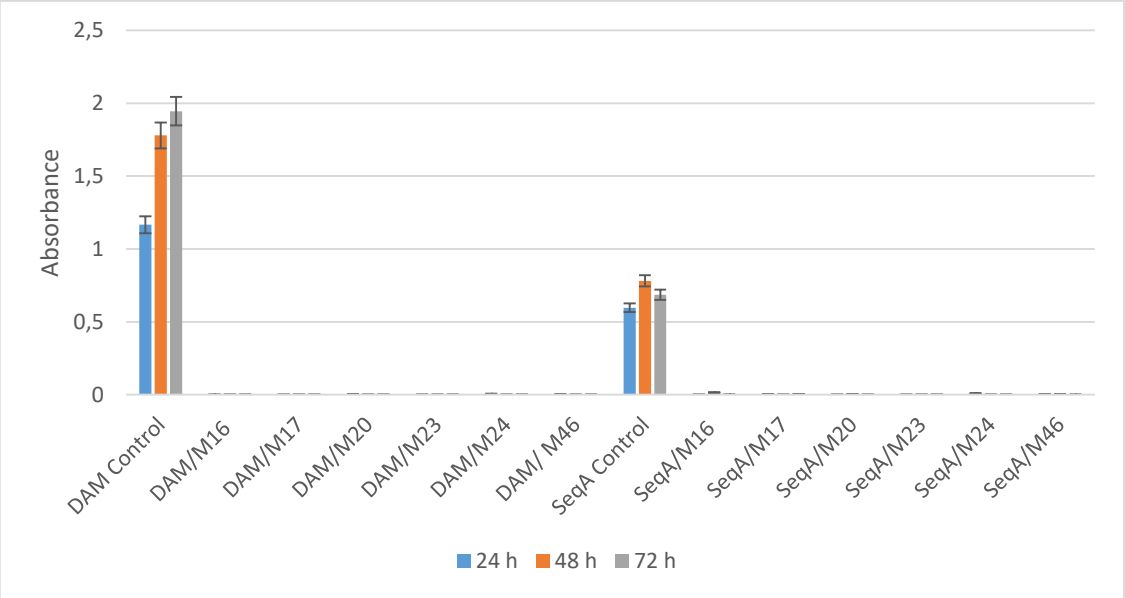


Figure 3. Effect of lactic acid bacteria culture filtrates on biofilm formation of *dam* and *seqA* gene mutants of *S.* Typhimurium 14028 wild type strain

These findings are essential physiological data supporting the literature data (Uğur et al., 2018) that deleting *dam* and *seqA* genes abolishes membrane stability in *Salmonella.* In addition, these data indicate that the strategy of combining culture filtrates of lactic acid bacteria and inhibitors of Dam and SeqA proteins can be used in clinical and industrial agent design against wild strains with intact *dam* and *seqA* genes. No eradication efficiency could be determined when the same culture filtrates were applied on mature Salmonella biofilms (Figure 4).

Although lactic acid bacteria completely prevent the formation of culture filtrates in mutant strains, their inability to show any activity against mature biofilm structures is probably due to the structural stability of the mature biofilm matrix (Hsu et al., 2002; Devlieghere et al., 2004; Akçelik et al., 2006; Afdora et al., 2010; Almasoud et al., 2016; Pelyuntha et al., 2019; Çelik et al., 2020). This can be overcome by combining lactic acid bacteria with agents that weaken the biofilm matrix.

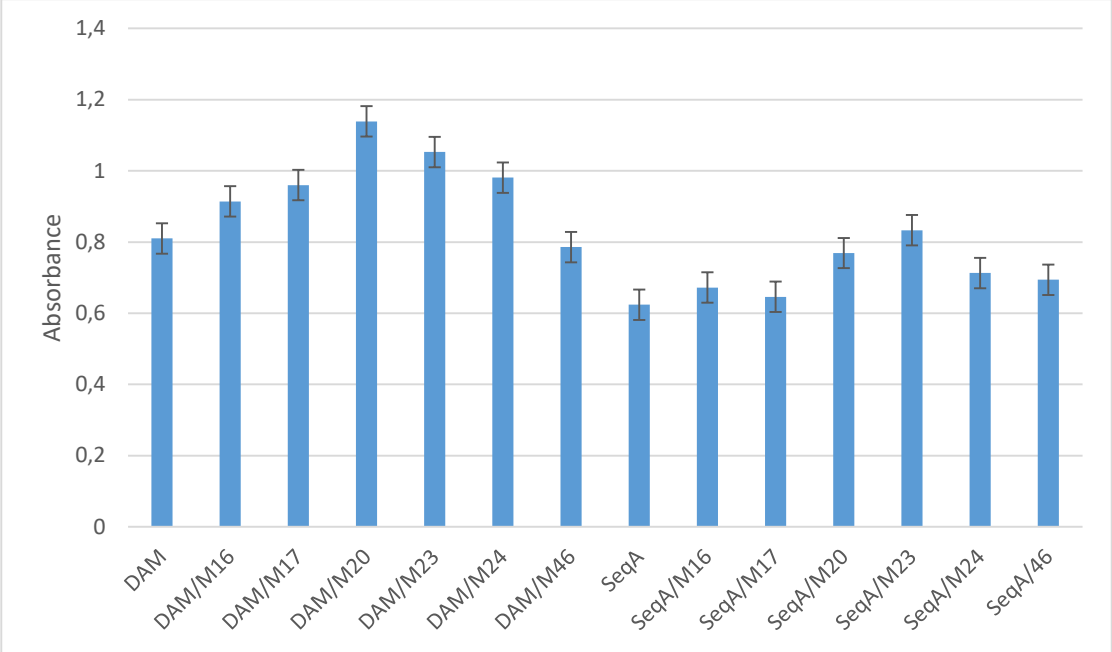


Figure 4. Effect of culture filtrates of lactic acid bacteria on biofilm eradication of *dam* and *seqA* gene mutants of *S.* Typhimurium 14028 wild type (24 hours)

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OAK TREES IN THE OULED BECHIH FOREST (EAST ALGERIA)

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ABSTRACT

The establishment and interpretation of diameter and height structures are essential for forest management decisions. The objective of this study is to describe the effect of altitude on the diameter structure, height structure and spacing factor of *Q. suber* and *Q. canariensis* populations in the Ouled Bechih state forest (Eastern Algeria). Dendrometric parameters such as diameter at 1.30m from the ground and total height of cork oak and zeen oak individuals were measured on 4 plots of 900m2 (30m×30m). The diameter and height structures were fitted to the theoretical Weibull distribution. According to the Weibull fit test, the Ouled Bechih forest is characterized by a stand dominated by large-diameter individuals, which translates into low regeneration of the species. Small diameter individuals are almost absent in the different stands studied; thus, the silvicultural analysis provided knowledge on the spacing between trees which is high and marked by the high intensity of a thinning. Finally, this state will constitute a reference for future monitoring results and decisions for foresters in the framework of management.

KEYWORDS: diametric structure, spatial structure, demographic structure

INTRODUCTION

Mediterranean forests represent a fragile natural environment that is deeply disturbed by human activity. Barbero (1990), underlines that these forest ecosystems are characterized by two types of criteria: their spatial heterogeneity and their vulnerability due to their irregular exploitation by man.

In Algeria, cork oak and zeen oak forests are particularly important because they constitute an essential element of the physical, climatic and especially socio-economic balance of rural areas (Benachoura, 1999). From an ecological point of view, the cork oak and the zeen oak are the most important forest formations in Algeria and cover more than 278,000 ha (Djema and Messaoudene, 2009).

The diametric structure of trees, their density, basal area and height are influenced by several factors: environmental, land use types or plant formations, also natural factors (Alves *et al*., 2010; Rahaingoson *et al*., 2013; Lee *et al*., 2014).

The present study focuses on the Ouled Bechih forest, located in eastern Algeria. The objective of this study is to describe the structure as a function of diameter and height of cork and zeen oak trees and their effects on natural regeneration for future development and sustainable forest management.

MATERIAL AND METHODS

Study site

Ouled Bechih forest located in the highlands of eastern Algeria, north of the Souk-Ahras region, near the Tunisian-Algerian border. The geographical coordinates are 36° 21′ 26″ N, 7° 50′ 08″ E (Fig. 1). This forest covers an area of 6582 ha and is composed mainly of cork oaks and zeen oaks. It is characterized by a mountainous relief, being part of the Tellienne chain, with very steep slopes ranging from 15% to over 20%. The region has a subhumid climate, the extreme altitudes of the forest are varied between 790-1050m, an average annual temperature of 16 ° C, average annual precipitation of 625 mm and a high atmospheric humidity of 68% (Ganaoui *et al*., 2019).

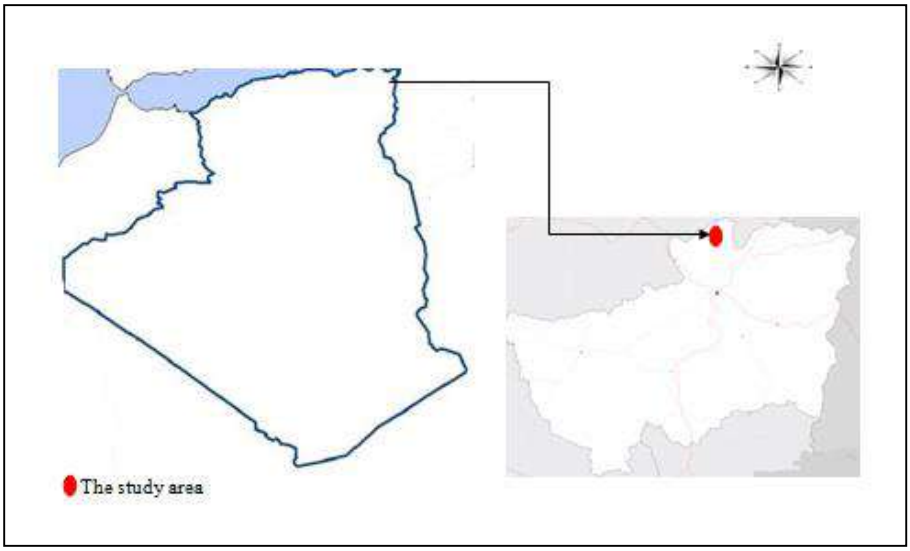

Figure 1. The study area

Methods

4 plots were randomly selected with an area equivalent to 900m2 (30m x 30m), within each plot all individuals were inventoried (Table 1) (Rached-Kanouni *et al*., 2019). For each plot, diameter at breast height (DBH, i.e., 1.30m from the ground) was measured for two species. Total height was measured using a smartphone with the Measure Height app. This positioning highlighted the spatial distribution of trees in the study area.

Table 1. Characteristics of the study plots.

Plot	Species	Latitude	Longitude	Altitude (m)
P1	Cork oak	36°23'04.641"	7°51'18.359"	957
P2	Cork oak Zeen oak	36°23'56.218"	7°55'13.556"	862
P3	Cork oak Zeen oak	36°24'24.764"	7°55'24.467"	890
P4	Zeen oak	36°22'52.264"	7°52'13.140"	1038

Demographic structure

Demographic structure was analysed using the distribution of woody individuals into diameter and height classes. A test of fit to the theoretical Weibull distribution (Hamidou *et al*.,

2017) was performed using Minitab 18 software. The theoretical Weibull distribution with three parameters (position a, scale or size b, and shape c) was used to characterize stand structure. Parameter (a) corresponds to the threshold value, that is the smallest diameter (respectively height) value used for the constitution of histograms. Parameter (b) is related to the central value of the diameter and height class distribution. Finally, parameter (c) is related to the observed structure and, depending on its value, leads the Weibull distribution to take several forms depending on the value of this parameter. A value of c < 1, an "inverted J" distribution, is characteristic of multi-species or uneven-aged stands, whereas a value of c > 3.6 is characteristic of stands with a predominance of old individuals. On the other hand, if 1 < c < 3.6, this indicates stands with a predominance of young or small diameter individuals. Its probability density function f (x) is of the following form (Kotz and Johnson, 1970):

$$f(x) = \frac{c}{b}\left(x - \frac{a}{b}\right) \mathrm{c} - 1\exp[-\left(x - \frac{a}{b}\right) c]$$

With x the diameter (cm), the height of the trees (m), f (x) its probability density value generated from the centers of the diameter or height classes and the parameters a, b and c. To check the significance of the fit under the null hypothesis of equality between the observed frequencies of the diameter (respectively height) class considered and the theoretical frequency expected according to the Weibull function (Agresti, 2010), a log-linear analysis, an iterative method of analysis of variance of the logarithm of the class densities, was performed.

Spacing factor

Hart Becking's spacing factor (S) is used primarily to specify the degree of vigor of a thinning; it gives a relationship between the average tree spacing 'a' and the dominant height (Hd) of the stand. The average tree spacing (a) is calculated with the formula below where N is the stand density in number of stems per hectare.

$$\boldsymbol{a = \sqrt{10000}/(N * 0.866)}$$

According to Hart-Becking (Merino et al., 2007), the spacing factor accurately defines the density of each stand in relation to its vigor and growth rate, and therefore the thinning regime to which it is subjected. It is therefore calculated with the following formula:

$$\boldsymbol{S\% = \left(\frac{a}{Hd}\right) * 100}$$

Hd: Dominant height, the average height of the tallest trees in the stand (Parde 1956). A spacing factor of 16% corresponds, for example, to weak thinnings, 20% to moderate thinnings, and 25% to very strong thinnings (Johnson and Kotz, 1970).

RESULTS

Our results indicate that the stand density is low. The diameter of plot 1 is characterized by very large wood (68.71cm) and of P4 is characterized by large wood (54.45cm). The lowest average diameter ($17.5 < d \leq 27.5$) is from plot 3; these stands have more developed characteristics at the perch to young forest stage. The height of trees in Ouled Bechih forest exceeds 7m, indicating that the majority of trees are mature and the average maximum height is 19.11(P4). Basal area and volume are high in P1 compared to the other plots (Table 2).

Table 2. Dendrometric characteristics

Plot	Species	A (ha)	D (cm)	H(m)	G (m^2/h)	V (m^3)	S (%)
P1	QS	89	68,71	14,18	3,09	39,57	10,64
P2	QC	111	36,78	11,55	1,09	7,00	67,51
	QS	78	39,12	11,05	0,88	5,39	99,66
P3	QS	111	27,13	7,05	0,62	2,69	111,71
	QC	67	48,51	13,25	1,12	8,30	87,30
P4	QC	133	54,45	19,11	2,95	31,42	40,72

Demographic structure

Figure 2 shows the height structure of the cork oak and zeen oak populations in the Ouled Bechih forest; the Weibull parameter (c) is between 1 and 3.6 for plot 1, so the distribution has a bell-shaped appearance. This is a positive asymmetric distribution, characteristic of stands with a dominance of individuals of high height.

For the other plots, the distribution of parameter (c) is greater than 3.6 and reveals a predominance of individuals of medium height.

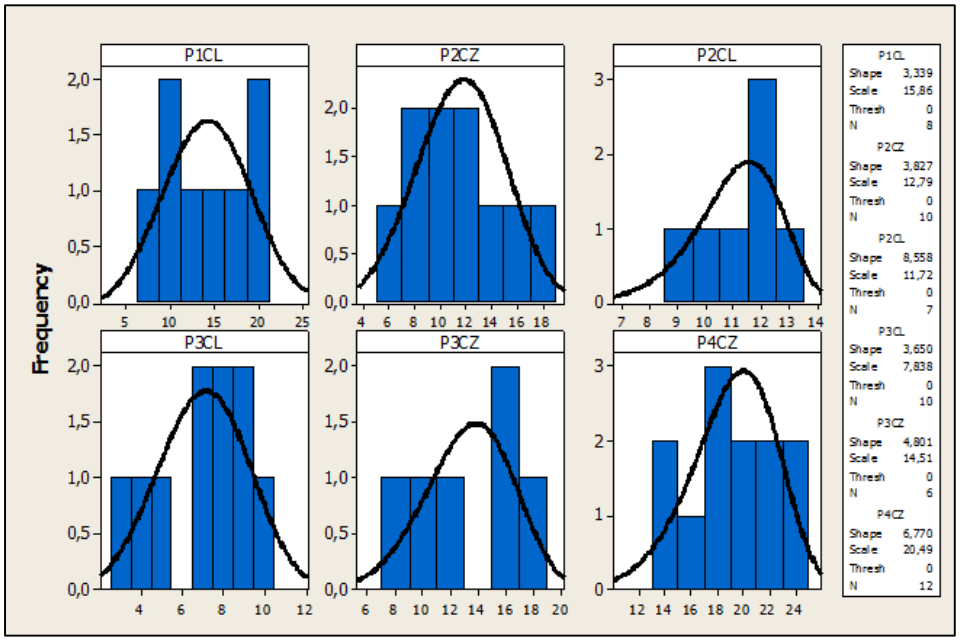

Figure 2. Height structure of QS and QC according to Weibull

Figure 3 shows the diameter structure of individuals in the cork oak and zeen oak populations in the Ouled Bechih forest. With the exception of plot 3 where the value of c is between 1 and 3.6 (this value indicates that the individuals have average diameters and the stand is young); it is higher than 3.6 for the other plots and indicates that the stand is predominantly composed of large diameter and old individuals.

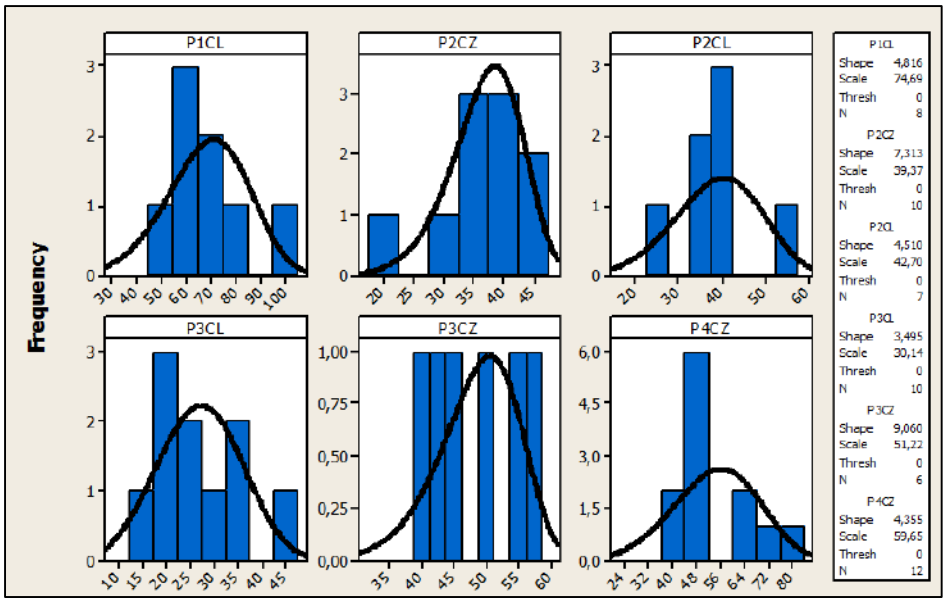

Figure 3. Diameter structure of QS and QC according to Weibull

Spacing factor

The spacing factor is used to quantify the high intensity of a thinning in a forest stand (Parde and Bouchon, 1988). Figure 4 summarizes the results obtained for the 4 plots of cork oak and zeen oak; the higher the spacing factor (S%), the more open the stand and the greater the thinning (Forster et al., 2001). In general, the plots studied had a high spacing factor value >25%, indicating that the cork and zebra oak stand is very open and sparsely populated with a large distance between trees.

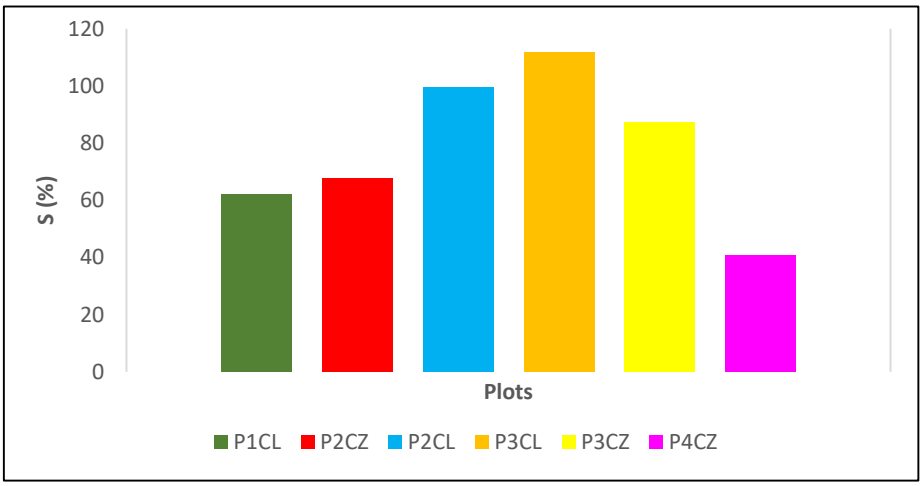

Figure 4: Spacing factor of 4 plots

CONCLUSION

Monitoring a forest allows us to detect changes over time. Any living environment is in perpetual change. The study of the dendrometric characteristics of CL and CZ along the study plots showed that the density increased with the altitudinal gradient. According to the Weibull adjustment test, the Ouled Bechih forest is characterized by a stand dominated by large-diameter individuals, young or small-diameter individuals are almost absent, which translates into a low regeneration rate for these two species.

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EVALUATION OF SOILWAT MODEL FOR SOIL PHYSICAL PROPERTIES IN THE DIFFERENT LAND USE

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Abstract

In sustainable soil management, it is necessary to determine the physical properties of the soil and to provide ideal conditions. Aeration, root development, water, and nutrient movements are closely related to soil physical properties. In this study, the estimation accuracy of the SOILWAT model (version 6.1.52) in the Soil-Plant-Air-Water (SPAW) program developed by the USDA Agricultural Research Service was calculated for different land-use types (dry farming, irrigated farming, pasture) in the territory of Isparta-Güneykent town. Observed and predicted values of saturation (ST), field capacity (FC), wilting point (WP), available water content (AWC), and bulk density (BD) were compared by using sand, clay, organic matter, EC, and penetration resistance values of the soils. In the study, it was determined that there were no significant changes in the predictive accuracy of the model according to different land-use situations. The mean absolute error (MAE) values for the examined properties were determined as 4.93%, 5.27%, 3.83%, 3.53% and 0.13 g cm^{-3}, respectively. The R^2 values obtained in the comparison of the linear relationship between the actual and predicted values of the field capacity and wilting point properties were found to be 0.54 and 0.68, and the R^2 values for the other properties were obtained quite low. The lowest mean absolute percent error (MAPE) was obtained in the bulk density estimation (9.74%). The highest error rate was determined with 27.26 % in the available water content of the soils. The RMSE values obtained as a result of the estimation of the field capacity and wilting point of the soils were found as 6.23% and 4.51 %. As a result of the study, it has been revealed that the SOILWAT model can be used successfully in the prediction of ST, FC, WP, and BD properties of soils under different land use, and the error rate in the estimation of AWC is higher than other properties.

Keywords: SPAW, moisture constants, soil physical properties

1. Introduction

Optimum efficiency needs to ensure the appropriate air and water balance, as well as the presence of plant nutrients in the soil in sufficient quantities, For plants to grow in an ideal environment. Ensuring this balance is closely related to the physical properties of the soil. Soil physical properties are quality indicators that directly and indirectly affect plant yield and yield components (Şenol et al., 2020). The physical properties of soils such as water content, air-filled porosity, temperature, and penetration resistance directly affect plant growth, while other properties such as bulk density, texture, aggregate stability, and pore size distribution affect indirectly (Letey, 1958). The productivity ability of the soil, which is a plant growth environment, is closely related not only to its nutrient content but also to its physical properties. In addition to the necessity of irrigation, it is also a necessity to know the amount of water to be applied for optimum efficiency in plant production. Therefore, it is very important to determine the field capacity and wilting point constants of the soils. Accurate estimation of soil moisture status and scheduling of irrigation will have an important place in drought management. To evaluate how much of the water given in irrigation programming will wet how many cm of the soil or how much will move away from the root zone, it is necessary to know the amount of soil moisture held at certain tensions.

Determining some soil properties is time-consuming, difficult, and costly. In recent years, prediction model studies on soil properties have attracted the attention of researchers. Pedotransfer functions (PTFs) are defined as mathematical models created for the prediction of soil properties measured by laborious, time-consuming, and expensive methods, usually using easily measured soil properties (McBratney et al. 2002; Pachepsky and Van Genuchten, 2011). Many parametric and point models have been developed to predict soil physical properties (Alaboz and Işıldar, 2019). One of them is the Soil-Plant-Air-Water (SPAW) Soil Water Characteristics Program, the SOILWAT model and has been used for many years. The Soil and Water Assessment tool (SOILWAT) is a modeling software package for analyzing water, soil, agriculture, and nutrient interactions in watershed modeling. A soil-water (SOILWAT) model that can simulate soil structure, soil hydrological properties helps provide an important dataset to better understand our soils for better soil management. These data are highly effective requirements by decision-makers aiming to achieve optimum results. In the SOILWAT model, predictions are made through the equations developed by Saxton and Rawls, (2006).

It has been stated that the bulk density of sandy soils can be successfully estimated with the SOILWAT model, while the predictive power of other features is weak (Aliku and Oshunsanya, 2016). In the estimation of Saturated hydraulic conductivity with the SOILWAT model, $R^{2'}$ was determined as 0.92 and RMSE was determined as 0.03, and the estimation accuracy was found to be high (As et al, 2019). Different estimation accuracies were obtained in studies in which the SPAW model was evaluated. The validity of the models created is not successful for every soil feature. More accurate estimations are made especially in soil properties, which are similar to the data set that created the model (Alaboz and Işıldar, 2019). For this reason, after examining the suitability of the program and model according to regions and different soil groups, it becomes necessary to use it.

This study; It is aimed to evaluate the estimation of some soil physical properties with the SPAW model of soils in different land-use types (dry farming, irrigated farming, and pasture) in Güneykent town of Isparta province.

2. Material and Method

2.1. Studying area

The study area is located within the borders of Güneykent Town of Gönen District of Isparta Province in the Western Mediterranean Region. Güneykent town is located in the west of the district, 20 km away from the Gönen district center. Its location is between the coordinates X: 265733-277030, Y: 4198561-4212827 (UTM WGS 1984 Zone 36N). According to Corine (2018) land classification, the land use within the boundaries of the study area is forest and semi-natural areas 85.21% (8415.49 ha), agricultural areas 13.89% (1371.88 ha), water bodies 0.29% (28.86 ha), and artificial areas 0.61% (It covers an area of 59.83 ha). Isparta oil rose cultivation is widely practiced in the district. Fruit and vegetables are grown in irrigated lands. Dry farming is practiced in areas where irrigation is not possible. Sampling was made from 47 points with different textures in the study area (Figure 1). The land uses of the sampling points are dry farming (16 points), irrigated farming (18 points), and pasture (13 points).

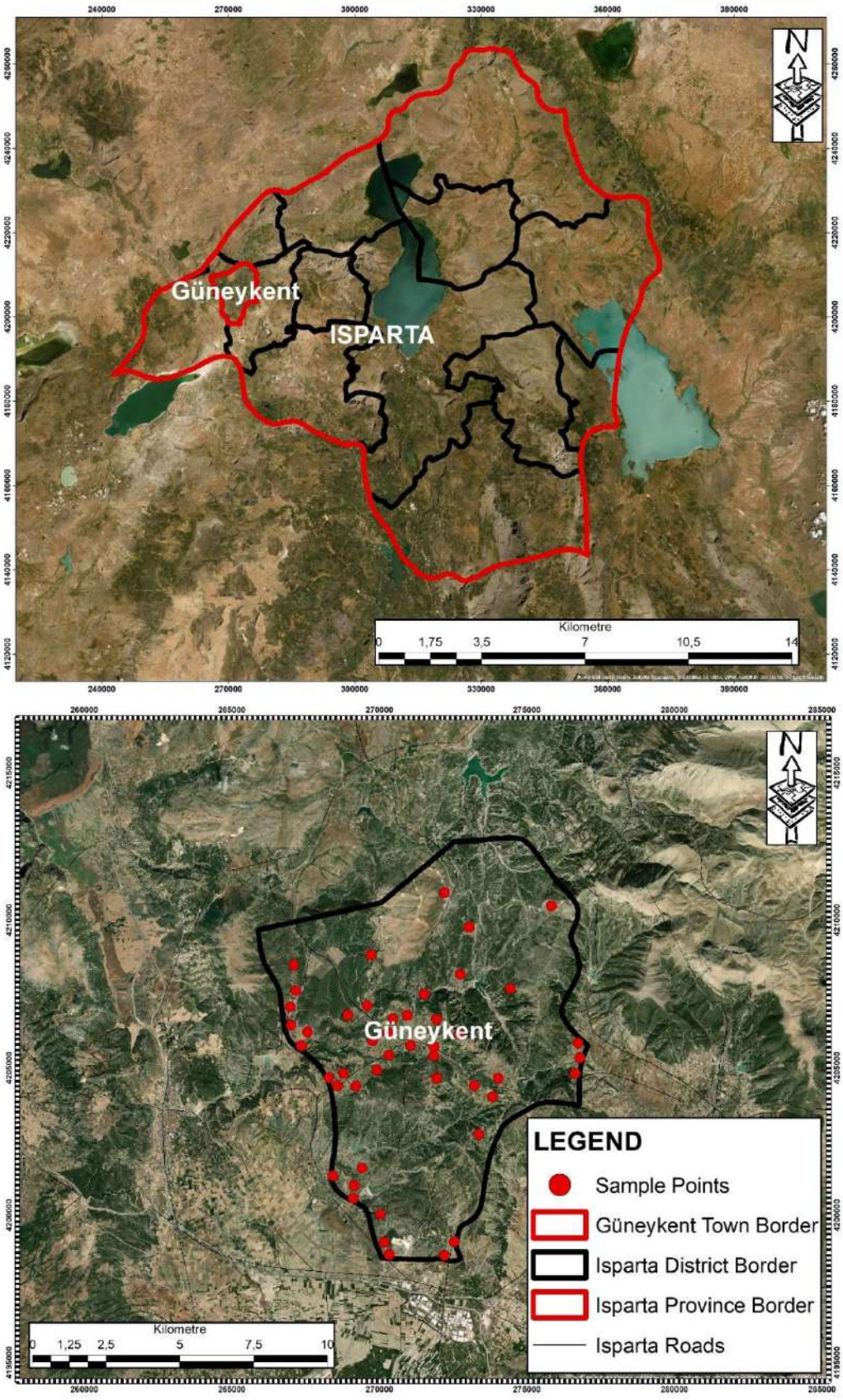


Figure 1. Study area

2.2. SPAW model

Soil-Plant-Air-Water (SPAW), Soil Water Characteristic Program (SOILWAT model) is a predictive system programmed for a graphical computer model to provide easy application and quick solutions in hydrological analysis (Saxton and Rawls, 2006). The estimation equations used for the SOILWAT model are a comprehensive laboratory data set obtained from the USDA/NRCS National Soil Characterization database (Soil Survey Staff, 2004). The data were initially estimated based on the sand, silt, and clay contents and organic matter contents of soils to estimate the soil water content, bulk density at 33 and 1500 kPa tensions (USDA-SCS, 1982). Then, according to Saxton and Rawls (2006), regression equations were developed for retained moisture at tensions of 1500, 33, 0 kPa, and air intake value. While air intake values are estimated using the exponential form of Campbell's equation (Rawls et al, 1992), Saturated moisture (θs) values are estimated according to 2.65 g cm^{-3} particle density value and bulk density values (Saxton and Rawls, 2006). Finally, the moisture tension equations were developed by Rawls (1998) conductivity equations. Organic matter, gravel, and salinity effects were also included in the model (Figure 2) (Saxton and Rawls, 2006).

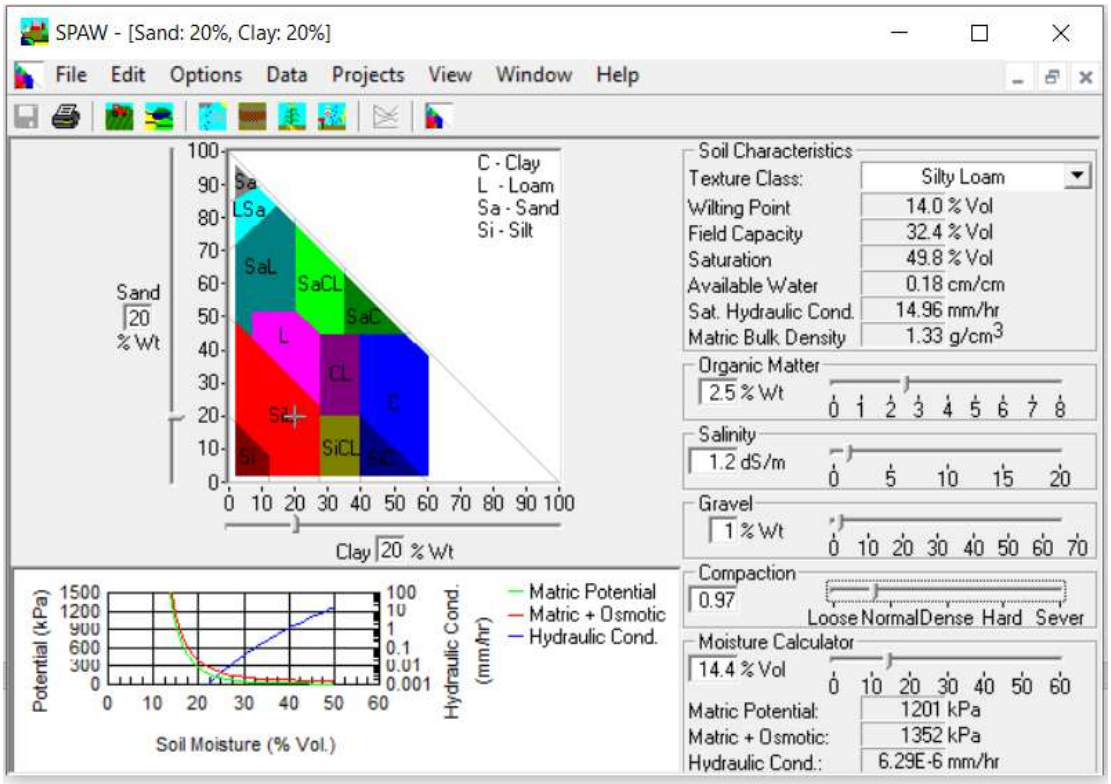


Figure 2. SOILWAT assesment tool

2.3. Soil analyzes

Within the scope of the study, pH and EC were determined according to Kacar (2016) and US Salinity Laboratory Staf (1954). The organic matter content of the soils was found using the modified Walkey-Black method and % $CaCO_3$, Scheibler calcimeter method (Kacar, 2016). Mechanical analysis was determined by hydrometer method (Demiralay, 1993), penetration resistance was determined using a digital penetrologer (1 cm^2 cone tip). The bulk density was determined by making gravel corrections in undisturbed soil samples of 100 cm^{-3} volume. Soil water characteristics; in undisturbed soil samples, starting from saturation, the 0.33 and 15 bar moisture contents were determined in a pressure plate (Klute, 1986).

2.4. Testing the accuracy of the model

The root means square error (RMSE), mean absolute error (MAE), and mean absolute percentage error (MAPE) parameters were used to examine the relationships between predicted and observed values. Estimates were determined using the following formulas (Eq. 1, 2, 3).

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|Zi - Z| \text{ (Eq. 1)}$$

$$\text{RMSE} = \sqrt{\frac{\sum (\text{Zi-Z})^2}{\text{n}}} \text{(Eq. 2)}$$

$$\text{MAPE} = \frac{1}{n}\sum_{i=1}^{n}\left|\frac{Zi-Z}{Z}\right| * 100 \quad \text{(Eq. 3)}$$

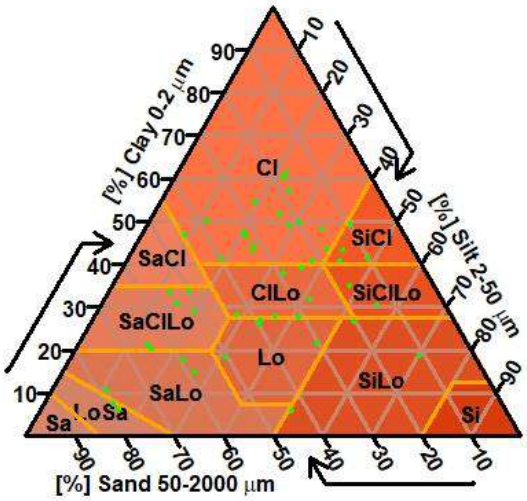
Zi: predicted value, Z: observed value, n: number of observations.

TUKEY, one of the multiple comparison tests, was used. IBM SPSS 23 was used in statistical approaches.

3. Results and Discussion

Descriptive statistics of soil properties in the study area are given in Table 1. The sand (Sa), silt (Si), and clay (CI) contents of the soils were determined in the range of 9.37-78.4%, 9.03-70.41%, 5.69-61.18%, respectively. Depending on the ratios in the textural fractions, the texture classes of the soils showed a distribution as in Figure 3. Soils have texture classes of clay, clay loam, loam, sandy clay loam, silty clay, silty loam, sandy loam, silty clay loam, and loamy sand. According to Hazelton and Murphy (2016), the organic material (OM) contents of the soils were very low (0.1%) and high (3.985%), and salt contents (EC) were between 39.2-1236µs cm^{-1}. There is no salinity problem in the soils. According to Schoeneberger et al.(2012), penetration resistance (PR) values were found to be between moderate (0.46 MPa) and high

(2.35 MPa). The lowest bulk density (BD) is 1.05 g cm^{-3}. The water retention of the soils at the saturation (ST) level varied between 28.39-60.36%. Soil properties that affect penetration resistance are frequently cited as texture, structure, porosity, water content, cementing agents, and compaction (Grunwald et al., 2001). Stewart and Hartge (1995) stated that compaction affects plant root development and distribution, and also changes the water and air balance by causing a decrease in the amount of pores. Field capacity (FC) and wilting point(WP) varied between 14.79-50.115% and 6.72-35.69%, respectively, depending on the differences in soil properties. The available water content (AWC) varies between 4.88% and 26.10% depending on the soil texture and other factors.



Sa:Sand, Lo:Loam, Si: Silt, CI:clay

Figure 3. Soil texture triangle of the soils

When the coefficient of variation (CV) of soil properties was examined, the lowest CV value was determined in the BD feature. The narrow range of change in BD resulted in a low CV. The BD values showed a change of 11.63% compared to the mean. According to Wilding (1985), CV values were classified as low (< 15%), moderate (< 35%) and high (> 35%).

Table 1. Descriptive statistics of soil properties

Variable	Mean	StDev	CoefVar	Minimum	Maximum	Skewness	Kurtosis
CI -%	34.8	14.55	41.8	5.69	61.18	-0.25	-0.7
Si-%	30.22	13.86	45.86	9.03	70.41	0.63	0.05
Sa-%	34.97	18.95	54.19	9.37	78.4	0.76	-0.22
OM -%	1.41	0.893	62.91	0.10	3.98	0.84	0.42
EC-µs cm^{-1}	237.1	205.6	86.72	39.2	1236	2.88	11.42
PR-MPa	1.13	0.37	33.28	0.46	2.35	1.00	1.76
BD-g cm^{-3}.	1.40	0.16	11.63	1.05	1.89	0.27	0.71
ST-%	46.82	6.18	13.21	28.39	60.36	-0.27	0.71
FC-%	34.36	7.99	23.26	14.79	50.15	-0.16	-0.23
WP- %	20.73	7.29	35.17	6.72	35.69	0.16	-0.47
AWC-%	13.62	4.43	32.58	4.88	26.10	0.80	0.93

CI: clay, Si: silt, Sa: Sand, OM: organic matter, EC: electrical conductivity, PR: penetration resistance, BD: bulk density, ST: saturation moisture content, FC: field capacity, WP: wilting point, AWC: available water content

Soil properties FC, AWC, and WP have medium coefficients of variation and other soil properties have high coefficients of variation. Significant deviations from the mean were determined since the properties examined showed great variability depending on the textural fractions. This resulted in a high CV value. Rawls et al., (1982) stated that FC and WP are highly variable according to the textural fractions, while FC can be determined between 1.8-16.4% in sandy texture and 32.6-46.6% in clay texture. At the wilting point, these values were determined as 0.7-5.9% and 20.8-33.6%. Although the field capacity varies significantly depending on the texture, organic matter, and structure (Karahan et al., 2014), the variety and amount of clay minerals are more effective on the change in wilting point. (Lal and Shukla, 2004). In addition, with compaction, the field capacity contents of the soils may vary depending on the change in the pore structure (Negiş et al., 2020). The skewness and kurtosis coefficients being close to 0 indicates a normal distribution. Negative skewness indicates left skewness, positive skewness indicates right skewness. While CI, ST, and FC were skewed to the left and the other properties were skewed to the right, the EC contents of the soils were the property that showed the furthest distribution from the normal. Obtaining the highest value for the coefficient of variation in EC is a reason for its abnormal distribution. Soil properties varied from sandy texture to heavy clay texture. The variability in the structure causes changes in the adhesion of ions and salt in the soil. In addition, the different land-use conditions of the study area samples also led to variability in soil properties.Within the scope of the SOILWAT model, the sand, clay, organic matter, EC, and gravelly conditions of the sampling points were used. The linear relationship between the Saturation, FC, WP, AWC, and BD values determined by the analysis of the soils and the estimated values BD is given in Figure 4. The R^2 values showing the linear

relationship between the observed and predicted values of the soils were determined as 0.0876, 0.5497, 0.6837, 0.1668, 0.0545 for Saturation, FC, WP, AWC, and BD, respectively. The highest R^2 values were determined for wilting point and field capacity. The wilting point can be predicted with an accuracy of 68% and field capacity with an accuracy of 55%. R^2's of the distributions of actual and predicted values of saturation, AWC, and BD values were determined to be low. The R^2 values obtained in the equations vary depending on the linear relationship. However, the linear equation R^2 values obtained were found to be low, since the errors in the estimations did not always show a negative or positive change. As et al(2019) determined the R^2 value as 0.02 in the estimation of volumetric moisture content with SOILWAT.

The RMSE, MAE, and MAPE values obtained for the evaluation of the SOILWAT model are given in Table 2. The low level of RMSE, MAE, and MAPE values indicates that the model prediction accuracy is high. The lowest RMSE and MAE values were determined in bulk density and the highest in field capacity estimation. The fact that the error in BD is low compared to other features is due to the fact that the BD value is lower than other features and the variation range is narrow. The error in the saturated state estimation was determined as ± 4.9% on average. The mean error was ± 5.27 %, 3.83 %, 3.53 %, 0.13 gr cm^{-3} in FC, WP, AWC, and BD respectively. According to Lewis (1982), the model with a MAPE value has been classified as below 10% is “very good”, between 10-20% “good”, between 20-50% “acceptable” and 50% “wrong and faulty”. The MAPE value was highest in AWC (27.269%) and lowest in BD (9.749%). With the SOILWAT model, the BD estimation was determined as “very good”, ST, FC, WP as “good” and AWC as acceptable. AWC is calculated from the FC and WP properties of soils. AWC's error was found to be higher than the other two features. When calculating AWC, the MAPE value of the AWC estimation is higher as a result of the combination of the error caused by the two features. The lower determination of the RMSE and MAE values of AWC is entirely due to the values in the data set.

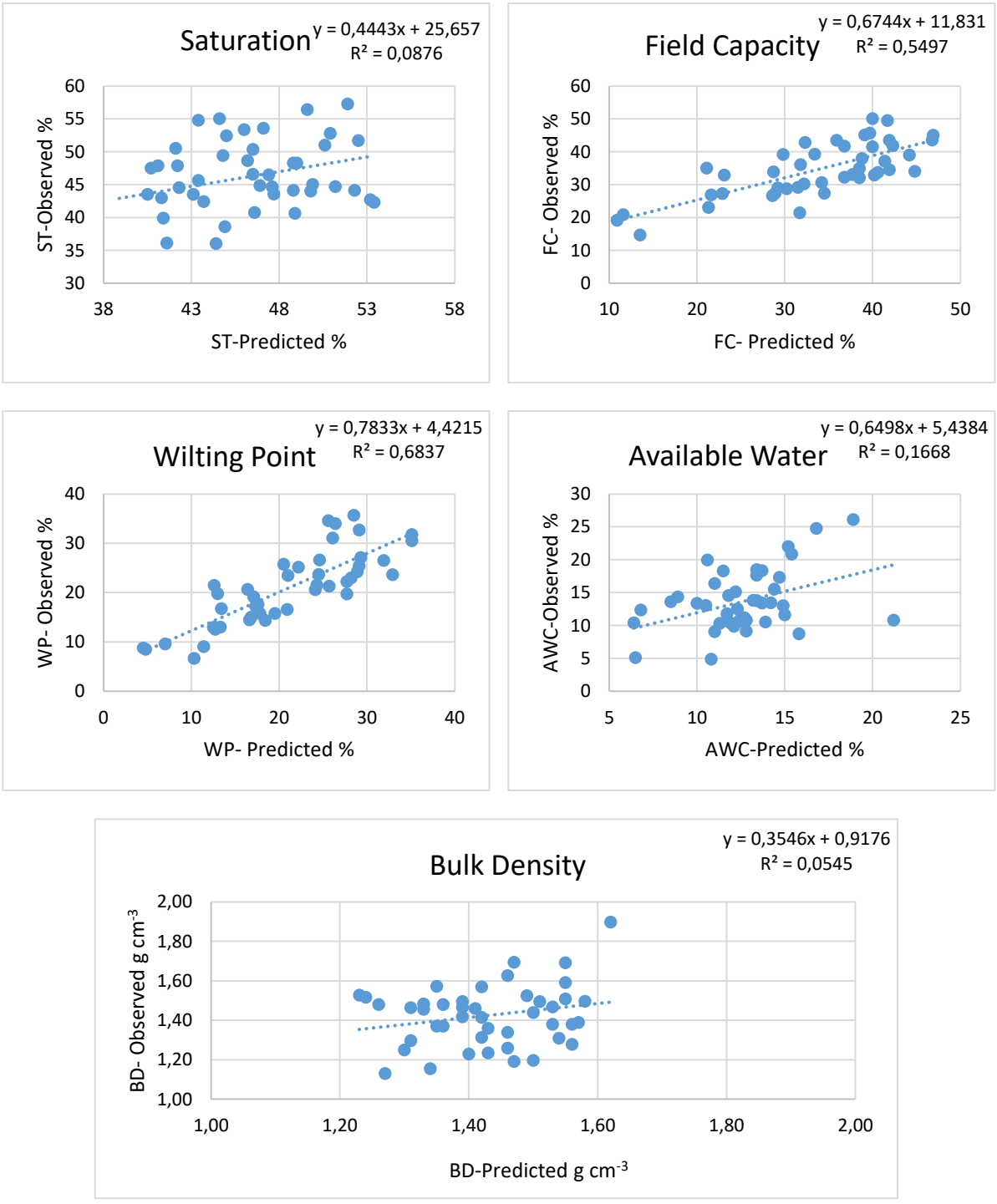


Figure 4. Distribution of observed and predicted values of soil properties

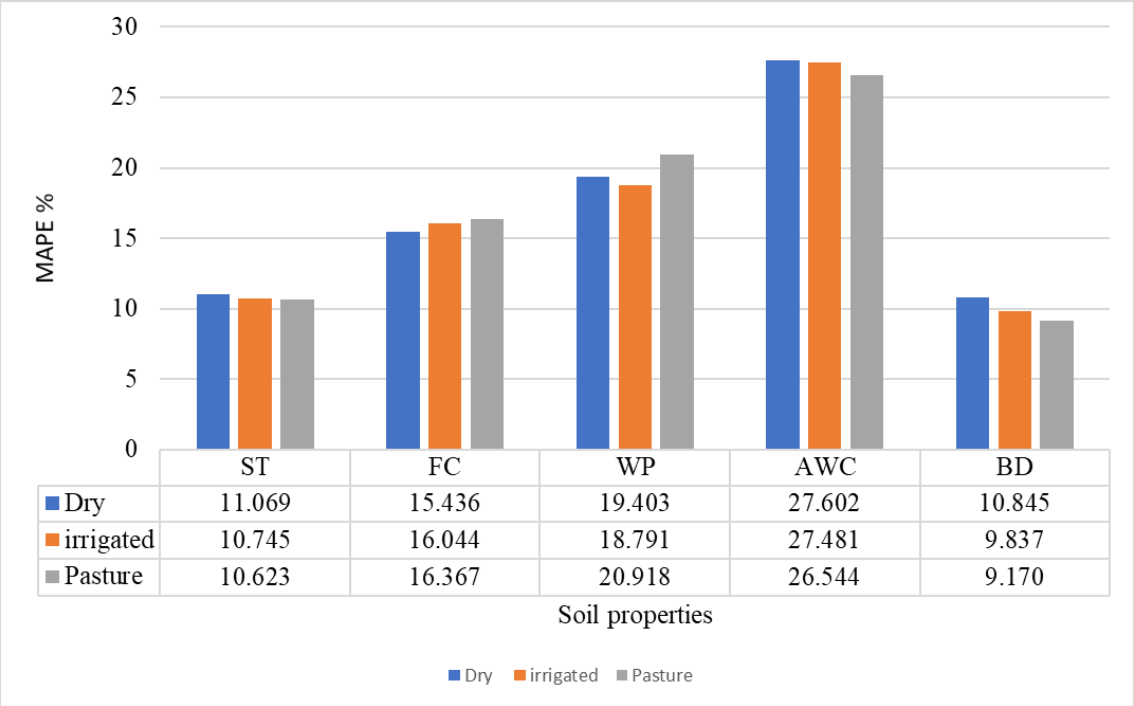
The MAPE value provides a clearer explanation in evaluating the predictive power of different features. While RMSE and MAE depend on the dataset, MAPE reveals the % error. Gijsman et al. (2002) reported that the performance of SOILWAT was not effective for every soil group. As et al. (2019) found that BD was significantly estimated for Sandy loam and sandy clay loam soils among the observed and simulated values with SOILWAT (RMSE-0.033 gr cm^{-3}). It is thought that the sources of error in the water retention rates of the soils may also be due to the clay type differences. Although the clay content is high, it has been revealed by the studies that there are differences in the water holding capacity of the soils depending on the clay type (Tunçay et al., 2020).

Table 2. Evaluation of SOILWAT model prediction accuracy

	RMSE	**MAE**	**MAPE (%)**
ST (%)	5.914	4.934	11.011
FC (%)	6.235	5.274	16.093
WP (%)	4.517	3.838	19.594
AWC (%)	4.352	3.533	27.269
BD (gr cm^{-3})	0.162	0.137	9.749

ST: Saturation moisture content, FC: field capacity, WP: wilting point, AWC: available water content, BD: bulk density

The results of MAPE values obtained according to different land-use types are given in Figure 5. The differences between the predicted values based on land use were not found to be statistically significant ($P>0.05$). The estimation accuracy obtained for all features did not change significantly from the values specified in Figure 5, where the general averages are present. Depending on the change in land use, dynamic characteristics usually change. However, the model takes into account textural fractions, one of the stable genetic traits. Variations in textural fractions are not expected depending on land use.



	ST	FC	WP	AWC	BD
Dry	11.069	15.436	19.403	27.602	10.845
irrigated	10.745	16.044	18.791	27.481	9.837
Pasture	10.623	16.367	20.918	26.544	9.170

Figure 5. SOILWAT model prediction accuracy (MAPE %) for different land use

4. Conclusion

This study, it is aimed to investigate the usability of the SOILWAT model for soils in different land-use types. According to the results obtained, ST, FC, WP, and BD properties can be estimated reliably using the SOILWAT model, while the error rate in the estimation of AWC has been determined at higher levels than the others. In addition, there was no significant difference in the accuracy of the estimations due to different land uses. This study has demonstrated that the SOILWAT model can be used successfully in defining the general physical properties of the study areas by using the textural fractions of the soils, OM, penetration resistance, and EC contents.

Agriculture is widely practiced in the study area. With the increase in closed irrigation systems, it is important to determine the amount of irrigation required for drip irrigation and the irrigation time. In areas where irrigation infrastructure has been completed, drip irrigation projects are being prepared. It has been revealed that the SOILWAT program can be used in these projects for the town. This study is important in programming the irrigation amount and irrigation time in the region. The use of the SOILWAT model in determining soil physical parameters is a reference for producers and users in public institutions.

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MICROBIAL BIOREMEDIATION OF PESTICIDES FROM CONTAMINATED ENVIRONMENTS

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ABSTRACT

With the increase in population all over the world, the need for all foodstuffs, especially plant-based, has increased day by day, and this has brought along the intensive use of pesticides in today's modern agriculture. Due to all these reasons, this increase in the use of pesticides has reached levels that will adversely affect the environment and human health. While receiving environments such as air, soil and water are polluted, pesticides accumulate as a result of their vital activities and the effect of the food chain in living things these environments. These pesticides, which accumulate in living things, become threatening the living elements of the ecosystem over time, as they reach concentrations that will create toxic effects. Regarding this situation, if necessary precautions are not taken, serious ecological crises in many ecosystems will become inevitable. Today, the use of some pesticides is prohibited, while the use of others is restricted. However, this is insufficient and the necessary action plans should be determined and put into practice urgently. Today, many physical and chemical processes are already used for the removal of pesticides. However, such methods have many disadvantages due to their high cost and the possibility of creating secondary pollutants in the environment. Biological processes, especially improvement studies carried out using microorganisms, have many advantages in terms of both cost and environmental friendliness. Considering the negative environmental effects of pesticides, it is of great importance to improve the environments contaminated with these pollutants with appropriate and effective methods. This study aimed to compile recent scientific studies on the microbial bioremediation of pesticides.

Keywords: Pesticide, microorganism, bioremediation

INTRODUCTİON

With the development of agriculture and industry, soil pollution has become a serious problem due to the large-scale and excessive use of harmful wastes. Both inorganic and organic-based products, including pesticides, can affect soil quality and agricultural function (Das et al., 2016). The use of pesticides is actually low, and most pesticide residues penetrate the environment through precipitation and runoff (Sultana et al., 2005). Improper disposal of pesticide stocks has resulted in many long-term contaminated sites containing high concentrations of pesticides (Alvarez et al., 2017).

Pesticides are natural or synthetic products used in a variety of agricultural applications to control pests, weeds and diseases found in plants. Pesticides includes., herbicides, insecticides, fungicides, rodenticides, nematicides, etc. Due to advances in agriculture, pesticides have become a vital tool for plant protection and increasing crop yields. About 45% of annual food production is lost due to pest infestation; Therefore, effective pest management

using a wide variety of pesticides is required to combat pests and increase crop production (Abhilash ve Singh, 2009).

The biological remediation approaches, phytoremediation (Del Buono et al., 2020) and bioremediation (Sun et al., 2018), are the most popular for in situ treatment of agricultural soils due to their efficiency, low cost, and environmental friendliness. Sustainable farming techniques and improvement of agricultural areas is a pioneering research area and has attracted attention in recent years. Bioremediation methods have also been developed in recent years by utilizing the diversity of microorganisms.

In order to prevent the use of wrong pesticides by the Ministry of Agriculture and Forestry, to expand the integrated control studies, which are accepted all over the world in the fight against harmful organisms in plant products, to carry out pre-harvest pesticide control studies, to support and expand the biological and biotechnical control methods from alternative control methods, such as farmer field applications. Studies such as focusing on non-formal and applied education extension studies are continuing. In Turkey, the ratio of the area where production is made with the principles of integrated agriculture to the total production area is 44 % as of 2018, and it is aimed to increase it to 50% in 2023. In figure 1, Total pesricide usage in Turkey is shown

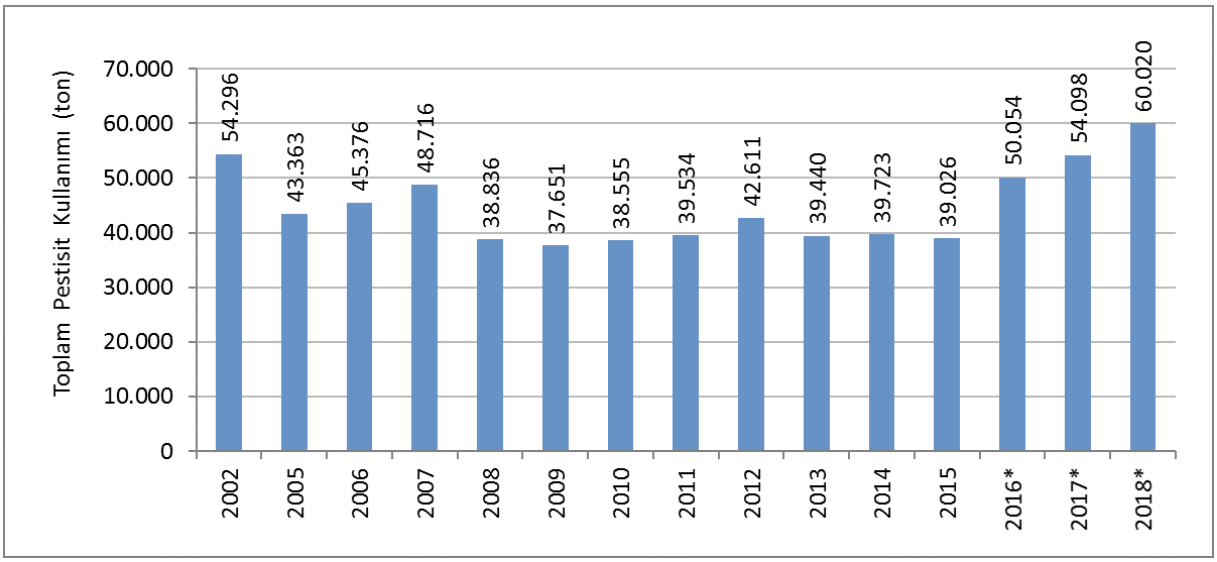

Figure 1. Total Pestıcıde Use Amounts By Years (Ministry of Agriculture and Forestry, 2019).

Pesticides

Pesticide using in World

Pesticide production in the world pesticide market is expected to increase by 1% each year. According to the work of Tiryaki et al. (2010), herbicides are in the first place with a share of 47%. While insecticides take the second place with 29%, the share of fungicides is about 19%. Rotendicide, molluscide, nematicide and acaricide, which are among the other pesticide groups, are in the lower ranks with a 5% share. Looking at the world market, 31% of pesticide use is insecticide, 26% is herbicide and 20% is fungicide. Worldwide pesticide usage shown in Figure 2.

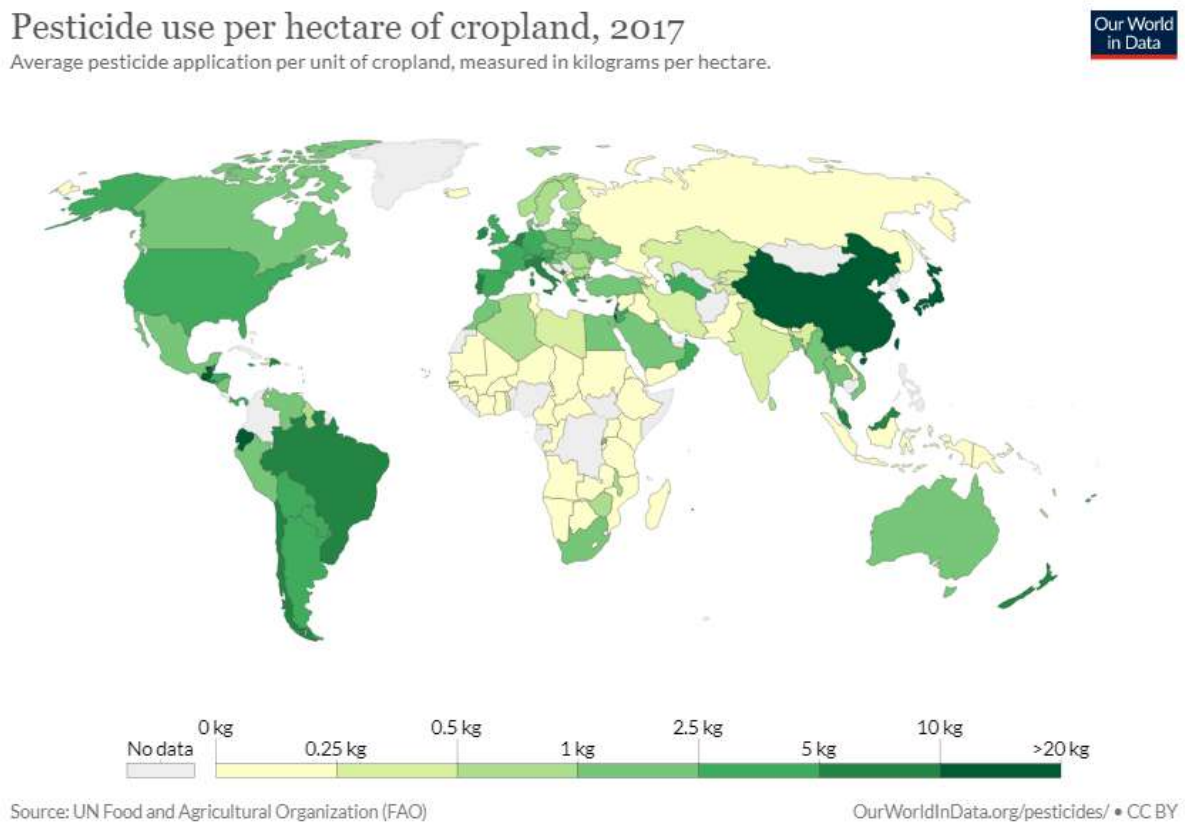

Figure 2. Pestcice use per hectare of cropland, 2017 (https://ourworldindata.org/grapher/pesticide-use-per-hectare-of-cropland)

Pesticide using in Turkey

The total amount of pesticide use in Turkey in 2018 increased by 10.9% compared to 2017 and reached 60,020 tons. When the amount of pesticide use is examined on the basis of groups, fungicides constituted the largest group in our country as well as in the world. In 2018, 38.4% of the total pesticide use was fungicides, 24.6% herbicides, 22.6% insecticides, 4.1% acaricides, 0.5% rodenticides and 9.6% others (plant activator, plant growth regulator, insect attractant, fumigant, nematocide, sulfur, mineral oils) (Ministry of Agriculture and Forestry, General Directorate of Food and Control, 2019).

In our country, pesticides were used the most in the Mediterranean Region (28.7%) in 2018. This is followed by Aegean, Marmara and Central Anatolia Regions, respectively. Eastern and Southeastern Anatolia Regions constitute only 11.1% of Turkey's consumption. The Black Sea Region is in the last place with 4.1%. As of 2018, our top 5 provinces with the most pesticides are; Antalya with 8.8%, Manisa with 8.1%, Adana with 7.4%, Mersin with 6.2% and Aydın with 5.8% (Ministry of Agriculture and Forestry, General Directorate of Food and Control, 2019). The status of pesticides used in Turkey shown in Figure 3.

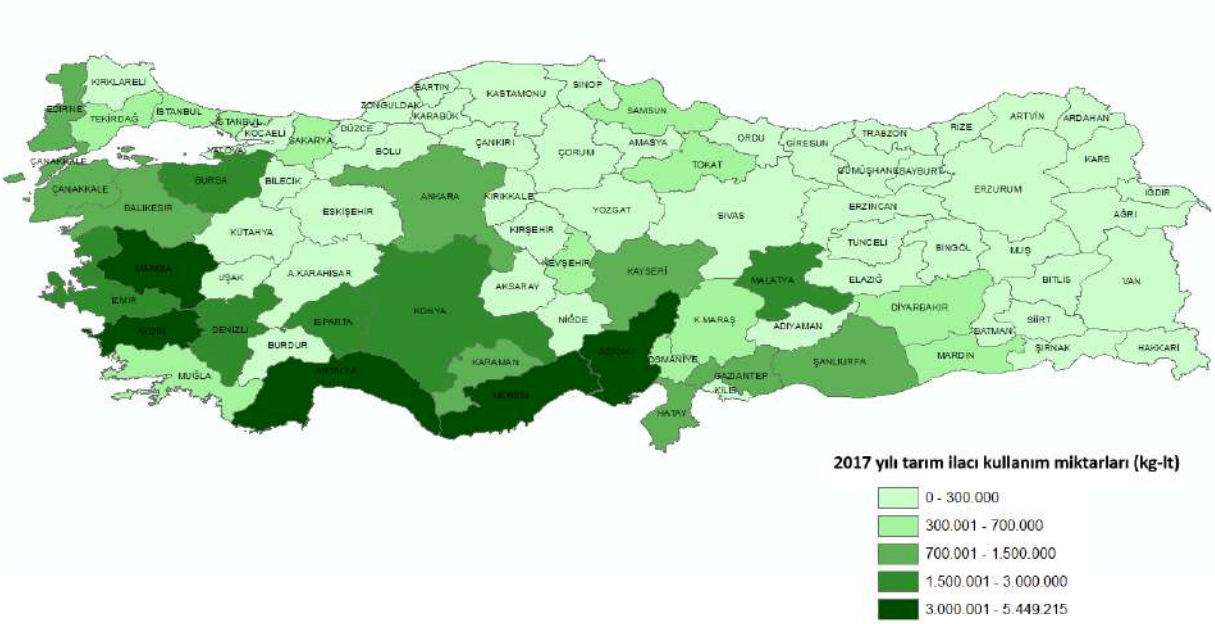

Figure 3. Pesticide use amounts in Turkey (Ministry of Agriculture and Forestry, General Directorate of Food and Control, 2019)

Environmental effects and fate of the pesticides

After the pesticide is applied to an agricultural field or receiving environment, it may behave differently. These may include evaporation to the atmosphere, runoff and erosion to surface waters, or photodegradation by sunlight. Pesticides can be taken up by plants from the soil, biodegraded to other chemical compounds, or they can also leach into the water and groundwater below the plant root zone. The amount of some chemicals that evaporate, leak, decompose or take place in the stream depends on the geographical conditions of the region, climate, soil and pesticide properties (active substance structure, residence time in the soil, half-life). The persistence of a pesticide depends on whether it has undergone chemical or biological degradation. Another factor affecting the potential of pesticides to contaminate groundwater is pesticide mobility. Conditions affecting them; soil properties such as water and organic carbon content, excessive irrigation and precipitation, volumetric density and pesticide retention and adsorption (Pierzynski et al., 1994). The environmental effects and fate of the pesticides are shown in figure 4.

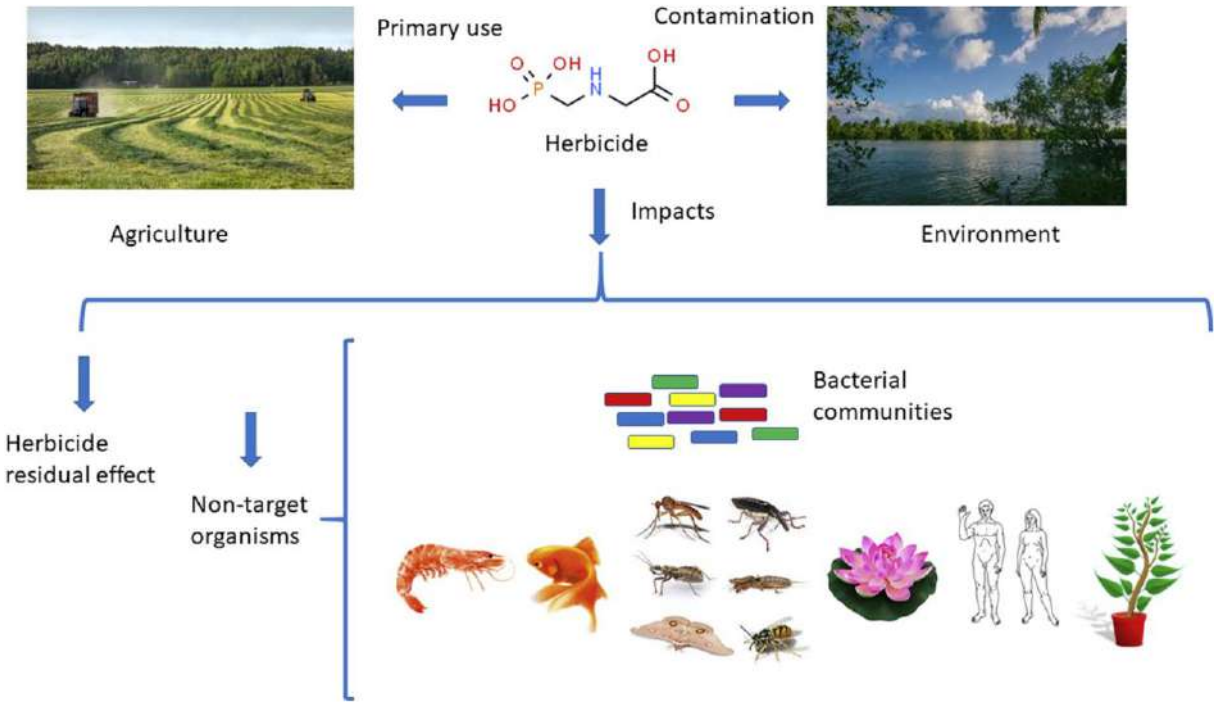

Figure 4. Impacts of the overuse of herbicides on agriculture and the environment affecting non-target organisms (Pileggi et al., 2020)

Evaporation of pesticides from the soil where they are applied and from the plant surface is one of the main reasons for their release into the atmosphere (Yeo et al., 2003). Rapid evaporation; temperature increase also occurs due to direct exposure to sunlight and excessive soil moisture (Otieno et al., 2013). After evaporation, pesticide compounds can spread from areas of high concentration to areas of low concentration (van Dijk and Guicherit, 1999) and can spread widely at low concentrations by air intake or as wet deposition in raindrops (Bloomfield et al., 2006).

Bioemediation methods

Bioremediation is the conversion of many harmful pollutants, especially pesticides, into non-toxic or less toxic compounds by living organisms (Asha et al., 2013). As a result of bioremediation, the process of returning contaminated areas to their original conditions is completed without any other adverse effects on the receiving environments (Jobby et al., 2018). Among living organisms, actinomycetes, fungi, bacteria and plants are capable of regenerating receiving environments contaminated with heavy metals and pesticides (Figure 5). The soil properties for effective bioremediation process isshown in Table 1.

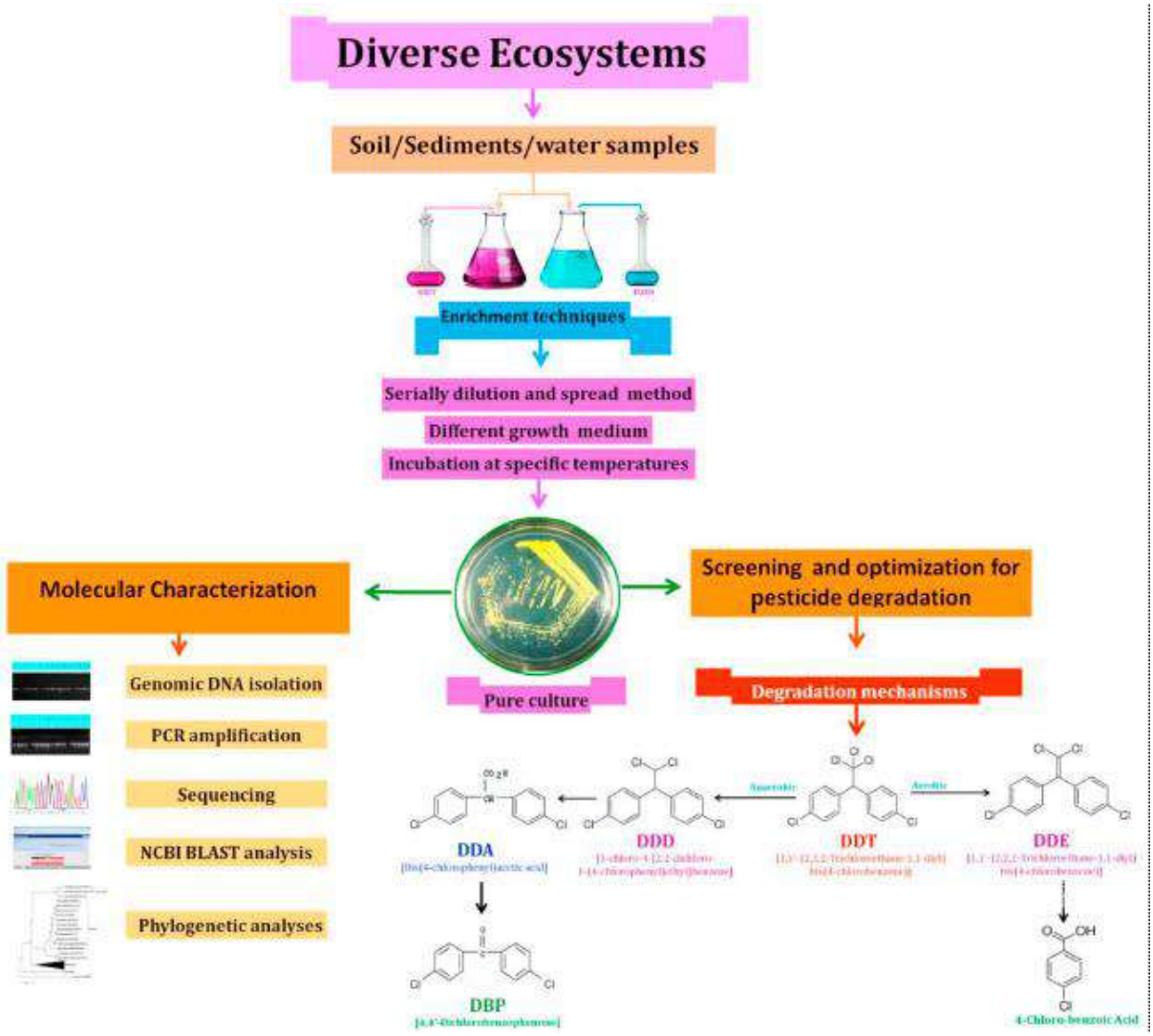

Fig. 5. Flow diagram representing the isolation and identification of pesticide degrading microbes (Kumar et al., 2021).

Table 1. The conditions of bioremediation of microorganisms in soil (Shanahan, 2004)

Environmental Factors	**Optimal Conditions**
Soil moisture	25-85% Water holding capacity
Oxygen	>0.2mg/L D.O, >10% interparticle air space for Oxygenated Fragmentation
Redox Potantial	Eh> 50milivolt
Nutrients	C:N:P = 120:10:1 Molar rate
pH	5.5-8.5
Temperature	15-45^{0}C

Microorganisms are used to remove or minimize the toxic effects of pollutants and plants are preferred to stabilize or remove pollutants from the soil. According to the type of living organisms involved in bioremediation, microbial remediation can also be divided into plant remediation and combined remediation.

Daily literature

Bacteria are generally known as bioremediators and are isolated from pesticide-exposed agricultural areas. It has been determined that agricultural areas contain too many bacteria to reduce the negative effects of pesticides (Erguven ve Yildirim, 2019). It has been observed that the consortia formed individually or together by *O. thiophenivorans* and *S. melonis* isolated from the cotton cultivation area of Adana can decompose imidacloprid with high efficiency in liquid medium. According to the results of bioremediation studies of individual and mixed cultures in liquid medium, *S. melonis* bacteria showed a more successful removal efficiency than *O. thiophenivorans* in the COD parameter; however, it was determined that *O. Thiophenivorans* was more successful than *S. melonis* by about 5 percent in BOD5 and TOC parameters. In consortia of two bacterial species, the yield was over 93% for all three parameters (Erguven and Demirci, 2021).

Many strains of bacteria have biopremediation capabilities in bioreactors. Because the strains remain active until the biodegradation stages (Lin et al., 2015). Tatar et al. (2020) investigated metomyl bioremediation by a consortium of *O. thiophenivorans* and *S. melonis* and found removal efficiencies for BOD_5 and COD parameters between 97% and 95% in eight days.

In another study on the bioremediation of herbicide with imidacloprid active ingredient, Ergüven and Yıldırım (2019) determined that the COD parameter of *Methylobacterium radiotolerance* and *Microbacterium arthrosphaerae* consortium was 53% and 99% with 20, 40 and 80 ml at the end of 18 days. 79 and 50%. Yang et al. (2014) found a bacterial strain that can use chlorsulfuron-ethyl (herbicide) as a carbon source in a phosphatebasal minimal environment. They found that chlorimuron-ethyl was provided as a carbon source, the growth rate of the microbial strain was accelerated by chlorimuron-ethyl biodegradation, and more than 95% of 50 mg/L chlorimuron-ethyl was degraded initially.

Erguven et al. (2017) studied the removal of malathion active ingredient insecticide with *P. chrysosporium*. They performed a COD-based removal follow-up at 50, 100 and 150 ppm malathion concentrations and 130 rpm under shaken culture conditions. At the end of two weeks, they determined a removal efficiency of over 97% at three different concentrations, respectively. According to these results, they determined that P. Chrysosporium is an elite microorganism in the remediation of recipient environments exposed to malathion.

In 2018, Yildirim et al. investigated the reduction rates of malathion on COD and BOD_5 parameters. Their study focused on different results depending on the concentration differences in the submerged culture medium and the incubation period. According to the COD results; in the medium with 50 ppm malathion, approximately 15% reduction was observed at the end of the 8th day, and this reduction increased to 52% after 15 days. The 10% reduction value at 100 ppm increased to 27% and finally the removal rate increased from 23% to 38% in 8 days to 15 days, respectively, in the medium with 150 ppm malathion. According to these results, the best reduction performance on COD occurred at 50 ppm concentrations after the 15^{th} day. For BOD_5 experiments, the best removal efficiency was seen as 81% after 15 days at 150 ppm malathion concentrations.

Conclusions

Remediation of contaminated soils is complex. Bioremediation is environmentally friendly and has great potential as a remediation method for co-contaminated soil. To promote contaminated receiving environments with bioremediation, future research should consider the following:

(1) Most reported studies were performed under arithmetically controlled conditions in the laboratory. The design of the parameters used in the experiment should be studies to simulate natural conditions, including climate, weather and polluting conditions. More work should be done to determine bioremediation efficiency in field conditions rather than in laboratory simulated conditions. Field studies should also be carried out in different locations.

(2) Among the purposes of bioremediation, recovery strategies should be developed not only for organic pollutants but also for their metabolites. We should be mindful of the situation where some metabolites of some organic pollutants have a long half-life or are more toxic than the main pollutants.

(3) Microorganisms that have been used for bioremediation of contaminated soil then need to be purged from the system to prevent secondary contamination due to the death of organisms or changes in the environment. Unusable biomass should be disposed later as hazardous waste.

(4) The interaction mechanisms of different pollutants need further investigation. Meanwhile, research is needed to elucidate the mechanisms of bacteria-bacteria interactions under pollutant stress.

(5) Pre-bioremediation soil pollution assessment methods and post-bioremediation efficacy assessment should be higher sensitivity, integration, capability, low energy consumption and speed.

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USE OF OXIDATIVE BIOMARKERS IN THE EVALUATION OF BIOREMEDIATION EFFICIENCY

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ABSTRACT

Bioremediation of pesticide contaminated sites with bacteria that are adapted to these pesticides or genetically engineered for biodegradation of persistent organic pollutants are a study area that is currently being explored as an alternative remediation techniques. Biomarkers have been developed to track the survival and efficiency of specific agricultural soil bacteria that are used as inocula for bioremediation of pesticide contaminated agricultural fields. Examples of biomarkers like catalase (CAT), glutathione S-transferase.(GST), cytochrome P4501A1 (CYP1A1) activities in *Gammarus pulex* can be useful for these kind of studies. Other biomarkers can also be used for monitoring of microbial inocula used for bioaugmentation or bioremediation of pesticide contaminated receiving environments. The choice of biomarker and monitoring system depends on the particular site, bacterial strain and sensitivity and specificity of detection required. According to the results of the most studies, CYP1A1, CAT, and GST activities in *G. pulex* sanctioned the capability of soil *bacteria* in bioremediation of pesticides. Isolated and enriched bacteria and microbial consortia can be helpful for decrease of the chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5), pH and dissolved oxygen parameters of the pesticide contaminated aquatic environments. This means these parameters can give a useful alternative opinion for screening the active material of the pesticide. As we know, monitoring the active material of the pesticides with chromotographic methods and using these devices are very expensive. At the final phase of the bioremediation step, it was determined that these bacteria have efficient bioremediation properties at a rate of up to 80%. According to recent studies soil bacteria can be used for bioremediate the receiving environments that are polluted by pesticides. The treatment efficiency of these bacteria can be determined by monitoring changes in some biochemical biomarkers in *G. pulex*.

Keywords: Bioremediation, pesticide, *Gammarus pulex*, chemical oxygen demand

INTRODUCTION

The chemical and physical remediation methods are not considered or preferred for treatment of wastewater as these methods have low removal efficiency, leave a high mass of sludge, need extra efforts to decompose sludge, require high energy input, and are time-consuming processes (Capodaglio and Olsson 2020). Therefore, bioremediation techniques that use microorganisms are considered safe and sustainable methods to bioremediate toxicants from contaminated water. The most important factor for this process to occur is the availability of contaminants for microbes or their enzymes so that the metabolism of contaminants can occur (Boudh and Singh 2019). The process of bioremediation uses different microbes such as bacteria, algae, fungi, and yeast to treat oil spills, contaminated soil, contaminated water, and many more. The microbes' selection depends on the contaminated area because every microbe

needs different pH, temperature, and moisture for its activation. Microbes used in this process are also called bioremediators (Rusten and Sahu 2011).

Among several processes, protein phosphorylation, various activation of transcriptional factors, immunity, differentiation, and apoptosis depend on an adapted production of ROS and the presence of cells that need to be at the lower level (Rajendran et al., 2014). Depending on the increased production of ROS, harmful effects may be seen on important cellular structures such as lipids, proteins, and nucleic acids (Wu et al., 2013).

Oxidative stress biomarkers are widely used to evaluate the effects of many classes of chemical pollutants on organisms. One of the important biomarkers in environmental studies is the use of changes in key enzymatic activities of sentinel species after contact with contaminated waters (Nunes et al., 2006). ROS, which can be detoxified by enzymatic and non-enzymatic cell defense systems, can also be measured as biomarkers of xenobiotic-mediated oxidative stress, as well as changes in enzymatic and non-enzymatic biochemical parameters.

Advocates against ROS include compounds such as GSH, SOD, CAT, GSH-Px, and GST. Since differences in cell antioxidant defense mechanisms indicate exposure to pollutants and/or toxicity of these pollutants, all of these may be useful biological indicators in the identification of aquatic environments (Almeida et al., 2007: Bouraoui et al., 2008; Ruas et al., 2008; Monteiro et al., 2010). Pollutant parameters and factors can accelerate the production of ROS (Livingstone 2001). During oxidative stress, cells generally increase antioxidant enzyme levels such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) (Livingstone, 2001; Valavanidis et al. 2006).

The use of lipid peroxidation and antioxidant defense systems as oxidative stress biomarkers in studies integrated with environmental engineering has produced successful results in recent years and has been preferred in evaluating the effects of polluting parameters in aquatic ecosystems (Livingstone 2001; Valavanidis et al. 2006).

Gammarus species are more sensitive to contamination in water than fish. The greater sensitivity to various polluting parameters, their faster reproduction, and greater abundance have made the use of this genus more attractive in toxicological studies (Arthur 1980). Gammarus sp. is a freshwater species and is suitable for ecotoxicological measurements of environmental pollutants due to its high ecological suitability and important properties in the food pyramid. These species, which are a source of energy for organisms such as fish, birds, and frogs, are preferred as an organism suitable for high-level ecotoxicological studies due to their ecological and ecotoxicological importance and sensitivity (Geffard et al. 2007; Tatar et al. 2018).

The aim of this study is to summarize some previous studies in which bioremediation efficiency was evaluated with some oxidative biomarkers.

Oxidants and free radical production

Radicals symbolize species that contain at least one unpaired electron in shells around the atomic nucleus and arise independently. The high reactivity of these radicals is due to an unpaired electron which can acquire another electron to stabilize it. ROS production mainly depends on enzymatic and non-enzymatic reactions. Enzymatic activations that can produce ROS are associated with the respiratory chain, prostaglandin synthesis, phagocytosis, and the cytochrome P450 system (Halliwell, 2001; Kumar and Pandey, 2015).

Oxidative stress biomarkers measured bioremediation studies

Catalase

Catalase (EC 1.11.1.6) has a molecular weight of 240 kD and contains tetrameric protein. This enzyme catalyzes the breakdown of hydrogen peroxide into water and oxygen (Chelikani et al., 2004) (Fig. 1). Hydrogen peroxide is also considered a harmful byproduct of many normal

metabolic processes. To cope with the damage caused by this, it must be rapidly converted to less toxic substances such as oxygen and water (Gaetani et.al., 1996). In addition, catalase has a peroxidative function that converts peroxides (ROOH) to alcohol (ROH) and water. Thus, cells are also protected from the negative effects of lipid peroxidation (Chelikani et al. 2004).

Superoxide dismutase

The first antioxidant enzyme to act against ROS is superoxide dismutase. SOD is a metalloenzyme that dismutes the superoxide anion into hydrogen peroxide and molecular oxygen (Fig. 1). There are three types of SODs that differ in amino acid sequence, active metal site, and cellular distribution. The first is Mn-SOD localized in mitochondria, the second is Cu-ZnSOD localized in the cytosol, and the third is CuSOD bound to the vascular endothelium, which metabolizes superoxide radicals in plasma (Young and Woodside, 2001). Since the SOD-CAT system is the first defense system against oxidative stress, an increase in CAT and SOD activity is also detected against environmental pollutants in general (McCord, 1996).

Glutathione peroxidase

Glutathione Peroxidase (EC 1.11.1.9) are homotetrameric water-soluble enzymes found in the mitochondrial and cytosolic portions of cells (Epp et al., 1983). GSH is an enzyme that catalyzes the reduction of hydrogen peroxides to water and alcohols (Figure 1). Another task of GPx is to protect the organism from oxidative damage (Ursini et al., 1995). GSH is also an important intracellular antioxidant. GSH in its reduced form is involved in inhibiting free radicals, stabilizing reduced sulfhydryl groups, and regenerating tocopherol and ascorbate. Another task is to be a cofactor of GSH-Px (Ulakoğlu et al., 1998; Armstrong, 1998).

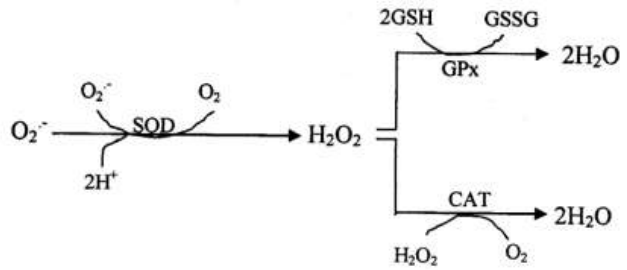

Figure 1. Scavenging of free radicals (Gregus ve Klaassen, 1996).

Barim et al. (2009) argued that the low activity of GSH-Px may be a determinant of the inability of this animal to neutralize the effects of peroxides, which may result in increased lipid peroxidation. Also, decreased GSH-Px activity may be associated with the decreased availability of GSH required to reduce the effect of ROS.

Glutathione S-transferase

Glutathione S-transferase (GST) is a phase II enzyme that catalyzes the detoxification of electrophilic and hydrophobic compounds with reduced glutathione (Ye and Song 2005) (Fig. 2). Glutathione S-Transferase (GST) is a multi-substrate enzyme (Anton et al, 1990). GST enzyme is present in mammals, insects, fish, birds, annelids, mollusks and many microorganisms (Habig et al., 1974). Glutathione Stransferases are involved in many catalytic and non-catalytic functions. GSTs perform both detoxification and have intracellular binding

and transporter functions. GSTs, also known as one of the natural protective formations, are herbicides, pesticides, anticancer drugs, chemical carcinogens and environmental pollutions etc. They also perform a powerful function in the detoxification of electrophilic xenobiotics. GST takes place in many organisms from *E.coli* to mammals and has been isolated from the liver, erythrocyte, lung, placenta and intestinal mucosa of animals such as humans, rats, mice, and cattle (Gyamfi et al, 2004).

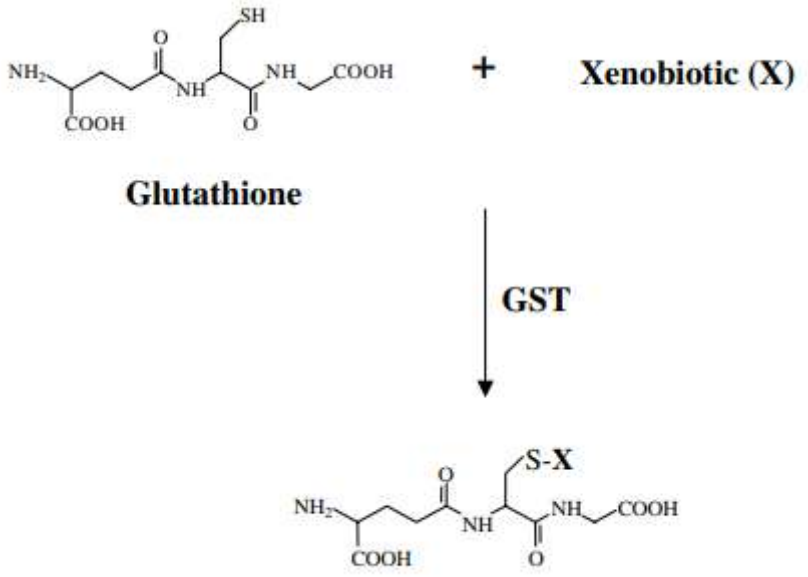

Figure 2. Glutathione conjugation to a generic xenobiotic (X) via GST results in the formation of a glutathione-S conjugate (Townsend and Kenneth 2003).

Previously, Kaur and Kaur (2015) found that the azo dye Acid Black caused oxidative stress in freshwater Labeo rohita by increasing GST activity. Excess ROS production on zebrafish exposed to textile industrial wastewater also reduced GST activity, which is an indicator of oxidative damage (Zhang et al., 2012).

Malondialdehyde

Polyunsaturated fatty acids in the membrane structure are oxidized by the effect of free radicals on the tissues, thus the lipid peroxidation process begins (Fig. 3). As a result of the conversion of lipid hydroperoxides to aldehyde and carbonyl compounds, the metabolic product MDA, which is an indirect marker used in determining the level of oxidative damage in the systemic circulation, is formed (Kose, 1992). Since MDA has a long life and high reactivity, its levels in tissues have been used to determine the severity of peroxidation since the 1960s (Ogus et al. 2004, Kacmaz 2013). MDA can be preferred as an indicator of lipid peroxidation. Lipid peroxidation of MDA produces a by-product that reacts with thiobarbituric acid and as a result; level directly reflects the degree of oxidative damage caused by pollutants (Ortega-Villasante et al. 2005).

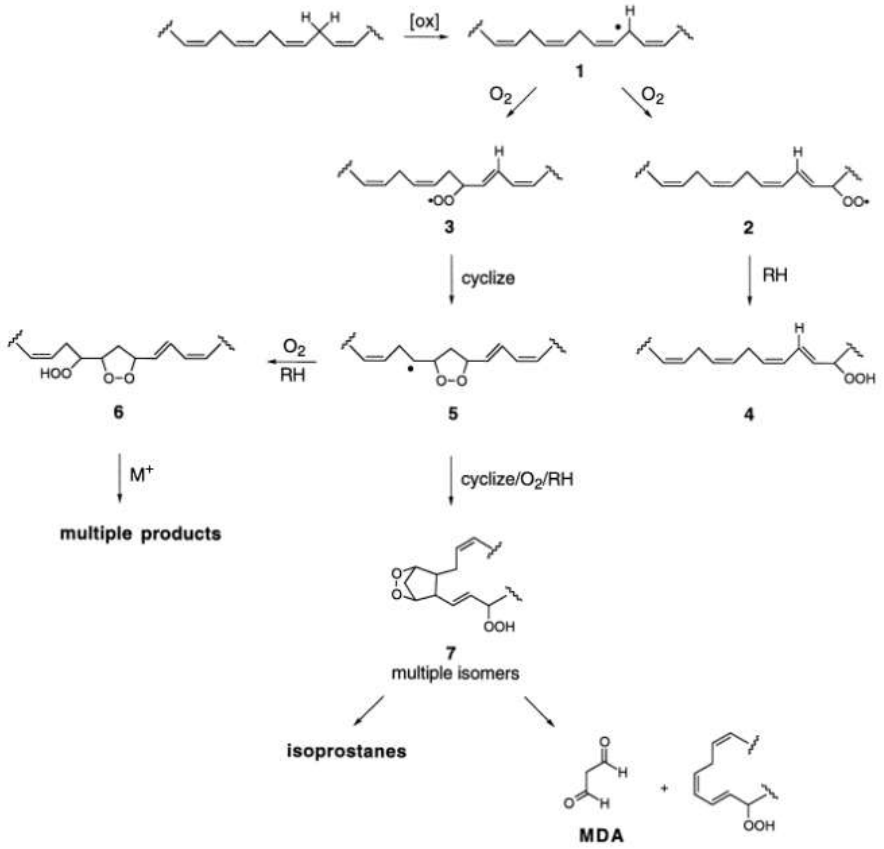

Figure 3. Pathways of lipid peroxidation (Marnett 1998).

Studies evaluating the bioremediation efficiency with oxidative markers

There are various mechanisms to eliminate the damage caused by free radicals in the living body and to prevent the damage. The most basic and important defense mechanism of the living body is antioxidants. They are water or fat soluble vital biomolecules that defend against free radicals and oxidative stress that have harmful consequences for the body. This system is called 'antioxidant defence systems' and the molecules used by the system are called 'antioxidants' (Nice, 1997). Antioxidant enzymes play an important protective role against ROS and, as in other biochemical systems, their activity may vary according to the development period and other physiological aspects of the organism. (Livingstone, 2001).

The literature on this species in amphipods is not sufficient. It is known that gammarids are highly sensitive organisms to contaminants. Many environmental polluting parameters can increase oxidative stress in living organisms living in receiving environments (Lopez-Lopez et al. 2011). Antioxidant enzyme activities such as CAT, superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) have been proposed as biological indicators of a pollutant in various marine and freshwater organisms, and their induction creates a response to pollutants (Borkovic et al. 2005).

Some previous studies evaluating bioremediation efficiency with some oxidative biomarkers are as follows. Yildirim and Yaman (2019), who conducted a study on for *G. pulex* exposed to methyl orange. Similarly, they found that the MDA levels decreased after bioremediation with *Coriolus versicolor.* Phugare et al. (2011) determined a significant increase in lipid peroxidation before treatment with bacterial consortium. They suggested that this increment was due to the reactive oxygen intermediates reactive oxygen species (ROS) generated by the chemicals and other toxic compounds present in the effluent. This situation can be explained by the excessive production of ROS after textile dye exposure. Tatar et al.

2018, found that GSH levels increased in *G. pulex* exposed to secondary effluent from municipal wastewater treatment plants after a 96 h exposure period. Demirci et al. (2018) investigated the toxic effects of the combined use of the atrazine and the insecticides endosulfan, indoxacarb, and thiamethoxam on *Gammarus kischineffensis*. CAT activity was reduced after exposure to the thiamethoxam and atrazine mixture during all application period.

Tatar et al. (2020) studied the bioremediation of methamyl insecticide with bacteria isolated from soil. At the end of the study period, they determined that the *Ochrobactrum thiophenivorans* and *Sphingomonas melonis* consortium provided a reduction of 94.7% and 96.8% via the COD and BOD_5 test results. *Ochrobactrum thiophenivorans* and *Sphingomonas melon*is can remediate Methomyl, indicating a significant effect of this bioremediation on the oxidative stress of *G. pulex*. The upregulation in the GPx enzyme is thought to be the initial response to exposure to toxic compounds that have the potential to induce oxidative stress (Lindesjoo et al. 2002). GPx activities decreased in G. pulex exposed to lead acetate (Kutlu and Susuz 2004). On the other hand, Serdar et al. (2018) exposed *G. pulex* to untreated leachate and stated that GPX activity increased

CONCLUSION

All these previous studies have shown that MDA levels and GPx, CAT, GST and SOD activities of *G. pulex* are useful biomarkers for evaluating the efficiency of bioremediation. As a result, we can have an idea about the efficiency of bioremediation by evaluating the changes in oxidative biomarkers measured as a result of exposure of various pollutants to *G. pulex* before and after bioremediation.

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COMPARISONS OF HIGH YIELDING GENETICALLY DIVERSE WHEAT LINES IN CROATIA

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ABSTRACT

Wheat is one of the most common and important cereals in Croatia. Therefore, high selection pressure at wheat breeding program is continually placed on disease, drought and lodging resistance, heading date and end-use quality. The usage of genetically resistant wheat lines to abiotic and abiotic stresses can be useful in controlling of wheat diseases and food contamination. The aim of the current study was to evaluate the wheat grain productivity and quality, as well as response of wheat seedlings to drought. In the current study, five lines of winter wheat with referent variety were used for evaluation of desired traits. In general, investigated wheat lines had such good characters as early maturity, high yield, but they were smaller in test weight and protein content, but with higher sedimentation value, dough energy and extensibility, compared to referent variety Kraljica. Overall, lines Osk.4.330/6-18, Osk.3.530/59-18, Osk.4.354/12-18 out yielded the referent check with regard to the grain yield. According to the results collected in this research, wheat lines differences in germination energy, and seedling growth affected by drought were obtained. As planting conditions are not always ideal, all lines could offer farmers tolerance to mild drought during sowing, and will achieve high yields. Nevertheless, stability and drought tolerance of investigated winter wheat lines at different environments needs to be checked at multi-location trials.

Keywords: Drought, Yield performance, Quality traits, Wheat

INTRODUCTION

Increased biotic and abiotic stresses occurring as a consequence of climate change are a threat to the already decreasing wheat productivity (Suzuki et al., 2014). According to Liu et al. (2016) for every 1°C increase in temperature, global wheat yields are predicted to decline by 4.1–6.4%. By 2050 increase of more than 60% in global grain production will be needed, as a consequence of food demand by population growth (Godfray et al., 2010). Therefore, the main targets of wheat breeding programs should be for increasing grain production limits. The main objectives of the winter wheat breeding program at Agricultural Institute Osijek are good quality and high yields, early maturity including some other various characteristics such as disease and drought resistance. The major threat in yield production is various pests and pathogens which cause considerable losses every year where fungal diseases cause the highest damage. The genetic basis of defence mechanisms to biotic stress is stored in the plant's genetic code (Singla and Krattinger, 2016). Despite the fact that the most important is the resistance of the genotype, most of wheat varieties were produced with high inputs of fungicides for plant defense against biotic stress.

Besides biotic stress, the concurrent occurrence of abiotic stresses such as drought and heat is more and more common due to global warming (Mittler, 2006) where increasing drought

also threatens agricultural productivity and food security (FAO, 2018). Hence, these climatic changes will have a dramatic effect on agriculture especially at the regions with water shortage and high temperatures (Rinaldi 2009). Nevertheless, drought is a global problem, which leads to osmotic stress with huge impacts on global wheat production (Daryanto et al., 2016). Therefore, the development of new wheat varieties with high water use efficiency is crucial for producing optimum yield under marginal rainfall conditions. According to Mickky and Aldesuquy (2017) in wheat certain developmental stages appear to be more sensitive to osmotic damage. However, it was demonstrated that wheat genotypes that showed drought tolerance at the germination stage exhibited the same tolerance to water deficit under field conditions (Khakwani et al., 2011).

In order to obtain correct levels of yield and quality, investigated winter wheat lines and referent variety were screened without usage of fungicide to find the best suited varieties for disease stressed conditions, as well as seedling test for drought resistance was performed in controlled conditions.

MATERIAL AND METHOD

Plant material and field experiment

Five winter wheat lines with referent variety (Table 1) have been studied in the field trial in vegetative season 2019/2020 at Osijek (45°27' N, 18°48' E) in Croatia, where soil type is eutric cambisol. The plants were sown in October in the experimental plot area of 7.56 m2 in four replications. The average annual precipitation in vegetative period in 2019/2020 was 408.6 mm and the average annual temperature was 11.1˚C. Insecticides and herbicides were applied as needed, without usage of fungicides. In the field trial, traits as heading date, resistance to lodging and plant height were evaluated. After harvest at the beginning of July, grain yield (t ha^{-1}), test weight (kg hl^{-1}) and 1000 kernel weight (g) were obtained.

Table 1. Investigated five winter wheat lines and referent variety Kraljica

No.	Variety/Line
1	Kraljica
2	Osk.4.324/5-18
3	Osk.4.312/10-18
4	Osk.4.330/6-18
5	Osk.3.530/59-18
6	Osk.4.354/12-18

Technological and rheological quality

The quality tests described in this section are standardized testing procedures commonly used for quality control purposes. After milling the grain samples protein and wet gluten content, gluten index, zeleny sedimentation volume and falling number were obtained by ICC method No. 155, 116/1 and 107/1, respectively. Farinograms were obtained using method for using the Brabender Farinograph (HRN ISO 5530-1:1999). Extensogram test was conducted using method for using the Brabender Extensograph (HRN ISO 5530-2:1999).

Experiment with drought on seedlings

The growth chamber experiments were conducted to evaluate drought resistance in seedlings stage by usage of polyethylene glycol-6000 (PEG6000) solutions as the moisture stress inducing media. Seedlings were grown in trays and water stressed up to seven days with 16 h light period at 25˚C and 8 h dark period at 20˚C, with constant relative humidity of 60%. Drought-exposed plants were watered daily with 10 ml of 10 and 20% PEG solution, while in the control treatment only distilled water was applied. After seven days germination energy, growth parameters, as well as relative water content (RWC) were calculated. Germination energy was defined as the percentage by number of seeds in a given sample which germinate within 7 days. Growth parameters (root and shoot length) were recorded in mm with a ruler after seven days of different treatments. RWC was calculated by the formula: RWC (%) = (FW-DW)/(TW-DW)*100 where FW was a fresh weight of leaf tissue which upon weighting was submerged in distilled water for 24 h to reach turgid weight (TW). Dry weight (DW) of tissue was obtained by drying leaf discs at 105°C for 24 h.

Statistical analysis

All recorded values for agronomical and quality parameters represent the means of the results of four replications. For drought experiment, 15 replicated seedlings were taken for investigated traits as a mean value.

RESULTS AND DISCUSSION

The investigated winter wheat lines were selected by the pedigree method over few years of selection and tested in varietal experiments in trial in vegetative season 2019/2020 at one location. Climate changes enhance the importance of drought stress in wheat growing regions of the world where drought stress can lead to a high yield decline in recent years. We want to create drought-tolerant varieties and therefore, it is essential to primarily understand response of wheat plants in water-deficient conditions at different stages of growth.

Agronomical and morphological traits

The highest grain yield had line Osk.3.530/59-18 (12.75 t ha^{-1}). The lowest grain yield was recorded for line Osk.4.312/10-18 (11.55 t ha^{-1}) with the lowest test weight. Test weights ranged between 78.5 (Osk.4.312/10-18) to 82.2 kg hl^{-1} (Kraljica). The tallest line Osk.4.354/12-18 had the highest 1000 kernel weight (43 g). Currently, referent variety such as Kraljica (a compromise between yield and proteins, with good baking quality) perform excellent in Croatia, Slovenia, Bosnia and Herzegovina, Slovakia, Kosovo, Macedonia and Romania. Although referent variety Kraljica had the highest test weight, it did not give the highest yields, where the lowest resistance to lodging could be one of the reasons for not performing the best yields. Feng et al. (2019) reported that changing weather patterns such rain, wind, and hail storms have made the current varieties more susceptible to lodging, leading up to 80% yield losses. Test weight as the specific volume was in good range in all wheat lines. According to Mecha et al. (2017) by selecting for this trait together with grain filling period, number of productive tillers per plant, spike length, number of spikelets per spike, number of kernels per spike, 1000 kernel weight, biomass yield per plot and harvest index, there is also possibility to increase grain yield of bread wheat.

Five wheat lines had the similar heading date as Kraljica, except line Osk.4.330/6-18 with three days later heading date. Investigated five lines developed were an early maturing,

similar as Kraljica, making it well suited to short seasons. It is more and more important to grow winter wheat varieties that are not late-maturing as harvest delays or terminal heat stresses can often occur (Mondal at al., 2016). Among wheat lines, the maximum plant height (96 cm) was recorded in line Osk.4.354/12-18, and minimum plant height (85 cm) was recorded in line Osk.4.312/10-18, the same as for referent variety Kraljica. All lines together with Kraljica had red coloured seed (Table 2). Plant height was measured from soil surface to the base of the ear head of main shoot at maturity stage. It should be in relation to the plant architecture, lodging resistance and yield performance (Wang et al. 2017). In the current research, only one wheat line was taller than 90 cm, which potentially could be resource of feed for ruminants.

Table 2. Agronomical and morphological traits of five winter wheat lines and referent variety Kraljica

Variety/Line	GY*	TW	1000KW	HD	LOD	PH	SC
Osk.3.530/59-18	12.75	79.5	40	5.05.2020.	1	86	Red
Osk.4.330/6-18	12.62	81.6	39	8.05.2020.	0	89	Red
Osk.4.354/12-18	12.62	81.9	43	5.05.2020.	0	96	Red
Kraljica	12.54	82.2	40	5.05.2020.	1,5	85	Red
Osk.4.324/5-18	12.52	80.1	37	5.05.2020.	0	89	Red
Osk.4.312/10-18	11.55	78.5	37	6.05.2020.	0	85	Red

*GY-grain yield in t ha^{-1}, TW-test weight in kg hl^{-1}, 1000KW-1000 kernel weight in g, HD-heading date, LOD-lodging evaluated as 1-9 (0-no lodging, 9-fully lodged plants at the plot), PH-plant height in cm, SC-seed colour (soaked in 1 M NaOH)

Table 3. Technological and rheological parameters of of five winter wheat lines and referent variety Kraljica

Variety/Line	P	SED	VG	GI	FN	WA	QG	E	Ext
Osk.3.530/59-18	13.2	49	26.6	97	463	55.5	B2	90	155
Osk.4.330/6-18	13.1	42	26.9	93	369	56.1	B2	-	-
Osk.4.354/12-18	14.3	46	24.6	97	376	58.2	A1	98	159
Kraljica	14.5	44	28.7	99	450	58.3	A2	86	155
Osk.4.324/5-18	12.8	49	22.9	99	354	51.2	B1	96	160
Osk.4.312/10-18	13.2	48	24.4	99	374	53.1	B2	91	175

*P-protein content in %, SED-sedimentation value in ml, VG-wet gluten content in %, GI-gluten index, FN-falling number in s, WA-water absorption in %, QG-quality group (ranking A1-C1), E-energy in cm2, Ext-extensibility in mm

Technological and rheological quality

To meet specifications of mill and bakery industry in grain and flour quality testing is necessary to evaluate dough and gluten strength properties. The farinograph test is used to measure the resistance of dough to mixing, while extensograph test is used to measure the resistance of dough to stretching. Results from these tests have a direct relationship to finished product quality. In the current research, the lowest protein content and wet gluten content were recorded for line Osk.4.324/5-18 (12.8 and 22.9%), while referent variety Kraljica had the highest values of those two parameters (14.5 and 28.7%). The same line Osk.4.324/5-18, together with line Osk.3.530/59-18 had the highest sedimentation value (49 ml). Gluten index

ranged between 93 (Osk.4.330/6-18) to 99 (Kraljica, Osk.4.324/5-18 and Osk.4.312/10-18). The highest falling number had line Osk.3.530/59-18 (463 s), followed by Kraljica (450 s).

Line Osk.4.354/12-18 had a high yield potential and also maintained a higher grain protein content than other lines with similar yield potential. Four wheat lines had very good sedimentation values, compared to referent variety, thus showing good gas retention in dough stability and baking volume. Usually, sedimentation value of flour depends on the wheat protein composition and is mostly correlated to the protein content (Hrušková and Faměra, 2003), which was not the case in the current study. It was observed that referent variety Kraljica had the highest wet gluten content. It was previously concluded that the higher a flour's protein content, the higher the gluten formation (Baslar and Ertugay, 2011). All tested wheat lines were selected for low pre-harvest sprouting, where falling number as the level of alpha amylase activity, was within acceptable limits. Values below 300 seconds are indicatives of pre-harvest sprouting (grain may start to germinate before harvest) which will result in poor quality (Kiszonas et al., 2018).

Knowing the technological properties of the flour is also not enough to fully characterize end-use quality of wheat. Therefore, it is useful to obtain rheological properties of the dough where in the current study we obtained water absorption and quality group from farinograph measurements, as well as energy value and dough extensibility from extensograph tests. The best water absorption had Kraljica (58.3%) and line Osk.4.354/12-18 (58.2%), belonging to quality groups A2 and A1 thus showing good end-use quality properties. Line Osk.4.330/6-18 exhibited the lowest dough energy and extensibility. The highest dough energy had line Osk.4.354/12-18, and the best extensibility was obtained in line Osk.4.312/10-18 (Table 3). The flour with good bread-making properties has higher water absorption, takes longer to mix and is more tolerant of over-mixing than poor-quality bread flour (William, 2001). All lines, except Osk.4.330/6-18, had higher energy values showing the greater gas holding capacity and fermentation tolerance of the dough where no difficulties in bread making should be obtained. Similar behaviour of those lines was obtained for dough extensibility. According to Anderssen et al. (2004) rheological parameters can be used to determine the processability of wheat to different products.

Drought-related traits in seedling

During germination wheat plants are very sensitive and drought stress can delay or inhibit germination processes, leading to potential yield loss due to reduced cropping density (Almansouri et al., 2001). In general, both solutions (PEG10 and 20%) significantly affected germination energy and seedling growth parameters (shoot and root lengths) compared to controlled treatment. Relative water content (RWC) was influenced in lesser extent.

The highest germination energy had Kraljica and Osk.4.330/6-18 (91.25%) in controlled treatment, Kraljica in PEG10 treatment (92.5%) and line Osk.4.330/6-18 in PEG20 treatment (81.25%) (Figure 1). Germination energy which is used to determine the speed of germination at two various concentrations of PEG differed among wheat lines, where Osk.4.530/6-18 showed the lowest value in PEG10% treatment. The highest germination energy in PEG20% treatment had line Osk.4.330/6-18, compared to other lines. According to the results of Duan et al. (2017) germination of wheat seeds could be inhibited by drought stress, where germination energy significantly decreased with the increase of drought degree in most wheat lines. In the current research the germination energy of Osk.4.330./6-18 and Kraljica was stronger compared to other lines.

The highest reduction of shoots in 10% PEG solution, compared to controlled treatment, was recorded in line Osk.4.354/12-18 (13.04%) (Figure 2), while the length of the roots was increased in all wheat varieties, where line Osk.3.530/59-18 was showing the highest increase among other varieties (67.33%), followed by line Osk.4.324/5-18 (58.36%) (Figure 3). The highest reduction of shoots was recorded in line Osk.3.530/59-18 (37.54%) in 20% PEG treatment, while the highest reduction of the roots had line Osk.4.354/12-18 in 20% PEG treatment (27.5%). Lines Osk.4.330/6-18 and Osk.3.530/59-18, as well as Kraljica increased length of the roots in 20% PEG treatment (17.13, 14.41 and 1.39%, respectively), compared to seedling in controlled conditions. Liu et al. (2013) reported that wheat seedling growth indices decreased under drought stress. In the current research it was different where lines Osk.4.330/6-18, Osk.3.530/59-18 and Osk.4.324/5-18 showed the increase of shoots length in PEG10% treatment, with the highest reduction of shoots in PEG20% treatment, compared to controlled treatment. The same lines had the highest increase of root length in PEG10% treatment with lower increase in PEG20% treatment, except Osk.4.324/5-18 line which showed root reduction in PEG20% treatment. According to Xu et al. (2015) alterations in root system architecture aids in short-term adaptation to water deficit. Root length at the seedling stages of the plant is a key genetic trait for increasing yield under drought conditions (Shahbazi et al., 2012).

Relative water content (RWC) ranged between 88.0% (Osk.4.324/5-18) to 104.7% (Osk.4.312/10-18) in controlled treatment, while in 10% PEG treatment that range was from 82.4 (Osk.4.354/12-18) to 89.6% (Osk.4.312/10-18), and in 20% PEG solution it ranged from 70.02 (Osk.4.324/5-18) to 95.5% (Kraljica) (Figure 4). The line Osk.4.312/10-18 (with the lowest yield) had the highest RWC in controlled and PEG10% treatment, but not in PEG20% treatment thus showing that increased drought stress will significantly influence RWC. Almeselmani et al. (2011) concluded that RWC is a good criterion for the selection of drought-tolerant wheat varieties at the seedling stage. Datta et al. (2011) applied both normal and water-deficient conditions to wheat genotypes and observed that genotypes performed better under environments which had optimum RWC and root and shoot length, which were considered drought-tolerant genotypes. In the current research all varieties retained good RWC when grown under mild drought conditions (PEG10%). Similar results were obtained by Tahara et al. (1990) in winter wheat varieties as the high-yield selections maintained a significantly higher RWC than the low-yield selections.

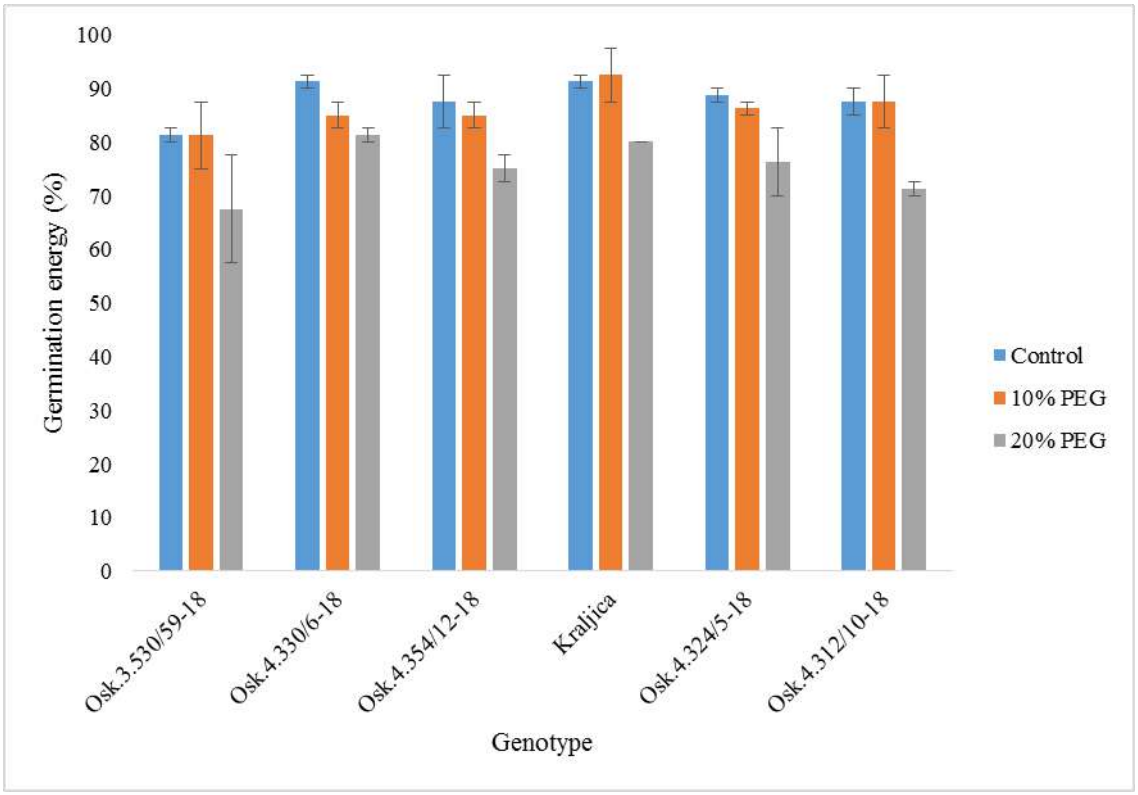

Figure 1. Germination energy of five winter wheat lines and referent variety Kraljica

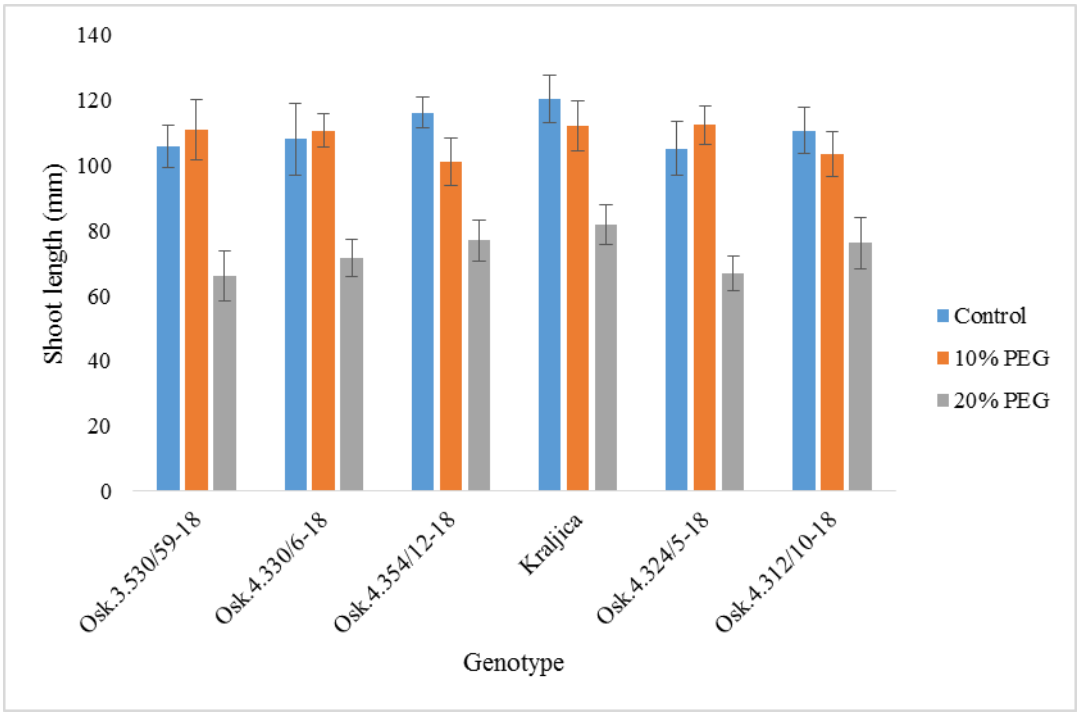

Figure 2. Shoot length of five winter wheat lines and referent variety Kraljica at 7th day of experiment

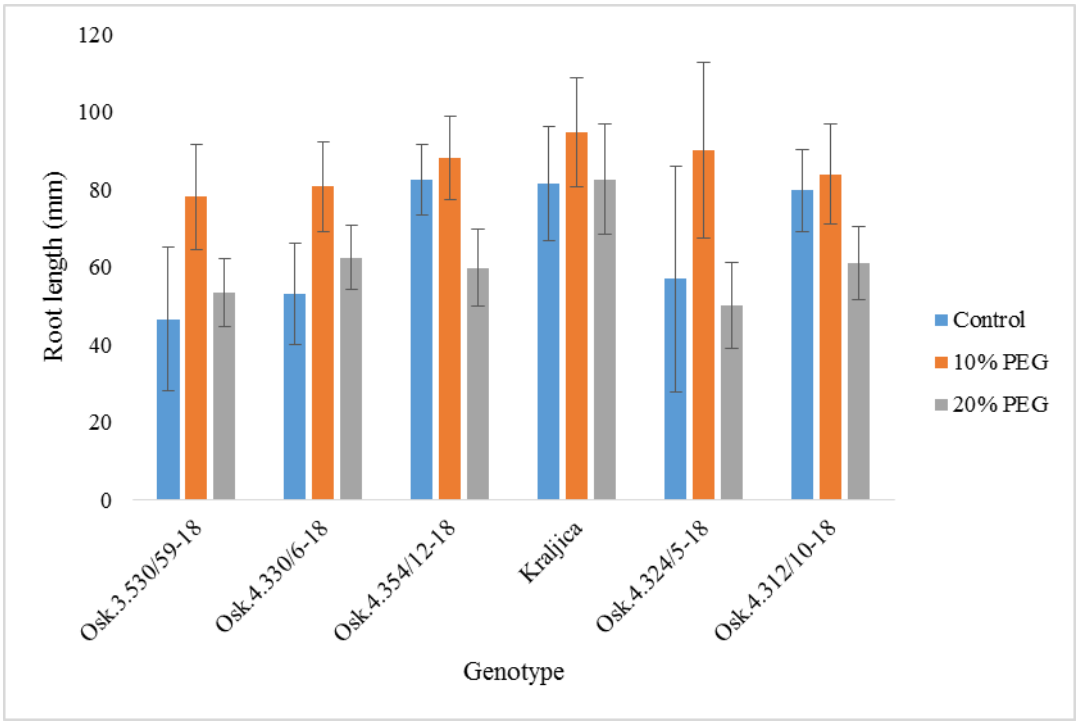

Figure 3. Root length of five winter wheat lines and referent variety Kraljica at 7th day of experiment

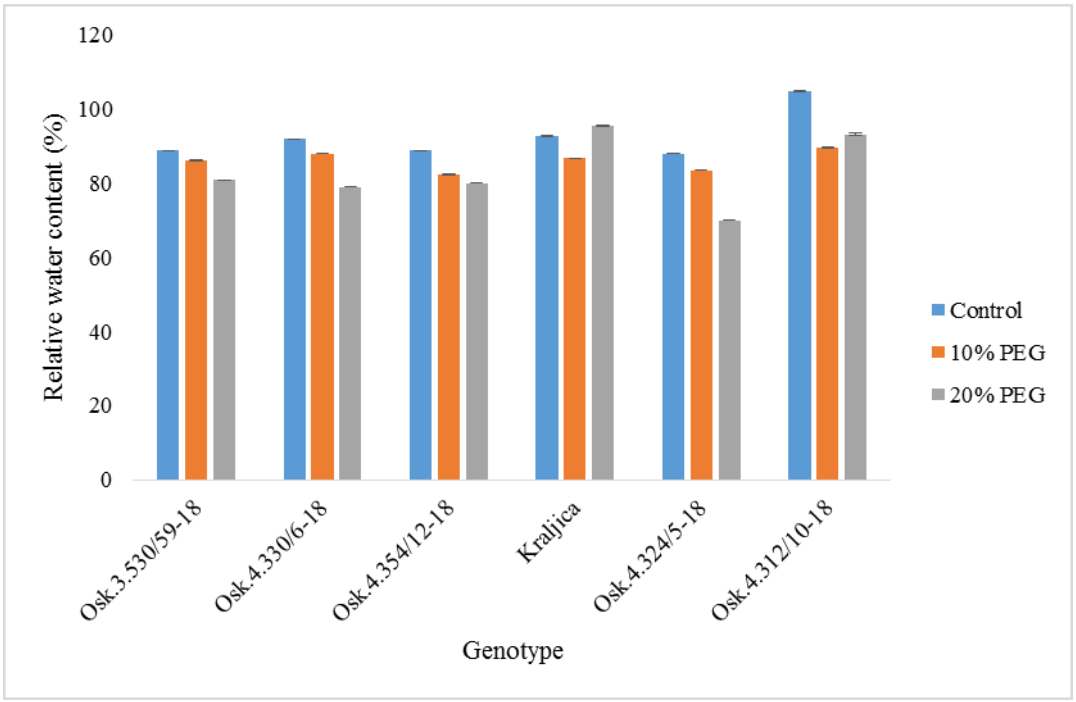

Figure 4. Relative water content of five winter wheat lines and referent variety Kraljica

Although wheat plants with early maturity can contribute to drought escape and could be suitable for environments subjected to late season drought stress, the line with the latest heading date exhibited the best germination energy in PEG20% treatment. These results indicate that breeding efforts to produce drought-tolerant wheat can benefit current climate

change adaptation. According to Bernier et al. (2008), there is the possibility to combine high yield potential and good yield under drought successfully. Nevertheless, in the current research all tested lines having good characteristic for grain yield and quality still need to be checked how they are broadly adapted.

CONCLUSIONS

Although, created winter wheat lines have been tested for production in Croatia, it is desired and anticipated that those lines have good yield and quality performance at different countries. Therefore, multi-location trials need to be set up in next seasons. Multi-location testing of those lines will allow a better understanding of the genotype×environment (G×E) interactions related to grain yield. The current results demonstrated that advanced winter wheat lines perform well compared to referent variety with good levels of yield and ground cover; baking quality data are good up till now, but have yet to be completed this year. Continued emphasis has been placed on selecting breeding lines with superior quality and disease resistance where line Osk.4.354/12-18 showed good performance. We expect that most of the lines are high yielding, by showing good emergence in more dry soils.

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DISTRIBUTION OF NOSEMA SP (CAUSATIVE AGENT OF NOSEMOSIS IN HONEY BEES) IN ALGERIA

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ABSTRACT

The bee is an indispensable part of the environmental balance in the world as a pollinator of many species. It also has other interests including the production of honey, propolis, royal jelly and wax. Unfortunately, this species is threatened by several factors, the most important of which is the presence of pathogens that cause pathologies in the colonies. One of the most dangerous conditions is nosemosis. The interest of this present work is to assess the health situation of bee colonies in some regions of central Algeria (Boumerdès, Bouira and Tizi ouzou). The comparison of the prevalence shows that the apiaries located in the Boumerdès area have the highest rate of infestation (21%). This high prevalence of nosemosis in this area is linked to particular climatic conditions such as the presence of high humidity and a long cold period. Symptoms have been detected in a few apiaries but no correlation exists between the presence of the signs and the rate of contamination.

Key words : Bee - Nosemosis - Center of Algeria - prevalence - climate.

1. Introduction

The bee is a social insect with a very important role in pollination and in agriculture. A third of the food consumed in the world is linked to the pollinating activity of bees (Gallai et al. 2009). For several years, a noticeable decrease in honey bee populations has been reported in many countries by beekeepers and scientists. Several risk factor hypotheses have been put forward to try to explain this phenomenon (VanEngelsdorp et al., 2008; Guzman-Novoa et al., 2010; Currie et al., 2010; Fries, 2010)

In Algeria, many cases of bee colony mortality have been observed since 2007. The presence of pathogens in these colonies as well as their health status are the main causes of this lethality (Adjlane, 2009). Varroasis is the most well-known pathology in Algeria. However, the reader has very little information on other diseases, especially nosemosis. Indeed, few studies have been carried out in Algeria to determine the prevalence of this disease in bee colonies. This apparent gap justifies the present work. The causative agent of nosemosis is Nosema sp., A single-celled microorganism that infects the epithelium of the mesenteron wall of the worker bee (Faucon, 2005). Nosema forms resistant spores which remain viable for long periods of time.

2. Materials and Methods

Place and period of work:

The work was carried out on 21 apiaries. At the level of each apiary, sampling is carried out on 5% of the hives. Samples are taken in 2019 at the end of winter and at the beginning of spring in 3 agricultural areas: Boumerdès, Bouira and Tizi Ouzou with 7 apiaries in each region.

Bee sampling method

Worker bees of undetermined age are collected from the 4th or 6th frame of the brood of colonies of the race *Apis mellifera intermissa*. After sampling, the bees are kept in boxes containing 95 ° ethanol and at a temperature of -20 ° C until the day of the analyzes.

Figure 1: Location of the study area.

Method of statistical analysis of the results

The data obtained are analyzed with Statistica version 5.0 software following the analysis of variance (ANOVA) process.

3. Results and discussion

Upon observation with the light microscope, Nosema spores appear transparent with a very distinct dark outline, measuring between 5 and 7 µm long and 3 to 4 µm wide.

The comparison of the percentage of contamination between the three zones studied shows that the apiaries located in the zone of Boumerdès record the highest rate of contamination (21%) (Figure 2). This rate is higher than those noted in the other two zones, those of Bouira (08%) and Tizi ouzou (10%). Analysis of variance shows a highly significant difference in the prevalence results between the areas studied. Analysis of the climatic data mentioned for the studied region shows that the Boumerdès area corresponds to a prolonged winter and high humidity. There appears to be a relationship between the increased prevalence and particular climatic conditions such as high humidity and long cold spell. Indeed, it is generally accepted that Nosema sp. is prevalent in cold areas and the severity of infection is limited to areas with long winters (Moeller, 1978). Bailey (1981) indicates that the causes which favor the development of this pathology are mainly linked during long winters to the prolonged confinement of the bee inside the hive, which favors an active dissemination of Nosema.

According to Barbançon and L'Hostis (2007). Research on Nosema also indicates that the infestation reaches its highest rate during the wet seasons of the year (Fries, 1988; Huang et al. 2007). In Mediterranean regions where humidity is high in summer, infection is also high during this same period (Martin-Hernandez et al. 2007). The maximum infestation is recorded in spring and the lowest rate in summer (Higes et al. 2006).

On a worldwide scale, several research works have focused on the distribution of nosemosis within bee colonies, particularly in Europe and Spain (Higes et al., 2008), in the North-West of Turkey. (Aydin et al., 2006), France (Chauzat et al., 2007), Denmark, Finland, Germany, Greece, Hungary, Italy and Serbia (Klee et al., 2007) and Asia among others in Taiwan (Huang et al., 2007). Research on Nosema indicates that the infestation reaches its highest rate during the wet seasons of the year (Fries, 1988; Huang et al., 2007). Specifically, in research conducted in Turkey, 23.8% of 168 colonies studied are infected with Nosema during the spring period (Aydin et al., 2006). In Mediterranean regions where humidity is high in summer, infection is also high during this same period (Martin-Hernandez et al., 2007).

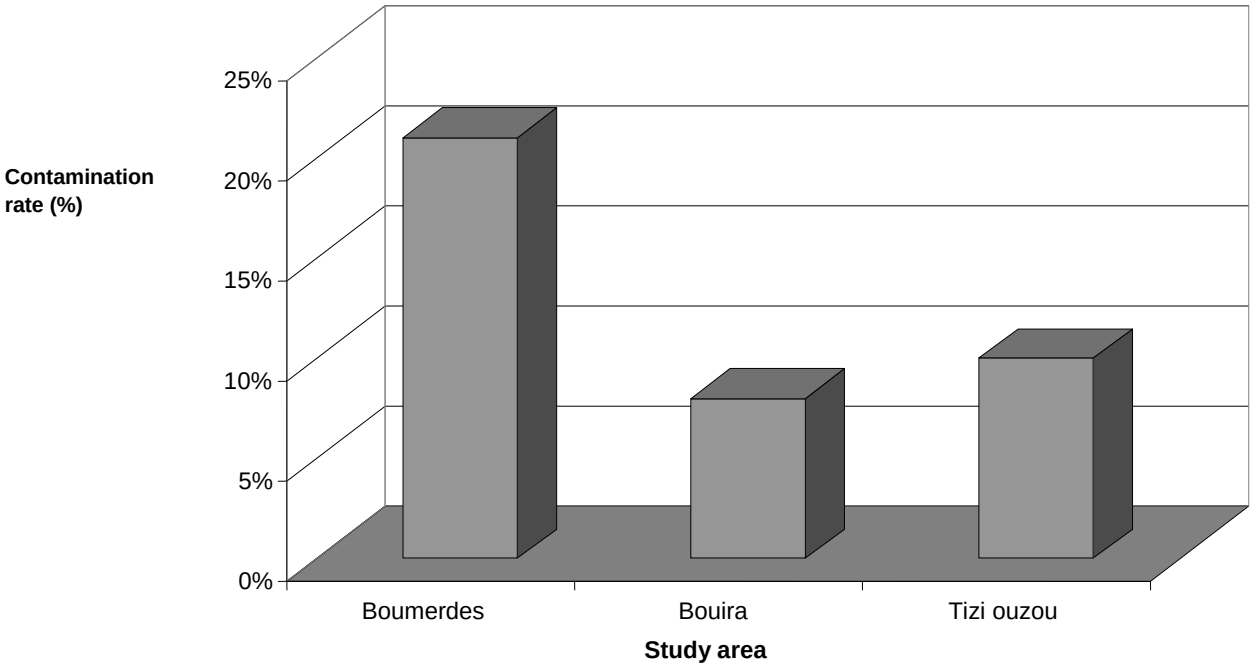

Figure 2: histogram of the percentage of contamination by nosemosis in the 3 areas studied.

Table 1 : Search for a significant difference by an ANOVA between the rate of prevalence of nosemosis between the areas studied

Source of variations	Sum of squares	Degree of freedom	Mean of squares	*F*	Probability	*Valeur critique pour F*
Between Groups	686	2	343	25,9411765	0,000	3,55455715
Within groups	238	18	13,2222222			
Total	924	20				

In Iran, infection of bee colonies with Nosema reaches its highest level in the spring (Lotfi et al., 2009). There is a time lag in the Mediterranean Basin since in Spain the maximum infestation is recorded in spring and the lowest rate in summer (Higes et al., 2006).

Recently, Copley and Jabaji (2012) reported through a study done in 2009 and 2010 in Canada that the Nosema apis infestation rate is 29% with seasonal peaks detected in spring and fall. A typical Nosema infection results in a low level of spores, even undetectable during the summer (Fluton, 2007). Mention should be made of the low level of infection in the fall, followed by a slow increase at first in winter, then rapid in spring when brood feeding and cleaning intensify as the temperature rises in the colony. . In addition to the climatic conditions favorable to the increase in the prevalence of nosemosis, other factors may explain this heavy infestation of the colonies, such as the honeydew produced by Homoptera and brought back to the hives which leaves residues in the intestine of each bee in winter, which favors the development of Nosema (Faucon, 1992).

The results of the presence of symptoms in the colonies studied are presented in (Fig 3) we observed in the field that the area of Tizi ouzou did not show symptoms (0%), so nosemosis can go unnoticed for a long time when the infestation is low because there are no observable symptoms. Nosema sp is called the silent killer, due to its insidious development (Aurière, 2001). Bees die, most often in the wild, which causes progressive depopulation which may go unnoticed by the beekeeper (Dottin, 1986). Note that the analysis of variance shows a highly significant difference in the presence of symptoms between the study regions.
On the other hand, the agricultural study zone of Boumerdès presented 5% and that of Bouira (1%). So only a microscopic examination can provide a definite laboratory diagnosis that really indicates the presence of Nosema in bee colonies..

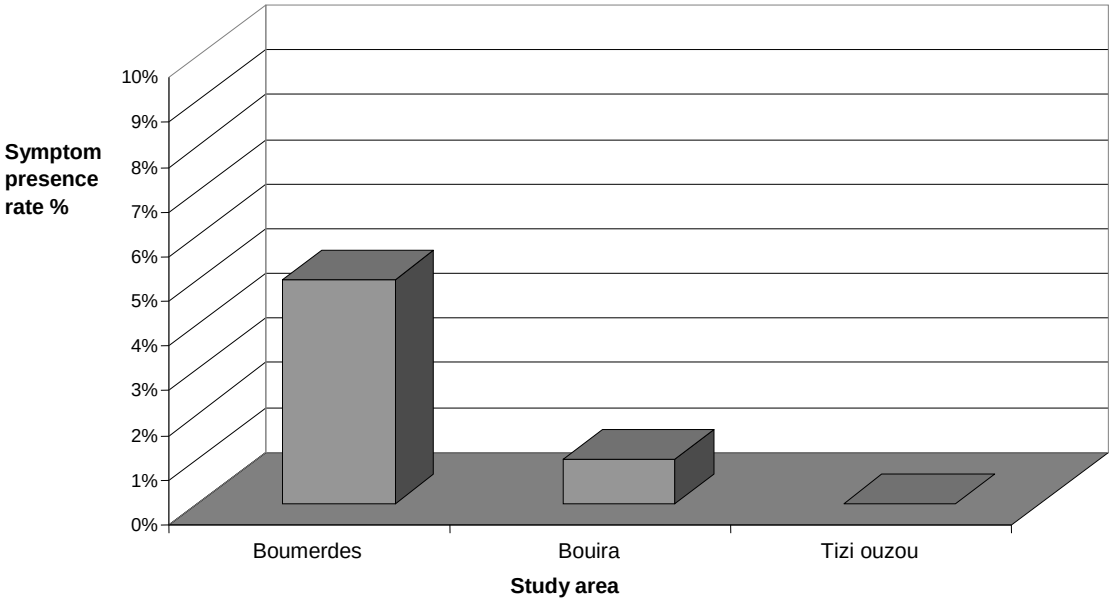

Figure 3: Symptom presence rate in the areas studied

Tableau 2: Recherche de différence significative par une ANOVA entre le taux de présence des symptômes de la nosémose entre les zones étudiées

Source of variations	Sum of squares	Degree of freedom	Mean of squares	*F*	Probability	*Valeur critique pour F*
Between Groups	98	2	49	11,025	0,00074822	3,55455715
Within groups	80	18	4,44444444			
Total	178	20				

A typical Nosema infection results in low or even undetectable spore levels during summer (Fluttom, 2007). In addition to the climatic conditions favorable to the increase in the prevalence of nosemosis, other factors may explain this heavy infestation of the colonies, such as the honeydew produced by Homoptera and brought back to the hives which leaves residues in the intestines of the colonies. bees in winter, which favors the development of Nosema (Faucon, 1992). The heavy infestation of the colonies may be due to the susceptibility of certain breeds of bees. This is the case for the Italian and Caucasian races (Faucon, 1992). Yet according to Malone and Stevanovic (1999) there is no difference between the Italian race and the black race in the face of Nosema infection. No study has yet been carried out on the susceptibility of the two local bee breeds, Tellian and Saharan, to nosemosis. A colony heavily parasitized by Varroa destructor constitutes a favorable field for the development of nosemosis (Orantes Bernejo & Garcia Fernandez, 1997; Barbançon & L'Hostis, 2007; Colin et al, 2007).

In addition, poor farm hygiene resulting from prolonged use over several years of the same frames is another source of disease development (Fries, 1988). Finally, the low presence in the hive of pollen, a source of proteins, also favors the development of the disease (Fries, 1993).

Heavy infection of bee colonies with nosemosis causes a drop in honey production. According to Hucorne (2002) a 50% drop in honey production is observed in a hive where the Nosema infestation rate is 5 to 25%. This reduction reaches 80% in an infested hive between 30 and 50%. Indeed, Nosema sp. reduces the lifespan of workers by more than half (Kleinschmidt and Fergusson, 1989). This fungus disrupts the physiology of the bee by reducing the development of the hypopharyngeal glands (Dottin, 1986), by causing atrophy of the ovaries and by reducing the development of the wax glands (Faucon, 1992). It also causes a rapid decrease in the proteolytic capacities of the bee (Malone and Gatehouse., 1998). The impact of this infection is considered to be equal to or greater than the losses caused by all other diseases combined, including the more easily diagnosed brood diseases (Mussen et al. 1975).

In recent years, the parasite Nosema ceranae, which was then only known from the Asian species Apis cerana, appeared in Apis mellifera for the first time noted in 2005 (Huang et al., 2007). The disease caused by Nosema ceranae is now called nosemosis type C and is considered to be one of the major threats to bee colonies. Mayack and Nuag (2009) hypothesize that the presence of Nosema in the colony is one of the explanations for the absence of dead bees around hives affected by CCD (Colony Collapse Disorder). Unlike Nosema apis, Higes et al. (2009) have shown that factors such as access to fresh pollen, of various origins, or the variation of humidity and temperature conditions throughout the year do not seem to influence the development of the parasite within beehives.

4. Conclusion

The results of our study show that this pathology is present in all the areas studied, with a difference in the rate of infection. The fact that the Boumerdès area is characterized by infestations by Nosema sp. significantly higher than in other regions shows the important influence of climatic factors in particular high humidity following heavy rainfall and lengthening of the winter. Thus, humidity and low temperatures favor the development of nosemosis.

The observation of the symptoms of the pathology in the apiary does not in any way constitute proof of the presence or absence of Nosema. Indeed, the results obtained show the presence of Nosema spores without any presence of typical symptoms. Probably, these signs of the disease appear when the rate of presence of the spores in the bees is very high. Hence the need to plan in the future work to study a possible correlation between the rate of the presence of spores and the onset of symptoms.

Early detection of the disease is essential to avoid colony loss. It can help prevent the infection from spreading to healthy bee colonies. When colonies are affected by this disease, production becomes a secondary problem due to the need to urgently treat the infected colony to save it. It would be useful to take 4 samples per year, ie 1 per season. This precaution could help beekeepers determine the trend of infection with Nosema sp. and to evaluate the effectiveness of control strategies for this disease. It is also necessary to determine the occurrence of the two species, Nosema apis and Nosema ceranae using the tools of molecular biology.

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ENTRERIC METHANE MITIGATION OPTIONS IN RUMINANTS

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ABSTRACT

We have been facing a problem that has been threatening our planet and our future for years, global warming. Besides various factors, livestock activities and especially ruminants cause global warming. Greenhouse gases such as methane, carbon dioxide and nitrous oxide, which are produced as a result of livestock activities, are the main threats to our planet. Ruminants are responsible for 93% of animal-based greenhouse gas emissions in a year. In this amount, enteric methane emission is important as it constitutes 47% of the livestock sector. It is also estimated that the number of ruminants will increase in the future with the increase in the human population. Therefore, methane emissions must be reduced immediately. Scientists working on this subject suggested that many substances such as oil, oil seeds, various algae, plants, methane inhibitors, nitrate, sulfate, saponin, tannins and ionophore reduce gas emission. Among them, algae such as Asparagopsis taxiformis, plants containing various phyto-chemicals, and methane inhibitors have been reported to reduce methane emission up to 95%. Therefore, it is necessary for researchers to conduct more studies on these products. Increasing the usability of products that are economical and not harmful to animal health is important for our planet and our future.

Keywords: Ruminant, greenhouse gases, methane, enteric, climate change

INTRODUCTION

Livestock activities (animals, feeding, housing, manure storage, manure application, soil) contribute to global climate change by causing greenhouse gas (GHG) emissions such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4; enteric CH_4 in this review) (Rotz, 2018; Haque, 2018). Among animals, ruminants transform plants that cannot be consumed by humans into foods such as milk and meat, with enteric fermentation (Hristov et al., 2013; Gerber et al., 2015). However, with the enzymes secreted by microorganisms (bacteria, archaea, protozoa, yeast, fungi) in the rumen, gases such as CO_2, N_2O and CH_4 are produced while the feed is fermented (Moss et al., 2000; Boadi et al., 2004; Martin et al., 2010; Rotz 2018; Haque 2018). In addition, H_2 and CO_2 produced as a result of rumen fermentation are converted to methane by methanogens (methanogenic archaea) (McAllister and Newbold, 2008; Sethy et al., 2021). Except causing global warming, it is stated that 6% of the gross energy that should be used by animals is lost as CH_4 (Appuhamy et al., 2016).

It has been reported that animal production is responsible for 14.5% of the total greenhouse gas emissions caused by human activities in a year. Ruminants are responsible for 93% of this amount (Gerber et al., 2013; Watts et al., 2021). In addition, in the total GHG emissions, enteric methane constitutes 47% of the total livestock sector (Opio et al., 2013), and approximately 6% of the total anthropogenic emissions (Beauchemin et al., 2020). It is also reported that enteric fermentation constitutes more than 90% of the total CH_4 originating from ruminants (Opio et al., 2013). Therefore, in this review, strategies for reducing enteric methane emission from ruminants will be examined.

OPTIONS TO REDUCE METHANE FORMATION

Fat

It has been reported that the supplementation of fat (Sethy et al., 2021) and oilseeds (Beauchemin et al., 2008; Martin et al., 2010) to ruminant diets decrease methane emissions. Use of fats decreases the free hydrogen in the rumen during the biohydrogenation of polyunsaturated fatty acids, thus reduced methane production in the rumen (Johnson and Johnson, 1995; Sethy et al., 2021). Because hydrogen is used in methane production (Lan and Yang, 2019). In addition, some researchers reported lipids inhibit methanogenesis by reducing the number of rumen methanogens and protozoa (Nogueira et al., 2020). Because protozoa supply H_2 to methanogens for ruminal CH_4 production (Newbold et al., 2015).

Nogueira et al. (2020), reported cottonseed supplementation to cattle diet reduced methane emission by 42%. In other studies, it has been reported that there is a 27% (Martin et al., 2008) and 18% (Beauchemin et al., 2009) reduction in methane emission with the use of cotton seed in ruminant diets. In a study, 29.5% reduction in methane emission was reported with the supplemented of 60g/kg dry matter (DM) camelina oil (Bayat et al., 2015). According to a meta-analysis study, 9% reduction in methane production was reported with the addition of fat in dairy cow diet (Eugène et al., 2008). In a study, it was reported that the use of 50 g/kg of dietary DM of sunflower oil in dairy cow diets reduced methane production by 18% (Bayat et al., 2017). Mata e Silva et al. (2017), reported when the amount of sunflower oil given to grazing animals was increased (from 3% to 5% DM), daily methane production decreased by 20%. In an in vitro study using oils (olive oil, soybean oil, palm oil, sunflower oil) in soap form, it was determined that olive, soy and sunflower oil reduced methane production (Blanco et al., 2012). Another in vitro study, it was reported that coconut oil reduced methane production by 15% and decreased the number of protozoa (Kim et al., 2014). Chung et al. (2011), reported that methane emission decreased by 36% when flaxseed was supplemented (150 g/kg diet DM) to the cow diet. Similarly, the use of 4% linseed oil (diet DM) in cows has been reported to reduce methane release by 17% (Guyader et al., 2015). Bayat et al. (2018), reported when rapeseed oil was added (50g/kg DM) to the dairy cow diet, decrease in methane release by 22%. However according to another study, rapeseed oil did not supress methane emission (Chagas et al., 2019). Villar et al. (2020), reported when canola oil (50g/kg feed basis) is added to beef cattle diet, methane release decreased by 6.1%. Similarly, it has been reported that there is a 10.8% decrease in methane emission with the use of canola oil (0.80kg/d) in cow diets (Alvarez-Hess et al., 2019). Studies suggest, 8% (Nogueira et al., 2020), 3.77% (Patra, 2013) and 4.30% (Patra, 2014) reduction in methane emission for each percentage of lipids supplemented in the diet.

Organic Acids

Dicarboxylic acids such as fumarate and malate when used in diets, propionic acid increased and methane production decreased (Sethy et al., 2021). Because hydrogen is used for the production of propionic acid, the amount of hydrogen to be used in methane production decreases (Boadi et al., 2004).

In a study conducted in vivo and in vitro in sheep, it was reported that there was a 76% reduction in CH_4 emission with the use of encapsulated fumaric acid (Wood et al., 2009). In another study, use of encapsulated fumarate in dairy cattle had no significant effect on methane emission (McCourt et al., 2008). Foley et al. (2009), reported the use of 3.75-7.5% DM malic acid in beef cattle diet reduced methane emission by 16%. In another in vitro study, it was reported that propynoic acid and p-coumaric acid significantly reduced methane emission (64% and 37.7% respectively) compared to the control group (Chagas et al., 2019). Similarly, it was

reported that propynoic acid reduces methane production (70-99%) in vitro study (Zhenming et al., 2011).

Ionophore

Ionophores change the bacterial population from gram positive to gram negative, reducing the amount of acetate and increasing the amount of propionate. Thus, the hydrogen used by methanogens for methane production is reduced (Patra, 2012). Monensin is the most widely used ionophore antibiotic. Other antibiotics used are laidomycin, lasalocid, narasin, lysocellin, salinomycin and tetronasin (Boadi et al., 2004).

Vyas et al. (2018), reported supplementation of monensin (33mg/kg DM) to beef cattle diet reduces methane emision by 27.1%. Capelari and Powers (2018), used high (100:0 roughage:concentrate) and low (10:90 roughage:concentrate) roughage in an in vitro study. They reported that the supplementation of monensin (3-6mg/L) to these groups reduced methane release by 55% and 50%, respectively. The same authors reported methane emission was found 5% less daily, when monensin (33 mg/kg DM basis) was supplemented to the beef cattle diet (Capelari and Powers, 2018). According to a meta-analysis study, the use of 32mg/kg dry matter intake (DMI) monensin in beef cattle and 21 mg/kg of DMI monensin in dairy cows has been reported to reduce methane emissions (Appuhamy et al., 2013). However, ionophores have been banned in European Union countries and Turkey since 2006 due to residue in food and development of resistant microorganisms (Demirtaş et al., 2020).

Probiotic

According to Mamuad et al. (2019), supplementation of probiotics can mitigating methane production.

Chen et al. (2020), were use thirty-one propionic acid bacterial strains and was reported that methane release decreased up to 20% in in vitro study. Among them, P. jensenii LMGT2826, Propionibacterium thoenii LMGT2827 and T159 decrease CH_4 production are 18%, 8% and 20% respectively (Chen et al., 2020). According to Mamuad et al. (2019), increase in propionate level and a decrease in methane level were found with the use of Enterococcus faecium (0.1%) in in vitro study. However, in a previous study, it was reported that the use of inoculants such as Lactobacillus plantarum, Lactobacillus fermentum and Enterococcus faecium in grass silages had no effect on methane emission (Jalč et al., 2009). According to an in vitro study, Fusobacterium sp. reduced methane emission by 38% compared to the control group (Paul et al., 2011). Sethy et al. (2021), reported Aspergillus oryzae reduces the methane release by 50% by reducing the protozoan population. It has been reported that Saccharomyces cerevisiae reduces methane production by 10% in studies conducted in in vitro systems (Sethy et al., 2021). In a meta-analysis study observed no significant decrease in CH_4 production through the use of probiotics (Darabighane et al., 2019).

Nitrate-Sulphate

Supplementation nitrate and sulfate to the diet reduces methane production due to it reduces the amount of free hydrogen. Hydrogen is used during the reduction of nitrate to nitrite and then to ammonia (Honan et al., 2021; Black et al., 2021).

According to an in vitro study, calcium ammonium nitrate decahydrate (1.25 and 2.5 g/100g DM) reduced methane release by 54% to 71% (Capelari and Powers, 2018). The same researchers reported that the addition of calcium ammonium nitrate decahydrate (1.5% DM) to the beef cattle diet reduced methane release by 9% (Capelari and Powers, 2018). In a meta-analysis study, it was reported that nitrate reduces daily methane release by 15% (Ungerfeld, 2020). Similarly, in another meta-analysis study, nitrate was reported to reduce enteric methane emission in ruminants by up to 30% (Lee and Beauchemin, 2014). Chagas et al. (2019), reported

the use of nitrate in an in vitro study reduced methane emissions by 45%. According to van Zijderveld et al. (2010), the addition of nitrate (2.6% DM) and sulfate (2.6% DM) to lamb diets, decreased methane emissions compared to the control group (sulfate: -16%, nitrate: -32% and nitrate+sulfate: -47%). Similarly, use of nitrate, sulfate and saponin decrease methane emission (three inhibitors combined: -45.7 %, sulfate: -5.4%, nitrate: -30.5%) was reported in in vitro study (Patra and Yu, 2014). Pesta et al. (2016), reported supplementation nitrate (2.0% diet DM) and sulfate (0.54% diet DM) to beef cattle diet had no effect on methane emission. Lee et al. (2017), observed that the addition of encapsulated nitrate (1.25% and 2.5% DM) and unencapsulated nitrate (2.3% DM) instead of urea (0.94% DM) to beef cattle diet had no effect on methane release. When nitrate (85 g/d) is supplemented to beef cattle diet instead of urea (125 g/d), it has been reported 32% reduction in methane emission (Hulshof et al., 2012). Villar et al. (2020), reported when nitrate (20 g/kg feed basis) and nitrate+canola oil (20 g/kg + 50 g/kg feed basis) supplemented to beef cattle diet, methane emissions decreased by 8.7% and 25%, respectively. In a study conducted in dairy cows, it was reported that there was a 16% reduction in methane emission with nitrate (21 g of nitrate/kg of DM) supplemented to the diet (van Zijderveld et al., 2011). Similarly, it has been reported that the supplementation of 21g/kg DM nitrate to dairy cow diet reduces methane emission by 27.6% (Klop et al., 2016). Olijhoek et al. (2016), determined that 24.8% decrease in methane production in dairy cows with the supplementation of nitrate (21.1g/kg of DM). In a study, it was reported that supplementation 3% (diet DM) calcium nitrate to cow diet reduces methane release by 22% (Guyader et al., 2015). In a study, it was determined that the number of methanogenic microorganisms decreased as a result of supplementation nitrate to goat diets (Asanuma et al., 2015). However, it has been reported that the risk that nitrate can be reduced to nitrite should not be forgotten (Van Zijderveld et al., 2010).

Algae

Machado et al. (2016b), suggested algae such as Asparagopsis taxiformis suppress methane emission and archaea (metanogenic) with secondary metabolites.

Chagas et al. (2019), reported Asparagopsis taxiformis significantly reduced methane emission (0.20 mL/g DM) compared to the control group (38.7 mL/g DM). Similarly, when use of %5 organic matter (OM) A. Taxiformis in vitro, methane production was decreased by 95% (Roque et al., 2019a). In another in vitro study, A. taxiformis (1% DM) reduced methane yield by 98% (Stefenoni et al., 2021). It has been reported that with the supplementation of Asparagopsis taxiformis (0.25-0.5% OM intake basis) to the beef steers diet, daily methane emissions decreased between 36.46% and 81.9% (Roque et al., 2021). In a study conducted in beef cattle, it was reported that the supplementation of 3.26g/kg DM A. Taxiformis reduced methane emission up to 98% (Kinley et al., 2020). In an in vitro study, it was reported that dichloromethane (in the biomass of Asparagopsis) reduced methane production by 79% (Machado et al., 2016a). Li et al. (2018), found that methane emission was reduced by up to 80% by supplementing 5 different concentrations of Asparagopsis taxiformis (0-3% OM basis) to sheep diets for 72 days. In addition, as a result of the study, a decrease in the amount of acetate and an increase in the amount of propionate were reported.

When A. Armata (0.5% and 1% OM basis) was supplemented to the dairy cow diet, it was reported that there was a 26.4% and 67.2% reduction in methane release, respectively (Roque et al., 2019b). Similarly, 1% Asparagopsis armata was supplemented to the diet, and it was reported that there was a decrease in methane emission in animals (Searby, 2019). Stefenoni et al. (2021), reported 65% reduction in methane emission with the use of in vivo A. Taxiformis (0.50% DM). In a study, it was reported that there was an 80% reduction in methane emission with the supplementation of A. Taxiformis (0.5% DM) to the diet in dairy cows (Stefenoni et al., 2019). In an in vitro study, 10 different doses of Asparagopsis taxiformis

(ranging from 0 to 16.7% of OM) were used. Researchers reported methane emission was reduced by 84.7% when 1% was used, and more than 99% at 2% and higher doses (Machado et al., 2016b). Kinley et al. (2016), reported Asparagopsis taxiformis (1% OM basis) had little effect on methane emissions in vitro. In the same study was reported use of 2% is the minimum dose that prevents methane production, but when used at 5% and 10%, it significantly suppresses methane production. In an in vitro study, it was reported that A. taxiformis with varying amounts of bromoform (1-4.39 mg/g DM) reduced methane release by 100% in all groups (Vucko et al., 2017).

Plant Secondary Metabolites and Essential Oils

Tannins reduce methane release by suppressing ruminal methanogens and protozoa (Bhatta et al., 2009; Jayanegara et al., 2015). As a result of studies on this subject, it has been confirmed that condensed tannins suppress methanogenic archaea (Fagundes et al., 2021). In addition, condensed tannins have been reported to suppress methane production by increasing propionate synthesis and reducing the amount of metabolic hydrogen needed by archaea (Valencia Salazar et al., 2018).

In an in vitro study, it was found that the use of purified hydrolyzed and condensed tannins (0.5, 0.75 and 1.0 mg/ml) decreased methane concentration, methanogen population and protozoa number in all groups (Jayanegara et al., 2015). In a study, it was reported that when dried Leucaena leucocephala leaves containing tannin were supplemented to the diet (12%, 24%, 36% of DM intake) methane emission was reduced by 6.2%, 11%, and 19.6%, respectively (Montoya-Flores et al., 2020). Similarly, dried Leucaena leaf (6kg/head/day) containing condensed tannin (CT) (36g/kg DM) reduced methane emission by 19.5% (Phesatcha and Wanapat, 2017). As a result of using alfalfa hay, dried, pelleted grape marc (contains condensed tannins) and ensiled grape marc as roughage source in dairy cows, methane emission was determined as 470, 375 and 389 g/day, respectively. It has been reported that the decrease in methane emission is due to the decrease in the number of ruminal bacteria and archaea (Moate et al., 2014). In a study, methane emission was found to be 14% less daily when ensiled crimped grape marc (300 g/kg of containing 31.2 g/kg of condensed tannins in diet DM) was supplemented to beef cattle diet (Caetano et al., 2018). Chen et al. (2021), reported when tannin acid is supplemented to the diet containing silage, there is a decrease in ruminal methane emission. In an in vitro study, condensed tannin-rich ten legumes were analyzed. The CT content of these plants varies between 72g/kg and 203g/kg DM. As a result of the study, L. Leucocephala plant decreased methane emission (18.27 mL/g). Other plants caused methane emission similar to alfalfa (30 to 40 mL/g) (Fagundes et al., 2021). In the in vitro study, plants containing phyto-chemicals (saponin, flavanoid, tannin and triterpenes) such as S. saman, A. lebbeck, A. indica, T. diversifolia, C. alba, L. leucocephala, P. dulce and M. Oleifera were analyzed. Methane production was found to be 93.3%, 91.3%, 86.4%, 85.7%, 82%, 74.8%, 69.5% and 61% lower, respectively, compared to the control group (Galindo et al., 2014). In a study, it was reported that with the addition of Pods of Samanea saman (containing tannin) to the diet (from 0 to 30%), methane production decreased up to 50.9% per day at the highest dose (Valencia Salazar et al., 2018). The same authors reported an increase in rumen propionic acid level and a decrease in acetic:propionic acid ratio (Valencia Salazar et al., 2018). Similarly, when pods of S. saman (60g/kg DM) added to dairy cow diet, methane release decreased an average 8.75% (Anantasook et al., 2014). In an in vitro study, it was reported that the use of 50-100 g/kg condensed tannin-rich acacia and quebracho and hydrolyzed tannin-rich chestnut and valonea reduced methane production by 40% (Hassanat and Benchaar, 2013). Akçil and Denek (2013), reported the use of eucalyptus leaves (0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5%) in vitro, decreased methane production at all doses and 46% decrease in the using 2.5%.

Saponins are glycosides found naturally in various plant species and reduce CH_4 production in the rumen (Patra and Saxena, 2009).

It is stated that saponins have antiprotozoal effects (Goel and Makkar, 2012), reduce the amount of ruminal H_2 and suppress methane-producing microorganisms at high doses (Bodas et al., 2012).

In a study conducted in sheep, it was stated that daily addition of 3 g saponin to the diet reduced ruminal methane emission (Zhou et al., 2011). In one study, garlic oil (0.25 g/L), nitrate (5 mM), and saponin (0.6 g/L) were used alone or in combinations. As a result of the study, it was determined that there was a decrease in methane emission in all groups except the groups that only used saponin. The triple combination of garlic oil, nitrate and saponin has been reported to reduce methane production by 65% on day 2 and 40% on day 18 (Patra and Yu, 2015). In a study, it was reported that tea saponins reduced methanogenesis by 8% and the protozoal population by 50% (Guo et al., 2008). However, some studies have reported that saponins have no effect on methane release (Yuan et al., 2007) and protozoa population (Ramírez-Restrepo et al., 2016; Valencia Salazar et al., 2018).

In a study, it was reported that adding pods of E. cyclocarpum (0.15, 0.30, and 0.45 kg of DM) to the sheep diet as a saponin source reduced methane emissions by 36% (Albores-Moreno et al., 2017). Mao et al. (2010), added 3 g saponin per day to sheep diets and reported that methane production in the rumen decreased by 27%.

In a study conducted with the in vitro gas technique, it was reported that the use of peppermint oil (0.33, 1.0 and 2.0 µl/ml) reduced methane release by 19.9%, 46.0% and 75.6%, respectively (Agarwal et al., 2009). According to Carvalho (2018), Eucalyptus citriodora essential oil 500mg/kg of DM, Brazilian peppertree essential oil 500mg/kg of DM and Lemongrass essential oil 500mg/kg of DM supplemented to cow diet, had no effect on methane release in three groups. In an in vitro study, the use of 300 mg/lt thyme oil, peppermint oil, orange oil, clove oil and cinnamon oil significantly reduced methane release (18.6, 17.1, 20.4, 18.9 and 21.1 mmol/L respectively) compared to the control group (28.7 mmol/L) (Canbolat, 2012). In a study conducted in sheep, it was stated that the addition of 5 g/kg DM garlic oil or 2 g/kg DM dially disulfide did not affect methane release (Klevenhusen et al., 2011). In the other study, it was reported that the use of encapsulated garlic oil at different doses reduced methane production between 56% and 80% and increased the acetate/propionate ratio (Blanch et al., 2016). In a study, it was determined that the use of essential oils obtained from thyme and green tea in lactating cows reduces methane gas emissions (Kolling et al., 2018). However, in another study, no effect of thyme essential oil on methane emission was observed (Olijhoek et al., 2019).

Chitosan

It has been reported that chitosan has antimicrobial effects against bacteria, fungi, yeasts and viruses (Wencelová et al., 2014; Divya et al., 2017). Chitin and chitosan can be used to reduce methane, because of they are able to antimicrobial effect (Haryati et al., 2019).

Belanche et al. (2016), reported chitosan reduced methane production 42%, increased propionate production, decreased cellulolytic bacteria and hemicellulolytic bacteria. Supplementation of 100mg/L chitisan in vitro reduced methane emission by 21% in the trial with low roughage content, but it was reported that there was no significant effect in the study using high roughage (Wencelová et al., 2014). In an in vitro study, it was reported that the use of 1% and 2% chitosan did not have an effect on methane release (Haryati et al., 2019) However other in vitro study, it was stated that the use of 750mg/L chitosan reduced methane release by 10-30% (Goiri et al., 2009).

Methane Inhibitors

Methane inhibitors reduce methane release by suppressing the rumen archaeas.

Zhenming et al. (2011), reported, ethyl-2-butynote and 2-nitroethanol reduced methane production by 23% and 70-90%, respectively in vitro study. In an in vitro study, it was reported that 2-nitroethanol and bromoform (1.30 and 2.00 mL/g DM, respectively) reduced methane release significantly compared to the control group (38.7 mL/g DM) (Chagas et al., 2019). In a study, it was reported that supplementation of chloroform–cyclodextrin (1.6-2.6 g/100 kg liveweight) to the steer diet reduces the methane emission by 30-35% (Martinez-Fernandez et al., 2016). In a another study, reported daily supplementation of bromochloromethane (0.30 g/100 kg of body weight) to goats reduced methane production by 33% (Abecia et al., 2012). In a meta-analysis study, it was stated that methanogenic archaea population and methane release were decreased with the addition of 3-nitrooxypropanol (3-NOP) in ruminants. In addition, use of 3-NOP at 100 mg/kg DMI has been reported to reduce methane emission by 19.2% (Jayanegara et al., 2018). Similarly, according to another meta-analysis study, CH_4 emissions in both beef and dairy cattle significantly decreased with increasing 3-NOP supplementation (Kim et al., 2020).

Martinez-Fernandez et al. (2014), reported the use of ethyl-3-nitrooxy-propionate and 3-nitro-oxypropanol, reduced methane emissions by up to 95%in vitro. Also decreased 29% in vivo study. Hristov et al. (2015), reported supplementation of 3-NOP (40-80mg/kg DM) to lactating cows reduced enteric methane emission by 30%. It was determined that methane release was decreased by 17.5% and 10.5%, respectively, in cattle with 3-NOP (100mg/kg DM) added to high forage and high grain diets (Kim et al., 2019). In a study, it was reported that with the supplementation of low (100 mg/kg DM) and high (200 mg/kg DM) doses of 3-nitrooxypropanol to beef cattle at the backgrounding phase, 16.6% and 37.6% decrease in methane emission, respectively. With the addition of low (100 mg/kg DM) and high (200 mg/kg DM) doses of 3-nitrooxypropanol at the finishing phase of the same study, it was reported that methane emissions decreased by 12.5% and 84.3%, respectively (Vyas et al., 2016).

Vyas et al. (2018), reported, 3-nitrooxypropanol supplementation in beef cattle diet in both backgrounding phase (200mg/kg DM) and finishing phase (125mg/kg DM) decreased CH_4 production by 53-54%. In a study conducted in dairy cows, adding 2,500 mg/d of 3-nitro-oxypropanol to the diet reduced methane release by 60% (Reynolds et al., 2013). In a study, it was reported that there was a 38% decrease in CH_4 production in 0.30 g/kg DM 3-NOP supplemented diet compared to the control group in calves (Martinez-Fernandez et al., 2018). Similarly, Lopes et al. (2016), reported 3-NOP (60mg/kg DM) supplementation to lactating cows reduced methane release by 31%. A meta-analysis study reported that the use of 123mg/kg DM 3-NOP reduced enteric methane release in dairy and beef cattle by 39% and 22.2%, respectively (Dijkstra et al., 2018).

Biochar

Biochar is produced by burning organic matter (typically plant material) in the absence of oxygen (Hansen et al., 2012). Some researchers suggested biochar as a feed additive with the potential to support rumen fermentation and reduce enteric CH_4 release (Leng et al., 2012).

Biochar reduced methane emissions between 11% and 17% in the in vitro system (Hansen et al., 2012). Similarly, biochar (0.5% DM) in the artificial rumen system reduced methane release by 25% (Saleem et al., 2018). Leng et al. (2012), reported rice husk biochar decreased CH_4 production by 22% and reduced 41% used with nitrate. It has been reported that the addition of biochar (0%, 0.8%, or 3% of DM) to beef cattle diet reduces methane emissions between 9.5% and 18.4% (Winders et al., 2019). However, some researchers reported it has no effect on methane release (Terry et al., 2019).

CONCLUSIONS

It is necessary to reduce enteric methane emission because it causes global warming. Especially, algae such as Asparagopsis taxiformis, plants containing various phyto-chemicals, and methane inhibitors have been reported to reduce methane emission up to 95%. Therefore, it is necessary for researchers to conduct more studies on these products. Increasing the usability of products that are economical and not harmful to animal health is important for our planet and our future. If this cannot be achieved, future generations will have to live on an unhealthy planet due to the increasing greenhouse gas effect.

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EFFECT OF CHROMIUM ON PARENTAL STRAIN ALTERNARIA ALTERNATA AND ITS THREE COLOR MUTANTS STRAINS

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ABSTRACT

In this study, minimal inhibitory concentrations (MICs) of sodium chromate were determined for wild type (w.t) AA_1 strain of *Alternaria alternata* with its three derivatives strains are SW_1, SW_2 and SW_3 which have color mutation with lacking melanin genes. MIC of AA1 was reached to 10 mM that less than MICs of other color mutants strains. However, SW_1, SW_2 their MICs were reached to 20 mM unlike MIC of SW_3 strain that was 14 mM. Stability of growth also was occurred on certain different concentrations of chromate for each strain. Reason of variation in chromate MICs for these strains is still unknown. Probably, because of loss melanin genes that bind or control other physiological properties that related on trace element resistance. Furthermore, 14 resistant strains were distributed between UV induced and spontaneous mutations of chromate were isolated from AA_1 and SW_2. Reverse mutation also was tested, 10 reverse mutation are reverse whereas 4 strain are forward mutants. Morphological changes also were recorded in these mutants.

Keywords: *Alternaria alternate*, Color mutations, Chromate, Resistant mutants.

INTRODUCTION

Alternaria alternata is an opportunistic plant pathogen that tops of the list plant pathogens which affect various species of plants and causing different economic damages (Hadi, 2019; Rathod, 2012); therefore controlling process is difficult except using fungicides that are a more widespread and cheaper means. However, adding some heavy metals to these chemicals may make their effectiveness more efficiency (Shoaib et al., 2015).

On the other hand, the release of these heavy metals to the environment, whether from these fungicides or various other industries, is a frightening matter; therefore many articles were looking for organisms those capable of removing these pollutant metals either by resistance or tolerance of their high concentrations (Ezzouhri et al., 2009).

Therefore, it became necessary to measure the pathogen tolerance levels for these heavy metals, so the *A. alternata* fungus was used in the current study which have the black pigment known as melanin (Shoaib et al., 2015). It is known by high resistance and protection properties, especially when it is exposure to external chemical agents (Fernandes et al., 2016).

Consequently, the current research aims to measure the tolerance and resistance of the wild strain *A. alternata* fungus (have black-colored conidia) and their derivatives color mutants SW_1, SW_2 and SW_3 strains (lacking melanin) of the chromium element. As well as the genetic effect of chromium with or without of the ultraviolet radiation in its laboratory form, with studying

the effect of presence or lack melanin on the tolerance these strains to the heavy metal, which is chromium.

Material and methods

Test organism: The study was carried out on w.t strain of *A. alternata* (named AA_1) with black color conidia. With three color mutant strains are SW1, SW2 and SW3 which it's colonies color were white to pale pink their source is from (Hadi and Dhahi, 2012) article.

1. Media and chemicals

A. Minimal Medium (MM): attended this medium as (Caten, 1979).

B. Potato Dextrose Agar (PDA) and Potato Sucrose Agar (PSA) media: They were used for obtaining intensive and sustaining growth (Pitt and Hocking, 2009) . Whereas PSA medium in which, the dextrose replaced by equivalent sucrose (Hadi and Dhahi, 2012).

C. Sodium deoxycholate solution (D): a final concentration 400mg/ml of this solution was used to obtain single colonies which is suitable for this fungus as in (Hadi and Dhahi, 2012).

D. Sodium chromate stock solution: The stock solution was prepared from the Sodium chromate powder with a concentration of 1 Molar from the Department of Chemistry / College of Science / University of Mosul, which was supplied from Sigma-Aldrich Company.

2. Preparation of the Spore Suspension

It was prepared by growing the test strain for 5-7 days on the PDA media, then the conidia were washed by sterile lope under sterile conditions; dilutions (10^{-6}-10^{-7}) were prepared that were give an appropriate conidia that can be counted in control plates.

3. Culture and Incubation Conditions

The strains under study were cultured and incubated in the aforementioned media under sterile conditions and at 28 ± 2° C temperature.

4. Minimal inhibitory concentrations (MICs) Measurement

MICs of sodium chromate was measured by adding ascending concentrations to MM media for reaching concentrations at which a 100% inhibition rate occurred and these concentrations were considered the MICs.

5. Isolation of Chromate Resistant Mutants

The induced resistant mutants to chromate were isolated by exposing the conidia to UV rays with lethal intensity according to (Hadi and Dhahi, 2012), and then it was cultured on MM media containing MIC concentrations according to each strain, and at the same time, control media were cultured for finding the living counts. Spontaneous resistant mutants were obtained by culturing the conidia on media containing MIC of chromate without exposing it to any mutagen and leaving it until the well-developing chromate-resistant colonies.

6. Reverse Mutants Test

Chromate resistant mutants were cultured on the MM media plus MIC of chromate at the same time they were inoculated on the control media. To track the mutant's growth and their

resistance to chromium, we compare the growth of the resistant mutants on the media with or without MIC of chromate. The inoculation process was carried out by making a master plate of resistant mutants and inoculating the aforementioned media using a pin replica method.

Results and discussion

1. MICs of Chromate

It is noticed from (Table 1) that the MIC of the SW_1 strain is 20 mM, which is the same MIC for the SW_2 strain. The inhibition percentage 40% for SW_1 strain was stable at different concentrations of chromate were the 2, 4, 6 mM. This is what happened with the inhibition percentage of the SW_2 strain, which was stable for two times, once at the 6,8,10 Mm concentrations at 66.6% inhibition percentage and at the 14, 16 mM concentrations, when the inhibition percentage reached 83.3% (Table 2).

Although both SW_1 and SW_2 strains have the same final MICs 20mM but inhibition percentage was constant at different concentrations (Tables 1, 2). The MICs stability of the SW_1 strain occurred at low concentrations 2, 4 and 6 mM, while the MIC of SW_2 strain was stable at higher concentrations and reached at the first time to the 10 mM then to 16mM. This indicates that the genes or gene which responsible of chromate resistance or tolerance in these two lacking melanin strains (that carry the color mutation) may be found in two different locations. The 50% inhibition percentage of two above-mentioned strains on the at 4-8 mM was higher than the inhibitory effect of chromium at 47%, that reached to 1 mM on wild type (w.t) strain of the same fungus in another study (Ezzouhri et al.,2009).

Table 1: MIC of chromate on SW_1 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
SW_1	0	1	0
	2	0.6	40
	4	0.6	40
	6	0.6	40
	8	0.5	50
	10	0.4	60
	14	0.3	70
	20	0.0	100

Table 2: MIC of chromate on SW_2 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
SW_2	0	1.2	0
	2	0.7	41.6
	4	0.6	50
	6	0.4	66.6
	8	0.4	66.6
	10	0.4	66.6
	14	0.2	83.3
	16	0.2	83.3
	20	0.0	100

The chromate MIC of SW_3 strain was 14 mM (Table 3), which is lower than the MIC of the previous two strains SW_1 and SW_2 by the 6 mM. This strain did not show a stability in the inhibition percentage as happened with the SW_1 and SW_2 strains because the inhibition percentage continued to increase exponentially until it reached 100% at a 14 mM concentration. This also indicates genetic variations in the chromium resistance among the three strains that lacking melanin genes.

Table 3: MIC of chromate on SW_3 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
SW_3	0	1.3	0
	2	0.5	61.5
	4	0.4	69.2
	6	0..3	76.9
	8	0.2	84.6
	10	0.1	92.3
	14	0.0	100

The inhibition percentage of AA_1 (w.t strain) was 100% at 10 mM concentration, which in all cases is less than reached MICs by other studies such as (Zafar et al.,2007), but it is similar to another study that found 50% inhibition percentage was occurred at 1 mM of chromate (Ezzouhri et al.,2009).

The MIC of the w.t strain less than the MICs of three white strains, this means chromium resistance isn't related with melanin presence or lacking in this fungus (Tables 1, 2, 3 and 4). All of the melanin lacking strains has higher MICs that reached to 20 mM in the SW_1 and SW_2 strains, whereas 14 mM in SW_3 strain when they compared with MIC of AA_1 strain that reached to 10 mM.

Table 4: MIC of chromate on AA_1 strain

strain	Sod. Chromate conc. mM	Average of colony diameter cm	Inhibition precentage
AA_1	0	1.5	0
	2	0.5	66.6
	4	0.2	86.6
	8	0.2	86.6
	10	0.0	100

The variation of chromium resistance between MICs of the lacking melanin strains (have a color mutation) and w.t strains can only be explained by one reason, which is the genes or gene controlling the melanin pathway are able to impair or change other functions (to the better or worse) such as resistance to heavy metals (Fetzner et al., 2014), which is actually happened in our current study.

However, the targeting of the responsible genes for melanin not only affects the physical characteristics, but the morphological characteristics are also affected in the fungus *Sclerotinia*

sclerotium (Liang et al.,2018) in addition to the increased production of exonzymes such as cellulase, protease, amylase and pectinase to compensate the deficiency of melanin mutants in *Bipolaris sorkiniana* (Chand et al.,2014).

We conclude from the foregoing that it is possible to take advantage of the chromate resistance characteristic of the three strains with color mutation SW_1, SW_2 and SW_3 which were reached to 20 mM (Table 1 and 2) and 14 mM (Table 3). To benefit from them it in ridding the environment from heavy metals more than using the same wild strain. However,this happened when isolating a wild strain of *Aspergillus flavus*, which has a high chromat-tolerance (Kumar and Dwivedi, 2019). Anywhere, it is less tolerant than the strains with the color mutation in *A. alternata* in our current study. The mutants may be similar to the parental strain in terms of pathogenicity but they are different in other traits, as X-ray resistant strains of *Alternaria sp.* (Babalola, 2009).

In the current study, the chromates MICs were not measured at different environmental conditions, as in (Kumar and Dwivedi, 2019).Furthermore, despite the stability of the environmental conditions for conducting the experiments, the white mutants (lacking melanin) strains were showed high resistance to chromium, this may belong to the included excess of heavy metal in their subcellular parts (Cornejo et al., 2013), these subcellular parts are melanin-free. This confirms the possibility of using these color mutants to withdraw chromium pollution from the environment, which is researchers are trying to obtain it as well as mention in (Chen et al., 2019). The current study also enhances another aspect which is the possibility of using chromium by adding it to some natural or industrial pesticides as a fatal component for controlling the wild strain *A. alternata* fungus due to its sensitivity to it (the present study) this need extensive prospective studies.

2. Isolation of Chromate Resistant Mutants

The total number of chromate resistant mutants from the two strains w.t and SW_2 was 14 (Table 5), which were distributed between 8 resistant mutants from w.t black isolates of *A. alternata* and six resistant mutants from the SW_2 strain, while no resistant mutants were obtained from the remaining strains such as SW1 and SW3.

Table 5: resistant mutants of chromate with reverse and forward mutants count

strain	Treatment type	Mutants count	Reverse mutants	Forward remaining mutantss
W.t	0	2	1	1
	U.V	6	4	2
SW_2	0	0	-	-
	U.V	6	5	1

Indeed, many experiments failed, which we didn't mention in Table 5, because no chromate-resistant mutant was obtained, which were 10 experiments distributed between UV or spontaneous treatment (0) which were performed on all strains .The reason was not known, this may be due to the fungal spores influencing by the chemicals toxicity (Bhajbhuje, 2014) that coming from the chromate with the killing intensity of UV rays with the (Hadi and Dhahi, 2012). Thus, spores of treated strains did not germinate therefore; I sometimes reduced the MIC to obtain more mutants. Two spontaneous mutants (Table 5) were also obtained from w.t strain.

The continuous growth of resistant mutants on chromate media with lethal concentration for preserving mutants, as well as control media without adding chromate in order to study them intensively. We noticed there are mutants that could not grow and resist on the chromate medium, but rather became growing on the control media only. Thus these mutants were considered are reverse or recurrent mutations, and this may be due to one of the following reasons: either because of the duplications or their changes in specific locations of the chromatids and along the chromosome, leading to an opposite direction of the mutation or due to certain replications of the DNA (Holliday, 1964).

Or, the reversal of the mutation may occur at a finer level at the gene level, by substituting a specific gene as an alternative for the defective gene or within the gene itself by replacing the healthy allele for the damaged allele. In both cases shows phenotype to the examiner, it is in the fact not the phenotype because its genotype remains mutations containing that are not visible in its phenotype (Clark. and Pazdernik 2013).

However, it may be due to the phenomenon of multinucleate conidium in in this fungus, which is the reason that I see more likely because of the conidium contains more than one nucleus (Knox-Davies, 1979). So that the mutation effect of one nucleus that present in the conidium is covered by effect of other perfect nuclei that are not mutated and thus the wild phenotype returns to the mutant strain.

3. Morphological Changes of Resistant Mutants

There are morphological changes that occurred to chromate-resistant mutants (fig 1), which corresponds many studies in it the fungi changed morphologically when exposed to certain chemical pressures (Fernandes et al., 2016; Kumar and Dwivedi, 2019). They were characterized by being very dense, coherent and not widespread even if the color of the mycelium has become lemon colored (whether the resistant mutants are isolated from the w.t black strain or the mutant lacking melanin (Table 5), possibly due to the taking of morphological changes of the resistant strains from as a defense means to preserve their cells.

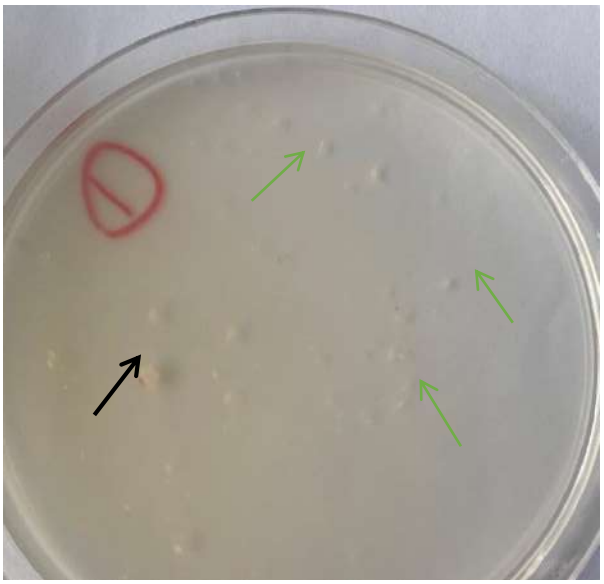

Figure 1: morphological changes of chromate resistant mutants. Orange arrows indicate poor resistant that ignored while black arrow refer to strong resistant that was completed tests on it.

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TRANSFER LEARNING BASED DEEP NETWORKS FOR THE COVID-19 DIAGNOSIS

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ABSTRACT

This paper presents a transfer learning-based approach for the detection of COVID-19 and distinguishing it from normal and pneumonia cases in X-rays radiographs. The study involves the implementation of 3 different deep networks that use SVM classifier to classify x-ray images into COVID-19, Normal, and Viral Pneumonia. Experimentally, the deep networks were all trained and tested on x-rays dataset and the results showed that a VGG16 with SVM classifier can outperform other employed models in detection the COVID-19.

Keywords: COVID-19, pneumonia, deep networks, VGG16, SVM.

INTRODUCTION

In 2019, Wuhan, China, Coronavirus (COVID-19) has been detected, and then it was declared as a dangerous health issue worldwide. Short time later, COVID-19 was declared as pandemic by the world health organization (WHO), in March 2020. Unfortunately, the spread of COVID-19 is exponentially, and the transmission process is not clearly understood. Today, dealing with Coronavirus is an important healthcare challenge for humanity all over the world. Even though the Coronavirus infection in common incorporates small or no side effects, it causes lethal pneumonia in 2:8% of the infected patients [1,2,3]. Normally it takes 5:6 days from the disease day with the infection for indications to appear. However, it may take up to 14 days in some cases. As shown in the figure 1, without rapid testing, the number of COVID-19 cases could increase beyond the healthcare system's capacity to deal with serious cases (red curve in relation to healthcare system capacity line). Fast and accurate diagnosis reduces the daily rate of new cases.

Since its spread, COVID-19 has been detected using the PCR technique. However, this technique showed some low sensitivity and effectiveness according to some reports [4,5]. Thus, it is essential to use other techniques to diagnose such virus. X-ray radiographs have been seen as effective tools to diagnose COVID-19 as it has direct effects on the lungs. Hence, analyzing X-rays of patients may lead to detection of COVID-19 and other diseases such as pneumonia. Hence, in this paper we develop transfer learning based deep networks for the detection of COVID-19 and distinguishing it from normal and viral pneumonia cases, in x-rays images obtained from a public dataset [6].

Traditional deep networks reduce partial information when extracting features, and this affects their capacities for detection. The residual neural-network structure of the low complexity dilated bottleneck adopted in this paper avoids the computational lag associated with network depth and the problems involved in the large numbers of parameters associated with network width and integrates into the FPN [7] network. Extracting deep-feature semantic information from an image, thereby avoiding loss of information.

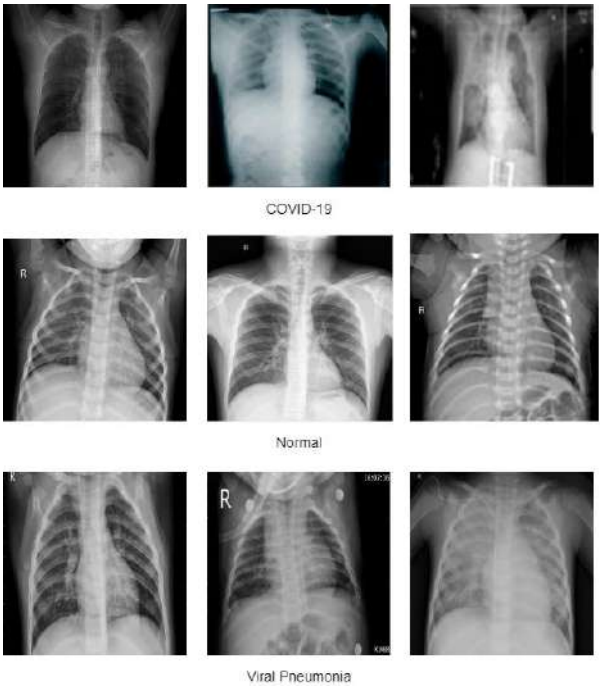


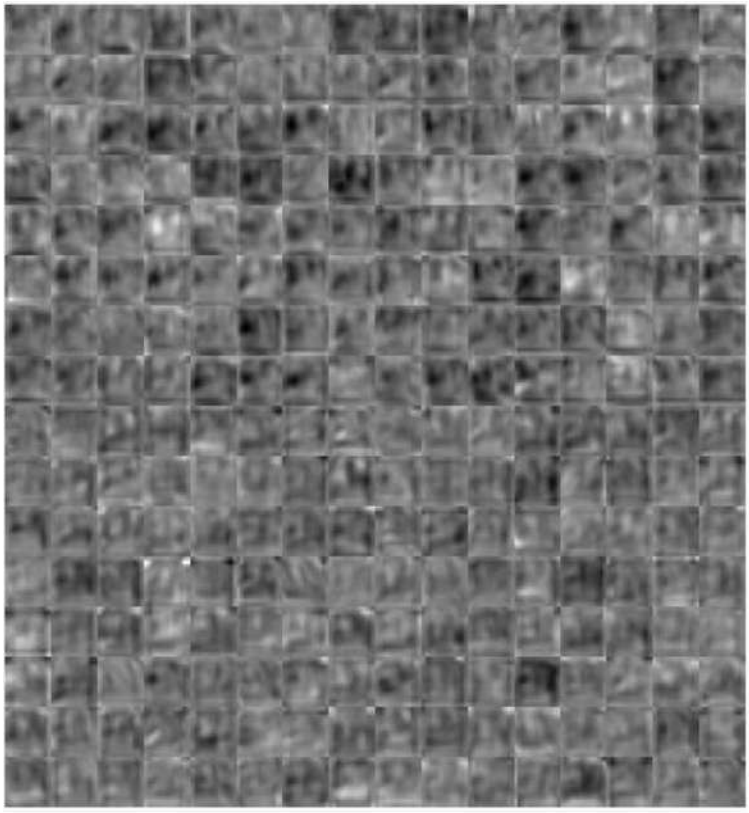
Figure 1: Sample of the dataset X-rays

Discussion

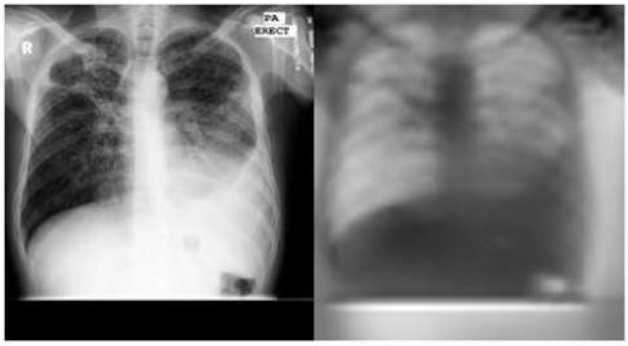
2.1 Original models performance discussion

Upon training, both AlexNet and VGG-16 pre-trained models are examined on the same amount of data, i.e., 40% of the readily available data. Table 2 demonstrates the performances of each of the model during training. As shown, AlexNet achieved 93.5% accuracy, while VGG-16 was capable of achieving a higher recognition rate of 95.6%. Also, AlexNet required 150 epochs to attain such accuracy, which is lower than that required for VGG-16 to achieve its higher accuracy. Moreover, it is noted that VGG-16 achieved a lower mean square error (MSE) (0.035) than that achieved by AlexNet (0.097); nonetheless, this needed lengthier training time. Figures 3 and 4 show the learning curves of the models. Those statistics show the error variations with respect to the Epochs mounting during training of both networks, AlexNet and VGG-16, correspondingly. It can be seen that all the networks are trained well; though, the increase of depth in VGG-16 results it into more training time, i.e., it required longer time and more epochs to reach the minimum square error (MSE) and converge. Furthermore, it is important to denote that this difference in time and epochs number of VGG-16 ended up with a lower MSE than that reached by AlexNet.

For more understanding of the networks learning performance and to have understanding into the different level's features learned by the deployed network models, we sought to visualize the learned kernels or features of the convolutional layers.



(a) Learned Features at Convolutional layer 5



(b) Features learned in specific channels

Figure 5: Learned features of AlexNet

Figure 5 shows the learned features of AlexNet, fine-tuned to classify chest X-rays into three different chest related conditions. From Figure 5(a), it is shown that various levels of features are deducted at the convolutional layer 5. Figure 5(b) shows an example of one chest X-ray that was investigated to visualize the activations of AlexNet in specific channels. Each square of the image on the right is an activation which is an output of a channel in the convolutional layer 1. Positive activations in this image are represented by white pixels, while black pixels depict negative or poor activations. Note that a mostly gray channel does not activate as strongly on the input image.

Figure 6 shows the visualizations of extracted features of one chest X-rays by the VGG-16 pre-trained model. It is shown that different levels of abstractions are deducted during each layer which helps the network in learning the exact and appropriate features that discriminate the 12 different classes of chest X-rays.

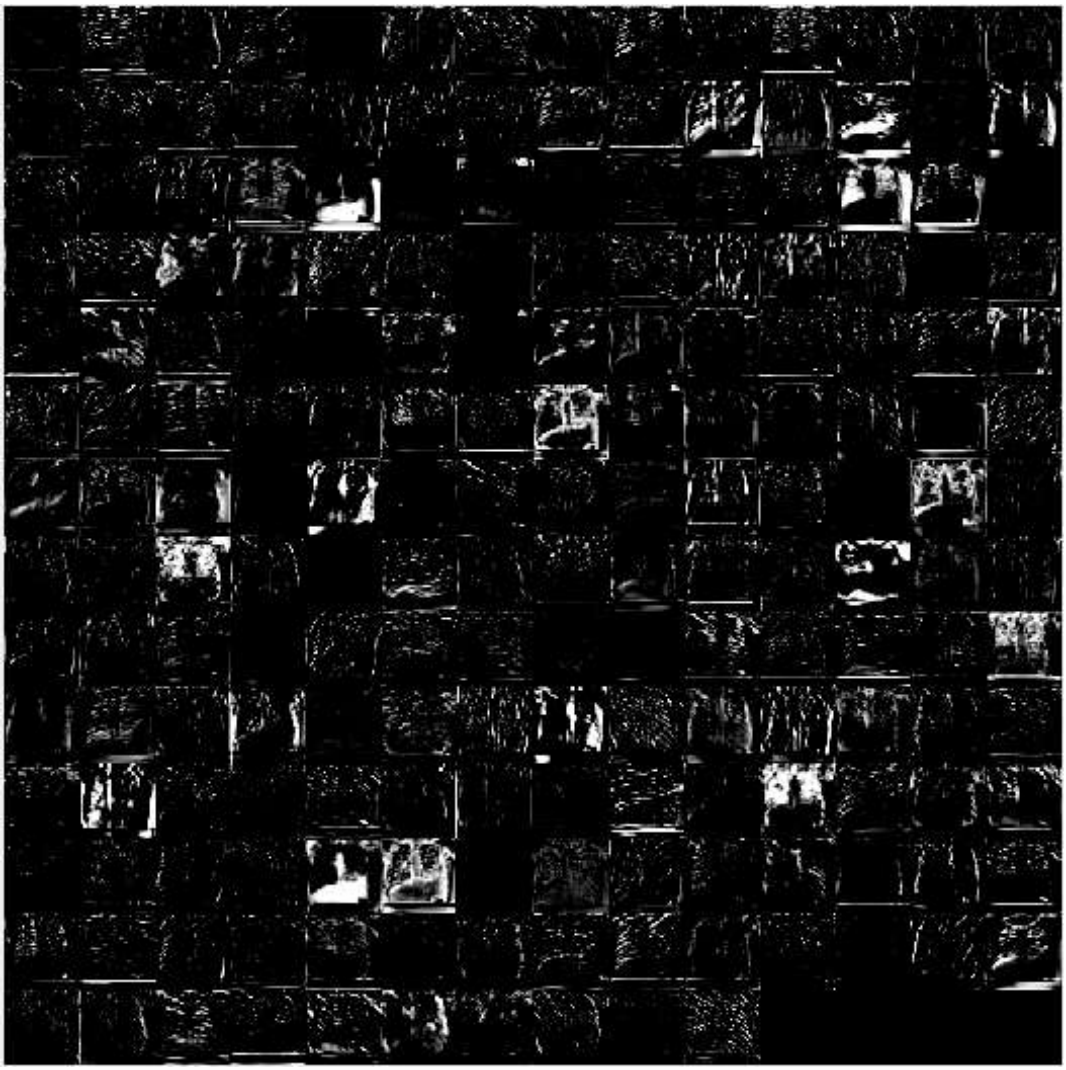


Figure 6: Learned features of VGG16

Table 2 shows the accuracies achieved by the employed pre-trained models for the classification of chest X-rays into COVID-19, normal, and viral pneumonia. Note that the networks are tested in 40% of the data. Moreover, the accuracy is calculated as in Equation 1, explained in the previous section. The table shows that VGG-16 achieved higher chest X-ray's classification accuracy during testing, than that obtained by AlexNet. It is important to denote that the VGG-16 was predicted to perform better than AlexNet owing to the variance in depth of both the networks, which pave the way for VGG-16 to deduct more useful features than the AlexNet, and consequently this results in a superior performance. In addition, VGG-16 has achieved a lower MSE compared to that of AlexNet; but this was not achieved without cost, it required a lengthier training time and more iterations. Furthermore, from Figure 5, it is seen that AlexNet learned some unrelated background features and information, in contrast to VGG-16. Note that this usually has negative effects on the final prediction [8-11].

Table 2: Original networks performance

	AlexNet	VGG-16
Training accuracy	93.5%	95.6%
Testing accuracy	89%,	93.2%
MSE	0.097	0.035

4.2 Modified models performance discussion

Table 3 shows the comparison of AlexNet-SVM and VGG16-SVM performances in relation to accuracy and mean square error (MSE). It is shown that VGG16-SVM outperformed the AlexNet as it achieved a higher training and testing accuracy of 91.34% and 94.13% respectively. As these two networks use the same classifier, it seems that this outperformance is due to the deeper architecture of the VGG16 network and the smaller kernel size that it uses compared to that of AlexNet. The small kernel sizes results in multiple non-linear layers and this consequently increases the depth of network, which gives it the power of extracting more complex features.

a. Comparison between modified and original models' performance

In this work, the fusion of pre-trained models (AlexNet and VGG-16) and SVM classifier was used for comparison. The combination of AlexNet and VGG-16 with SVM is more than sufficient, where AlexNet and VGG-16 perform the high-level feature extraction while SVM carried out the classification. From table 4, it is seen that adding SVM contributed to boosting the performance of the AlexNet and VGG-16. This slight boost was not only terms of accuracy; however, SVM allows networks to converge in shorter time while reaching smaller errors.

4.4 Models' comparison with earlier works

An assessment of the developed network models employed in this work with some previous works is shown in Table 5. Firstly, it's seen that the employed pre-trained CNNs with SVM attained higher accuracies during testing, compared to other deep neural networks, which might be due to their powerful efficiency in deducting the important features from input images. The convolutional neural networks (CNNs) employed within this work achieved higher accuracies than other earlier work that used a multi-layer neural network with two hidden layers and synthetic features, to distinguish the COVID-19 versus non COVID-19 images [12]. Furthermore, it is important to note that our employed networks, gained a better generalization in diagnosing capability COVID-19, compared to other deep convolutional networks such as Xception and inception [13].

Note that only works which deliver overtly achieved accuracies and number of data used for train and test are considered for comparison. Our results can show that applying transfer learning deep CNNs combined with SVM can solve the problem of COVID-19 diagnosis.

Table 3. Modified networks performance

	AlexNet-SVM	VGG16-SVM
Training accuracy	94.13%	96.27%
Testing accuracy	91.34%	94.13
MSE	0.076	0.017

Table 4. Modified and original models' performance

	AlexNet	VGG-16	AlexNet-SVM	VGG16-SVM
Training accuracy	93.5%	95.6%	94.13%	96.27%
Testing accuracy	89%,	93.2%	91.34%	94.13
MSE	0.097	0.035	0.076	0.017

Table 5. Network's performance comparison with earlier works

Parameters	AlexNet	VGG16	AlexNet-SVM	VGG16-SVM	MLNN [14]	Xception [15]	Inception [15]
Testing Accuracy	89%	93.2%	91.34%	94.13	94%	90.71%	89.38%

Conclusion

In this study, transfer learning based neural networks were deployed. AlexNet and VGG-16 are both used. Their features learned on a source task are transferred to a new task, chest X-rays dataset, in order to learn the diagnosis of COVID-19 by classifying the chest X-rays into three different conditions. Also concluded that VGG-16 is a well-designed and deeper architecture of sufficient complexity, was capable of achieving significantly higher classification accuracy when distinguishing between three different chest x-ray classes, as compared to that of AlexNet. Furthermore, VGG-16 network learned features visualization demonstrates that mid and high-level features are learned effectively by the model.

Overall, it can be stated that the transfer of knowledge from a well-trained convolutional network to learn a new task can work accurately with a small margin of error, even when trained on a comparatively small database. It is significant to note that the depth of VGG-16 network added to a enhanced understanding of the input images by deducting various levels of abstractions, which helped in achieving higher recognition rates; however, required a longer time than that of AlexNet.

It can be concluded that transfer of knowledge learned by a well-trained convolutional network to learn a new classification task is possible, regardless of small margins of errors when trained using a relatively small database. Moreover, it is important to conclude that depth of the convolutional networks can help in a better understanding and analysis of the images, which helps in extraction different levels of abstraction through the network's convolutions and pooling layers. Furthermore, it is found that replacing Softmax neural network with SVM contributes to a slight boosting of the network performance and reducing its training time. Lastly, our research shows that implementing deep pre-trained CNN models combined with SVM instead of Softmax, for the tasks of COVID-19 diagnosis, is favorable in a way that similar or confusing chest radiograph can be recognized or correctly classified with good recognition rates. Convolutional neural networks benefit since the hierarchical learning and representations of data which have been found to contribute to obtain superior performances in various applications and therefore in this research.

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ORGANIC FARMS IN AGRICULTURAL TOURISM

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ABSTRACT

In recent years, there have been changes in people's tourism preferences. Ecotourism has become a popular and preferred type of tourism. As people become more conscious, they reach saturation in a short time from crowded and usual tourism centers, ecotourism is starting to be preferred because of its serenity and nature's ability to treat people. Especially people living in big cities and families with children prefer ecotourism alternatives. Agritourism, which is one of the ecotourism alternatives; it not only creates an alternative job opportunity for farmers but also becomes a stop for people who like to spend time in nature. It is one of the places where families can have a good time with their children in organic farms, which are included in agricultural tourism. organic farms; In addition to giving people the opportunity to examine organic cultivation on-site and reach organic products, it is the most ideal place for children to spend time in nature and see the products grown on the farm, pick from the branch and consume healthy products. In our study; By giving information about organic farms, which plants are grown in which season, marketing opportunities of these products grown in organic farms, and life in organic farm where visitors can have a good time are mentioned.

Key words: Organic, farm, agricultural tourism

INTRODUCTION

Various practices and many theories, principles, and models have been developed and tried to be implemented to solve environmental problems that put living spaces at risk (Kılıç et al. 2019). Organic agriculture, which respects nature and aims to grow without polluting and exploiting the soil, is one of them. Organic agriculture is an agricultural production system in which controllable, sustainable, plant and animal production are integrated. Organic farming is an environmentally, socially, and economically sustainable farming approach, advocating that the dependence on non-farm agricultural inputs, whether of chemical or organic origin, should be reduced as much as possible (Lampkin, 1994). Ecological farms are farms that aim to do agriculture without harming nature, where recycling is possible, water and energy resources are used consciously, and all elements of ecological life philosophy are included (Akçay Özkan et al., 2019).

Organic products, which are included in the shopping bags of many consumers around the world, continue to increase rapidly in terms of both production area and market size. According to 2017 data announced in 2019, 97 billion dollars of organic product sales were realized in the world. The latest published information has indicated that organic production and total area have increased in many countries and reached 70 million ha with 2.9 million producers (Anonymous, 2019).

Organic farming and agrotourism are closely linked and there is no doubt that gastronomy

and natural tourism play an important role in the future development of rural areas (Privitera, 2010). The greatest support for agricultural tourism comes from farms. In recent years, farms have become stops for nature lovers. Among the farms, the type that arouses curiosity is organic farms. Especially the pandemic has pushed people to seek healthy food to strengthen their immune systems. This state has made organic producers and organic farms sought after on the internet and social media. This study aims to give information about organic farms and examine marketing possibilities of products grown in organic farms, life issues in organic farms, and contributions of organic farms to agricultural tourism.

MATERIAL AND METHOD

In the study, a literature review on organic farms and their contributions to agricultural tourism was conducted and the subject was examined in detail.

RESULTS AND DISCUSSION

Cultivation on an Organic Farm

Soils, on which 95% of our food resources depend, are non-renewable resources. Chemical manure applications that save the day in conventional agriculture are depleting soil with organic matter at an alarming rate. Organic farmers, instead of using strong chemicals that cause soil erosion, turn their direction to soil by maintaining soil fertility and health for future generations (Veselý et al., 2016). An organic farmer produces vegetables, fruits, cereal products, or livestock without using chemical manures, pesticides, or herbicides (Meena et al., 2013). The phrase "Feed the soil to feed the plant" is one of the most fundamental principles of organic farming and gardening. Healthy food comes from healthy soil. The health of the soil is not only related to food, but is directly related to the health of the entire ecosystem. The basic principles of soil and water systems are the same on all farms, as are the nutrient cycle of carbon, nitrogen, and phosphorus. Soil plays an important role in many vital natural biological and chemical cycles (Veselý et al., 2016).

The use of crop varieties grown in organic systems and adapted to conditions will increase the ability of organic farming to perform. Murphy et al. (2007) stated that while choosing genotypes more suitable for organic conditions in organic wheat, they chose genotypes that could take and use nutrients more efficiently due to the limited nitrogen supply. Since organic farmers often have to deal with a relatively poor nutrient source, they must prefer techniques, breeds and practices that are beneficial for resource efficiency (Kölling, 2010). Duman et al. (2011) initiated the "Organic Production Project" on Ege University Faculty of Agriculture, Menemen, Research, Application and Production Farm. The project included the research, production and application studies of the fields of 20 decares of vegetable, 55 decares of vineyard, 35 decares of olive, and 406 decares of pine. In the study, they aimed to find solutions to the problems observed in practice by switching to controlled and certified organic production in different types of fruits, vineyards and vegetables, and some and also cereals and forage crops, thus optimizing the production methods and they reached the expected targets within 6 years. The common view is that in case of switching from conventional parcels to organic production, some problems may be encountered in the early production years, but these problems are declared to decrease or disappear after the 3rd and 4th years, and generally a positive balance is achieved in yield and quality.

Especially olives and products produced from olives, medicinal and aromatic plants such as thyme, sage, laurel, and rosemary and local products such as carob are among the products frequently encountered in farms in the Aegean region of Turkey. Considering the products

produced in the farms located in other regions, although the product variety is very high, there are no specific products concentrated in the farms (Dalgın et al., 2020).

The most attractive thing in organic farms is to see, consume, and buy only seasonal products. Not using genetically modified organisms, hormones, chemical pesticides and chemical fertilizers in any way, and encountering only seasonal foods in the environment prevent many possible health problems from the beginning. Being the most important step of healthy nutrition, feeding with seasonal food is a must for organic farms. This situation is most important for families with children who prefer organic farms in terms of supporting children's healthy nutrition gains.

Life on an Organic Farm

Organic farms are a settlement model that combines human behavior with nature, supports healthy individual development, and provides a self-sufficient life plan at human scale (Kılıç et al., 2019). Farm tourism is based on agricultural production and traditional lifestyle (food, health, handicrafts, householding and housekeeping) (Ahmadova et al., 2016).

Organic farms are the application areas where agricultural tourism activities are carried out. Besides offering accommodation and eating and drinking opportunities to its guests who want to experience the farm life by making only daily visits, they also offer the opportunity to participate in various outdoor activities and their main business field is agriculture and livestock. The products offered on the farms can be found in farm activities such as sowing, planting, and harvesting, processing, making by-products such as jam, oil, and soap, local handicrafts, making local products, caring for small cattle and bovine livestock, as well as accommodation in the farmhouse (Akçay and Özkan et al., 2019). Foreign and domestic tourists visiting the farms generally have common features: those who want to have unique experiences different from the classical holiday concept, those who aim to contribute to the farm works with a nature-friendly social responsibility project, those who stay on the farm for a fee, or volunteer visitors, etc. (Ahmadova et al., 2016).

TA TU TA Activities on an Organic Farm

Ecotourism farms operating within the scope of farm tourism in Turkey, continue their activities with the project known as "Agricultural Tourism in Ecological Farms and Voluntary Information and Experience Exchange" and the short name is "TaTuTa/AgToEx" (Agriculture-Tourism-Exchange). TaTuTa project, which has been operating since 2004, is also the Turkey representative of the WWOOF (Worldwide Opportunities on Organic Farms) network, which has more than 11,000 ecological farms in nearly 100 countries around the world (Ahmadova et al., 2016).

Within the scope of the TaTuTa project, a visitor who wants to be an agricultural tourist chooses the farm he/she wants to go as a "guest" or "volunteer" according to the acceptance periods of each farm. Accommodation, food and beverage needs of the volunteers are met by the farm and no fee is charged. It is sufficient to work six hours a day in farm works related to agriculture and animal husbandry (Selim Selvi et al., 2012).

Conducting the exchange of knowledge and experience, TaTuTa Project organizes many meetings on ecological life for its members and volunteers every year, publishes in this field, and makes radio programs. TaTuTa, which also supports the ecological literacy project, provides information to the participants on farms about what can be done for a more ecological life. At the same time, the project teaches ecological living practices by introducing the farms, which are good examples of ecological life, to the participants (Şahbudak et al., 2017). The

regions where TaTuTa farms are concentrated in Turkey are the Aegean, Mediterranean, and Marmara regions (Dalgın et al., 2020).

Table 1: Regional Distribution of Organic Farms in Turkey

Region Name	Number of Farms
Mediterranean Region	25
Black Sea Region	25
Marmara Region	15
Aegean Region	14
Eastern Anatolia Region	6
Central Anatolia Regio	5
Southeastern Anatolia Region	2

Source: Akçay özkan et al., 2019

Marketing of Products on an Organic Farm

One of the most significant problems in organic production is to market the grown products healthily. In the marketing of organic products, the market is divided into segments first and the target market is determined. Then, marketing components suitable for the target market are determined and presented to the organic market (Kurt, 2006). Organic farms may choose to negotiate with retailers with a certain recognition or create their own online sales channels to market and sell the local products they produce to large masses (Dalgın et al., 2020).

Based on her research, Arabska (2014) suggests forming marketing strategies in organic production enterprises (farms) by considering the four elements of the marketing mix - product, price, place, and promotion.

Most of the farms are engaged in organic farming and marketing and sales of products at the same time. The results of the survey revealed that 9 out of 17 farms sell organic products produced in the farm. Others do not sell, but only offer their products to visitors to the farm. The products are offered for sale not only in organic market places but also online channels and on the farm. The most significant problems related to the marketing of farm products are intense competition and the statement of most of the farms that they do not invest heavily in marketing activities and that marketing activities increase the financial burden of the farm (Ahmadova et al., 2016). Civelek Oruç et al., (2015) indicate that an examination of the websites created by countries exhibits that the enterprises operating in agricultural tourism are mostly gathered under one roof, this allows all activities to be announced and promoted, social media is used as a marketing tool, and information about the products produced by the enterprises is included in the websites so that there is easier consumer access to these products.

The best way to market products on farms is both the natural market where the grown products are sold fresh, and the restaurant and cafe where the grown products are processed and served by preparing food, fresh salad, etc. At the same time, when the guests are at the farm, the best step to establish a marketing network and win every guest permanently for the sale of products can be by giving them brochures with information about the address and online sales.

Contribution of Organic Farms to Agricultural Tourism

Agricultural tourism is the total of initiatives initiated to contribute to the development of farm businesses, and it plays an active role in rural development by diversifying tourism for economic purposes, creates an additional source of income for local businesses in the region, provides job opportunities, and helps to stop the outward migration of people in that region (Köroğlu et al., 2019). In recent years, there has been an increasing interest in the conservation of natural resources and ensuring sustainability. The concept of farm tourism is a type of

tourism that reduces waste and prevents environmental damage. It also promotes the preservation of plant and animal diversity and the preservation of natural and cultural diversity. Farm tourism provides cross-cultural exchange as it offers the opportunity to experience rural life by living on site. It also makes economic contributions to the local people living in the countryside (Savgın, 2016). At the end of their survey Köroğlu et al., (2019) stated that the most important factor for the participants to visit ecological farms is to relax mentally, get away from city life, enjoy the view, and relax physically.

CONCLUSIONS

In recent years, there have been changes in people's tourism preferences. Ecotourism has become a popular and preferred form of tourism. Agritourism, which is one of the ecotourism alternatives, creates an alternative job opportunity for farmers and also becomes a stop for people who like to spend time in nature. Agritourism is actually one of the initiatives initiated to contribute to the development of farm businesses. Being in the Ta-Tu-Ta project, farm tourism also allows those who come to visit the farms to have a holiday in the farmhouse feeling a part of nature. Especially organic farms allow tourists to come and spend time in nature and to watch or participate in farm works. It also provides the opportunity to consume organic products in the farm restaurant and leave the farm after buying fresh products from the farm market. Thanks to organic farms, the farmer has the opportunity to deliver the product he/she has grown to the consumer at first hand with its true value. Most importantly, the markets and restaurants in organic farms provide the farmer with hot money flow throughout the year, and in fact, organic farms contribute much more to both agriculture and agricultural tourism than is thought.

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PROBLEMS AND PROPOSAL OF SOLUTIONS IN ORGANIC FARMING

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ABSTRACT

Interest in organic farming is increasing day by day in our country. The fact that people have to keep their immune systems strong, especially during the Covid-19 pandemic process, has directed people to organic products. Due to the increasing demand for organic products, it should be required to gain momentum in its cultivation. However, organic farming growers in our country face various problems at the beginning of their work. These is generally organic seed and organic seedling supply, organic fertilization, organic spraying. This is followed by the marketing of organic products grown under very difficult conditions and informing the consumers in order to eliminate the problems in marketing. The dissemination and sustainability of organic agriculture is not possible only by increasing the production areas and amount. It is important to ensure that consumers also prefer organic products and to be informed for this purpose. Informing producers and consumers will increase the supply and demand of organic products, thus increasing both commercial production and the production of products that are beneficial to the environment, nature and human health. The aim of the study; It is to offer solutions by considering the problems faced by organic agriculture growers from the first time they start to work, until the harvest and sales point.

Key Words: organic production, problem, solution

INTRODUCTION

Organic agriculture is a form of controlled and certified agricultural production in every stage that does not harm human health and the environment and does not use chemical inputs in production, from production to consumption. It is a production method that aims to protect vital resources such as air and water and natural life by preserving the natural balance (anonymous, 2021).

Organic farming first started in Europe and the United States (USA) and then spread to other countries. As a result of the increase in consumer demand for organic agriculture and food products, naturally, the number of farmers adopting organic agriculture has increased. The growth of this demand has also enhanced international trade. In Turkey, the organic farming movement was initiated through the representatives of European organic farming companies. This situation emerges as a result of the demand for Turkey's classical agricultural export products that cannot be grown in Europe. For example, organic farming activities were first introduced to a limited number of grape producers in the Aegean Region of Turkey by the representatives of European organic farming companies (Demiryürek, 2011).

According to the 2018 reports of IFOAM, India has the largest share in world organic agriculture production with 1,149,000 producers. Uganda (210,000 people) and Ethiopia (204,000 people) follow India. Australia is the country with the largest organic farming area, with an area of 35.7 million hectares. Argentina with 3.6 million hectares and China with 3.1 million hectares follow Australia (Willer, 2020). In fact, Turkey has not reached the point it

deserves, although natural resources such as soil and water have not been polluted yet and it is in an advantageous position in terms of appropriate ecology and organic agriculture. Turkey has an important potential in the field of organic agriculture due to the diversity in crop production, the availability of natural meadows and pastures, the presence of cattle and ovine animals, and sufficient workforce (Bayram et al., 2011).

As a result of the study, they carried out to determine the problems experienced by enterprises producing organic and natural products, Bozyiğit and Doğan (2015) indicated that except for the certificate issue, the problems of the companies producing organic and natural products are similar and the main problems experienced by the enterprises producing organic products are the lack of customer perception and consumer information, the small and fragmented enterprises, and the low state support.

To increase the organic product market share, some problems need to be solved urgently regarding the organization of farmers, the structural characteristics of organic farming enterprises and accredited laboratories (Bayram et al., 2011). The purpose of the study is to propose solutions by considering the problems faced by organic agriculture growers from the first time they start to work, until the harvest and sales point.

MATERIAL AND METHOD

In the study, a literature review was conducted on the problems and solution proposals in organic farming, and the subject was examined in detail.

RESULTS AND DISCUSSION

Organic Seed Supply

Organic seed is the starting material of organic plant production. It is foreseen that the seeds and other production materials to be used in organic production will be produced organically (Anonim, 2010). However, as in many countries, the implementation of this obligation is postponed to a later date every year, since organic seed production in Turkey is far below the producer's demand (Duman 2009). One of the most important reasons for the difficulty in obtaining organic seeds is that in seed production, it is necessary to keep the plant healthy for a longer period (Başay, 2018). The farmer, who cannot obtain organic seeds, has to use the non-organic but pesticide-free seed at the beginning of production, based on the regulation allowing the use of pesticide-free seeds for a certain period.

Problems with organic seeds are not only due to insufficient production. The fact that Turkey does not have varieties that perform well in organic farming rules, there is lacking of the genetic purity of the seeds produced and the low quality of the seeds, and the contamination with diseases and pests are among the important problems (Saygılı and Yanmaz 2015).

In a survey conducted in Europe, the use of organic seeds by European farmers was investigated and it was found that European organicists were not very consistent in this regard. In terms of organic seed utilization rate, it was observed that the farms in the North and Central regions were higher than the farms in the South and East regions (Orsini et al., 2020).

Winter et al., (2021) proposed a cross-sectoral pool financing strategy among all value chain partners of the organic sector to join forces to invest in organic breeding. In organic seed production, due to the low number of domestic varieties, it is necessary to define Turkey's native genotypes and quickly develop the types with a potential in organic farming conditions (Duman, 2009, Başay, 2018). Also, it is necessary to accelerate the development of Turkey's domestic hybrid varieties adapted to organic conditions, and then the seed production works (Yanmaz, 2016). If these works are carried out quickly, the problem of Turkish farmers in the supply of organic seeds will be solved.

Organic Seedling Supply

The article 10-clause a of the "Regulation on the Principles and Implementation of Organic Agriculture" defines the characteristics of organic agricultural multiplication materials and mentions seedling as something in which no external intervention has been made, chemical inputs are not used, artificial radiation and microwaves are not applied, and complies with the organic agriculture regulation (Anonymous, 2010). As can be understood from the clause of this article, in organic vegetable cultivation, the seedling must be produced certified under organic conditions (Duman, 2009). Although seedling production companies have increased in recent years, there are very few companies producing organic seedlings. Organic seedlings are produced by some companies only if they are ordered well in advance, and they are usually grown by the producer himself (Tan, 2014). There is almost no practical application of the ready-made seedling industry in organic seedling production (especially due to difficulties such as the control of seedling growth, the necessity of producing organic seedlings in special greenhouses separately from other seedlings, and the demand for organic seedlings not yet at the desired level). In this case, the organic seedling needs are met by the producers themselves (Tüzel et al., 2015; Özbay, et al. 2015, Fadıllıoğlu et al. 2021).

What organic farmers, who have to grow their own seedlings, should do are as follows: using locally available wastes directly or by composting, using different vegetable types for seedling production, preferring slightly wider viol chambers, regular maintenance conditions, obtaining tea by keeping farm manure and worm manure, which are allowed by the control and certification body, in water for a certain period, and using them in watering by diluting them. By doing these, it seems possible to achieve success in organic seedling.

Organic Manuring

The vast majority of Turkey's soils contain very little organic matter due to the scarcity of natural vegetation under arid climatic conditions. The fact that Turkey is a very old settlement and its use and exploitation as agricultural land for many years is one of the important reasons for the lack of organic matter (Aygün et al., 2019). To eliminate this negativity, organic manures have an extremely important role (Yetgin, 2010). Organic manures are continuous sources of nutrients due to their slow release during degradation (Singh 2012). As a result of their study on different organic manure doses, Kılıç et al. (2019) stated that while different organic manures were effective in pH, EC, organic matter, N, P, K, Ca, Zn, and Cu contents in soil properties, chicken manure and farmyard manure were the most effective manures in these parameters.

Organic manures are offered to producers under various names and ingredients on the market. Later, this range was expanded and in addition to organic materials such as compost, humic and fulvic acids, and leonardite, manures containing various types of microorganisms, enzymes, and algae extracts began to be produced commercially (Okur et al., 2007). Azotobacter, Rhizobium, Azospirillum, Mycorrhiza and Pseudomonas can be counted among the main microorganisms used in microbiological manures (Anaç, 2017). In their study, Şener et al. (2020) used a day-neutral variety 'Albion' as a plant material, and commercial preparations 'SimBacil', 'SimDerma' and 'OrgaStar', which are preparations with microbial content, as plant growth and development regulators. A comparison of the results of the applications with the check plots revealed statistically significant differences and they concluded that plant growth regulator applications with microbial content can be recommended in strawberry cultivation.

In organic farming, producers can use natural manures that they can easily obtain in the region they live in, by making preliminary tests in small areas first. In case of need, in addition to organic materials such as compost, humic and fulvic acids, and leonardite, it is also possible

to use commercial manures, which contain various types of microorganisms, enzymes, and algae extracts, for the manuring process.

Organic Spraying

The use of certain products in powder or liquid form, which are allowed to be applied to protect cultivated plants from pests, diseases, and weeds in organic agriculture, in pest, disease and weed control, increasing the resistance of the plant, or cleaning and disinfecting animal buildings and shelters, is the basis of plant protection (Anonim, 2010; Önen, 2014). According to organic production standards, producers can use biotechnical control methods using cultural, mechanical or physical, biological, non-synthetic attractants, repellants, and traps in the management of pests (Tuncer, 2014). Moreover, weeds can become a big problem in organic agriculture, unlike traditional agriculture, since the use of pesticides is not allowed and the use of herbicides in weed control is also limited. In organic agriculture, the aim is not eliminating weeds, but rather suppressing weeds. This is achieved by keeping weeds under pressure by increasing the competitiveness of crop plants or by increasing their phytotoxic effects (Allelopathy) by keeping weed populations below the level that they will be harmful (Önen, 2014).

It is desirable to reduce the applications of synthetic pesticides in agriculture. One of the ways to achieve this goal, and the most promising, is to use new techniques for disease control based on biological control agents (BCAs) and, accordingly, to minimize the harmful effects of chemicals on the environment. In recent years, biological manures, plant stimulants, and biological pesticides, which are microbiological factors, are considered as sources that will meet the nutrients needed by the plant (Küçük, 2009; İmriz, 2019; Aydın, 2015).

There are various biological, microbial, bio technically produced insecticides, acaricides, nematicides, and fungicides with organic licenses. These are organic drugs with active substances of copper, sulfur, and various bacteria (bacillus, paecilomyces, etc.) (Kaya, 2017). In Turkey, organic poisons such as garlic, euphorbia, and yellow soap are made by producers and used by them in organic agriculture. Licensed products to be used in organic agriculture have started to be present in the market in recent years. The effect of the licensed products, their way of use, the duration of the effect, and the effect on the environment are mostly known and can be used safely accordingly (Sullivan, 2021).

While planting seedlings on the organic producer's land, it can benefit from the repellent properties of medicinal and aromatic plants by planting mixed with medicinal and aromatic plants. When faced with a problem in terms of plant protection, homemade pesticides can be used, if the problem persists, biological control agents and finally organic pesticides allowed by organic farming regulations should be used.

Marketing

One of the most important problems in organic production is to market the grown products properly. In the marketing of organic products, the market is divided into segments and the target market is determined. Then, marketing components suitable for the target market are determined and presented to the organic market (Kurt, 2006). Although it is thought that high prices limit marketing at the point of insufficient demand for organic products, it is possible to mention that the main factor is the inadequacy of distribution channels (Ayla and Altıntaş 2017). Also, marketable yields of organic horticultural crops are generally below the yields of non-organic crops (Cihanban and Davari, 2013).

According to Korkmazyürek (2020), the organic agriculture market in Turkey cannot develop as fast as the world organic markets. He stated that the most important reasons for this are the lack of marketing strategies and the insufficient development of export products.

Acarsoy Bilgin, et al. (2019) indicate that current market conditions do not provide an income advantage for producers to turn to organic agriculture. For this reason, they stated that increasing the existing subsidies and arranging continuous publications are seen as the most important factors that can enable the producers in the region to turn to organic agriculture. The organic products export of Turkey is mostly to European countries. To pave the way for production, it is necessary to open up to new markets such as the USA, Japan, and Australia, which are mostly consumers. The development of the domestic market remains very limited. The primary reason for this is that the economic condition of Turkish people is not as good as those of developed countries (Demir, 2006; Ayla et al., 2017).

The most important solution to the problems experienced in marketing is to reduce the costs by supporting the inputs of the organic producer by the state and ensuring that the producer of organic products works in wider areas. Afterward, a safer environment for organic products should be provided to the consumer, and the awareness of the consumers should be increased with the necessary publication network so that they prefer organic products for their own health and do not pollute the environment.

CONCLUSIONS

The interest and demand for organic agriculture are increasing day by day, but organic products are offered to the market at more expensive prices since the inputs are higher and organic production is less than conventional production. As a result, not everyone has access to organic products. Only people whose economic level is above a certain level can reach them. In terms of producers, high costs (pesticides, manures, etc.), the long procedure, and very low support reduce the interest in organic production. There is information pollution on organic products for consumers.

As a result, what to do are as follows: first of all, education programs to be prepared for consumers to eliminate information pollution about organic products can contribute to the rapid development of the organic production system in Turkey. Organic producers need to adopt a consumer-oriented form of marketing that will include all consumers. By increasing state supports considerably, organic production will be encouraged and it will be made in large areas. The increase in the organic seed and organic seedling needs of the producers will encourage organic seed and seedling companies to produce. Large-scale production as a result of government supports will reduce the prices of organic products to more reasonable figures. By selling organic products at more reasonable prices, more people will have the opportunity to consume them, and by ensuring that organic production is out of the vicious circle it is currently in, production and consumption will increase day by day.

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RHIZOBIA INOCULATION AND DMPP NITRIFICATION INHIBITOR HAD EFFECT PHENOLOGICAL AND MORPHOLOGICAL CHARACTERS OF BEAN (Phaseolus vulgaris L.)

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ABSTRACT

The field experiment was conducted during 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey. The experiment was designed as factorial arrangement in the complete randomized block design with four replications. In this study, rhizobia and non-rhizobia were investigated at different nitrogen fertilizer types and doses (control, 25 kg ha^{-1} AS, 25 kg ha^{-1} DMPP, 50 kg ha^{-1} AS and 50 kg ha^{-1} DMPP). The effects of the year were significant for all of the investigated characters except for emergence time. Bacteria inoculation was increased all of the morphological characters and grain yield and decreased phenological characters. 50 kg ha^{-1} DMPP nitrogen fertilization types and doses were provided the highest values for investigated characters and grain yield. The use of nitrogen ihnibatörs may be an important practice to improve the bean crops. With the use of DMPP, the amount of nitrogen fertilizer and the number of applications of nitrogen fertilizers can be reduced.

Key Words: Bean, DMPP, phenological properties, morphological properties, yield

INTRODUCTION

N plays an essential role in plant production, regarding both its economic and ecological aspects. On the one hand, N affects yield level and quality like no other plant nutrient, and on the other hand, nitrogenous compounds in the hydrosphere (NO_3^- accumulation in groundwater) and atmosphere (release of nitrous greenhouse gases) may have numerous unwanted effects on the environment (Zerulla et al., 2001)

The microbial mechanisms underlying the key biogeochemical processes of the N cycle in soil have significant environmental and ecological effects, both through nitrification, where ammonium (NH_4^+) is converted to NO_3^-, a major potential source of N loss to surface and groundwater, and denitrification, where NO_3^- is converted to the potent greenhouse gas N_2O and other gasses (Myhre et al., 2013; Qin et al., 2017). An efficient strategy to reduce the N losses and pollution associated with fertilization is the use of additives that block or delay nitrogen-associated microbial processes such as nitrification inhibitors (Rodrigues et al., 2019). Therefore, the use of nitrification inhibitors (NIs) has become an increasingly important strategy in recent decades to help reduce N_2O emissions (Smith et al., 2015). Hundreds of nitrification inhibitors are known, but only a few so far have gained commercial importance for practical use, such as dicyandiamide (DCD) and 3,4-dimethylpyrazole phosphate (DMPP) (Liu et al., 2013). Among nitrification inhibitors, 3,4-dimethylpyrazole phosphate (DMPP) has been reported by many authors as the most efficient in slowing nitrification and reducing N_2O losses (Weiske et al., 2001a; Weiske et al., 2001b; Liu et al.,
2013). The duration of its action depends on temperature and humidity conditions (Pasda et al., 2001). It can remain effective in upper soil layers even after heavy rain (Fettweis et al., 2001).

Like other legumes, the common bean can benefit from BNF through symbiosis with nitrogen-fixing rhizobia (Hungria and Kaschuk, 2014). There are many works on growth and yield of bean in response to Rhizobia. Küçük ve Kıvanç (2008), Bulut (2013) and (Şen (2018) indicated that rhizobia bacteria inoculation and nitrogen fertilization increases the yield in beans. Odabaş and Gülümser (2001), Bildirici (2003) and Bilen (2003) reported that bacteria application and nitrogen fertilization increase plant height. Şahin (2018) and Şen (2018) reported that bacteria application and nitrogen fertilization increase flowering time, harvest maturity time, plant height, first pod height, main branch number and pod height.

This study aims to investigate the effects of rhizobia and different nitrogen fertilizer types and doses on yield and some phenological and morphological characters for bean.

MATERIAL AND METHOD

The field experiment was conducted during 2017 and 2018 at the experimental area of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey (39º48' N; 30º31' E, 798 m above sea level). Climatic data for long-term and experimental years are shown in Figure 1. Long-term annual total precipitation is 104.1 mm and it was 143.4 and 170.2 mm in the experimental years, respectively. The annual average temperature was 19.64 ºC in 2017 and 20.1 ºC in 2018. The soil of the experimental area was organic matter content of 1.44%, with lime 2.50% and pH of 7.61. Corresponding available P_2O_5, K_2O and N contents were 108.9 kg ha^{-1},1944.6 kg ha^{-1} and 0.07% in the first year, respectively. In the second year, it was organic matter 1.65%, with lime 7.56%, pH of 7.71, available P_2O_5 177.5 kg ha^{-1} K_2O 2450.0 kg ha^{-1} and N 0.08%.

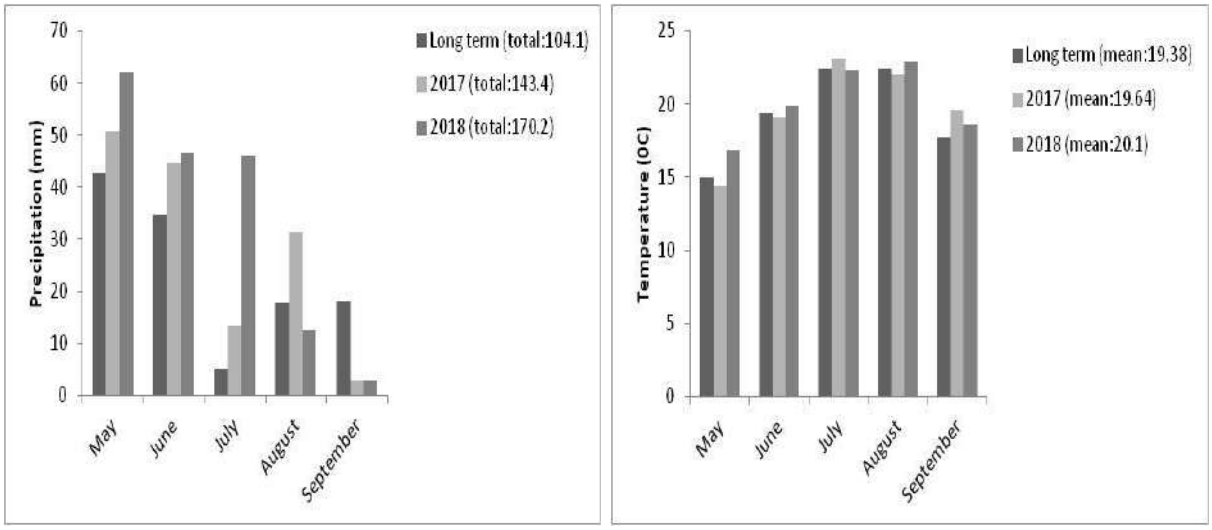

Figure 1. Climatic data of the research area

The experiment was designed as factorial arrangement in the complete randomized block design with four replications. In this study, rhizobia and non-rhizobia were investigated at different nitrogen fertilizer types and doses (control, 25 kg ha^{-1} AS, 25 kg ha^{-1} DMPP, 50 kg ha^{-1} AS and 50 kg ha^{-1} DMPP). Bean varieties Göynük-98 was used research material. Each plot was 7.2 m^2 (4 m x 1.8 m) and bean was sown 45 cm row spacing and seeding rate was 26 seeds m^{-2}. The sowing time was 03 May and 04 May in 2017 and 2018, respectively. All of the nitrogen fertilizers were applied at sowing time. Seeds were inoculated with *Rhizobium endophyticum* (formed colonies at 10-8 level) bacteria at the recommended rate (100 kg seed to 1 kg peat inoculant) before sowing in rhizobia plots. Application of the peat inoculant on the

seeds was carried out by water, which contains 2% sugar. Peat inoculation was provided by the Soil, Fertilizer and Water Central Research Institute. It was kept in a refrigerator at + 4°C until use**.** The basal fertilizer application of 60 kg ha^{-1} TSP (triple super phosphate (43-44 P_2O_5%) was given to each plot at the sowing. The harvest time of chickpea was on 13 September 2017 and 27 August 2018 in the first and second years, respectively. Emergence time (day), flowering time (day), pod formation time (day) and harvest maturity time (day) were determined in all the plots. The plant height (cm), first pod height (cm), main branch number, main branch diameter (mm) and pod height (cm) were evaluated on 5 randomly selected plants in each plot. Each plot was harvested, blended and grain yield (kg ha^{-1}) was estimated.

The variance analysis was subjected to based on General Linear Model using the Statview package (SAS Institute). Means were compared by Least Significant Differences (LSD) test.

RESULTS AND DISCUSSION

The effects of the year were significant for all of the investigated characters except for emergence time (Table 1,2). Differences between the rhizobia were significant were all of the investigated characters except for flowering time and first pod height. All investigated characters were significant for nitrogen fertilization except for pod formation time and first pod height. While flowering time had higher values in all of the plots in 2018, these traits showed lower values in 2017 (Figure 2B). For this reason, year x nitrogen fertilization interaction was significant. While pod formation time higher Rh^- in 2017, Rh^+ showed the lower values (Figure 3A). For this reason, year x rhizobia interaction was significant. While emergence time higher Rh^- on control plots in both of the years, Rh^+ showed the lower values (Figure 2A). While harvest maturity time, plant height, main branch diameter and grain yield higher Rh^+ on 50 kg ha^{-1} DMPP plots in 2017, Rh^+ showed the lower values on control plots in 2018. (Figure 3B, 4A, 5A, 6). While main branch number and pod height were higher Rh^+ on 25 kg ha^{-1} AS plots in 2018, Rh^- showed the lower values on same plots in 2018. (Figure 4B, 5B). For this reason, year x rhizobia x nitrogen fertilization interaction was significant.

Table 1.Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	ET (day)	FT (day)	PFT day)	HMT (day)	PH (cm)
2017	13.03	49.52 B	61.97 b	134.87 A	52.31 A
2018	13.26	52.32 A	62.75 a	132.15 B	48.72 B
Mean	13.14	50.92	62.36	133.51	50.51
Rh^-	13.74 A	51.07	62.75 A	132.80 B	48.11 B
Rh^+	12.55 B	50.77	61.97 B	134.22 A	52.92 A
Mean	13.14	50.92	62.36	133.51	50.51
Control	13.02 B	51.18 A	62.18	131.18 E	42.41 E
25 kg ha^{-1} AS	13.25 A	50.93 A	62.87	132.68 D	45.96 D
25 kg ha^{-1} DMPP	13.10 AB	51.00 A	62.31	133.18 C	51.36 C
50 kg ha^{-1} AS	13.25 A	51.50 A	62.18	134.50 B	54.96 B
50 kg ha^{-1} DMPP	13.12 AB	50.00 B	62.25	136.00 A	57.88 A
Mean	13.14	50.92	62.36	133.51	50.51
Year	ns	**	*	**	**
Rhizobia	**	ns	**	**	**
Nitrogen fertilization	**	**	ns	**	**
Year x rhizobia	**	ns	*	**	**
Year x nitrogen fertilization	**	*	ns	**	**
Rhizobia x nitrogen fert.	**	ns	ns	ns	**
Year x rhizobia x nitrogen fert.	**	ns	ns	**	**

ns: non-significant, *: $p \leq 0.05$, **: $p \leq 0.01$. Means in the same column with different letters are significant.ET : Emergence time FT: Flowering time PFT: Pod formation time HMT: Harvest maturity time PH: Plant height

Table 2.Effects of rhizobia and different nitrogen fertilization on some traits of bean

Treatments	FPH (cm)	MBN	MBD (mm)	Pod H. (cm)	GY (kg ha^{-1})
2017	12.26 b	2.92 a	7.12 A	12.28 A	1885 A
2018	14.61a	2.88 b	7.04 B	12.08 B	1425 B
Mean	13.43	2.90	7.08	12.18	1655
Rh$^-$	13.00	2.83 B	6.79 B	12.04 B	1312 B
Rh$^+$	13.87	2.97 A	7.38 A	12.33 A	1999 A
Mean	13.43	2.90	7.08	12.18	1655
Control	12.01	2.88 BC	6.02 E	12.11 BC	1162 E
25 kg ha^{-1} AS	14.63	2.83 C	6.56 D	12.05 C	1446 D
25 kg ha^{-1} DMPP	13.43	2.91 AB	7.18 C	12.15 ABC	1693 C
50 kg ha^{-1} AS	13.40	2.92 AB	7.42 BC	12.26 AB	1783 B
50 kg ha^{-1} DMPP	13.71	2.95 A	8.23 A	12.33 A	2193 A
Mean	13.43	2.90	7.08	12.18	1655
Year	*	*	**	**	**
Rhizobia	ns	**	**	**	**
Nitrogen fertilization	ns	**	**	**	**
Year x rhizobia	ns	**	**	**	**
Year x nitrogen fertilization	ns	**	**	**	**
Rhizobia x nitrogen fert.	ns	**	**	**	**
Year x rhizobia x nitrogen fert.	ns	**	**	**	**

ns: non-significant, *: p≤0.05, **: p≤0.01. Means in the same column with different letters are significant. FPH: First pod height MBN: Main branch number MBD: Main branch diameter Pod H: Pod height GY: Grain yield

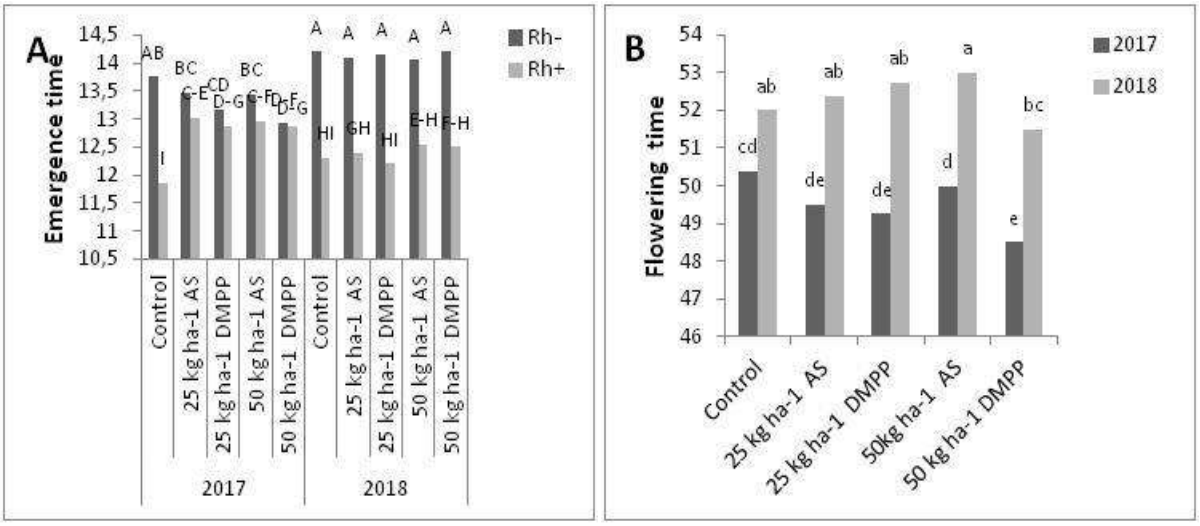

Figure 2.The interaction between rhizobia and different nitrogen fertilization on emergence time (A) and flowering time (B) of bean. Letters on each bar represent significance level at P < 0.05

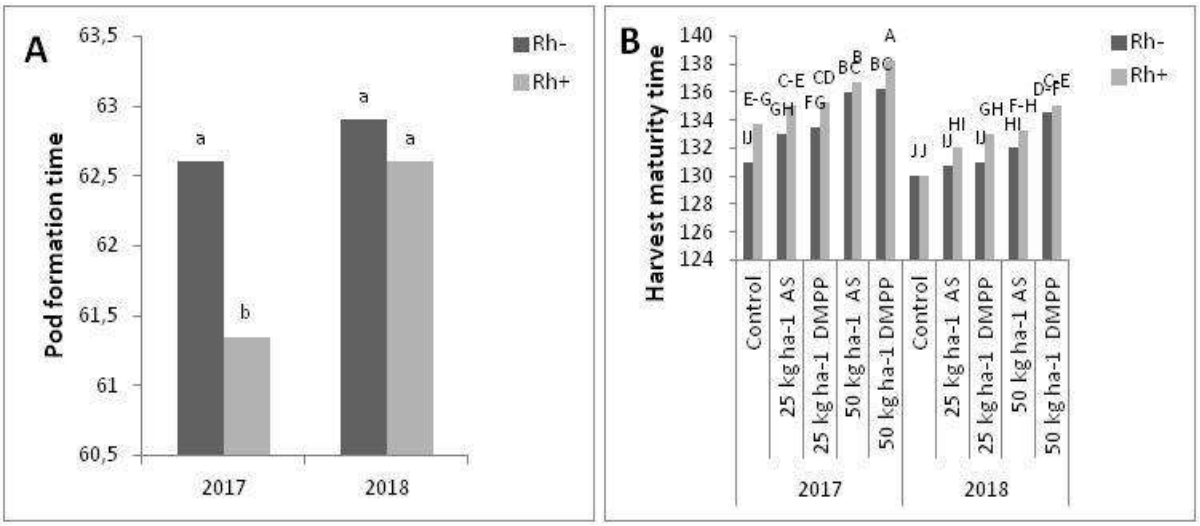

Figure 3.The interaction between rhizobia and different nitrogen fertilization on pod formation time (A) and harvest maturity time (B) of bean. Letters on each bar represent significance level at $P < 0.05$

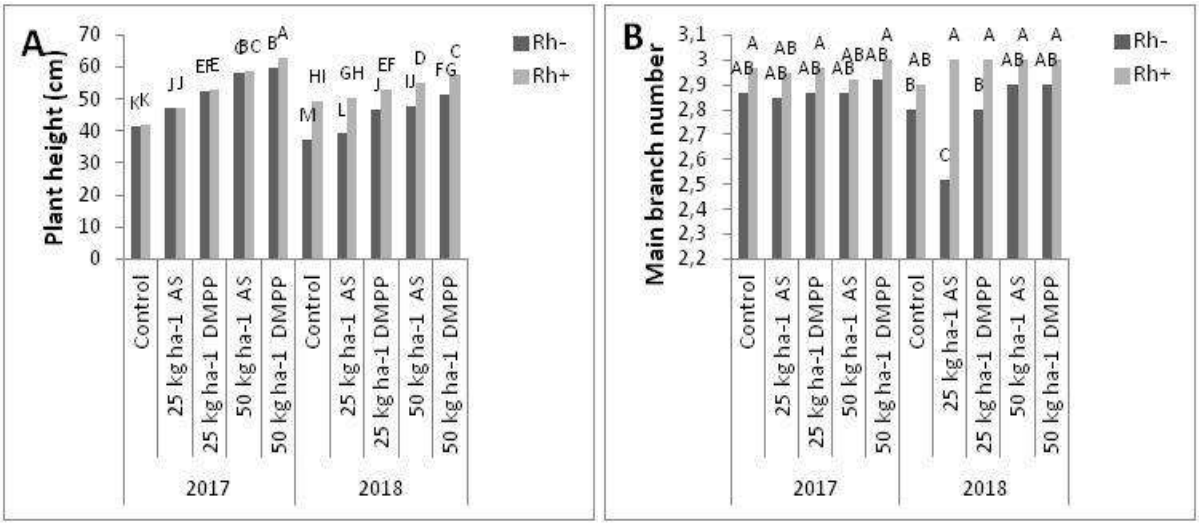

Figure 4.The interaction between rhizobia and different nitrogen fertilization on plant height (A) and main branch number (B) of bean. Letters on each bar represent significance level at $P < 0.05$

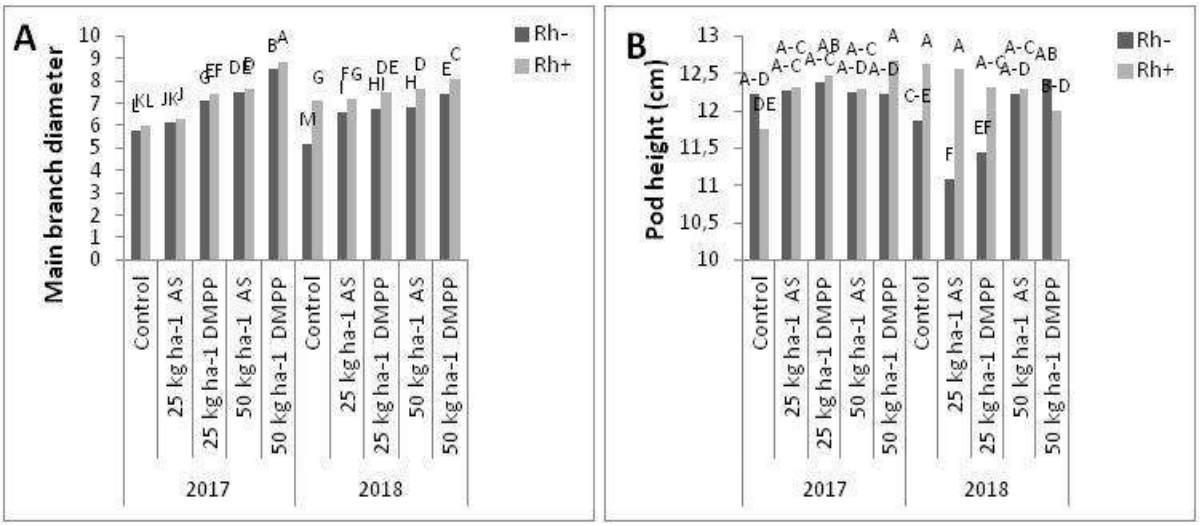

Figure 5.The interaction between rhizobia and different nitrogen fertilization on main branch diameter (A) and pod height (B) of bean. Letters on each bar represent significance level at $P < 0.05$

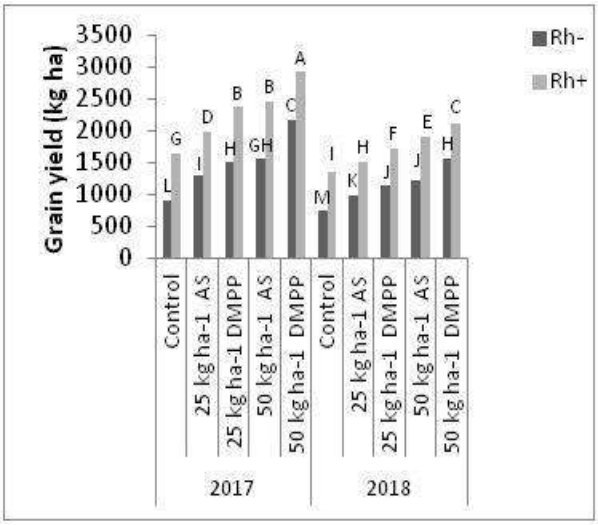

Figure 6.The interaction between rhizobia and different nitrogen fertilization on grain yield of bean. Letters on each bar represent significance level at $P < 0.05$

Flowering time, pod formation time and first pod height were higher second year but the other characteristics were a higher first year (Table 1,2). The flowering time and pod formation time were delayed due to more precipitation in May and June (especially in May) in the second year (Figure 1). Şehirali (1988) reported that more rainfall extends the flowering period in beans. Harvest maturity time was shorter in the second year. The higher temperatures in August was caused the plants to mature in a shorter time in the second year (Figure 1). High temperatures and drought after flowering were shortened the grain filling time (Wiegand et al., 1981). Plant height was higher first year than the second year but the first pod height was higher second year than the first year. Pekşen (2005) and Ülker (2008) reported that plant height was significantly affected by environmental conditions and significant differences may occur between the same genotypes in different years and locations. Main branch number, main branch diameter and pod height were higher in the first year. The main branch number depends on the genotype and growing conditions in beans (Aytekin and Çalışkan, 2015). Other researchers

were reported that significant differences between the years for main branch numbers due to climatic differences (Pekşen, 2005; Karakuş et al., 2005). Grain yield was lower due to total high temperature especially grain-filled period in the second experimental year (Figure 1, Table 2). Warland et al. (2006) reported that grain yield was reduced when the temperature is increased by 1.5 ^{o}C.

Rhizobia inoculation was decreased emergence time and pod formation time but harvest maturity time was increased by bacteria inoculation. Agba et al. (2013) reported that pod formation time was decreased rhizobia inoculation and Pekşen (1992) indicated that emergence time was decreased by rhizobia inoculation in chickpea. Plant height, main branch number, main branch diameter and pod height were increased by rhizobia bacteria inoculation. Rhizobia, which is one of the bacteria that plant growth-promoting rhizobacteria, increases plant growth by producing hormonal substances (Çakmakçı, 2005). Plant height, main branch number and diameter and pod length increased with the effect of bacteria in our study. Bildirici (2003), Bilen (2003) Uyanöz (2007), Altunkaynak ve Ceyhan (2018) ve Şen (2018) reported that nitrogen fertilization and rhizobia inoculation were increased plant height. Agba et al. (2013) indicated that rhizobia inoculation was increased main branch number and Ahmed et al. (2007), Rahman et al. (2008) and Akman (2017) reported that bacteria was increased pod height. Karadavut ve Özdemir (2001) indicated that rhizobia bacteria inoculation was increased plant height and main branch number in chickpea. Şahin (2018) reported that plant height, main branch number and pod length were increased by rhizobia inoculation in bean. Çakmakçı (2005), Bayraklı et al. (2017) and Barros et al. (2018) reported that grain yield was increased with rhizobia inoculation.

Emergence time and harvest maturity time were not affected by nitrogen fertilization types and doses. The shortest emergence time and harvest maturity time were observed in control plots. Zenawi and Mizan reported that emergence time was not affected by nitrogen fertilization. The shortest flowering time was observed on 50 kg ha^{-1} DMPP. Flowering time was shortened with DMPP application. The highest plant height, main branch number, main branch diameter, pod height and grain yield were obtained 50 kg ha^{-1} DMPP. Coelho et al. (2018) indicated that plant height was increased by nitrogen inhibatörs. The effectiveness of nitrogen inhibitors may vary depending on climatic conditions (especially precipitation and temperature), soil moisture, pH, soil texture and mineral N content. May, June and July were very rainy in both years of our study. Excessive precipitation causes nitrogen losses in soil and thus benefits of DMPP are more observed. Therefore, morphological characteristics and grain yield may be higher in 50 kg ha^{-1} DMPP plots in our study. Pasda et al. (2001) positive effect of DMPP on yield is more clear when precipitation was higher.

CONCLUSIONS

Flowering time, pod formation time and first pod height were higher second year but the other characteristics were higher first year due to climatic conditions. Phenological characters were affected differently by rhizobia and nitrogen fertilization application. Bacteria inoculation was increased all of the morphological characters and grain yield and decreased phenological characters. 50 kg ha^{-1} DMPP nitrogen fertilization types and doses were provided the highest values for investigated characters and grain yield. The effectiveness of nitrogen inhibitors may vary depending on climatic conditions. Excessive precipitation causes nitrogen losses in soil and thus benefits of DMPP might be more observed. The use of nitrogen ihnibatörs may be an important practice to improve the bean crops. With the use of DMPP, the amount of nitrogen fertilizer and the number of applications of nitrogen fertilizers can be reduced.

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TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITIES, AND POLYPHENOL OXIDASE (PPO) ENZYME INHIBITION OF TOTAL EXTRACTS OF WILD STRAWBERRY (*ARBUTUS UNEDO*) FRUIT AND LEAF

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ABSTRACT

Arbutus unedo L., the strawberry tree (Ericaceae family) fruit, has a great potential to serve as an important source of biomolecules known for panoply of applications in food and pharmaceutical industry. Arbutus unedo is a widespread shrub with economic importance, derived from the use of its berries in the production of alcoholic beverages and in folk medicine. In this study, total extracts were obtained from Wild Strawberry (Arbutus Unedo) Fruıt and Leaf. Their total phenolic content, ABTS and DPPH activities as antioxidant activity and inhibition effects on polyphenol oxidase (PPO) were evaluated.

Keywords: Antioxidant activity, PPO inhibition, Arbutus Unedo

Introduction

Arbutus unedo L., the strawberry tree (Ericaceae family) fruit, is an evergreen shrub or small tree and it is widely distributed in the Mediterranean region and North Africa. This fruit is suitable for the production of alcoholic beverages, jams, jellies and marmalades (Orak et al. 2021) and in some countries, such as Spain and Morocco, Arbutus unedo is frequently used in the traditional medicine (Orak et al. 2012). In traditional folk medicine, A. unedo has been used in antiseptics, diuretics and laxatives and to treat arterial hypertension. There are several qualities associated with this plant, such as ornamental, ecological and economical value, as well as therapeutic and medicinal properties (Olıveıra et al 2011).

The leaves of A. unedo L. have been reported as possessing several biological properties such as astringent, human platelet anti-aggregant due to its relative high amounts of tannins, urinary antiseptic, anti-inflammatory, anti-diarrheal, anti-hypertension and anti-diabetic (Miguel et al. 2014). Traditional medicinal uses of A. unedo leaves; Gastrointestinal disorders, urological problems, dermatologic problems, cardiovascular application, kidney diseases, hypertension, cardiac diseases, diabetes, antihae morrhoidal, diuretic, anti-inflammatory, anti-diarrheal. A. unedo fruit too; Gastrointestinal disorders, urological problems, dermatologic problems, kidney diseases, cardio-vascular application (Leonti et al. 2009).

In the literature, the strawberry tree fruits and leaves were characterized by the presence of bioactive substances such as polyphenols, aromatic acids, iridoids, monoter penoids, phenylpropanoids, sterols, triterpenoids and flavonoids (Salema et al. 2018)

In the leaves of A. unedo, different phytochemical compounds are present, such as terpenoids, α-tocopherol, essential oils and phenolic compounds (Elvira et al. 1997). Several components belonging to diverse phenol groups have been reported in Arbutus fruits: phenolic acids, flavonols, flavan-3-ols and galloyl derivatives, and anthocyanins (Miguel et al. 2019).

In this study, total extracts were obtained from Wild Strawberry (Arbutus Unedo) Fruıt and Leaf. Their total phenolic content, ABTS and DPPH activities as antioxidant activity and inhibition effects on Polyphenol Oxidase (PPO) were evaluated.

MATERIALS AND METHODS

Extraction

Fruit sample (1 g for lyophilized) was thoroughly mixed with methanol. The methanolic extract was filtered and evaporated to dryness at 40 °C in a rotary evaporator and dissolved in methanol. These solutions were used for the analysis (Silva et al. 2004).

Prior to the extraction of phenolic compounds, vegetable fats were removed from powdered leaves in a clean-up step. Accordingly, a prepared leaf sample (30 g) was extracted with n-hexane (200 mL) for 24 h in a Soxhlet apparatus. Then, the same leaf sample was air-dried again and phenolic compounds extracted with methanol under the same experimental conditions. The solvent extract was filtered and evaporated to dryness at 40 °C in a rotary evaporator. Finally, residual dry extracts were re-dissolved in methanol prior to analysis (Benzarti et al. 2015).

Phenolic content

The Folin–Ciocalteu reagent assay was used to determine the total phenolics content (Soong et al. 2004). An aliquot of the samples was mixed with of Folin–Ciocalteu reagent previously diluted with distilled water. The solution was allowed to stand at 25°C for 3 min before adding sodium carbonate solution in distilled water. The absorbance at 765 nm was read after initial mixing and up to 60 min until it reached a plateau. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as GAE (mg/g).

Antioxidant activity assay

It has been previously reported that antioxidant capacity determined by in vitro assays may differ from each other (Floege and Kim 2011). Differences between DPPH and ABTS radical scavenging activities can be ascribed to reaction media. The DPPH assay is conventionally conducted under 70% methanol/water, while the ABTS assay is carried out in aqueous conditions. Besides, compounds solubility in both media should be taken in consideration. Certain bioactive compounds may not soluble into reaction media and cannot express radical scavenging activities. Otherwise, the antioxidant capacities of the compounds depend on the mechanism of the assay. While the reaction runs on the cation radical in the ABTS assay, the

reaction runs over free radical in the DPPH assay. Consequently, in this study, these two different methods have been used for the determination of antioxidant capacity.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of fruit and leaf extract was measured according to the method described by Cadi et al. with a few modifications (Cadi et al. 20210). Briefly, different concentration of extracts (0,2 mL) were added to 3 mL of DPPH MeOH:water, 70:30 (v/v) solution. After gentle mixing and 30 min of standing at room temperature, the absorbance of the resulting solutions was measured at 517 nm. The antiradical activity is estimated according to this equation: % of antiradical activity =[(Abs control –Abs sample) / Abs control] 100. IC_{50} values of the extract i.e.the concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

ABTS Radical Scavenging Activity

$ABTS^{\bullet+}$ scavenging activities of the extracts were measured according to the method described by Sönmez et al.. The solution of $ABTS^{\bullet+}$ radical was generated by dissolving 19.2 mg of 2,20 -azino-bis(3- ethylbenzothiazoline-6-sulphonic acid) (7 mM ABTS) and 3.3 mg $K_2S_2O_3$ in distilled water (5 mL). This solution was kept in dark for 24 h at room temperature, and the absorbance of the solution was fixed to ±0.70 at 734 nm by dilution. The solutions of the samples were prepared in methanol at different concentration. The absorbance was measured at room temperature at 734 nm, after 6 min from $ABTS^{\bullet+}$ addition. The decrease in the absorption was used to calculate the activities. The results were expressed as IC_{50}.

PPO activity assay

Enzyme activity was determined; using catechol, by measuring the in crease in absorbance at 420 nm according to the method of Kamkaen et al. and all measurements were taken in duplicate and corrected for the non-enzymatic hydrolysis.

RESULTS AND DISCUSSION

The determined contents of phenolic compounds, antioxidant activity and PPO enzyme inhibition activitiy values are presented in table 1. Among fruit and leaf extracts, leaf extract had the highest phenolic content (226,95 GAE mg/ g extract), followed by fruit extract (49,89 mg/ g extract).

The ABTS method is based on the ability of hydrogen or electron-donating antioxidants to decolourize the performed radical monocation of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) generated due to oxidation of ABTS with potassium persulfate (Re et al. 1999). Leaf exhibited the strongest ABTS activity with IC_{50} values of 2,33 µg/ml.

Additionally, the methanol extract of leaf showed the highest antioxidant activity with IC_{50} value of 25,38 µg/ mL in DPPH assay in this report.

All extract obtained from Arbutus unedo cultivars inhibited the PPO enzyme inhibition activitiy. Additionally, extracts have higher inhibitory activity on leaf than fruit. Extracts (IC_{50}

= 16,90 µg/mL and IC_{50} = 19,25 µg/mL, for leaf and fruit, respectively) was found to guite hıgh inhibitor in this study.

Table 1. Total phenolic content (TPC) and IC_{50} values of DPPH, ABTS activity and PPO enzyme inhibition activity of Arbutus unedo leaf and fruit extracts

Extract	TPC (mg GAE/g extract)	DPPH assay (IC_{50} µg/ml)	ABTS assay (IC_{50} µg/ml)	PPO enzyme inhibition activitiy (IC_{50} µg/ml)
Fruit extract	49,89 ±1,35	53,42±0,06	68,02±7,9	19,25
Leaf extract	226,95±6,35	25,38±0,35	2,33±0,22	16,90

Conclusion

In conclusion, it was determined that the leaf of Arbetus Unedo had a significantly higher phenolic content than fruit. Moreover, the leaf extract showed higher DPPH and ABTS activity than the fruit extract. The leaf extract exhibited stronger PPO inhibitory activity than the fruit extract. The leaf extract have high total phenolic content (226.95 GAE mg/ g extract), strong ABTS (IC_{50}= 2.33 µg/ml) and DPPH (IC_{50} = 25.38 µg/ml) activities as antioxidant property and inhibitory activity against polyphenol oxidase (IC_{50}= 16.90 µg/ml). Therefore, the leaf extract of Arbetus Unedo may be used for protective additives in fruit after some further testing such as cytotoxicity etc.

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USING OF EXTERNAL SECRETIONS OF TRICHODERMA HARZIANUM AND T. VIRIDE AS ANTIFUNGALS TO DIFFERENT TYPES OF PATHOGENIC FUNGI

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ABSTRACT

The study aimed to assess growth inhibitory effect of *Trichoderma harzianum* and *Trichoderma viride* crude extracellular extract in a concentrations of (0.1, 0.5, 1, 2, and 3mg/ml) on common human and plant pathogenic fungi. The human pathogenic fungi (*Trichophyton equinum, Trichphyton simii, Microsporum audouinii, Microsporum boulardii, Microsporum gypseum*) were isolated from 50 patients with various ringworm infections attended the dermatology clinic at Al-Salam teaching hospital in Mosul between Sept. 2010 to Sept. 2011. The phytopathogenic fungi samples (*Alternaria sonchi, Aspergillus nigar, Aspergillus ustus, Cladosporium oxysporum,* and *Penicillium citrinum*) were gathered from black raisins purchased from the local market in Mosul city. The results show that dermatophyte growth rate inhibition began at a concentration of 1mg/ml and peaked at a concentration of 3mg/ml, almost equal to standard control antifungal drug (Ketoconazole). Plant pathogenic fungi was totally inhibited at lower concentration. In general, crude extract of *T.harzinum* is superior to *T. viride* at the same concentratin. In conclusion, crude extracellular extract of *T.harzianum* and *T.viride* inhibits growth rate of human and plant pathogenic fungi in vitro at concentrations ranging from 1-3 mg/ml. More research is required to establish the extract's safety and efficacy in vivo.

Keywords: *Trichoderma harzianum, Trichoderma viride,* crude extracellular extracts, Dermatophyte, plant pathogenic fungi.

INTRODUCTION

Advent of new antimicrobial agents are now widely acknowledged as an essential step to combat the threat of raising rate of antimicrobial resistance (**Clark, 1992**). Microbial resistance is described as the occurrence or development of an infection despite adequate antimicrobial therapy (**Pai *etal*, 2018**). Antifungal resistance continues to grow and evolve even after the advent of many organic antimycotic medications (**Prestinaci et al,2018**). This makes medical care more complex task, especially we are facing raising number of immune-compromised cases (**Schwartz & Patterson, 2018**).

The focus of research continues to be on developing novel antifungal medicines by looking at new potential targets (**Su *et al*, 2018**). The use of natural agents “enemies” to minimize the impact of unfavorable species and favour favorable organisms is referred to as “biocontrol”. This mode of management was used successfully in biological field studies. The application of biological control represents an important alternative approach for controlling many human and phytopathogenic fungi by interfering with their essential nutrient required for their development (**Junaid *etal*, 2013**).

Screening for natural or synthetic materials that suppress fungal growth and disrupting its viability, is a current techniques for finding new antimicrobial drugs (**Mukhopadhyay and Kumar, 2020**). The screening has yield two potentially effective agent (*Trichoderma Harzianum* and *Trichoderma viride*) which have the ability to inhibit other germs (**Gajera *et al,* 2013**). The Trichoderma fungus is soil Ascomycota fungus that can be grown in the lab using Potato Dextrose Agar (PDA) and Corn Meal Dextrose Agar (CMD) at temperatures ranging from 25 to 30 degrees Celsius.Humans started to use these fungi for the secondary metabolite it provides in the field of biological control, since it contains a variety of genes that help break down pathogen cell walls, production of DNA and protein. The releases of the extracellular materials "as biological control factors" protect the soil and plants from infection by a variety of other fungi such. A recent study conducted by Issa conclude that crude chitinase produced by *T. harzianum* was effective in controlling the growth of some pathogenic Dermatophyte like *Microsporum canis* and *Trichophyton mentagrophytes* .

The study aimed to assess growth inhibitory effect of *Trichoderma harzianum* and *Trichoderma viride* crude extracellular extract in a concentrations of (0.1, 0.5, 1, 2, 3mg /ml) on common human and plant pathogenic fungi.

MATERIALS AND METHODS

Human's pathogenic Dermatophyte source:

Between October 2011 and July 2012, Dermatophyte samples were obtained from 50 newly diagnosed patients with various ringworm infections of different cutaneous sites who attended the dermatology clinic at Al-Salam Teaching Hospital in Mosul. Scraped scales, nail fragments, and broken hair were collected from infected areas for direct microscopic identification of fungal elements. The material from confirmed cases were cultured on Sabaroud's dextrose agar (SDA).

Phytopathogenic fungi sources

The phytopathogenic fungi samples were gathered from black raisins. The Raisins were purchased from a local market in Mosul city. The plant surfaces were sterilized with NaOCl for one minute, washed three times with distilled water under aseptic conditions and stored in dry environment. The seeds were cultured on potato dextrose agar medium (PDA) and incubated for seven days with regular observations. Identification of plant fungi was based on taxonomic keys of Leslie et al 1990, Pitt and Hocking in 1985; Mengistu and Sinclair.1979.

Trichoderma harzianum and *Trichoderma viride* sources

Samples of the both fungi were obtained from college of agriculture and forestry/ University of Mosul. The fungi were cultured on a PDA medium, incubated, and multiplied for the current study.

Extraction of the crude extract of *Trichoderma harzianum* and *Trichoderma viride*

Both fungi were multiplied for 2 weeks using pota broth (PDB). Its crude extract were withdrawn from media using piece of quaze. The filtrate was placed in separate funnels using the method **Eziashi et al, 2007**. Then, with good shaking, the same amount (volume/ volume) of butanol is added and left to separate the two layer. The butanol layer containing the fungal

crude extract is removed. In order to extract the butanol, this process is repeated three times. After it has dried, weigh it and dissolve it in a known amount of Dimethyl sulphoxide (DMSO). Standard solutions with known concentration (weight / volume) were prepared from the SGA medium using the dilution rule $N_1V_1 = N_2V_2$ concentrations (0.1, 0.2, 1, 2, 3) mg/volume.

Evaluation of impact of Trichoderma crude extract on human and phytopathogenic fungi

A 7 days old fungal cultures incubated at a temp. (25±2Cº) were cultured for another two weeks and prepared for the in vitro evaluation of effectiveness of Trichoderma crude extract. They were incubated at 30 and for two weeks. A specimen of 5mm was taking from the outer edges of the fungal colonies using cork drill under aseptic conditions. Each colony specimen put it in the middle of dish containing Sabouraud dextrose agar (SDA). The agar was either mixed with one of the concentration of trichoderma extract, ketoconazole drug, or used alone as control group. Three replicates were used for each fungus and all steps were performed under sterilization conditions, then the dishes were incubated at 30 degrees for a period of 14 days(**Kwon-Chung and Bennett, 1992**).The results were taken by calculating the average measurement of each two perpendicular drops for each fungal colony **(Pitt and Hocking, 1997)**. Three replicates for each treatment concentration were prepared. Radial growths of the human and phytopathogenic fungi were recorded. Inhibition percent (%) of fungal growth was calculated as follows:

L= (c-t)/ c x 100

L = Inhibition percentage of radial growth
C = Measurement of radial growth of fungi in control (standard plate)
T= Radial growth of the investigated fungus in the presence of Trichoderma crude extract (Edington et al., 1971).

Statistical analysis

The analysis was performed by the use of software SPSS ver 26. Discreptive methods were used to tabulate and summarize data. ANOVA test was used to assess the significance of differences in radial growth diammeters of human and plant fungi at different concentration of crude extract. A p–value ≤0.05was considered significant.

RESULTS

Effect of *trichoderma harzianum* crude extract on human pathogenic fungi (Dermatophyte species)

Table 1 demonstrates the effect of various concentrations of *Trichoderma harzianum* crude extract on radial growth of different Dermatophyte species isolated from patients with a variety ringworm infections. With increasing crude extract concentrations, there was an apparent incremental increase in inhibitory impact on Dermatophyte colony diameters.

Table 1. Effect of different concentration of *Trichoderma harzianum* crude extract on average colony diameter (cm) of different Dermatophyte species

Dermatophyte spp.	*Trichoderma harzianum* crude extrate concentration					Standard x̄±SD	Keto conazole x̄±SD	P-value
	0.1mg/ml x̄±SD	0.5 mg/ml x̄±SD	1 mg/ml x̄±SD	2 mg/ml x̄±SD	3 mg/ml x̄±SD			
T. equinum	4.33±0.57	3. 30±0.26	1.66±0.51	0.13±0.15	0±0	8.66±0.57	0	<0.0001
T. simii	5.33±0.58	3.86±0.41	1.36±0.49	0	0±0	6.33±0.58	0	<0.0001
M. audouinii	1.70±0.43	1.23±0.49	1.33±0.66	0.70±0.10	0±0	5.03±0.16	0	<0.0001
M. boulardii	3.66±58	2.93±0.90	1.96±0.15	0.96±0.25	0.16±0.21	6.00±1.00	0	<0.0001
M. gypseum	1.90±0.90	1.77±0.71	1.33±0.66	1.13±0.15	0	6.33±0.57	0	<0.0001

Each value is mean & SD of three replica, T. denote Trichophyton, M denote Microsporum species

The total inhibition of each dermatophyte species under investigation is shown in Table 2. The results show that at a concentration of 3 mg/ml, total growth inhibition of three dermatophyte species (*Trichophyton equinum*, *Microsporum audouinii*, and *Microsporum gypseum*) occurred (100 percent inhibition); *T. simii* needed a lower concentration of 2 mg/ml to be fully inhibited; *M. boulardii*, on the other hand, was more resistant and only partially inhibited even at a concentration of 3 mg/ml (inhibition rate was 97.3 percent).

Table2. Effect of different concentration of *Trichoderma harzianum* crude extract on growth inhibition percentages of different Dermatophyte species

Dermatophyte spp.	*Trichoderma harzianum* crude extrate concentration				
	0.1mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml
T. equinum	50.0%	61.9%	80.8%	98.5%	100.0%
T. simii	15.8%	39.0%	78.5%	100.0%	100.0%
M. audouinii	60.2%	75.4%	73.3%	86.1%	100.0%
M. boulardii	39%6	51.1%	67.3%	84.0%	97.3%
M. gypseum	70.0%	72.0%	79.0%	97.9%	100.0%

Each value is % of growth inhibition of three replica, T. denote Trichophyton, M denote Microsporum species

Figure 1 shows how increasing the concentrations of crude extract of *T harzianum* causes a gradual decrease in the radial growth of fungal colonies. *T. simii, T. equinum, M. audouinii, M. boulardii*, and finally *M. gypseum* were the most sensitive to the extract as opposed to the control group. The inhibitory effect of 1 & 2 mg/ml *Trichoderma harzianum* crude extract on the radial growth of *Microsporum gypseum* compared to the standered is depicted in picture 1.

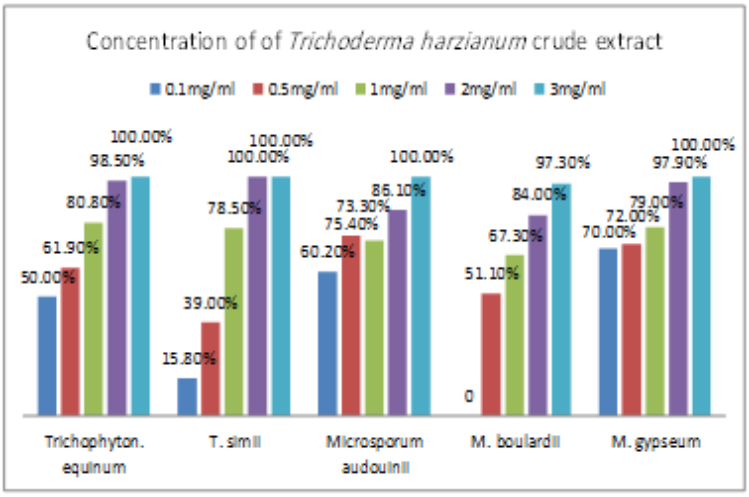
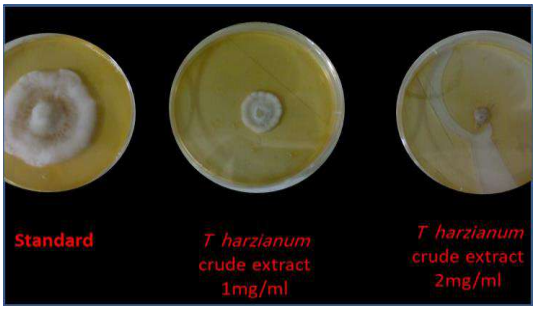


Figure 1. Effect of different concentrations of *Trichoderma harazianum* crude extract on growth inhibition percentages of different Dermatophyte species



Picture (1) The inhibitory effect of Trichoderma harzianum crude extract on the growth of Microsporum gypseum at concentrations of 1 & 2 mg / ml

1. Effect of *trichoderma viride* crude extract on human pathogenic fungi (Dermatophyte species)

Table (3) reveals that crude extract of the fungus *T. viride* have a growth inhibitory effect on the radial diameters of the investigated dermatophyte colonies. The percentage of inhibiting the growth of fungal colonies is shown in Table (4). The growth of the investigated fungal colonies were fully inhibited at a concentration of 3 mg/ml. The M. boulardii was more vulnerable to the inhibitory effect of crude extract of *T. viride* in comparison to *T. harzianum*. Figure 2 depicts that *M. audouinii* was the most sensitive to *T. viride* crude extract, followed by *T. equinum*, and finally *T. simii*. Both *M. boulardii and M. gypseum* were inhibited at the same degree (see picture 2). The findings indicate that *T. viride* crude extract has a stronger inhibitory effect on Dermatophyte species compared to that induced by *T. harzianum* crude extract. Table 3. Effect of different concentrations of *Trichoderma viride* crude extract on average colony diameter (cm) of different Dermatophyte species. . The inhibitory action of crude extract of both The *T. harzianum* has a stronger inhibitory effect on *T. simii* (100% inhibition at 2mg/ml) compared to *T. viride* (100% inhibition occurred at 3mg/ml).

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Table3. Effect of different concentration of *Trichoderma viride* crude extract on average colony diameter (cm) of different Dermatophyte species

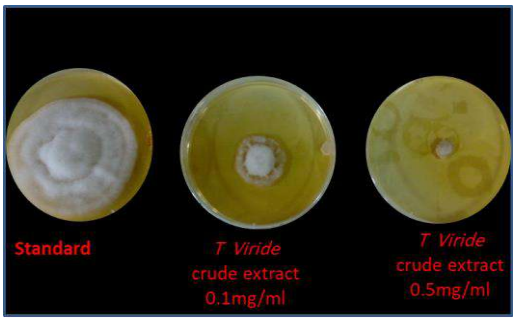
Dermatophyte spp	Trichoderma viride crude extrate concentration					Standard x̄±SD	Keto-conazole x̄±SD	P-value
	0.1mg/ml x̄±SD	0.5 mg/ml x̄±SD	1 mg/ml x̄±SD	2 mg/ml x̄±SD	3 mg/ml x̄±SD			
T. equinum	4.26±0.37	2.76±1.01	1.30±0.62	0.55±0.43	0	5.33±0.58	0	<0.0001
T. simii	3.36±035	1.80±0.72	1.20±0.46	0.93±0.45	0	6.33±0.57	0	<0.0001
M. audouinii	4.20±1.05	3.83±0.15	1.66±0.41	0.53±0.35	0	5.03±0.15	0	<0.0001
M .boulardii	4.1320	4.66±0.49	3.00±0.20	1.70±0.26	0	5.66±0.57	0	<0.0001
M. gypseum	4.13±0.32	3.53±0.50	1.80±0.20	1.03±0.45	0	2.93±0.11	0	<0.0001

Each value is mean & SD of three replica, T denote for Trichophyton, M denote Microsporum

Table 4. Effect of different concentrations of *Trichoderma viride* crude extract on growth inhibition percentages of different Dermatophyte species

Dermatophyte spp.	Trichoderma viride crude extrate concentration				
	0.1mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml
T. equinum	20.1%	48.2%	75.6%	89.7%	100%
T. simii	46.9%	70.3%	81.04%	85.3%	100%
M. audouinii	16.5%	23.8%	67.0%	89.4%	100%
M. boulardii	27.0%	17.6%	47.0%	67.0%	100%
M. gypseum	16.2%	28.4%	63.5%	79.1%	100%

Each value is mean of three replica, T denote Trichophyton, M denote Microsprum



Picture (2) The inhibitory effect of *Trichoderma viride* crude extract on the growth of Microsporum gypseum at concentrations of 0.1 & 0.5 mg / ml

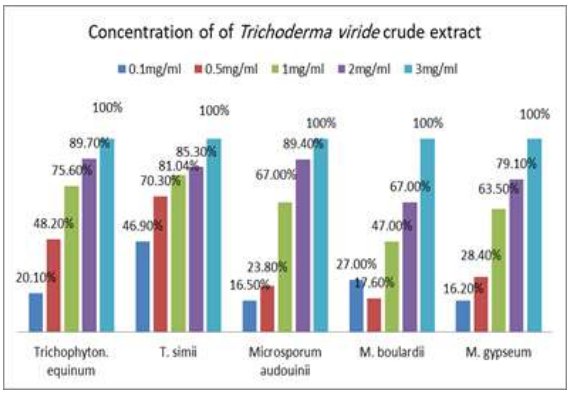
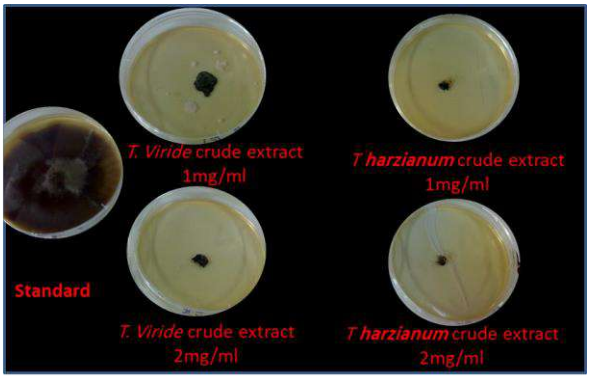


Figure 2. Effect of different concentrations of *Trichoderma viride* crude extract on growth inhibition percentages of different Dermatophyte spec



Picture (3): The inhibitory effect of crude extract of both *Trichoderma harzianum* and Tr*ichoderma viride* against the *T simii* at concentrations of 1 and 2 mg/ml.

2. Effect of *trichoderma harzianum* crude extract on phytopathogenic fungi

The effect of *Trichoderma harzianum*'s crude extract fungi on radial growth diameter of various fungi isolated from dried grapes (raisins) is shown in Table (5). Their percentage of inhibition is demonstrated in table (6). The results revealed that the growth of *Aspergillus niger* and *A ustus* were totally inhibited at a concentration of 1,g/ml. The rest of phytopathogenic fungi were inhibited at a concentration of 2mg/ml. The results further elaborate superior inhibitory impact of *T harzianum* crude extract against phytopathogenic fungi compared to its effect on human pathogenic Dermatophyte species.

Table 5. Effect of different concentrations of *Trichoderma harzianum* crude extract on average colony diameter (cm) of different phytopathogenic fungi.

Phtopathogenic fungi	*Trichoderma harzianum* crude extrate concentration					Standard x̄±SD	Keto-conazole x̄±SD	P-value
	0.1mg/ml x̄±SD	0.5 mg/ml x̄±SD	1 mg/ml x̄±SD	2 mg/ml x̄±SD	3 mg/ml x̄±SD			
A. sonchi	3.73±0.25	1.72±0.25	0.90±0.10	0	0	3.96±0.05	0	<0.0001
A. Nigar	1.63±1.10	1.50±0.40	0	0	0	4.66±0.76	0	<0.0001
A. ustus	3.26±0.75	2.66±0.35	0	0	0	5.73±0.23	0	<0.0001
C. oxysporum	1.70±1.37	1.40±0.52	0.63±0.35	0	0	4.13±1.00	0	<0.0001
P. citrinum	2.50±0.50	1.93±0.55	0.63±0.32	0	0	4.33±0.57	0	<0.0001

Each value is mean & SD of three replica, Firs A is for Alternaria , second A for Aspergillus , C for Cladosprium, and P for Penicillium

Table 6. Effect of different concentration of *Trichoderma harzianum* crude extract on growth inhibition percentages of different phytopathogenic fungi.

Phytopathogenic fungi	Trichoderma harzianum crude extrate concentration				
	0.1mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml
A sonchi	24.8%	53.7%	81.8%	100.0%	100.0%
A. Nigar	86.4%	67.8%	100.0%	100.0%	100.0%
A. ustus	43.1%	53.5%	100.0%	100.0%	100.0%
C.oxysporum	58.8%	84.7%	84.7%	100.0%	100.0%
P. citrinum	42.2%	55.4%	85.4%	100.0%	100.0%

Table (6) shows that *Aspergillus niger* and *Aspergillus ustus* were among the most susceptible fungi, as their radial growth was completely inhibited at 1mg/ml. Furthermore, as the concentration of the extract increased, the formation of black fungal spores decreased noticeably, and the colony's color became whitish.

Picture (4) The inhibitory effect of *Trichoderma harzianum* crude extract on the growth of *Aspergillus niger* at concentrations of o.1 & 0.5 mg / ml.

The growth of the rest phytopathogenic fungi (*A. sonchi*, *C. oxysporum* and *P. citrinum*) were totally inhibited at a concentration of 2 mg / ml. Figure (3) depict the inhibitory effect of

different concentration of *T. harzianum* crude extract concentrations compared to the control for different fungi.

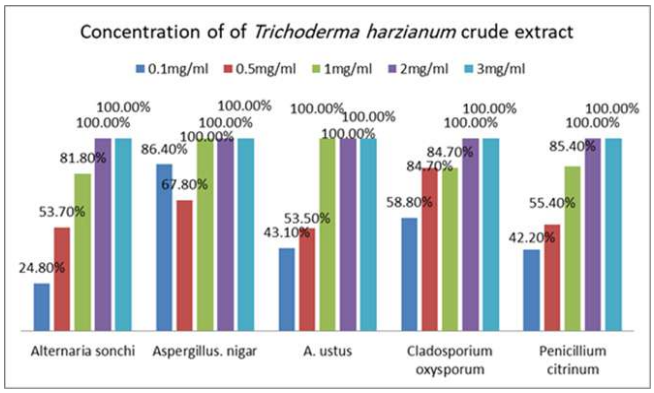


Figure 3. Effect of different concentrations of *Trichoderma harazianum* crude extract on growth inhibition percentages of different phytopathogenic fungi

3. Effect of *trichoderma viride* crude extract on phytopathogenic fungi

Table (7) reveals that the inhibitory action of *T. viride* on fungi isolated from raisins follows the same pattern as *T. harazianum*. The fungi *Aspergillus niger* and *Aspergillus ustus* were among the most susceptible, with their growth fully inhibited at 1 mg/ml. The remaining fungi need a concentration of 2mg/ml to be toatally inhibited. Figure (4) depicts the variations in the degree of inhibition of fungal colonies in different concentrations. The effect of crude extract of *T. viride* on the *A. sonchi* at concentrations of 0.1 and 0.5 mg / ml is shown in figure 5.

Table 7. Effect of different concentrations of Trichoderma viride crude extract on average colony diameter (cm) of different phytopathogenic fungi

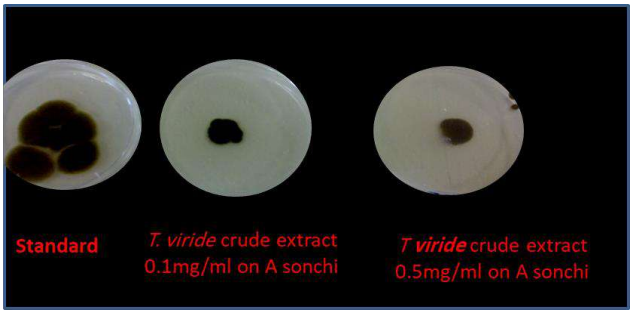
Phtopathogenic fungi	Trichoderma viride crude extrate concentration					Standard x̄±SD	Keto-conazole x̄±SD	P-value
	0.1mg/ml x̄±SD	0.5 mg/ml x̄±SD	1 mg/ml x̄±SD	2 mg/ml x̄±SD	3 mg/ml x̄±SD			
A. sonchi	1.06±0.22	0.76±0.32	0.46±0.25	0	0	4.66±0.57	0	<0.0001
A. nigar	2.60±0.60	2.40±0.52	0	0	0	5.66±1.15	0	<0.0001
A ustus	2.90±1.10	2.40±0.52	0	0	0	5.70±1.00	0	<0.0001
C. oxysporum	1.83±0.56	0.96±0.05	0.56±0.05	0	0	4.66±1.52	0	<0.0001
P. citrinum	3.03±0.89	1.76±0.75	1.60±0.53	0	0	5.33±0.34	0	<0.0001

Each value is mean & SD of three replica, Firs A is for Alternaria , second A for Aspergillus , C for Cladosprium, and P for Penicillium

Table 8. Effect of different concentrations of *Trichoderma viride* crude extract on growth inhibition percentage of different phytopathogenic fungi

Phytopathogenic fungi	Trichoderma viride crude extrate concentration				
	0.1mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	3 mg/ml
A. sonchi	77.2%	83.7%	90.1%	100.0%	100.0%
A. nigar	54.1%	57.6%	100.0%	100.0%	100.0%
A. ustus	49.1%	57.9	100.0%	100.0%	100.0%
C. oxysporum	60.7%	79.4%	88.0%	100.0%	100.0%
P. citrinum	43.1%	67.0%5.	70.0%	100.0%	100.0%

Each value is % inhibition of growth of three replica



Picture (5) The inhibitory effect of *Trichoderma viride* crude extract on the growth of *Alternaria sonchi* at concentrations of o.1 & 0.5 mg / ml.

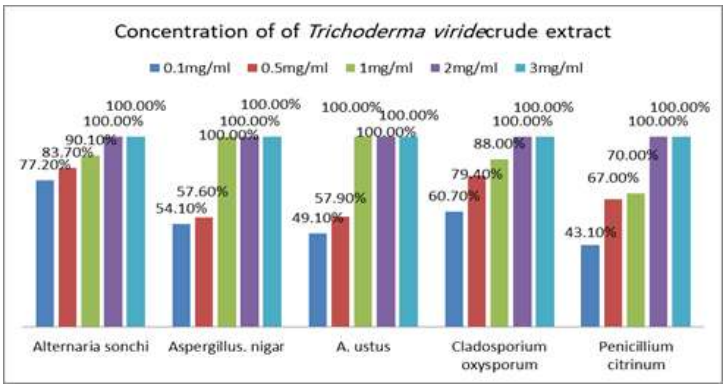
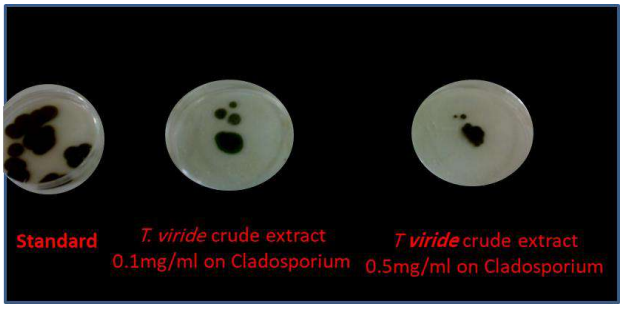


Figure 4. Effect of different concentrations of *Trichoderma viride* crude extract on growth inhibition percentages of different phytopathogenic fungi

Image (6) shows the effect of the Trichoderma viride crude extract against *Cladosporium oxysporum* at a concentration of 0.1 & 0.5 mg/ml. The picture show agradual raise in inhibition with increase concentration of the extract.



Picture (6) The inhibitory effect of *Trichoderma viride* crude extract on the growth of ***Cladosporium oxysporum*** at concentrations of o.1 & 0.5 mg / ml.

DISCUSSION

This research adds to the growing body of evidence that the "natural biocontrol" and echo-friendly Trichoderma species have antagonistic capacity against new series of both human and plant pathogenic fungi species like (*Trichophyton equinum, Trichphyton simii, Microsporum audouinii, Microsporum boulardii, Microsporum gypseum* as a common dermatohphytes) and (*Alternaria sonchi, Aspergillus nigar, Aspergillus ustus, cladosporium oxysporum,* and *Penicillium citrinum* as a common phytopathogens). The *T. harzianum* and *T. viride* antagonized the tested pathogens via a variety of pathways, one of them is the releasing of extracellular lytic enzymes thought to play a role in mycoparasitism. The inhibitory effect of crude extract of (*T. harzianum* and *T. viride*) against plant pathogenic fungi was tested by **Al-Obaidy and Al-Rijabo (2010)**. They observed the dominance of *T.harzianum* growth on *Alternaria alternate* as well as when grown with *Fusarium graminearum* and *Penovicillium*. They attribute the antagonistic capability to hydrolytic enzymes or compounds that may hydrolyse these phytopathogen. The also report the failure of inhibition when it was tested against *Aspergillus flavus* and *A.niger*. Later, researchers **Al-Rijabo and Al- Obaidy (2011)** looked at the impact of *T. harzianum* and *T. viride* on certain human dermatophytes in another study. *Trichophyton rubrumm, T. verrucosum, T. mentagrophytes, T. schoenleinii,* and *T. terrestre* were all inhibited by the their extract , but at different concentration.

The influence of *T. harzianum* and *T. viride* crude extract improved with rising concentrations of extract added to the culture of tested human and plant pathogenic fungi, which was consistent with the findings of **Kadhim et al. (2020)**. Furthermore, *T. harzianum* had better efficacy than *T. viride* in general, particularly at higher concentrations, which resulted in the best control.

CONCLUSION

Trichoderma harzianum and *T. viride* crude extract concentrations have a potent antifungal efficacy against human Dermatophytes (*Trichophyton equinum, Trichphyton simii, Microsporum audouinii, Microsporum boulardii,* and *Microsporum gypseum*) and phytopathogenic fungi (*Alternaria sonchi, Aspergillus nigar, Aspergillus ustus, cladosporium oxysporum,* and *Penicillium citrinum*). The results may open up new avenues for treating resistant cases of fungal infections using a "natural route" alone or in conjunction with recognized antifungal medications.

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Table 1. Formulation and ingredients inserted on the label of frying oil samples.

Samples	Type of oils	Antioxidants (ppm)		Citric acid	Anti-foam
		TBHQ	AP		
Palm-free samples					
PF1	CO,CNO,SFO	75	-	100ppm	Without
PF2	CO,SFO,HOSFO	75	-	100ppm	Without
PF3	SFO,SBO,CNO	75	-	100ppm	With 3ppm
PF4	SFO,SSO,CNO	70	-	50ppm	Without
PF5	SFO,CO,CNO	75	100	With (Uncertain)	With (Uncertain)
PF6	SSO,RBO,SFO,SBO,CNO	70	-	100ppm	With 3ppm
PF7	SFO,SBO,CNO	75	-	100ppm	With 3ppm
PF8	CO,SFO,HOSFO	75	-	100ppm	Without
PF9	SFO,HOSFO,CNO	75	-	100ppm	Without
PF10	SFO,HOSFO,CNO	75	-	100ppm	Without
Palm-based samples					
PB1	PO,SFO,CNO	75	-	100ppm	Without
PB2	PO,SFO,CNO,SBO	75	100	100ppm	Without
PB3	PO,SFO,CNO,SBO	75	-	100ppm	Without

SFO, sunflower oil; CNO, canola oil; COO, corn oil; HOSFO, high-oleic sunflower oil; SBO, soybean oil; SSO, sesame oil; RBO, rice bran oil; TBHQ, tertiary butyl hydroquinone; AP, Ascorbyl palmitate.

Table 2. Fatty acid composition, refractive index (at 40 °C) and iodine value of frying oil samples.

Fatty acid Composition (%)	*Frying oil samples*														
	PF1	*PF2*	*PF3*	*PF4*	*PF5*	*PF6*	*PF7*	*PF8*	*PF9*	*PF10*	*Mean* $_{PFs}$	*PB1*	*PB2*	*PB3*	*Mean* $_{PBs}$
C14:0	0.07±0.00	0.05±0.00	0.06±0.00	0.05±0.00	0.59±0.00	0.12±0.00	0.06±0.00	0.05±0.00	0.05±0.00	0.07±0.00	0.11	0.20±0.00	0.43±0.11	0.26±0.01	0.30
C16:0	5.81±0.12	5.73±0.08	6.32±0.17	10.82±0.15	8.45±0.23	9.32±0.10	6.05±0.22	6.07±0.11	5.30±0.13	5.45±0.11	6.93	10.83±0.10	18.37±0.25	12.77±0.11	13.99
C16:1c	0.14±0.05	0.03±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.03	0.16±0.00	0.14±0.00	0.18±0.01	0.16
C17:0	0.02±0.00	0.02±0.00	0.01±0.00	0.06±0.00	0.06±0.00	0.06±0.00	0.07±0.00	0.02±0.00	0.02±0.00	0.03±0.00	0.03	ND	0.06±0.00	0.05±0.00	0.06
C17:1c	0.05±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.04±0.00	0.02±0.00	0.03	ND	0.04±0.00	0.04±0.01	0.04
C18:0	2.73±0.08	2.55±0.04	2.95±0.04	3.23±0.08	3.48±0.07	3.66±0.13	3.40±0.07	2.82±0.10	2.66±0.08	2.69±0.08	3.01	3.13±0.10	4.02±0.10	3.50±0.10	3.55
C18:1t	0.22±0.09	0.06±0.06	0.06±0.00	ND	0.02±0.00	0.07±0.00	0.34±0.07	0.23±0.02	0.06±0.03	0.07±0.00	0.12	0.08±0.01	0.05±0.00	0.19±0.01	0.11
C18:1c	43.25±0.23	54.94±0.10	38.45±0.17	30.18±0.10	26.33±0.22	32.20±0.18	29.26±0.21	49.82±0.23	48.61±0.11	48.80±0.25	40.18	42.92±0.23	32.76±0.11	43.29±0.11	39.66
C18:2t	0.26±0.02	0.26±0.00	0.32±0.04	0.41±0.03	0.31±0.07	0.62±0.04	0.78±0.07	0.38±0.06	0.14±0.03	0.16±0.02	0.36	ND	0.36±0.09	ND	0.36
C18:2c	42.69±0.14	30.04±0.10	45.66±0.16	50.71±0.20	55.71±0.27	47.82±0.23	55.79±0.24	36.23±0.26	37.21±0.10	36.77±0.23	43.86	38.07±0.20	39.31±0.16	36.29±0.08	37.89
C20:0	0.21±0.02	0.39±0.03	0.42±0.08	0.45±0.04	0.35±0.04	0.47±0.03	0.41±0.02	0.54±0.04	0.40±0.08	0.41±0.10	0.40	0.40±0.06	0.30±0.02	0.53±0.02	0.41
C18:3t	0.16±0.03	0.28±0.00	0.24±0.00	0.30±0.02	0.25±0.02	0.23±0.01	0.32±0.04	0.13±0.02	0.20±0.00	0.18±0.02	0.23	ND	0.29±0.02	0.16±0.02	0.23
C18:3c	2.36±0.00	3.29±0.05	3.71±0.03	2.83±0.02	2.77±0.08	3.92±0.07	2.18±0.05	2.17±0.07	3.24±0.04	3.22±0.06	2.96	2.48±0.02	2.75±0.03	1.40±0.05	2.21
C20:1	0.59±0.07	0.63±0.02	0.61±0.02	0.15±0.07	0.21±0.00	0.44±0.05	0.64±0.04	0.67±0.07	0.65±0.06	0.06±0.00	0.46	0.49±0.02	0.51±0.07	0.44±0.07	0.48
C22:0	0.52±0.03	0.84±0.04	0.50±0.03	0.34±0.03	0.50±0.00	0.44±0.00	0.46±0.03	0.55±0.07	0.54±0.02	0.54±0.02	0.52	0.43±0.05	0.29±0.00	0.53±0.12	0.42
C24:0	0.21±0.00	0.30±0.04	0.14±0.02	0.13±0.03	0.18±0.06	0.17±0.04	0.17±0.02	0.18±0.03	0.19±0.03	0.19±0.05	0.18	0.13±0.00	0.09±0.00	0.15±0.00	0.12
TFA	0.64	0.60	0.61	0.71	0.58	0.92	1.46	0.74	0.40	0.41	0.70	0.54	0.69	0.35	0.53
SFA	9.57	9.88	10.40	15.08	13.61	14.24	10.62	10.23	9.16	9.38	11.21	15.12	23.78	17.26	18.72
Total	99.29	99.44	99.51	99.72	99.25	99.60	99.99	99.91	99.32	98.68	-	99.31	99.76	99.78	-
RI (at 40°C)	1.46607	1.46502	1.46647	1.46642	1.46707	1.46661	1.46659	1.46562	1.46566	1.46560	1.46611	1.46488	1.46189	1.46197	1.46291
IV	119.62	111.02	122.89	122.47	127.80	124.04	124.00	116.00	116.10	115.77	120.09	109.87	103.90	104.70	106.16

Values are shown as mean ± standard deviation (n=3); TFA, *trans* fatty acids; SFA, saturated fatty acids (sum of C14:0, C16:0, C17:0, C18:0, C20:0 and C24:0); USFA, unsaturated fatty acid (sum of C16:1c, C17:1c, C18:1c, C18:2c, C18:3c and TFA); RI, refractive index; IV, iodine value.

Table 3. Qualitative parameters of the frying oil samples.

Frying oil samples	Moisture content (%)	Soap content (ppm)	Lovibond Color				FFA (%)	PV (meqO2/kg)	p-AV	TV
			Red	Yellow	Blue	Chlorophyll				
PF1	0.08±0.01+	0+	3.80±0.10	55.00±0.20	0	0.02±0.00	0.046±0.030+	2.90±0.30-	5.77±0.20+	11.57
PF2	0.07±0.00+	0+	2.00±0.00	28.20±0.10	0	0.04±0.00	0.051±0.004+	1.65±0.24+	4.96±0.30+	8.26
PF3	0.09±0.02+	0+	2.60±0.20	27.00±0.00	0	0.02±0.00	0.038±0.020+	1.74±0.18+	5.29±0.10+	8.77
PF4	0.08±0.01+	0+	2.80±0.10	36.00±0.00	0	0.02±0.00	0.068±0.004+	2.65±0.30-	6.39±0.25-	11.69
PF5	0.09±0.01+	0+	3.00±0.10	48.20±0.30	0	0.03±0.00	0.046±0.005+	3.10±0.21-	6.84±0.11-	13.04
PF6	0.07±0.01+	3.00±0.0+	1.90±0.00	24.00±0.00	2.00±0.00	0.06±0.00	0.055±0.003+	2.36±0.10-	6.58±0.16-	11.30
PF7	0.08±0.00+	0+	1.50±0.00	22.00±0.00	0	0	0.068±0.005+	1.54±0.24+	3.30±0.14+	6.38
PF8	0.07±0.01+	0+	2.10±0.00	26.10±0.20	0	0.04±0.00	0.062±0.003+	1.28±0.04+	5.60±0.18+	8.16
PF9	0.06±0.01+	0+	2.10±0.00	32.50±0.30	0	0	0.046±0.002+	1.45±0.06+	4.55±0.30+	7.45
PF10	0.06±0.01+	0+	1.90±0.00	31.00±0.00	0	0	0.049±0.002+	1.42±0.17+	3.85±0.15+	6.69
Mean of $_{PFs}$	0.069	0.30	2.30	33.00	0	0.02	0.049	2.00	5.31	10.19
PB1	0.08±0.00+	0+	2.50±0.00	30.00±0.00	0	0	0.038±0.002+	1.02±0.11+	5.20±0.17+	7.24
PB2	0.06±0.01+	0+	2.00±0.00	35.00±0.00	0	0	0.058±0.003+	0.92±0.07+	7.54±0.14-	9.34
PB3	0.06±0.00+	0+	2.10±0.00	30.00±0.00	0	0	0.046±0.004+	0.96±0.03+	5.64±0.20+	7.56
Mean of $_{PBs}$	0.06	0	2.20	31.66	0.66	0	0.047	0.096	6.12	8.04

Values are shown as mean ± standard deviation (n=3); FFA, free fatty acids. PV, peroxide value; p-AV, p-Anisidine value; TV, TOTOX value. Symbols of + and – indicate that whether or not the parameters are in the standard rang.

Table 4. Synthetic phenolic antioxidants contents of the frying oil samples.

Samples	TBHQ (ppm)	BHT (ppm)	BHA (ppm)	Total synthetic phenolic antioxidants (ppm)
Palm-free samples				
PF1	113.18±0.05-	ND	ND	113.18+
PF2	68.95±0.03+	ND	ND	68.95+
PF3	105.97±0.02-	ND	ND	105.97+
PF4	98.50±0.07-	32.12±0.01+	92.5±0.06+	223.12-
PF5	121.97±0.00-	ND	ND	121.97+
PF6	84.69±0.01-	79.00±0.02-	89.47±0.01+	253.16-
PF7	110.23±0.08-	ND	ND	110.23+
PF8	72.30±0.03+	ND	ND	72.30+
PF9	74.01±0.04+	ND	ND	74.01+
PF10	75.09±0.03+	ND	ND	75.09+
Palm-based samples				
PB1	74.59±0.04+	ND	ND	74.59+
PB2	79.70±0.05-	ND	ND	79.70+
PB3	72.89±0.02+	ND	ND	72.89+

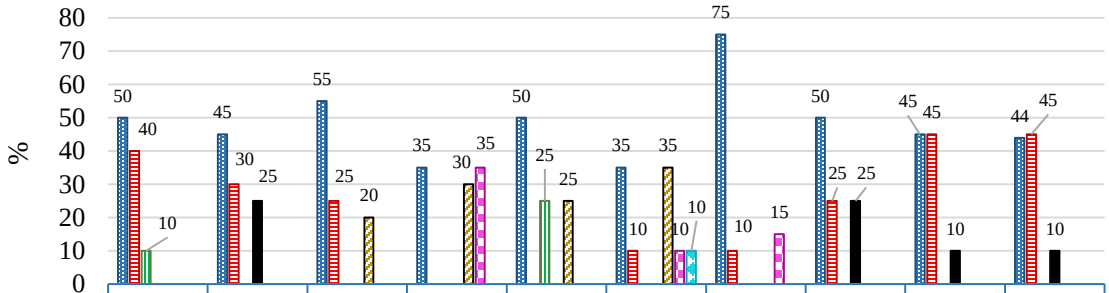
Values are shown as mean ± standard deviation (n=3). Symbols of + and – indicate that whether or not the parameters are in the standard rang. ND, not detected.

Table 5: Chemical properties of the frying oil samples after deep frying.

Samples	FFA (%)	PV (meq O2/kg)	p-AV	TV	TPC (%)
PF1	0.72±0.01+	8.25±0.09	16.89±0.81	33.39	12.36±1.12+
PF2	0.38±0.03+	7.51±0.08	11.46±0.95	26.48	10.16±2.23+
PF3	0.82±0.00+	13.24±0.06	16.47±1.14	43.95	17.57±3.07+
PF4	1.29±0.04-	15.62±0.11	21.72±0.86	52.96	19.44±2.34+
PF5	1.08±0.06-	16.20±0.09	24.52±0.71	56.92	20.11±1.76+
PF6	0.96±0.03+	14.05±0.08	15.20±0.63	43.30	17.19±2.61+
PF7	1.13±0.01-	13.46±0.07	17.28±0.76	44.20	17.65±1.88+
PF8	0.42±0.04+	8.53±0.09	12.23±1.21	29.29	11.44±1.43+
PF9	0.35±0.01+	9.23±0.12	12.41±0.60	30.87	12.23±1.36+
PF10	0.44±0.02+	8.46±0.06	11.94±0.70	28.86	11.73±2.01+
Mean of $_{PFs}$	0.76	11.45	16.01	39.02	14.98
PB1	0.63±0.04+	9.11±0.08	16.62±1.32	32.84	12.86±1.72+
PB2	0.52±0.03+	7.26±0.06	11.85±0.64	24.37	9.97±1.33+
PB3	0.47±0.02+	12.25±0.07	12.16±1.10	34.66	13.19±2.08+
Mean of $_{PBs}$	0.54	9.54	13.54	30.62	12.00

Values are shown as mean ± standard deviation (n=3). FFA, free fatty acids. PV, peroxide value; p-AV, p-Anisidine value; TV, TOTOX value. Symbols of + and – indicate that whether or not the parameters are in the standard. ND, not detected.

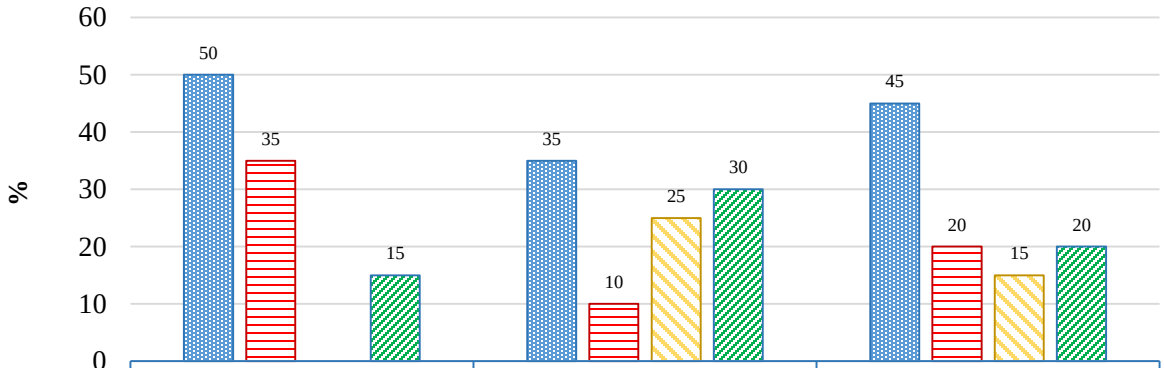
a



	PF1	PF2	PF3	PF4	PF5	PF6	PF7	PF8	PF9	PF10
SFO (%)	50	45	55	35	50	35	75	50	45	44
CNO (%)	40	30	25			10	10	25	45	45
COO (%)	10				25					
HOSFO (%)		25						25	10	10
SBO (%)			20	30	25	35				
SSO (%)				35		10	15			
RBO (%)						10				

Estimated Formulations

b



	FO1	FO2	FO3
SFO (%)	50	35	45
CNO (%)	35	10	20
SBO (%)		25	15
PO (%)	15	30	20

Estimated Formulations

Fig 1. Estimated formulations of palm-free (a) and palm-based (b) frying oil samples based on their fatty acid composition and iodine value.

SFO, sunflower oil; CNO, canola oil; COO, corn oil; HOSFO, high-oleic sunflower oil; SBO, soybean oil; SSO, sesame oil; RBO, rice bran oil; and PO, palm olein.

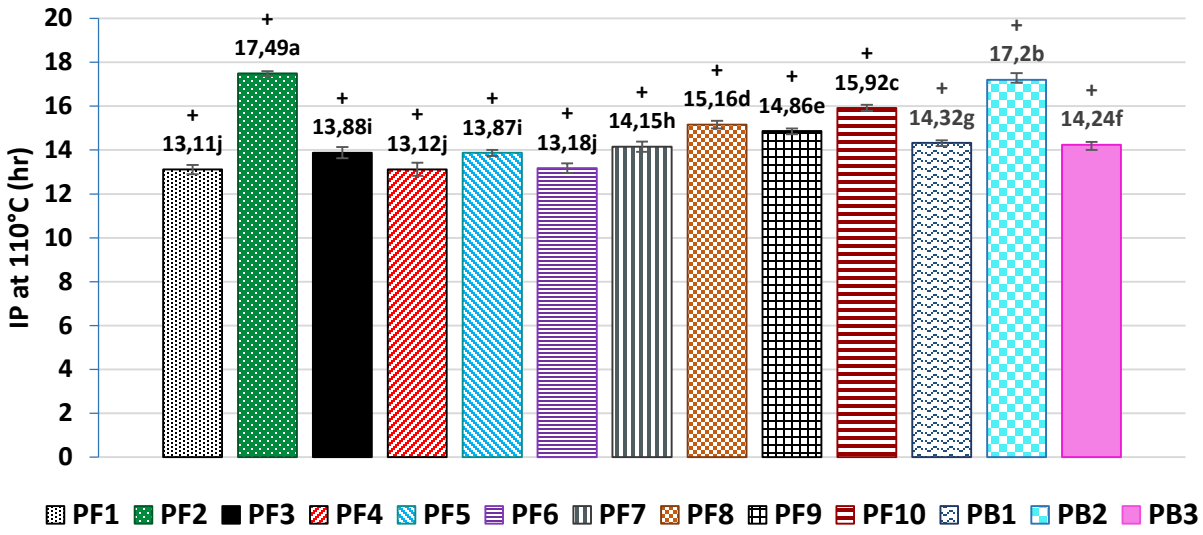
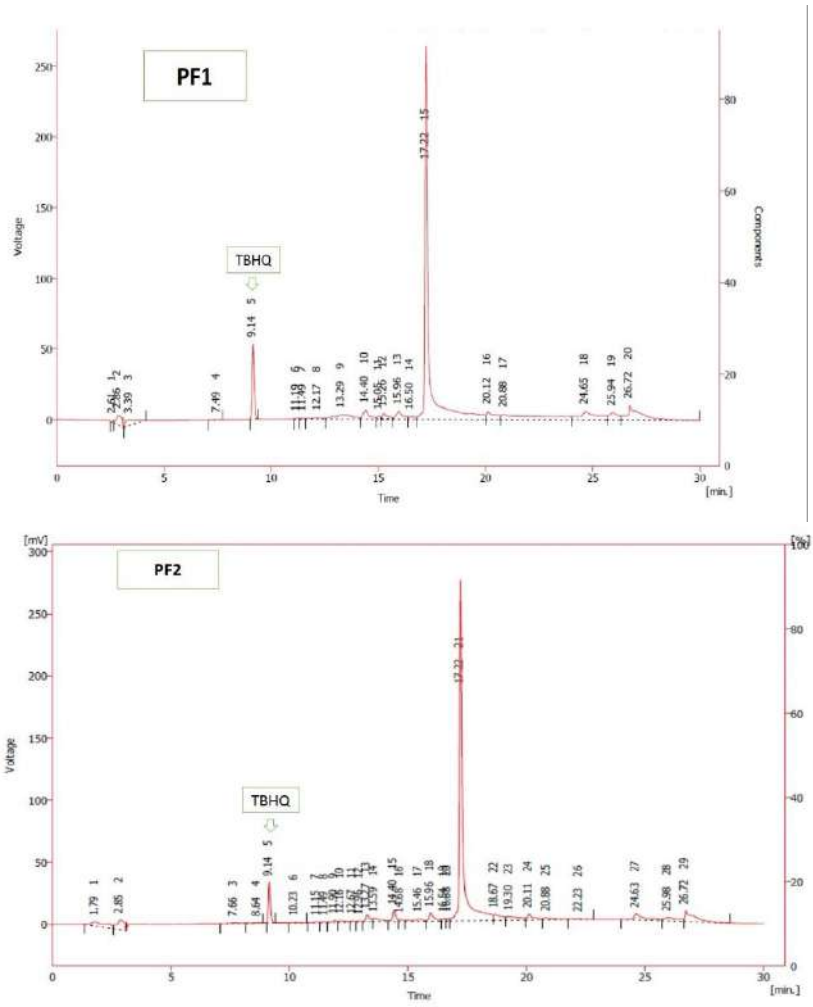
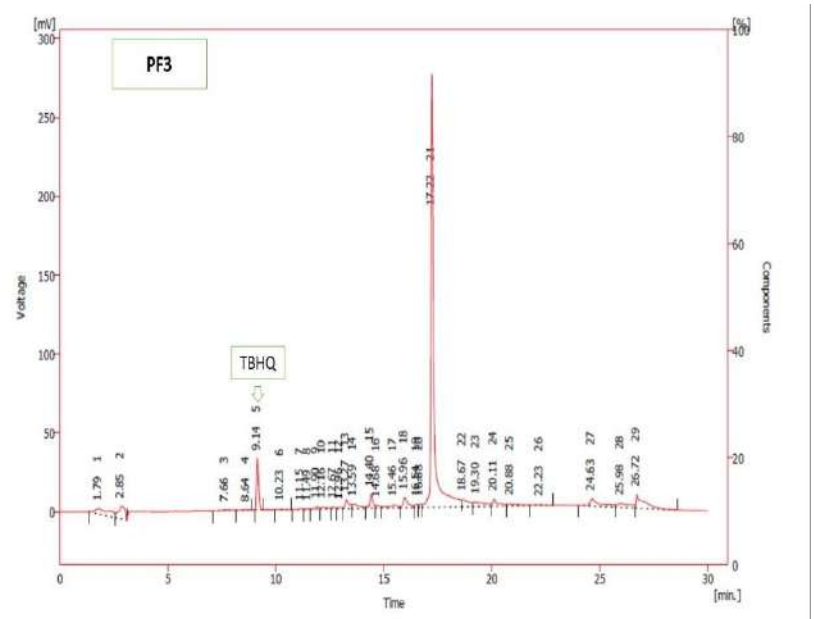
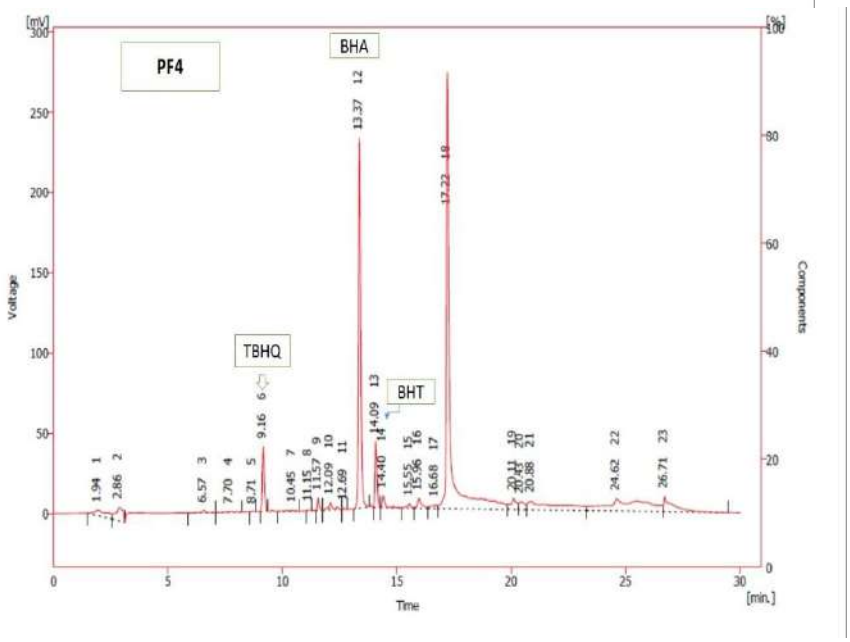


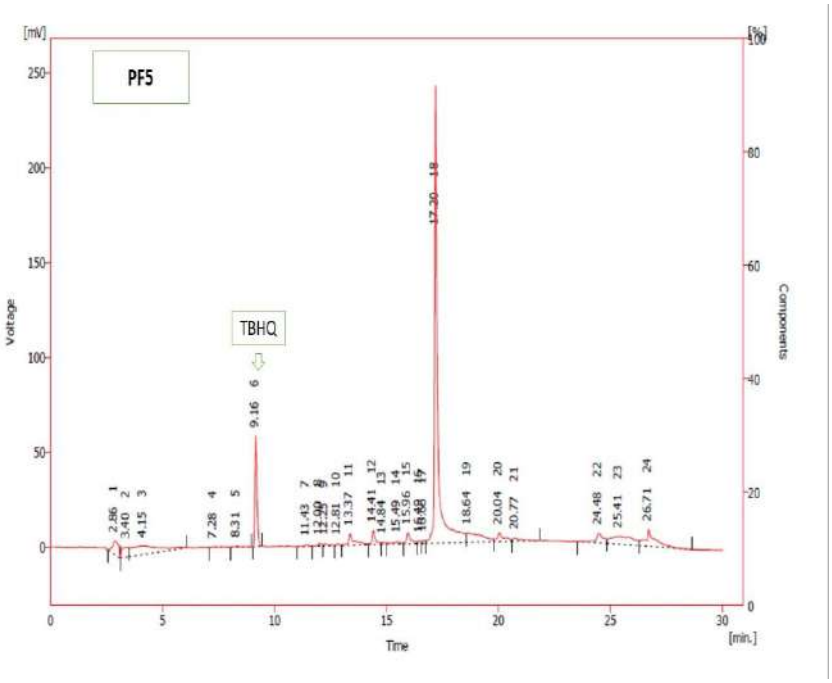
Fig 2. Induction period of oxidation (at 110 °C) of the frying oil samples.

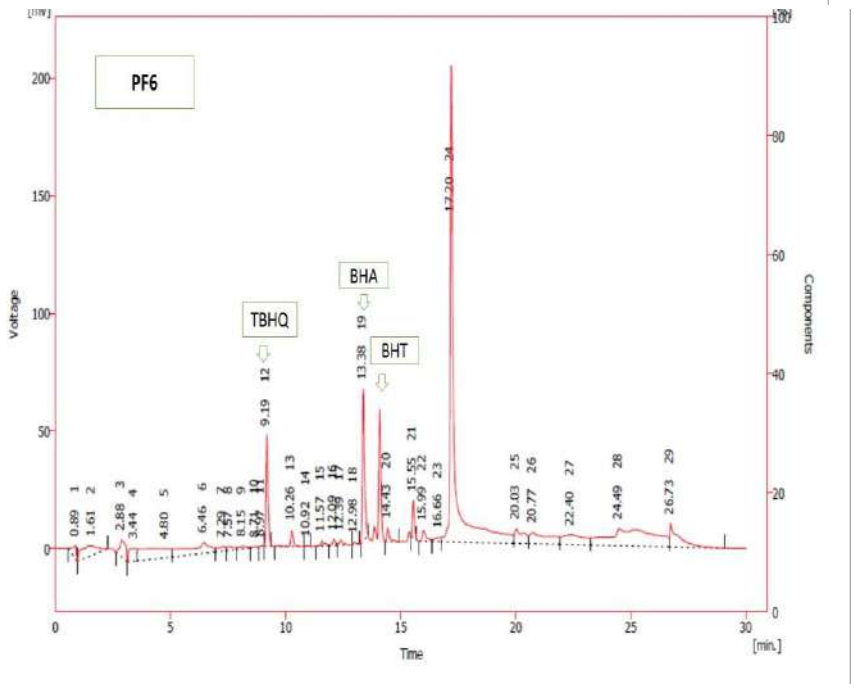
Different letters represent significance at $p < 0.05$. Symbols of + and – indicate that whether or not the parameters are in the standard rang.

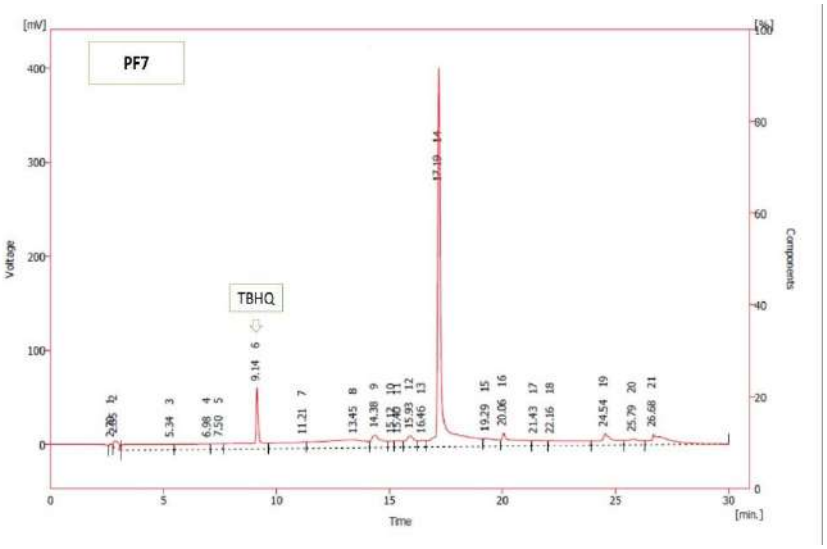

PF1
TBHQ
Voltage
Components
Time
[min.]
PF2
TBHQ
[mV]
[%]
Voltage
Components
Time
[min.]


PF3
TBHQ
Voltage
Components
Time
[min.]


PF4
BHA
TBHQ
BHT
Voltage
Components
Time
[min.]


PF5
TBHQ
Voltage
Time
[min.]
Components


PF6
TBHQ
BHA
BHT
Voltage
Time
[min.]
Components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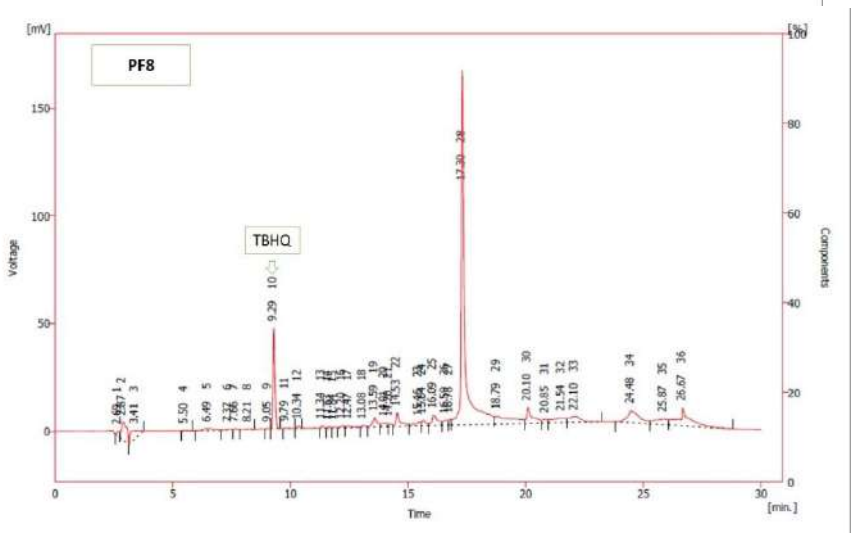
TBHQ
[mV]
[%]
Voltage
Compounds
Time
[min.]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TBHQ
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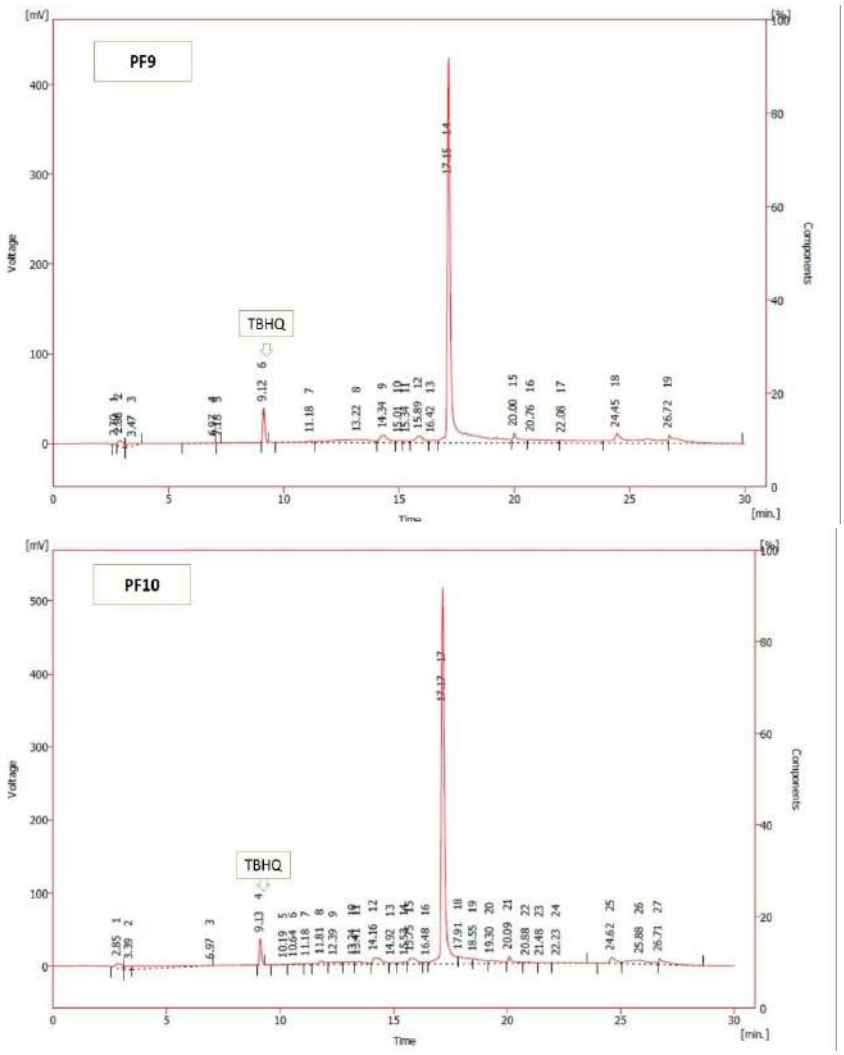
[%]
Voltage
Compounds
Time
[min.]


PF9
TBHQ
[mV]
[%]
Voltage
Components
Time
[min.]
PF10
TBHQ
[mV]
[%]
Voltage
Components
Time
[min.]

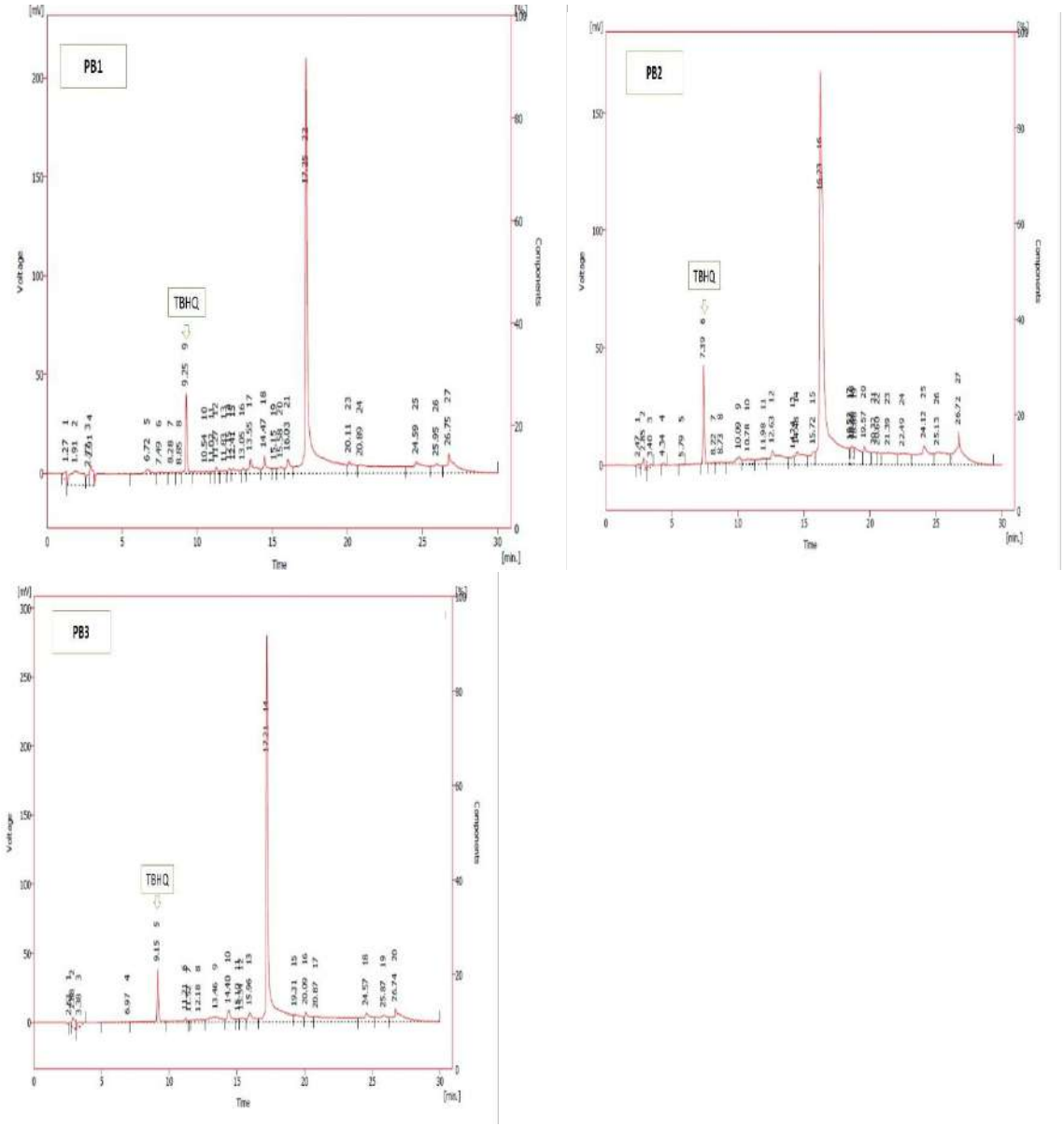


Fig 3: Chromatograms for the gradient elution of synthetic phenolic antioxidants of the frying oil samples.

A SIMULATION STUDY ON THE EFFECT OF LINKAGE DISEQUILIBRIUM ON GENOTYPIC VARIANCE AND ITS COMPENENTS: II- EFFECT OF LINKAGE DISEQUILIBRIUM ON POPULATION PARAMETERS IN A MODEL THAT DOMINANCE AT ONE LOCUS AND ADDITIVE GENE EFFECT AT THE SECOND

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ABSTRACT

In the first part of this study, to numerically demonstrate the effect of linkage disequilibrium on genetic variance and its components one in equilibrium and the other in disequilibrium 2 population were defined for the 2 gene effect models. So, 2*2=4 populations in total are defined and their parameters were calculated analytically using the MINITAB statistical package program. In the case of linkage disequilibrium, dominance deviation are observed at the three allelic locus with additive effects. At the second locus with two alleles, one allele is fully dominant over the other. It can be said that there may be a kind of quasi dominance caused by the linkage disequilibrium. Besides, it was understood that a covariance caused by linkage disequilibrium is effective in genetic variance and its components.In this second part of the study, it was clearly shown that the parameters were affected by the linkage disequilibrium in the population with linkage disequilibrium with full dominance at one locus and additive effect only at the other. In the case of linkage disequilibrium, the dominance deviation in the additive locus appears, and the effect of the covariance term on the genetic variance and its components resulting from the linkage disequilibrium was revealed by calculating numerically. In the next studies it is intended to emphasize the sampling distribution of the genetic variance and its components in the samples produced by simulation in the case of linkage disequilibrium.

Keywords: Linkage Disequilibrium (LD), Genetic Variance, Genetic Covariance

INTRODUCTION

Linkage disequilibrium (LD) is the dependence of the distributions of gene frequencies at different loci in a population. The effect of linkage disequilibrium on genetic variance and its components in terms of a quantitative trait has been studied theoretically for various gene effect models (Kavuncu 1983, 1984 ve 1987, Kavuncu ve Kesici 1982 ve Kavuncu ve Duzgunes 1983). In addition to these theoretical studies, two populations, one in linkage equilibrium and the other linkage disequilibrium, were defined numerically in order to understand the effect of linkage disequilibrium on genetic variance and its components more concretely. Accordingly, two populations in linkage equilibrium in table 1 and in linkage disequilibrium in table 2 are shown below. Accordingly, a two-locus model has A gene with three alleles and B gene with two alleles.

Table 1 (Linkage equilibrium)

B Gene A Gene	B1	B2	Total
A1	.24	.16	.4
A2	.24	.16	.4
A3	.12	.08	.2
Total	.6	.4	1.00

Table 2 (Linkage disequilibrium)

B Gene A Gene	B1	B2	Total
A1	.30	.10	.40
A2	.15	.25	.40
A3	.15	.05	.20
Total	.60	.40	1.00

If the tables above are examined, the gamete frequencies are found by multiplying the gene frequencies in the population in linkage equilibrium (Table 1). For example, when the frequency of the A1 gene is 0,4 and the frequency of the B1 gene is 0,6, the frequency of the A1B1 gamete is 0,24. On the other hand, the multiplying of gene frequencies in Table 2 is not equal to gamete frequencies. The 21 genotypes possible in case of random association of gametes in a two-locus structure as in Table 1 or 2 can be shown in Table 3 as follows.

Table 3 (21 genotypes)

	A3B2	A3B1	A2B2	A2B1	A1B2	A1B1
A3B2	A3B2/ A3B2	A3B2/ A3B1	A3B2/A2B2	A3B2/ A2B1	A3B2/A1B2	A3B2/A1B1
A3B1		A3B1/ A3B1	A3B1/A2B2	A3B1/A2B1	A3B2/A1B2	A3B1/A1B1
A2B2			A2B2/A2B1	A2B2/A2B2	A2B2/A1B2	A2B2/A1B1
A2B1				A2B1/A2B1	A2B1/A1B2	A2B1/A1B1
A1B2					A1B2/A1B2	A3B1/A1B1
A1B1						A1B1/A1B1

In these population structures, two quantitative models were developed by giving numerical values to the genes.

In the additive model, the values of the genotypes are formed in Table 4 as follows, assuming that A3 has 3, A2 has 2, A1 has 1, B2 has 2 and B1 has 1 effect on the fixed value of 5.

Table 4 (Additive effects model)

	A3B2 5	A3B1 4	A2B2 4	A2B1 3	A1B2 3	A1B1 2
A3B2 5	15	14	14	13	13	12
A3B1 4		13	13	12	12	11
A2B2 4			13	12	12	11
A2B1 3				11	11	10
A1B2 3					11	10
A1B1 2						9

For the second effect model in which alleles have additive effect in the A locus and B2 is fully dominant over B1 in the B locus, the values of the genotypes can be written as in Table 5 below.

Table 5 (At locus A, alleles are additive effect and at locus B, B2 is fully dominant to B1.)

	A3B2	A3B1	A2B2	A2B1	A1B2	A1B1
A3B2	15	14	14	14	13	13
A3B1		13	14	12	13	11
A2B2			13	13	12	12
A2B1				11	12	10
A1B2					11	11
A1B1						9

In this study, the second model was dealt with, and genetic variance and its components were calculated in populations with both linkage equilibrium and linkage disequilibrium. The genetic covariance between the effects of the two loci were also calculated and their contribution on the genetic variance were attempted to be shown.

MATERIAL AND METHOD

The possible genotypes, their frequencies, values, and the gametes that combine to form these genotypes are shown in Table 6 for the population in linkage equilibrium and in Table 7 for the population in linkage disequilibrium in the study, in which genes at one locus are additive and the other is complete dominance. From these tables, the means and effects of genes and gametes, genotypic values, additive effects, dominance deviations and their variances and covariances between loci were calculated with the formulas given in the section of results.

Table 6 (Linkage equilibrium)

Nu	A Locus		B Locus		AiBj	AkBl	P	Xijkl
	1	2	3	4				
1	A1	A1	B1	B1	A1B1 .24	A1B1 .24	0,0576	9
2	A1	A1	B1	B2	A1B1 .24	A1B2 .16	0,0384	11
3	A1	A1	B2	B1	A1B2 .16	A1B1 .24	0,0384	11
4	A1	A1	B2	B2	A1B2 .16	A1B2 .16	0,0256	11
5	A1	A2	B1	B1	A1B1 .24	A2B1 .24	0,0576	10
6	A1	A2	B1	B2	A1B1 .24	A2B2 .16	0,0384	12
7	A1	A2	B2	B1	A1B2 .16	A2B1 .24	0,0384	12
8	A1	A2	B2	B2	A1B2 .16	A2B2 .16	0,0256	12
9	A1	A3	B1	B1	A1B1 .24	A3B1 .12	0,0288	11
10	A1	A3	B1	B2	A1B1 .24	A3B2 .08	0,0192	13
11	A1	A3	B2	B1	A1B2 .16	A3B1 .12	0,0192	13
12	A1	A3	B2	B2	A1B2 .16	A3B2 .08	0,0128	13
13	A2	A1	B1	B1	A2B1 .24	A1B1 .24	0,0576	10
14	A2	A1	B1	B2	A2B1 .24	A1B2 .16	0,0384	12
15	A2	A1	B2	B1	A2B2 .16	A1B1 .24	0,0384	12
16	A2	A1	B2	B2	A2B2 .16	A1B2 .16	0,0256	12
17	A2	A2	B1	B1	A2B1 .24	A2B1 .24	0,0576	11
18	A2	A2	B1	B2	A2B1 .24	A2B2 .16	0,0384	13
19	A2	A2	B2	B1	A2B2 .16	A2B1 .24	0,0384	13
20	A2	A2	B2	B2	A2B2 .16	A2B2 .16	0,0256	13
21	A2	A3	B1	B1	A2B1 .24	A3B1 .12	0,0288	12
22	A2	A3	B1	B2	A2B1 .24	A3B2 .08	0,0192	14
23	A2	A3	B2	B1	A2B2 .16	A3B1 .12	0,0192	14
24	A2	A3	B2	B2	A2B2 .16	A3B2 .08	0,0128	14
25	A3	A1	B1	B1	A3B1 .12	A1B1 .24	0,0288	11
26	A3	A1	B1	B2	A3B1 .12	A1B2 .16	0,0192	13
27	A3	A1	B2	B1	A3B2 .08	A1B1 .24	0,0192	13
28	A3	A1	B2	B2	A3B2 .08	A1B2 .16	0,0128	13
29	A3	A2	B1	B1	A3B1 .12	A2B1 .24	0,0288	12
30	A3	A2	B1	B2	A3B1 .12	A2B2 .16	0,0192	14
31	A3	A2	B2	B1	A3B2 .08	A2B1 .24	0,0192	14
32	A3	A2	B2	B2	A3B2 .08	A2B2 .16	0,0128	14
33	A3	A3	B1	B1	A3B1 .12	A3B1 .12	0,0144	13
34	A3	A3	B1	B2	A3B1 .12	A3B2 .08	0,0096	15
35	A3	A3	B2	B1	A3B2 .08	A3B1 .12	0,0096	15
36	A3	A3	B2	B2	A3B2 .08	A3B2 .08	0,0064	15

Table 7 (Linkage disequilibrium)

Nu	A Locus		B Locus		AiBj	AkBl	P	Xijkl
	1	2	3	4				
1	A1	A1	B1	B1	A1B1 .3	A1B1 .3	0,0900	9
2	A1	A1	B1	B2	A1B1 .3	A1B2 .1	0,0300	11
3	A1	A1	B2	B1	A1B2 .1	A1B1 .3	0,0300	11
4	A1	A1	B2	B2	A1B2 .1	A1B2 .1	0,0100	11
5	A1	A2	B1	B1	A1B1 .3	A2B1 .15	0,0450	10
6	A1	A2	B1	B2	A1B1 .3	A2B2 .25	0,0750	12
7	A1	A2	B2	B1	A1B2 .1	A2B1 .15	0,0150	12
8	A1	A2	B2	B2	A1B2 .1	A2B2 .25	0,0250	12
9	A1	A3	B1	B1	A1B1 .3	A3B1 .15	0,0450	11
10	A1	A3	B1	B2	A1B1 .3	A3B2 .05	0,0150	13
11	A1	A3	B2	B1	A1B2 .1	A3B1 .15	0,0150	13
12	A1	A3	B2	B2	A1B2 .1	A3B2 .05	0,0050	13
13	A2	A1	B1	B1	A2B1 .15	A1B1 .3	0,0450	10
14	A2	A1	B1	B2	A2B1 .15	A1B2 .1	0,0150	12
15	A2	A1	B2	B1	A2B2 .25	A1B1 .3	0,0750	12
16	A2	A1	B2	B2	A2B2 .25	A1B2 .1	0,0250	12
17	A2	A2	B1	B1	A2B1 .15	A2B1 .15	0,0225	11
18	A2	A2	B1	B2	A2B1 .15	A2B2 .25	0,0375	13
19	A2	A2	B2	B1	A2B2 .25	A2B1 .15	0,0375	13
20	A2	A2	B2	B2	A2B2 .25	A2B2 .25	0,0625	13
21	A2	A3	B1	B1	A2B1 .15	A3B1 .15	0,0225	12
22	A2	A3	B1	B2	A2B1 .15	A3B2 .05	0,0075	14
23	A2	A3	B2	B1	A2B2 .25	A3B1 .15	0,0375	14
24	A2	A3	B2	B2	A2B2 .25	A3B2 .05	0,0125	14
25	A3	A1	B1	B1	A3B1 .15	A1B1 .3	0,0450	11
26	A3	A1	B1	B2	A3B1 .15	A1B2 .1	0,0150	13
27	A3	A1	B2	B1	A3B2 .05	A1B1 .3	0,0150	13
28	A3	A1	B2	B2	A3B2 .05	A1B2 .1	0,0050	13
29	A3	A2	B1	B1	A3B1 .15	A2B1 .15	0,0225	12
30	A3	A2	B1	B2	A3B1 .15	A2B2 .25	0,0375	14
31	A3	A2	B2	B1	A3B2 .05	A2B1 .15	0,0075	14
32	A3	A2	B2	B2	A3B2 .05	A2B2 .25	0,0125	14
33	A3	A3	B1	B1	A3B1 .15	A3B1 .15	0,0225	13
34	A3	A3	B1	B2	A3B1 .15	A3B2 .05	0,0075	15
35	A3	A3	B2	B1	A3B2 .05	A3B1 .15	0,0075	15
36	A3	A3	B2	B2	A3B2 .05	A3B2 .05	0,0025	15

RESULT

Population at Linkage Equilibrium (Model I)

The overall mean and the averages of the genes' effects in this population have been calculated as follows:

$$\mu = \sum_{i=1}^{36} p_i x_i = 11.88$$

$$\mu_{A1} = \left(\sum_{i=1}^{12} p_i x_i \right) \Big/ 0,4 = \frac{4,432}{0,4} = 11,08$$

$$\mu_{A2} = \left(\sum_{i=1}^{12} p_i x_i \right) \Big/ 0,4 = \frac{4,832}{0,4} = 12,08$$

$$\mu_{A3} = \left(\sum_{i=1}^{12} p_i x_i \right) \Big/ 0,2 = \frac{2,616}{0,2} = 13,08$$

$$\mu_{\mathbf{B1}} = \left(\sum_{i=1}^{18} p_i x_i \right) \Big/ 0,6 = \frac{6,84}{0,6} = 11,40$$

$$\mu_{\mathbf{B}2} = \left(\sum_{i=1}^{18} p_i x_i \right) \Big/ 0,4 = \frac{5,04}{0,4} = 12,60$$

and the additive effects of genes have been calculated as follows:

$$\alpha_{A1} = \mu_{A1} - \mu = 11,08 - 11,88 = -0,80$$

$$\alpha_{A2} = \mu_{A2} - \mu = 12,08 - 11,88 = 0,20$$

$$\alpha_{A3} = \mu_{A3} - \mu = 13,08 - 11,88 = 1,20$$

$$\alpha_{B1} = \mu_{B1} - \mu = 11,40 - 11,88 = -0,48$$

$$\alpha_{B2} = \mu_{B2} - \mu = 12,6 - 11,88 = 0,72$$

As expected, the mean of additive gene effects in linkage equilibrium is zero. At each locus, the means, frequencies, additive effects (breeding values) and dominance deviations of the genotypes have been calculated as follows:

Genotype Effects:	Frequency	Dominance
$\mu_{A1A1} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{1,6448}{0,16} = 10,28$	0,16	-1,6-2*(-0,8)=0
$\alpha_{A1A1} = \mu_{A1A1} - \mu = 10,28 - 11,88 = -1,6$		
$\mu_{A1A2} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{1,8048}{0,16} = 11,28$	0,32	-0,6-(-0,8+0,2)=0
$\alpha_{A1A2} = \mu_{A1A2} - \mu = 11,28 - 11,88 = -0,6$		
$\mu_{A1A3} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,08 = \frac{0,9824}{0,08} = 12,28$	0.16	0,4-(-0,8+1,2)=0
$\alpha_{A1A3} = \mu_{A1A3} - \mu = 12,28 - 11,88 = 0,4$		
$\mu_{A2A2} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{1,9648}{0,16} = 12,28$	0,16	0,4-2*0,2=0
$\alpha_{A2A2} = \mu_{A2A2} - \mu = 12,28 - 11,88 = 0,4$		
$\mu_{A2A3} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,08 = \frac{1,0624}{0,08} = 13,28$	0,16	1,4-(0,2+1,2)=0
$\alpha_{A2A3} = \mu_{A2A3} - \mu = 13,28 - 11,88 = 1,4$		
$\mu_{A3A3} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,04 = \frac{0,5712}{0,04} = 14,28$	0,04	2,4-2*1,2=0
$\alpha_{A3A3} = \mu_{A3A3} - \mu = 14,28 - 11,88 = 2,4$		
$\mu_{B1B1} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,36 = \frac{3,816}{0,36} = 10,6$	0,36	-1,28-2*(-0,48)=-0,32
$\alpha_{B1B1} = \mu_{B1B1} - \mu = 10,60 - 11,88 = -1,28$		
$\mu_{B1B2} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,24 = \frac{3,024}{0,24} = 12,6$	0,48	0,72-(-0,48+0,72)=0,48

$\alpha_{B1B2} = \mu_{B1B2} - \mu = 12,6 - 11,88 = 0,72$

$\mu_{B2B2} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{2,016}{0,16} = 12,6$ 0,16 0,72-2*0,72=-0,72

$\alpha_{B2B2} = \mu_{B2B2} - \mu = 12,6 - 11,88 = 0,72$

As a check of these calculations, and as expected, the means of additive effects and dominance deviations at both loci are zero. The variance of genotypic effects and its additive and dominance deviations have been calculated as follows:

$$\sigma_G^2 = E(x^2) - [E(x)]^2 = \sum_{i=1}^{36} p_i x_i^2 - \left[\sum_{i=1}^{36} p_i x_i\right]^2 = 143,176 - 141,1344 = 2,0416$$

$$\sigma_A^2 = 2 * [E(\alpha_A^2) + E(\alpha_B^2)] = 2 * \left[\sum_{i=1}^{3} p_{A_i} \alpha_{A_i}^2 + \sum_{i=1}^{2} p_{B_i} \alpha_{B_i}^2\right]^2 = 2 * (0,56 + 0,3456) = 2 * 0,9056 = 1,8112$$

$$\sigma_D^2 = \sigma_G^2 - \sigma_A^2 = 2,0416 - 1,8112 = 0,2304$$

From here, the genotypic variance was found to be 2, 0416. Additive variance at locus A was 1,12 and at locus B was 0,6912, resulting in a total additive variance of 1,8112. The dominance variance was calculated as 0.2304 as the difference of the additive variance from the genotypic variance, and the dominance variance from the dominance deviations was found also to be 0.2304. Here, the covariance between the effects of the loci is also zero as expected, so it has no effect on the total genotypic variance and its components.

Population at Linkage Disequilibrium (Model II)

In Model 2 there are linkage disequilibrium, and again at the B locus, B2 is fully dominant over B1. Table 7 (model 2) is shown from left to right alleles at locus A, alleles at locus B, gametes that combine to form genotypes, their frequencies and genotypic values.

Overall mean and gene means have been calculated as follows:

$\mu = \sum_{i=1}^{36} p_i x_i = 11.88$

$\mu_{A1} = \left(\sum_{i=1}^{12} p_i x_i\right) \Big/ 0,4 = \frac{4,36}{0,4} = 10,9$

$\mu_{A2} = \left(\sum_{i=1}^{12} p_i x_i\right) \Big/ 0,4 = \frac{4,94}{0,4} = 12,35$

$$\mu_{A3} = \left(\sum_{i=1}^{12} p_i x_i\right) \Big/ 0,2 = \frac{2,58}{0,2} = 12,9$$

$$\mu_{B1} = \left(\sum_{i=1}^{18} p_i x_i\right) \Big/ 0,6 = \frac{6,81}{0,6} = 11,35$$

$$\mu_{B2} = \left(\sum_{i=1}^{18} p_i x_i\right) \Big/ 0,4 = \frac{5,07}{0,4} = 12.675$$

Additive effects of genes from here:

$\alpha_{A1} = \mu_{A1} - \mu = 10,9 - 11,88 = -0,98$ $\alpha_{B1} = \mu_{B1} - \mu = 11,35 - 11,88 = -0,53$

$\alpha_{A2} = \mu_{A2} - \mu = 12,35 - 11,88 = 0,47$ $\alpha_{B2} = \mu_{B2} - \mu = 12,675 - 11,88 = 0,795$

$\alpha_{A3} = \mu_{A3} - \mu = 12,9 - 11,88 = 1,02$

As will be calculated, the means of gene effects are equal to zero at both loci, as expected.

Genotype Means have been calculated as follows:

$$\mu_{A1A1} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{1,58}{0,16} = 9,875$$

$$\mu_{A1A2} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{1,83}{0,16} = 11,4375$$

$$\mu_{A1A3} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,08 = \frac{0,95}{0,08} = 11,875$$

$$\mu_{A2A2} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{2,035}{0,16} = 12,71875$$

$$\mu_{A2A3} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,16 = \frac{1,075}{0,08} = 13,4375$$

$$\mu_{A3A3} = \left(\sum_{i=1}^{4} p_i x_i\right) \Big/ 0,04 = \frac{0,555}{0,04} = 13,875$$

$$\mu_{B1B1} = \left(\sum_{i=1}^{9} p_i x_i\right) \Big/ 0,36 = \frac{3,78}{0,36} = 10,5$$

$$\mu_{B1B2} = \left(\sum_{i=1}^{9} p_i x_i\right) \Big/ 0,24 = \frac{3,03}{0,24} = 12,625$$

$$\mu_{B2B2} = \left(\sum_{i=1}^{9} p_i x_i\right) \Big/ 0136 = \frac{2,04}{0,16} = 12,75$$

From here, the effects of genotypes and dominance deviations have been found as follows:

Genotype Effects:	Frequency	Dominance
$\alpha_{A1A1} = \mu_{A1A1} - \mu = 9,875 - 11,88 = -2,005$	0.16	-2,005-(-0.98*2)=-0,045
$\alpha_{A1A2} = \mu_{A1A2} - \mu = 11,4375 - 11,88 = -0,4425$	0.32	-0,4425-(-0.98+0.47)=0,0675
$\alpha_{A1A3} = \mu_{A1A3} - \mu = 11,875 - 11,88 = -0,005$	0.16	-0,005-(-0.98+1.02)=-0,045
$\alpha_{A2A2} = \mu_{A2A2} - \mu = 12,71875 - 11,88 = 0,83875$	0.16	0,83875-0.47*2= -0.10125
$\alpha_{A2A3} = \mu_{A2A3} - \mu = 13,4375 - 11,88 = 1,5575$	0.16	1,5575-(0.47+1.02)= 0,0675
$\alpha_{A3A3} = \mu_{A3A3} - \mu = 13,875 - 11,88 = 1,995$	0.04	1,995-1.02*2=-0.045
$\alpha_{B1B1} = \mu_{B1B1} - \mu = 10,5 - 11,88 = -1,38$	0.36	-1,38-(-0.53*2)=-0,32
$\alpha_{B1B2} = \mu_{B1B2} - \mu = 12,625 - 11,88 = 0,745$	0.48	0,745-(-0.53+0.795)=0.48
$\alpha_{B2B2} = \mu_{B2B2} - \mu = 12,75 - 11,88 = 0,87$	0.16	0,87-0.795*2=-0,72

Here, too, the accuracy of the calculations can be seen by calculating the mean of the effects to zero as expected. It can be noted that there is a dominance deviation at the A locus, although a dominance deviation is not predicted at the A locus, assuming the genes have additive effects.

The genotypic variance and the components of additive and dominance deviations were calculated as follows:

$$\sigma_G^2 = E(x^2) - [E(x)]^2 = \sum_{i=1}^{36} p_i x_i^2 - [\sum_{i=1}^{36} p_i x_i]^2 = 143,32 - 141,1344 = 2,1856$$

$$\sigma_A^2 = 2 * [E(\alpha_A^2) + E(\alpha_B^2)] = 2 * \left[\sum_{i=1}^{3} p_{A_i} \alpha_{A_i}^2 + \sum_{i=1}^{2} p_{B_i} \alpha_{B_i}^2\right]^2 = 2 * (0,6806, +0,42135) = 2 * 1,10195 = 2,2039$$

$$\sigma_D^2 = \sigma_G^2 - \sigma_A^2 = 2,1856 - 2,2039 = -0,0183$$

The genotypic variance in this model was found to be 2,1856. The additive variance in the A locus was calculated as 1,3612 and the additive variance in the B locus was calculated as 0,8427, resulting, the total additive variance was 2,2039 and the dominance variance as a meaningless value was found.

-0,0183 as the difference between the genotypic variance and the additive variance. If the dominance variance has been calculated from deviations directly, it is calculated as 0,00419524 at locus A and 0,2304 at locus B. Total dominance variance is the sum of the two is 0,23459524. At this point, the sum of the additive and dominance variances is greater than the genetic variance. This is due to the presence of covariance between the effects of the two loci, due to linkage disequilibrium. As a matter of fact, if the variances of the genotypic values of the two loci and the covariance between them were calculated and added together, it is seen that it would be equal to the variance of the sum of the genotypic effects.

V(GA)=1,3658

V(GB)=1,0731

Cov(GA,GB)=0,19138

V(GA+GB)=V(GA)+V(GB)+2Cov(GA,GB)=2,82166

Likewise, the variances of additive effects at both loci were calculated and a total of 2,2039 was found. The variance of the sum of additive effects is found as follows:

$Var(EA+EB)=2,4263-0,6992^2=1,93741936$

If we subtract 2,2039 from this value of 1,9374, half the difference is the covariance between the additive effects of the loci:

V(EA+EB)=V(EA)+V(EB)-2Cov(EA,EB)

Cov(EA,EB)=[V(EA+EB)-V(EA)-V(EB)]/2=(1,9374-2,2039)/2= - 0,13325

DISCUSSION AND CONCLUSION

In the case of linkage disequilibrium, dominance deviation occurs in the A locus, which has assumed to have only additive effects. So, there may be a kind of quasi dominance caused by linkage disequilibrium. In addition, it has been understood that a covariance caused by linkage disequilibrium is effective in genetic variance and its components. This is an issue that should be taken into account in breeding studies. It should be considered that a breeding study to be determined based on the assumption of dominance may not yield the expected result if this dominance is due to linkage disequilibrium.

In this study, first, this effect of covariance on genetic variance components due to linkage disequilibrium has been calculated. In the narrow sense heritability calculations, which show the percentage of the additive variance due to the breeding values in the total phenotypic variance, it is necessary to take into account that the covariance between these additive effects of the loci may cause a deviation. This deviation may lead to the failure of a selection program to be developed based on heritability in the narrow sense.

In the next study, it is planned to focus on the sampling distribution of the phenotypic variance and its components in the samples to be produced by simulation by adding an environmental effect produced from the normal distribution with a mean of zero and a variance of 12, for instance, on the genotypic values of the genotypes.

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ANALYSIS OF DIVERSITY AS A FACTOR OF RESILIENCE IN AGRICULTURAL SYSTEMS: THE CASE OF A SEMI-ARID ZONE IN MOROCCO

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ABSTRACT

The farms of the Tadla plain are characterized by a diversity of production strategies, that is considered one of the characteristics of richeness of the agriculture in the region of Beni Mellal-Khenifra, and the maintenance of this diversity in the ability to remain productive in the long term, is increasingly mentioned as an objective in itself. Nevertheless, the diversity of production systems of the farms makes the study of these units more complex. To this end, the objective of this work is to establish a structural typology that will make it possible to group the farms of the Tadla plain into types that are representative of the diversity observed, in order to guide studies and development actions according to the specificity of each type of farm. Based on data from a survey of 150 farmers, a statistical analysis was applied to the farms, taking into account three criteria: the useful agricultural area, the cropping system and the number of sheep/cattle. The typological approach allowed us to classify the 150 farms surveyed in 4 more or less homogeneous types. Type 1: large farms with a diversified production system (cereals, barley, fodder, citrus, sugar beet) and a very large sheep/cattle population (representing 6.7% of the farms surveyed). Type 2: large farms with a diversified production system (cereals, barley, fodder, sugar beet, market gardening) and a very low sheep/bovine population (representing 10.7% of the farms surveyed). Type 3: small farms with a production system based mainly on cereals and sugar beet. Type 4: small farms with a diversified production system (cereals, fodder, sugar beet, olive trees, citrus fruits, market gardening) and a significant presence of olive trees. This study appears to be sufficient to understand the diversity of farms in order to guide development efforts and ensure the resilience of agricultural activity.

Keywords: Agriculture, sustainable development, resilience of farming systems, typology, the Tadla plain, Morocco.

INTRODUCTION

Agriculture is an important strategic sector for Morocco, contributing to food security and employment, and to cope with climate hazards, the country has invested in the construction of irrigated perimeters including that of Tadla, at the foot of the Middle Atlas. The development of large irrigation perimeters, fed by dams, has been a central axis of economic and social development of this sector.

The Tadla plain is among the successful examples of this type of development. Irrigation of the irrigated perimeter began in 1935 in the Beni Amir, and in 1953 in the Beni Moussa with the impounding of the Bin El Ouidane dam (Hammani et al., n.d).

Indeed, modern production methods have been introduced since the creation of the Tadla irrigated perimeter, including industrial, tree and vegetable crops. This development of agriculture has given us a diversity of production strategies.
The diversity of production systems on the farms of the plain makes the study of these units more complex, hence the need to construct a typology that brings together the most homogeneous farms.

The purpose of this article is to analyze the diversity of farms and construct a typology of farms to ensure the success of research and rural development operations.
The typological tool represents a real investment for the development of the region because of the different functions it can fulfill, in particular it allows to move from individual or monographic analysis to a group analysis and to build a picture of the local or regional agricultural activity useful to orientate the development actions.

MATERIAL AND METHOD

PRESENTATION OF THE STUDY AREA:

1) Presentation of the Tadla Plain:

A preliminary phase to determine the geographical limits of the Tadla Plain.

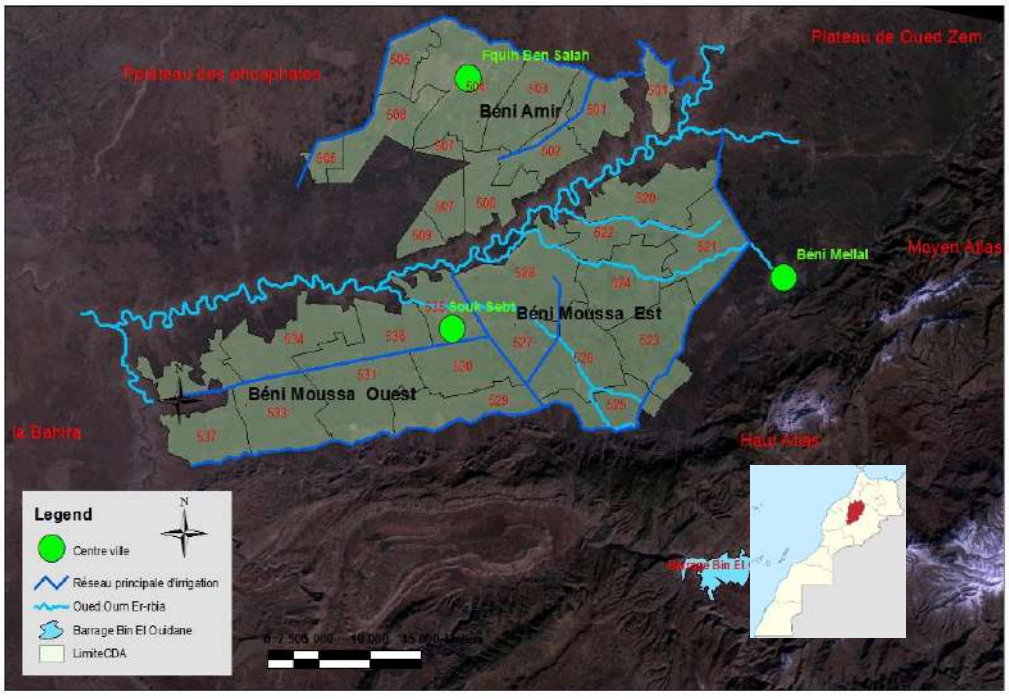

Figure 1: Situation of the study area (the Tadla plain) in the region of Beni Mellal-Khenifra

In central Morocco, in the region of Beni Mellal-Khenifra, the Tadla Plain covers an area of about 98,000 hectares, it is located south-east of the city of Casablanca and slopes from east to west. The hydraulic network of the Tadla irrigated perimeter is managed by the Tadla Regional Agricultural Development Office (ORMVAT).

It is limited in the North by the phosphate plateau, in the East the Tadla narrows in a corner between the Plateau of Oued Zem and the drop of the Middle Atlas, in the West it is limited by the Bahira at the level of Oued Elabbid and in the South the Plain is limited by the central High Atlas.
The Tadla Plain is crossed on all its length by the Oued Oum Er Rbia, one of the most important rivers of the country. It extends over a length of 160 km and divides the plain into two hydraulically independent sub-perimeters:

- The Béni Amir on the right bank of the Oum Er Rbia with a surface area of 27 500 ha (and soon
35,000 ha) irrigated from the Shahid Ahmed El Hansali- dam.
- The Béni Moussa on the left bank of a 69,500-ha area irrigated by the waters of the Oued El Abid from the Bin El Ouidane dam.

2) Sampling and surveys:

Following a survey carried out at the Institut National de la Recherche Agronomique, the objective of this work is to establish a structural typology and to group farms into types representative of the diversity observed on the basis of the information from these surveys.
The typology study covered 150 farms in the different municipalities of the plain. The holdings were selected from the 350 farms surveyed in order to cover the great diversity that currently exists in terms of the size of the structures and the choice of production.
The 150 farms were selected according to the availability of information on the variables studied in the typology.

Table 1: Breakdown of farms by reference municipality.

Municipality	Nbr of farms	municipality	Nbr of farms	municipality	Nbr of farms
Sidi jabeur	46	F.B.Saleh	5	El Khalifa	2
Bradia	30	B.Mellal	4	Lakrifat	2
Od M'Barek	16	Laasara	3	F.B.Saleh	1
Hel Merbaa	15	Od Gnaon	3	Beni Oukil	1
Od Zmam	15	Bni Aoun	3	Lahlalma	1
		Layaata	2	Od Boukhdou	1

STATISTICAL ANALYSIS:

In our study, a typology of structures was developed in two main steps:

- Principal Component Analysis (PCA) covered variables relating to the useful agricultural area, the cropping system and the size of the sheep/cattle herd on the farm
- An ascending hierarchical classification (AHC) of the evaluations described by the same quantitative variables was carried out. This classification makes it possible to identify well-defined types.

RESULTS AND DISCUSSION

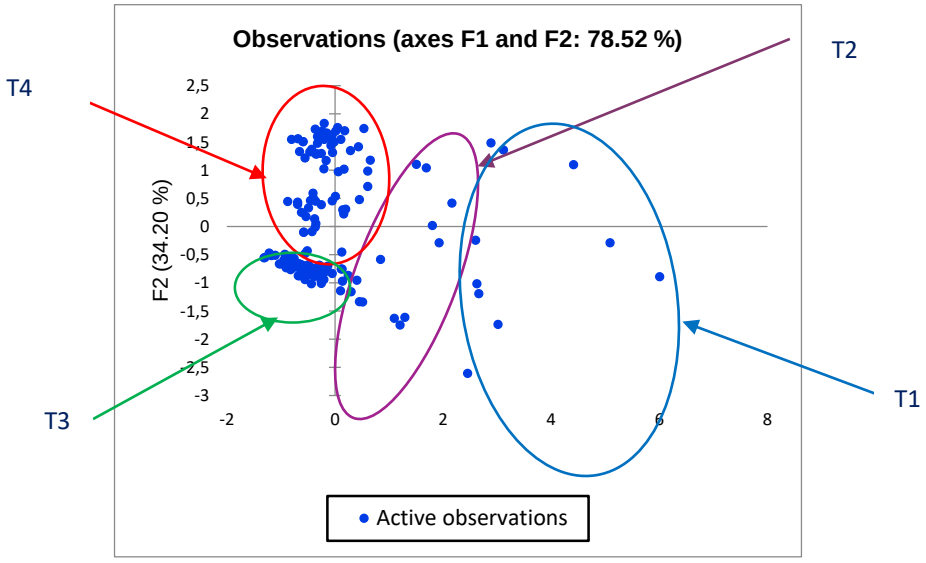

Figure 2: Principal component analysis /Coordinates of farms on Factors 1 and 2

In factorial terms, if two points are close to each other and are well correlated with the axes showing them close, it is very likely that the farm information represented by these two points is very similar. In addition, it should always be taken into account that there are very close points on one axis, while on another axis they are very far apart, or that they are not correlated with all axes. For this reason, we carried out another statistical analysis, using the same variables, but this time with the hierarchical ascending classification (HCA) to confirm the types of farms selected in the PCA

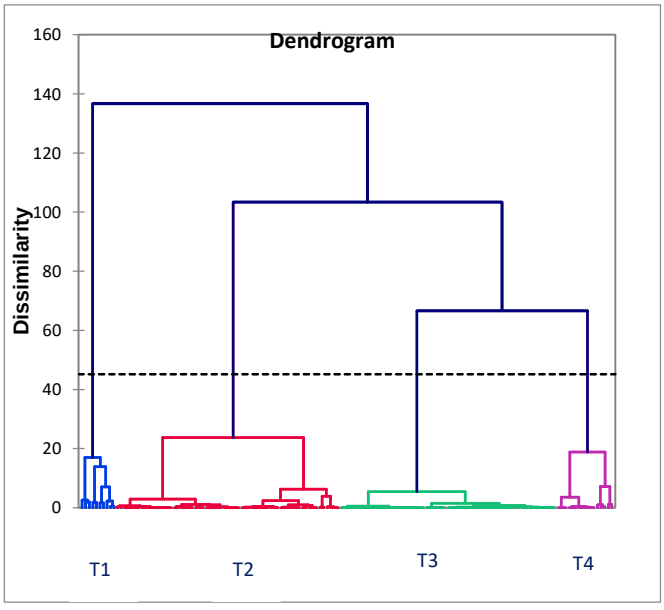

Figure 3: Distribution of the 150 farms into four types of farms with the ascending hierarchical classification [AHC].

On the basis of the variables used in the PCA, a categorization of the farms was made using a hierarchical bottom-up classification [HAC] to construct relatively homogeneous groups.

The schematic representation of the analysis allowed us to identify four types of farms that belong to the same group and each group contains similar farms.
The four types of farms identified by the HAC (T1, T2, T3, T4) are represented on the PCA graph by ellipses (Figure 5).

Table 2: Characteristics of the four types of farms.

	Average UAA/ha	Production system	Average, numb of sheep/bovine
T1	13,2	Cereal, barley, forage (alfalfa, corn), citrus, sugar beet,	143
T2	16,2	Cereal, barley, fodder (alfalfa, corn) Sugar beet, market gardening.	14
T3	4,2	Cereal, Sugar beet, alfalfa	10
T4	3,7	Cereal, fodder (alfalfa, corn), sugar beet, olive, citrus, market gardening	14

From Principal Component Analysis (PCA) and Hierarchical Upward Classification (CAH),
Four types of operations were established:

-T1: The first type is made up of 6.7% of the total number of farms. These are large farms with a mixed production system (crop-livestock).

The agricultural production system is mainly based on the following crops: Cereal, barley, fodder, citrus fruit, sugar beet.

-T2: With a percentage of 10.7% of the farms, type 2 groups large farms with a diversified agricultural production system (Cereal, barley, fodder, Sugar Beet, market gardening) and very low livestock farming.

-T3: Small farms with a production system oriented towards cereals and sugar beet, representing 40.7% of farms.

-T4: Type 4 represents the majority of farms with a percentage of 42%, it is small farms with a diversified production system (Cereal, fodder, sugar beet, olive, citrus, market gardening) and a high production of olive.

CONCLUSIONS

In sum, the study of the typology of farms is a real investment that helps to make decisions on development policies. It has made it possible to define target groups for more effective agricultural development interventions in the future.
Indeed, the above analysis revealed the existence of four main types of farms, and each type contains farms that are homogeneous with each other and heterogeneous with the farms of the other types, making it easier to read the diversity of farms in a more organised way.
Despite the semi-arid climate of the region, there is a great diversity of production systems in the farms of the Tadla Plain due to the easy access to groundwater.

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DETERMINATION OF GERMINATION TEMPERATURES IN SOME COOL AND WARM SEASON PLANTS

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ABSTRACT

Every day, new species are added to the plants cultivated in agricultural areas. In this study, it has been tried to determine the optimum germination temperature of some cultivated plants that have just started to be cultivated or are not very common. For this purpose, grass family *Eragrostis teff* (Zucc) Trotter), *Phalaris canariensis* L., *Panicum miliaceum* L. and *Lolium multiflorum* Lam., broadleaf species *Amaranthus* sp., *Chenopodium quinoa* Willd., *Fagophyrum esculentum* Moench. and *Salvia hispanica* L. were examined. 4x100 seeds of each species were monitored in germination cabinets at 0, 5, 10, 15, 20 and 25 ^{o}C for 14 days and their germination rates were determined. The research was carried out with 4 replications according to the completely randomized experimental design, and the differences between the means were evaluated with the LSD multiple comparison test.

No germination was observed in both groups at 0 ^{o}C. Germination rates increased depending on the increase in temperature, and this increase differed according to the species. In the grasses, *Lolium multiflorum* was the species that germinated the most at low temperatures and the earliest to reach full germination. *Eragrostis teff* started to germinate at 10 ^{o}C (7,2%) and reached 96,7% germination at 25 ^{0}C. Among broadleaf species, *Chenopodium quinoa* is the species that germinates more at low temperature. *Amarathus* sp. were able to reach high germination rates at 20 ^{o}C and 25 ^{o}C. These obtained data reveal important results for the cultivation of the new species and sowing times.

Keywords: Germination, Temperature, Alternative plants

INTRODUCTION

Every day, new crops are added to the cultivated plants. It is seen that some cool and warm season plants have started to become widespread in Turkey in recent years. Curious and innovative producers are trying to produce by planting new seeds they have obtained in different ways. However, attempts are often unsuccessful because there is not enough information on these alternative species. For this reason, it is useful to know the ecological demands of the species in order to make healthy production.

Quinoa (*Chenopodium quinoa* Willd.) is a plant that has become popular all over the world in recent years. Being resistant to salty soils and drought, high nutritional value and gluten-free seeds (Tan and Temel, 2019) have made it a sought-after plant in human nutrition. Many countries, especially the USA and European Union countries, have started studies to cultivate this South American origin plant. In Turkey, approximately 1.500 ha planting area has been reached and unions have started to be established among the breeders. Another plant originating from South America is *Amarathus* sp. These plants, which are grown for both their grains and leaves in human nutrition, are also used as ornamental plants and animal feed (Acar et al., 1999). These plants, whose seeds are very valuable for human nutrition, show resistance to high salinity and temperature (Keskin et al., 2021). Recently, researches on amaranth species have been carried out in Turkey. Another species that takes its place in markets and agricultural lands in Turkey is buckwheat (*Fagophyrum esculentum*

Moench.). This Far Eastern plant, which is also famous for its high nutritional value, attracts the attention of many researchers in Turkey (Kara and Gürbüzer, 2018; Polat, 2019; Güllap et al., 2021). Chia (*Salvia hispanica* L.) has also become popular in modern food diets due to its high omega-3 content (Çiçek and Özel, 2017). This valuable species of Salvia is called chia.

Alternative species are also grown among the Poaceae species, which are important in both human nutrition and animal nutrition. The annual ryegrass (*Lolium multiflorum* Lam.) cultivation areas are increasing in Turkey. In a few years, the cultivation areas reached 25.329 ha (TUIK, 2020). Annual ryegrass, which is important in animal nutrition, is a nutritious and productive plant. Species such as *Phalaris canariensis* L. (canarygrass), *Panicum miliaceum* L. (proso millet), which are also important as animal feed, are also grown on a small scale. These two plants are also important as bird food. One of the alternative species that has been heard a lot in recent years is teff (*Eragrostis teff* (Zucc) Trotter)). Teff, a plant of the African continent, draws attention with its resistance to high temperatures and quarks, and both roughage and grains are used in human nutrition (Geren et al., 2019).

The spread of alternative species in agricultural areas is suitable for polyculture applications. However, in order for the cultivation areas of these species to become widespread, their ecological demands and agromic characteristics should be known. The majority of the above-mentioned species are warm season plants and are therefore sensitive to low temperature. For this reason, this study was planned to determine the germination temperatures of the mentioned species. Thus, healthier decisions can be made about planting times.

MATERIALS AND METHODS

This research was carried out in Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum, Turkey. 8 species (*Eragrostis teff* (Zucc) Trotter), *Phalaris canariensis* L., *Panicum miliaceum* L. and *Lolium multiflorum* Lam., broadleaf species *Amaranthus* sp., *Chenopodium quinoa* Willd., *Fagophyrum esculentum* Moench. and *Salvia hispanica*) were subjected to germination tests in the grow cabinet. 4x100 seeds of each type were placed in petri dishes and between blotting papers and moistened with ionized water. Species were kept at 6 different temperatures (0, 5, 10, 15, 20 and 25 ± 1,0 ^{o}C) for 14 days (Sagsoz, 1995; Akçay ve Tan, 2108). Seeds with rootlets reaching 2 mm were considered as germinated and recorded and removed from the petri dishes (Prado et al., 2000). The research was carried out with 4 replications according to the completely randomized experimental design. The obtained data were subjected to variance analysis with the help of SPSS package program and the averages were compared with the LSD test. The aim of the research is to determine the optimum germination temperatures rather than comparing the species. For this reason, 8 species were divided into two groups as grasses and broad-leaved.

RESULTS AND DISCUSSIONS

The germination rates of 4 plants from the grasses group determined at different temperatures are given in Table 1. Among the plants in this group, ryegrass and canarygrass are cool season species, while the other two are warm season plants. None of the species showed germination at 0 ^{o}C ambient temperature. Cool season species started to germinate at 5 ^{o}C, and 37,7% germination was observed in ryegrass. At this temperature, canarygrass germinated at a rate of 15,6%, while there was no germination in warm season species. Ryegrass reached 98,1% germination at 10 ^{o}C, and 99,2% and 100% germinated at 15 and 20 ^{o}C, respectively. Similar germination tendency was also seen in canarygrass, reaching full germination at 20 ^{o}C. Proso millet reached 60,4% germination when the ambient temperature was 10 ^{o}C. As the temperature increased to 15, 20 and 25 ^{o}C, 73.2%, 82,1 and 100 % germinated, respectively. The slowest germination in this group is teff. This species showed only 7,2% germination at 10 ^{o}C, reaching 96,7% at the highest temperature (Figure 1).

Table 1. Germination rates of some grass species at different temperatures (%)*

Species	Temperature (°C)						Mean
	0	5	10	15	20	25	
Lolium multiflorum	0,0	37,7	98,1	99,2	100,0	100,0	72,5 A
Phalaris canariensis	0,0	15,6	70,0	91,2	100,0	100,0	62,8 B
Panicum miliaceum	0,0	0,0	60,4	73,2	82,1	100,0	52,6 C
Eragrostis teff	0,0	0,0	7,2	48,0	72,1	96,7	37,3 D
Mean	0,0 F	13,3 E	58,9 D	77,9 C	88,6 B	99,2 A	56,3

* Means marked with different letters are different at 0,05 probability level.
LSD, Species: 3,7, Temperature: 5,2, Species x Temperature: 8,6

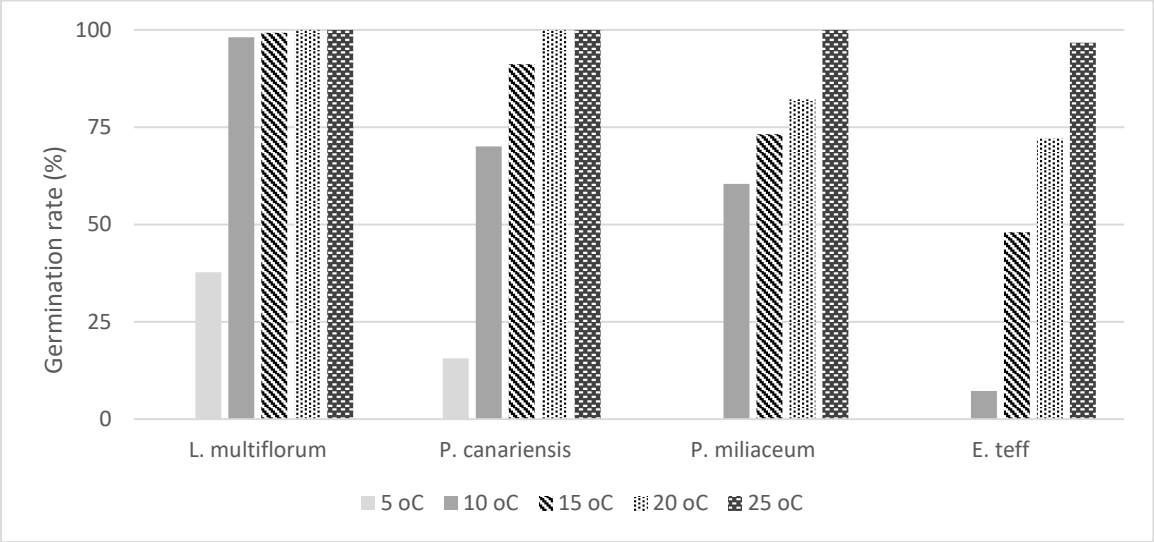

Figure 1. Germination rates of wheatgrass species at different temperatures

None of the broad-leaved species examined showed germination at that °C (Table 2). It was determined that the highest germination temperature requirement was in amaranth. While amaranth seeds did not germinate at 0 and 5 °C, they started to germinate at 10 °C. However, high germination rates were reached at 20 and 25 °C. The quinoa was the most germinated (48,0%) species at 5 °C. It reached high germination rates at 10 °C and higher temperatures. Buckwheat germinated at a rate of 25,2% at 5 °C. At 10, 15, 20 and 25 °C, germination rates were 86,2%, 90,0%, 99,2% and 100,0%, respectively. Chia started to germinate at 5 °C, but high germination rates (95-100%) occurred at 15-25 °C (Figure 2).

Table 2. Germination rates of some broad-leaved plant species at different temperatures (%)*

Species	Temperature (°C)						
	0	5	10	15	20	25	Mean
Amaranthus sp.	0,0	0,0	15,2	28,4	90,1	95,2	38,2 C
Chenopodium quinoa	0,0	48,0	93,2	98,2	100,0	100,0	73,2 A
Fagophyrum esculentum	0,0	25,2	86,2	90,0	99,2	100,0	66,8 B
Salvia hispanica	0,0	10,3	79,2	95,2	100,0	100,0	64,2 B

* Means marked with different letters are different at 0,05 probability level.
LSD, Species: 6,1, Temperature: 5,9, Species x Temperature: 9,7

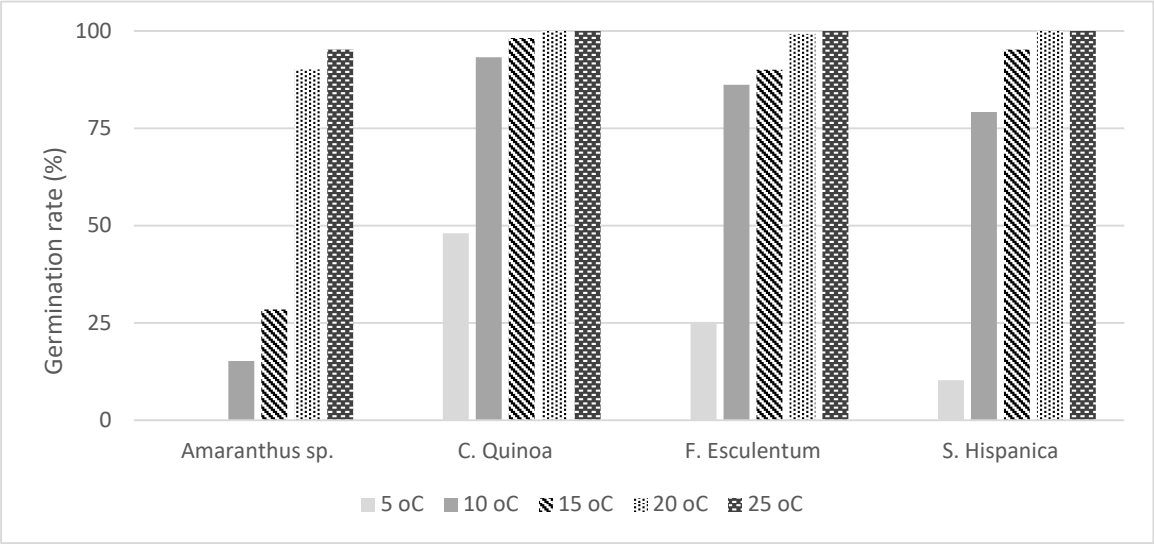

Figure 2. Germination rates of some broad-leaved species at different temperatures

The temperature demands of the species in their habitats are also reflected in their germination characteristics. The optimum temperature for growth in species grouped as cool season or C3 plants is around 20 ˚C. In warm season plants (C4), the best growth is around 30-35 ºC and active growth continues above 15 ˚C (Nelson and Volenec, 1995). Similar demands are also seen in the germination temperatures of species. In this study, ryegrass and canarygrass, which are cool season grasses, started to germinate at 5 ºC and reached maximum germination at 15-20 ºC. In this study, proso millet, teff and amaranth are warm season (C4) species. The germination of these species started with the ambient temperature reaching 10 ºC. Since teff, which is of Ethiopian origin, requires higher temperatures (Dumanlıoğlu and Geren, 2020), germination rates at 10 and 15 ºC remained below 50% (Figure 1). Nelson and Volenec (1995) reported that the germination temperature varies according to the species, however, a minimum temperature of 1-4 ˚C is required for the germination of cool-season plants, while a minimum of 8-12 ºC is required for warm-season plants. Ahmedi et al. (2013) found the highest germination in canarygarss at 20/10 ºC and 25/15 ºC temperature regimes. Okant (2014) reported that the minimum germination temperature of proso millet is 10-12 °C and the temperature should be around 20 °C for rapid germination. Among the broad-leaved (dicotyledonae) species examined in the study, Amaranth C4 is the species that uses the photosynthesis pathway and has the highest temperature demand (Dumanlıoğlu and Geren, 2020). Quinoa is a species that uses the C3 pathway in photosynthesis (Tan and Yondem, 2013). Germination characteristics of these two species showed great differences depending on the temperature. Quinoa seeds germinated at a rate of 48% at 5 °C, while amaranth seeds did not germinate at this temperature. Tan and Temel (2019) also reported that quinoa started to germinate around 5 ºC, germination above 70% reached at 10 ºC, and maximum germination at around 20-25 ºC. Chia grows optimally at 20 °C to 30 °C (Rodrigez-Abello et al., 2018), and requires a temperature of 15-25°C for high germination. Suzuki et al. (2021) reported high germination rates (85,5-98,9%) of buckwheat varieties at 35 ºC.

CONCLUSIONS

These results reveal important information about the climate demands and planting times of the species. Among the examined grasses, teff is the species that needs the most germination temperature. Annual ryegrass and canarygrass can germinate in cooler conditions. Among broad-leaved plants, amaranth needs at least 10 ºC to germinate. Other species start to germinate at 5 ºC. It is useful to consider these results in determining the planting time of the species.

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A STUDY ON DROUGHT ANALYSIS USING TIME SERIES, STANDARDIZED PRECIPITATION INDEX (SPI)AND STANDARDIZED PRECIPITATION EVAPOTRANSPIRATION INDEX (SPEI) IN BURSA REGION

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ABSTRACT

All agricultural activities are directly related to the climate. In recent years, temperature increases and changes in precipitation regimes adversely affect the Mediterranean Basin, which includes our country. While increasing temperatures and irregular precipitation increase the need for irrigation in crop production in some regions, excessive and irregular precipitation in some regions seriously damages production. Nowadays, climate change has been accepted by many climate scientists as a problem that cannot be ignored. In our country, drought is one of the natural disasters that will affect agricultural production the most. In this study, a drought analysis was made for Bursa, which is one of the important cities of our country in agricultural production. In addition, non-parametric Mann-Kendall and Sen's Trend Analyzes were conducted between 1990-2019 for precipitation and temperature values. According to results of the trend analysis, statistically significant trends could not be reached in the precipitation data, increasing trends were observed in the temperature data. SPI and SPEI methods were used for drought analysis, although extreme values were reached for the years 1960 – 2019 as a result of both methods, it was determined that normal drought levels were dominant in general.

Keywords: Drought, Precipitation, Temperature, Standardized Precipitation Index (SPI), Standardized Precipitation Evapotranspiration Index (SPEI), Trend Analyzes

INTRODUCTION

Climate is a phenomenon that will directly affect all agricultural activities. In recent years, temperature increases and changes in precipitation regimes negatively affect the whole world, especially the Mediterranean Basin, where our country is located. Temperature increases and rainfall irregularities increase the need for irrigation in crop production.

According to the Intergovernmental Panel on Climate Change; It is predicted that a global temperature increase of 1 to 3.5°C will occur on average by 2100 (IPCC, 2001). These predicted increases are expected to bring along extreme natural events and natural disasters. It is stated that events that will reach disaster dimensions such as floods, storms, drought and desertification will reach global dimensions and their incidence will increase (Öztürk, 2002).

Our country has a complex climate structure and therefore it is fragile in terms of climate change (Öztürk, 2002). Due to its different topography and complex climate, different regions of the country will be affected by climate change at different rates and in different ways (Öztürk, 2002). Decreases that may occur in water resources will be clearly felt in the field of agriculture and livestock. It is predicted that our country may be exposed to drought and forest fires along with heat waves (Şen, 2013).

Drought is a natural disaster that is the most difficult to predict but has wide-ranging effects (Anonymous, 2019). Even if its effect is not seen suddenly, it is one of the most harmful natural disasters (Gümüş et al., 2016). In order to minimize its effects, it needs to be monitored. If the drought can be determined and monitored beforehand, measures can be taken before its severity increases (Ilgar, 2010).

The order of importance and types of natural disasters may differ between regions. For example, in our country, especially for the Mediterranean Region, the threat of drought takes the first place (AFAD, 2016). Drought analysis is of great importance in order to minimize the effects of drought. There are many drought indices to define drought and determine its severity (Dinç et al., 2016). The results obtained with drought indices help us to obtain information about the climate of a country or region and the increase or decrease in drought (Ilgar 2010).

In this study; with the trend analysis applied to the average temperature and precipitation data for many years, temperature and precipitation trends were determined, thus it was observed whether there were precipitation and temperature anomalies. In addition, using the SPI and SPEI indices, drought analysis was carried out considering the 12-month time period, the annual drought characteristics of the obtained data were evaluated and the two methods were compared with each other.

MATERIAL AND METHOD

Bursa is located in the Southern Marmara Section of the Marmara Region, between 39°35′ - 40°37′ northern parallels and 28°5′ - 29°57′ east meridians. Bursa generally feature temperate climate characteristics. However, the climate varies according to the regions, with the mild and warm Mediterranean climate of the Sea of Marmara in the north and the harsh climate of Uludağ in the south. As of the 52-year observation period, the monthly average precipitation is 70.6 mm, and the annual total precipitation is 708.7 mm. The average relative humidity in the province is around 69%. The annual average temperature is 14.5 °C (Anonymous, 2019).

In this study; from 1960 to 2019, daily average temperature and precipitation data were used. Trend analysis was performed with Mann – Kendall and Sen's Trend Analysis methods, and MAKESENS program was used for these analyzes.

SPI and SPEI programs were used for drought analysis and the results obtained from both programs were compared. Standardized Precipitation Index (SPI), developed by McKee et al. (1993), is a time-flexible method that can be easily calculated, depends only on precipitation values and is related to probability, allows monitoring of water resources for all times (Sırdaş, 2002). Precipitation is the main factor affecting the presence of water. Therefore, it makes it possible to follow the increase or decrease in the drought situation in any region and at a certain time scale by using precipitation data (Dinç et al., 2016). The normal distribution of SPI allows to monitor humid periods as well as dry periods (McKee et al., 1993).

$$SPI = \frac{\mathrm{Xi} - \mathrm{Xort}}{\sigma}$$

Xi: total precipitation over a given period (mm)

Xort: Average total precipitation for the same period

σ: standart deviation

SPI is the most widely used method in drought analysis (Dinç et al., 2016). The dry period starts with the negative value of SPI and ends with its conversion to a positive value (Arslan et al., 2016). The World Meteorological Organization adopted the Standardized Precipitation Index (SPI) for measuring meteorological droughts. However, SPI is based only on precipitation data and does not take into account other drought factors such as temperature, relative humidity, evaporation, and wind speed (Çamalan et al., 2017; Sırdaş, 2002).

SPEI; it is a method based on precipitation and temperature data developed by Serrano, Beguveria and Moreno. It can evaluate drought with temperature variability. SPEI calculation is based on SPI calculation method. In the method, monthly precipitation and potential evapotranspiration values are used. Regular and complete temperature and precipitation data are needed (Fuchs, 2012). SPEI can explain the possible effects of variations and extremes in temperature (Çamalan et al., 2017).

$$SPEI = \mathrm{w} - \frac{C_0 + C_1 \mathrm{w} + C_2 \mathrm{w}^2}{1 + d_1 \mathrm{w} + d_2 \mathrm{w}^2 + d_3 \mathrm{w}^3}$$

$P \leq 0.5 \rightarrow \mathbf{w} = \sqrt{-2\ln(P)}$

$P > 0.5 \rightarrow$ Sign of the SPEI is reversed.

$C_0 = 2{,}515517$ $d_1 = 1{,}432788$

$C_1 = 0{,}802853$ $d_2 = 0{,}189269$

$C_2 = 0{,}010328$ $d_3 = 0{,}01308$

Values are the constant values of the formulation.

Standard Precipitation and Evapotranpiration Index, which is a method based on precipitation and temperature data and calculates based on water balance; it is a method that calculates with regular and complete precipitation and temperature data for periods of at least 30 years on different time scales.

RESULTS AND DISCUSSION

By applying trend analysis to the precipitation and temperature values used in this study, it was determined whether the change in temperature values was statistically significant. According to the trend analysis applied on the temperature data; for the time period studied, the change in temperature values in the Bursa region in March, April, May, June, July, August, September, spring, summer, autumn, winter seasons and at the level of years was found to be significant at the 5% level. It was concluded that the temperature has an increasing trend (Table 1).

Table.1 Temperature Trend Analysis Results

Time	Max.	Min.	Avg.	Changing (°C/year)	MK-Z (+/-1,96) %5 Significance Value	MK-Z (+/-2,57) %1 Significance Value
January	25.2	-19.2	5.35	0.0152	0.87	0.87
February	26.9	-16.8	6.41	0.0218	1.42	1.42
March	30.6	-10.5	8.65	0.0331	2.22	2.22
April	35.5	-3.1	13.01	0.0249	1.98	1.98
May	36.1	1.4	17.79	0.0350	3.74	3.74
June	41.3	4	22.34	0.0358	4.47	4.47
July	43.8	9	24.67	0.0423	5.14	5.14
August	41.9	8.6	24.36	0.0522	5.01	5.01
September	40.3	5	20.26	0.0362	4.16	4.16
October	37.3	-1	15.45	0.0180	1.84	1.84
November	31	-4.6	10.81	-0.0024	-0.01	-0.01
December	27.3	-16.3	7.32	-0.0215	-1.45	-1.45
Spring	36.1	-10.5	14.7	0.0310	3.25	3.25
Summer	43.8	4	13.15	0.0435	6.12	6.12
Autumn	40.3	-4.6	23.79	0.0173	2.42	2.42
Winter	27.3	-19.2	15.51	0.0019	2.42	2.42
Annual	43.8	-19.2	6.42	0.0243	4.14	4.14
1990 - 1999	40	-14.8	14.36	0.1377	1.25	1.25
2000 - 2009	43.8	-14	14.98	0.0532	0.45	0.45
2010 - 2019	40.3	-13.1	15.65	0.0990	1.07	1.07

Changes in May, June, July, August, September, spring, summer seasons and annual values were also significant at the 1% level.

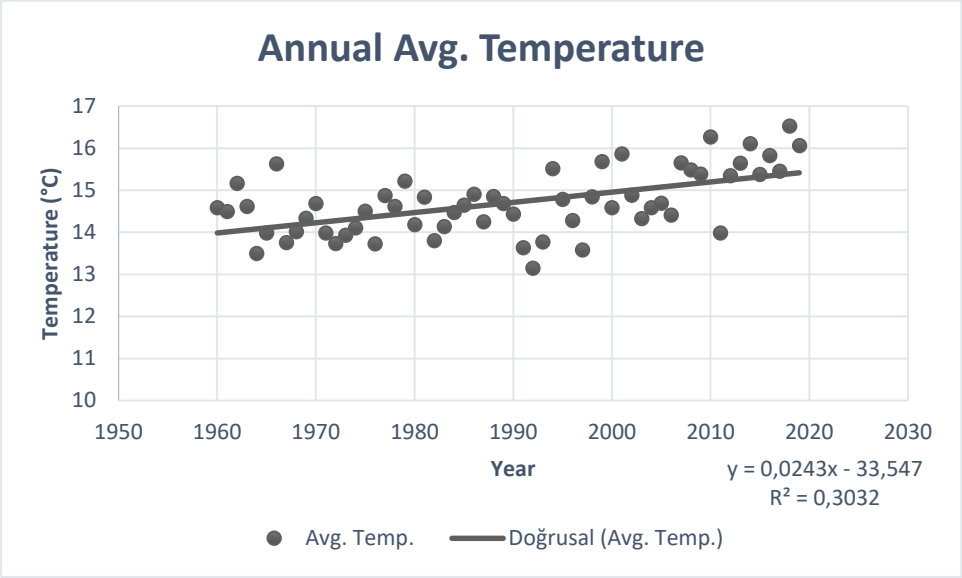

Figure 1. Average annual temperature trend

According to the Mann-Kendall and trend analysis applied to the annual average temperature data, it was observed that there was a significant increase of 5% and 1%. This increase is statistically significant. The value of the increase in the annual average temperature was calculated as 0.0243 ˚C/year.

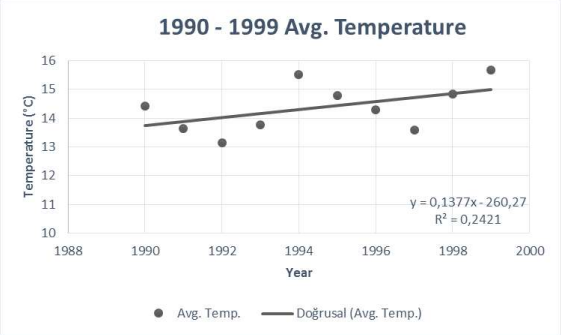

Figure 2. 1990 – 1999 average temperature

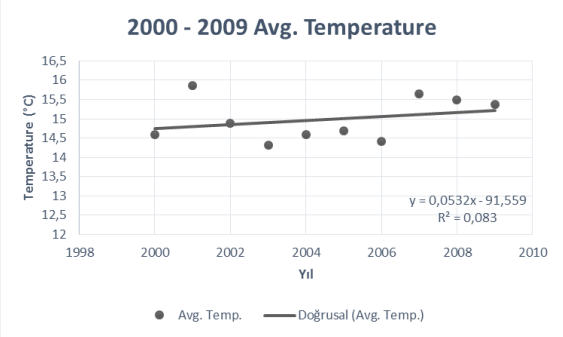

Figure 3. 2000 – 2009 average temperature

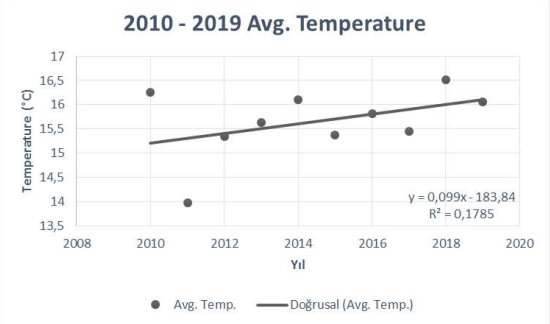

Figure 4. 2010 – 2019 average temperature

The Mann – Kendall test and trend analysis applied to the annual average temperature data were applied separately for the years 1990 – 1999, 2000 – 2009 and 2010 – 2019 in the form of 10-years averages. Although not statistically significant at the 5% and 1% significance levels, there is an increase of 0.1377 ˚C/year for 1990 – 1999, 0.0532 ˚C/year for 2000 – 2009, 0.0990 ˚C/year for 2010 – 2019. (Figure 1-4).

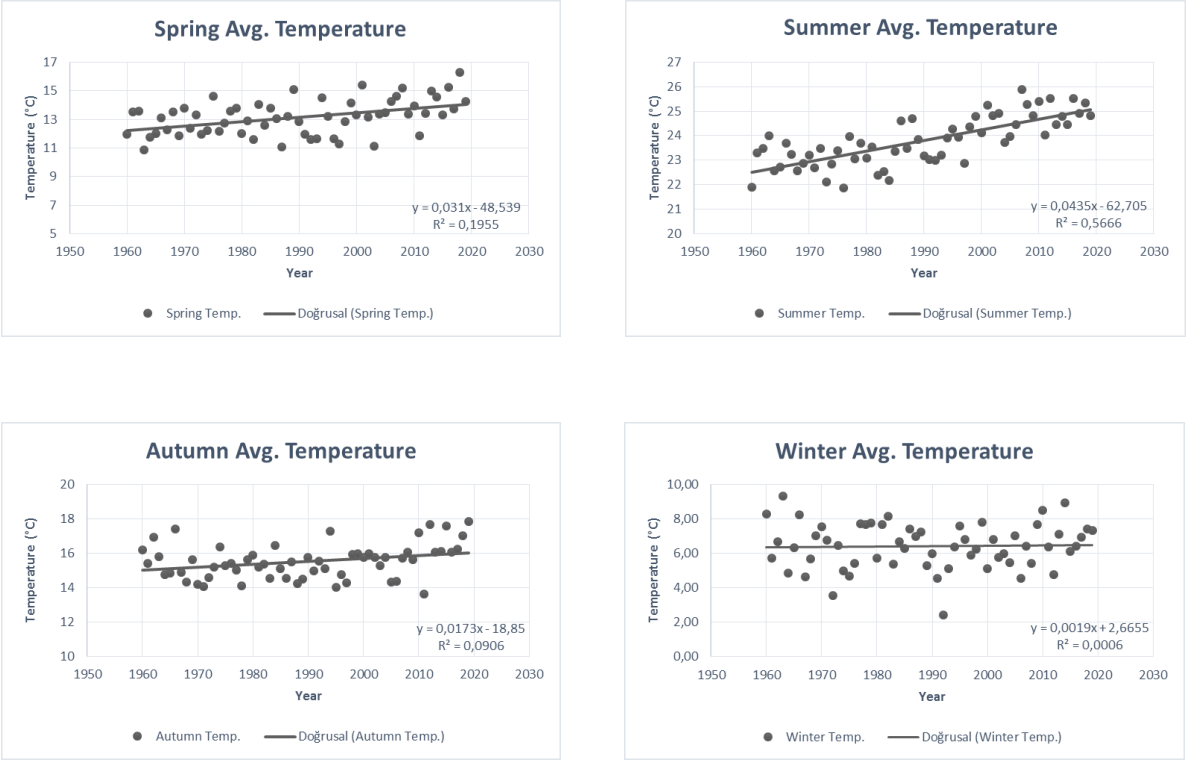

Figure5. Average temperature for four seasons

Trend analysis was applied to the average temperature data of spring, summer, autumn and winter seasons. There is an increase for all seasons. Statistically, while the change was significant for all seasons at the 5% significance level, the change in the spring and summer seasons was significant at the 1% significance level. At the 5% significance level, the highest increase was observed in summer with 0.0435 ˚C/year, and the lowest increase was observed in winter with 0.0019 ˚C/year. At the 1% significance level, while the increase was 0.0435 ˚C/year for the summer season, it was 0.0310 ˚C/year in the spring (Figure 5).

As a result of the trend analysis applied to the monthly average temperature data, there is a decrease of -0.0024 ˚C/year and -0.0215 ˚C/year in November and December, respectively. But in these months, the change in temperature was not statistically significant. Temperature increase is observed in the 10 months other than these months. Among the statistically significant values, at 5% significance level, the highest temperature increase was observed in August with 0.0522 ˚C/year, and the lowest temperature increase was observed in April with 0.0249 ˚C/year. At the 1% significance level, the highest increase was seen in August with 0.0522 ˚C/year, and the lowest increase was observed in May with 0.0350˚C/year.

According to the Mann – Kendall test and trend analysis applied to the annual average precipitation data, the change in precipitation is statistically insignificant. Although it is not statistically significant, it can be said that the annual average precipitation increased by 0.0577 mm/year (Table 2).

Table 2. Precipitation Trend Analysis

Time	Max. (mm)	Min. (mm)	Average (mm)	Changing (mm/year)	MK-Z (+/-1,96) %5 Significance Value	MK-Z (+/-2,57) %1 Significance Value
January	56.7	0	84.84	-0.1361	0.24	0.24
February	72.3	0	71.31	0.1348	0.62	0.62
March	41.4	0	68.99	0.0940	0.39	0.39
April	55	0	65.19	-0.0623	-0.57	-0.57
May	49.2	0	45.95	0.1186	0.35	0.35
June	47.2	0	36.61	0.3646	1.14	1.14
July	55	0	15.79	0.0190	0.18	0.18
August	68.9	0	16.39	-0.1464	-0.46	-0.46
September	79.4	0	40.4	0.4077	1.44	1.44
October	114.4	0	69.89	0.7666	1.49	1.49
November	79.7	0	74.89	-0.2355	-0.78	-0.78
December	89.2	0	108.58	-0.6432	-1.55	-1.55
Spring	55	0	60.04	0.0501	0.04	0.04
Summer	68.9	0	23.05	0.0874	0.76	0.76
Autumn	114.4	0	63.8	0.3717	1.61	1.61
Winter	89.2	0	87.52	-0.1757	1.61	1.61
Annual	114.4	0	58.21	0.0577	-0.16	-0.16
1990 -1999	79.7	0	56.96	0.9361	0.36	0.36
2000 - 2009	79.4	0	58.46	-0.8016	-1.25	-1.25
2010 - 2019	114.4	0	63.34	-4.1937	-1.61	-1.61

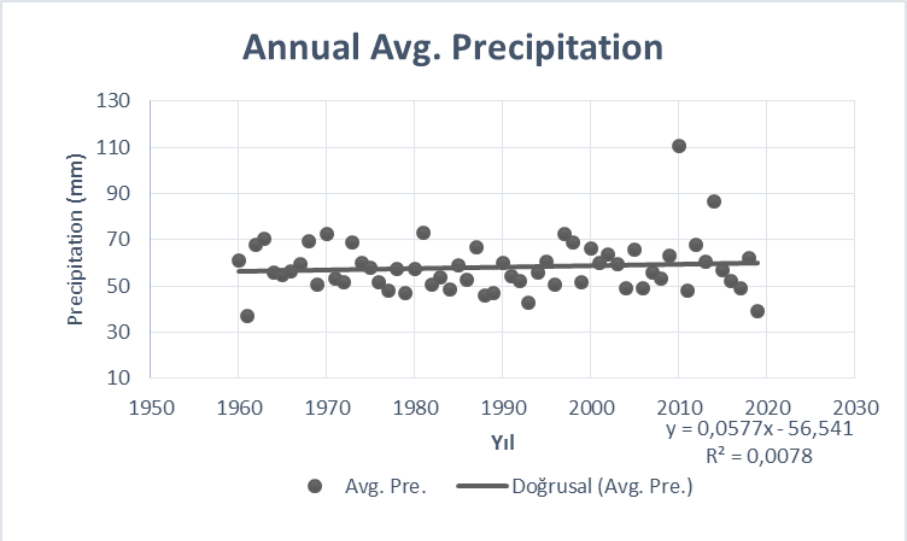

Figure 6. Average annual precipitation

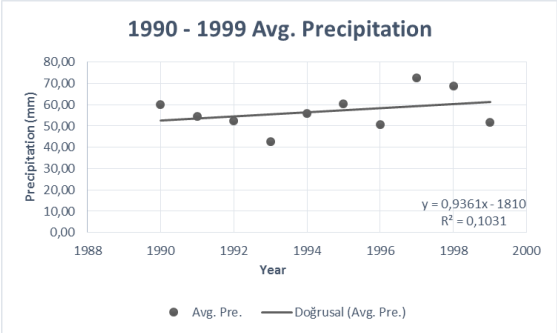

Figure 7. 1990 – 1999 average precipitation

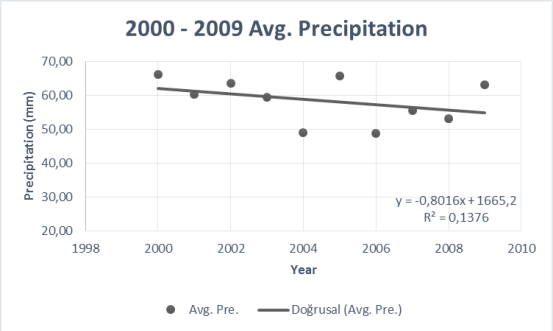

Figure 8. 2000 – 2009 average precipitation

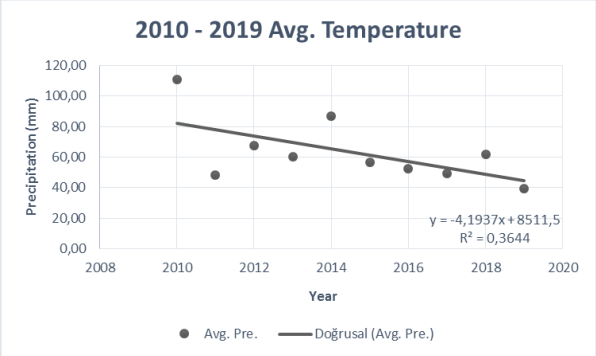

Figure 9. 2010 – 2019 average precipitation

The Mann – Kendall test and trend analysis applied to the annual average precipitation data were applied separately for the years 1990 – 1999, 2000 – 2009 and 2010 – 2019 as 10-year averages. No statistically significant value was found at the 5% and 1% significance levels (Figure 6-9).

Considering these insignificant results; while an increase of 0.9361 mm/year was observed between 1990 and 1999, there was a decrease of -0.8016 mm/year in precipitation between 2000 and 2009, and a decrease of -4.1937 mm/year between 2010 and 2019 decrease has occurred.

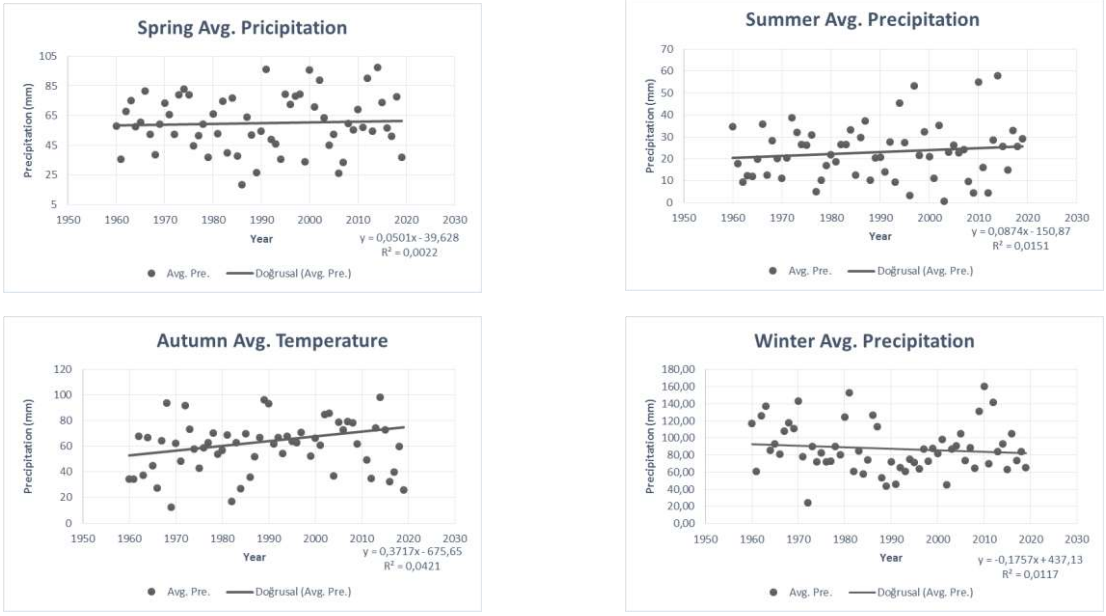

Figure 10. Average seasonal precipitation

Mann – Kendall test and trend analysis were applied to the average precipitation data of the spring, summer, autumn and winter seasons and no statistically significant result could be obtained (Figure 10). Although not statistically significant, for the spring, summer and autumn seasons, respectively; while there was an increase in precipitation at values of 0.0501 mm/year, 0.0874 mm/year and 0.3717 mm/year, a decrease of -0.1757 mm/year occurred for the winter season.

A statistically significant value could not be reached as a result of the Mann – Kendall test and trend analysis applied to the monthly average precipitation data. Among the results that were not found to be significant, the biggest decrease in precipitation was in December with a value of -0.6432 mm/year and respectively; the precipitation values decreased with -0.2355 mm/year in November, -0.1464 mm/year in August, -0.1361 mm/year in January and -0.0623 mm/year in April.

Although not significant, greatest increase in precipitation values occurred in October with 0.7666 mm/year, and this month was 0.4077 mm/year in September, 0.3646 mm/year in June, and 0.1348 mm/year, respectively. And February with 0.1186 mm/year, May with 0.0940 mm/year, March and finally July with 0.0190 mm/year.

2.00 > value	Extremely wet
1.50 - 1.99	Very Wet
1.00 - 1.49	Moderately Wet
(-0.99) - 0.99	Normal
(-1.00) - (-1.49)	Moderately Dry
(-1.50) - (-1.99)	Severely Dry
(-2.00) < value	Extremely Dry

Figure11. SPI – SPEI drought severity classification

According to the results of the SPI drought analysis, drought classification was made using the intervals in Figure 11. According to the results of drought classification, monthly and annual drought values are given in Table 3.

Table 3. SPI analysis over the 12-month time period 1960 - 2019

year	January	February	March	April	May	June	July	August	September	October	November	December	year
1960	-99.00	-99.00	-99.00	-99.00	-99.00	-99.00	-99.00	-99.00	-99.00	-99.00	-99.00	0.32	1960
1961	-0.09	-0.22	-0.30	-0.53	-0.74	-1.11	-1.15	-1.22	-1.07	-0.87	-1.21	-2.20	1961
1962	-2.84	-2.15	-1.41	-0.86	-1.07	-1.46	-1.28	-1.34	-1.18	-0.52	-0.42	0.91	1962
1963	1,87	1,52	1,25	1,11	1,58	1,82	1,64	1,68	1,61	1,11	1,04	1,14	1963
1964	0.02	0.18	0.30	-0.03	-0.23	-0.50	-0.49	-0.25	0.25	-0.04	0.44	-0.19	1964
1965	-0.13	0.59	0.23	0.67	0.62	0.79	0.97	0.81	0.10	0.24	0.33	-0.29	1965
1966	0.45	-0.84	-0.12	-0.01	-0.23	-0.12	-0.36	0.14	0.17	0.21	-0.28	-0.11	1966
1967	0.04	0.45	-0.08	-0.34	-0.23	-0.37	-0.23	-0.83	-0.66	-0.12	0.10	0.17	1967
1968	0.62	0.62	0.70	0.40	0.28	0.36	0.25	0.64	1,29	1,14	1,27	1,05	1968
1969	0.20	0.46	0.33	0.84	0.90	1,02	1,10	0.75	-0.17	-0.70	-1.25	-0.65	1969
1970	-0.64	-0.08	-0.06	-0.10	0.27	0.10	-0.03	0.06	0.40	0.82	1,16	1,31	1970
1971	0.87	0.17	0.76	0.32	-0.01	-0.05	0.10	0.21	0.03	-0.13	-0.12	-0.42	1971
1972	-0.50	-0.90	-1.89	-1.42	-1.18	-1.05	-1.06	-0.72	-0.04	0.30	0.35	-0.56	1972
1973	-0.47	0.08	0.39	0.62	0.68	0.80	0.71	0.55	-0.14	-0.15	0.13	0.98	1973
1974	0.99	0.58	0.58	0.27	0.64	0.43	0.49	0.53	0.76	0.28	0.17	0.21	1974
1975	0.33	0.59	0.72	0.56	0.48	0.88	0.77	0.49	0.14	0.30	0.15	0.02	1975
1976	0.06	-0.32	-0.85	-0.87	-1.17	-1.51	-1.24	-1.07	-0.60	-0.39	-0.63	-0.58	1976
1977	-0.81	-0.82	-0.47	-0.14	-0.57	-0.75	-0.95	-1.29	-1.43	-1.69	-1.15	-0.95	1977
1978	-0.58	-0.05	-0.05	-0.09	0.14	0.14	0.19	0.27	0.87	1,01	0.45	-0.00	1978
1979	0.05	-0.26	-0.60	-0.76	-0.79	-0.83	-0.80	-0.63	-1.24	-1.33	-1.05	-1.06	1979
1980	-0.76	-0.63	0.16	0.07	0.14	0.40	0.49	0.26	0.12	0.02	0.33	0.88	1980
1981	1,01	1,02	0.67	0.31	0.68	0.44	0.56	0.62	1,07	1,20	0.87	1,35	1981
1982	1,04	0.90	0.72	1,45	1,31	1,37	1,48	1,51	0.83	0.63	0.39	-0.67	1982
1983	-0.99	-0.44	-0.68	-1.09	-1.30	-1.08	-1.35	-1.34	-1.02	-0.85	-0.12	-0.38	1983
1984	-0.46	-0.85	-0.11	0.18	0.12	0.01	0.36	0.28	0.11	-0.14	-0.59	-0.88	1984
1985	-0.79	-0.72	-1.22	-1.84	-1.72	-1.72	-2.30	-2.41	-2.14	-1.37	-1.09	-0.87	1985
1986	-0.65	-0.44	-0.64	-0.60	-0.89	-0.46	-0.45	-0.47	-0.50	-0.65	-1.36	-0.47	1986
1987	-0.20	-0.87	-0.10	-0.04	0.30	0.08	0.26	0.48	0.45	0.51	0.83	0.80	1987
1988	-0.44	-0.17	-0.27	-0.12	-0.44	-0.66	-0.89	-1.17	-0.96	-0.98	-0.73	-1.19	1988
1989	-1.16	-1.70	-2.15	-2.70	-2.35	-2.26	-2.10	-2.09	-1.90	-0.84	-1.20	-1.09	1989
1990	-1.12	-0.70	-0.63	-0.14	0.04	-0.13	0.13	0.05	0.36	-0.23	-0.01	0.23	1990
1991	0.19	0.15	0.08	0.64	1,06	1,12	0.92	0.94	1.00	1,01	0.24	-0.30	1991
1992	-0.39	-0.16	0.37	-0.46	-1.34	-0.98	-0.96	-0.99	-1.56	-1.13	-0.84	-0.52	1992
1993	-0.30	-0.44	-0.81	-0.71	-0.47	-1.05	-1.10	-0.97	-0.56	-1.36	-1.28	-1.53	1993
1994	-1.58	-1.63	-1.61	-1.70	-1.81	-1.07	-1.06	-0.83	-1.09	-0.51	-0.47	-0.16	1994
1995	0.52	0.15	1,05	1,38	1,11	0.67	0.88	0.75	0.91	0.59	0.65	0.27	1995
1996	-0.62	-0.02	-0.42	-0.37	-0.16	-0.31	-0.56	-0.78	-0.32	-0.02	-0.78	-0.67	1996
1997	-0.77	-0.91	-1.09	-0.62	-0.69	-0.44	-0.10	0.51	-0.11	0.43	0.68	1,29	1997
1998	1,33	1,51	1,60	0.81	1,45	1,50	1,37	0.81	1,20	1,02	1,43	0.98	1998
1999	0.96	1,53	1,26	1,25	0.50	0.80	0.58	0.75	0.30	-0.29	-0.39	-0.57	1999
2000	-0.11	-0.74	-0.19	0.62	0.77	0.45	0.57	0.53	0.91	1,13	0.83	0.77	2000
2001	0.10	-0.14	-0.73	-1.20	-0.72	-0.95	-1.08	-1.01	-1.21	-1.97	-1.14	0.25	2001
2002	0.65	0.48	0.75	1,05	0.85	1,03	1,32	1,39	1,47	2,02	1,84	0.54	2002
2003	0.55	1,04	0.60	0.49	0.45	0.29	-0.11	-0.37	-0.34	-0.26	-0.34	0.16	2003
2004	0.83	0.60	0.80	0.34	0.16	0.45	0.47	0.67	0.16	-0.62	-0.47	-0.86	2004
2005	-0.97	-0.95	-0.75	-0.81	-0.68	-0.85	-0.40	-0.61	0.16	0.27	0.40	0.72	2005
2006	0.03	0.48	0.30	0.07	-0.14	0.20	-0.22	-0.23	-0.23	-0.25	-0.37	-0.86	2006
2007	-0.69	-1.80	-1.69	-1.51	-1.45	-1.66	-1.49	-1.56	-2.28	-1.49	-1.34	-0.19	2007
2008	-0.50	-0.29	0.22	0.27	0.35	0.22	0.12	0.11	1.00	0.58	0.08	-0.43	2008
2009	0.04	0.92	0.90	0.82	0.78	0.66	0.67	0.68	0.18	0.18	0.32	0.51	2009
2010	0.75	0.94	0.86	1,12	1,18	2,07	2,12	2,21	1,97	3,61	3,81	3,95	2010
2011	3,67	2,82	2,40	2,50	2,44	1,76	1,56	1,76	1,52	-0.41	-0.67	-0.94	2011
2012	-0.57	0.32	0.49	0.66	1,03	1.00	0.98	0.81	0.65	0.10	0.48	0.90	2012
2013	0.80	0.61	0.56	0.20	-0.23	0.23	0.34	0.34	0.33	0.96	1,19	0.26	2013
2014	-0.31	-0.96	-1.10	-0.46	0.17	0.61	0.46	0.84	1,57	1,13	1,33	2,37	2014
2015	2,90	3,28	3,21	3,04	2,68	2,34	2,31	2,09	1,69	1,67	1,56	0.05	2015
2016	0.12	0.18	0.16	-0.42	-0.23	-0.32	-0.43	-0.42	-0.92	-1.49	-1.48	-0.51	2016
2017	-0.94	-1.59	-2.00	-1.75	-1.63	-1.29	-1.07	-1.14	-1.17	-0.69	-0.92	-0.81	2017
2018	-1.00	-0.54	0.30	0.04	0.15	0.02	0.00	-0.02	0.21	0.30	0.45	0.40	2018
2019	0.35	0.16	-0.73	-0.46	-0.83	-1.08	-0.98	-0.76	-0.97	-1.30	-1.69	-1.92	2019

1961 was extremely dry with -2.20 spi, 1993 and 2019 were severely dry, 1979, 1988, 1989 were moderately dry, 2010, 2014 were extremely humid with 3.95 and 2.37 spi values, and 1963, 1968, 1970, 1981, 1997 years were found to be moderately humid. A very humid year was not encountered and the remaining 47 years were observed to be at normal drought levels.

SPEI drought analysis was performed for the same period and the results are given in Table 4. While 2019 was extremely dry with a value of -2,66751, 1961 was severely dry, 1979, 1988, 1989, 1993, 1999, 2008, 2016 and 2017 were moderately dry, 2010 was extremely humid with 2,56467,

1997, 2017 were very humid. The years 1963, 1968, 1970, 1973, 1980, 1981, 1987 were found to be moderately humid. It was observed that the remaining 40 years were at normal drought levels.

Table 4. SPEI analysis over the 12-month time period 1960 - 2019

year	January	February	March	April	May	June	July	August	September	October	November	December	year
1960												0,73572	1960
1961	0,458233	0,306099	0,13138	-0,26354	-0,51213	-0,95868	-1,04105	-1,14295	-1,052606	-0,76708	-1,031826	-1,924786	1961
1962	-2,85409	-1,83071	-1,32786	-0,7385	-0,9246	-1,18047	-1,04703	-1,11433	-1,126753	-0,60414	-0,565488	0,850875	1962
1963	1,664718	1,294554	1,196874	1,091603	1,611695	1,769841	1,556892	1,626764	1,703061	1,087449	1,112743	1,24408	1963
1964	0,316753	0,52432	0,547896	0,096788	-0,0376	-0,302	-0,18	0,21399	0,791271	0,502246	1,04543	0,529477	1964
1965	0,564694	1,163544	0,804596	1,247694	1,114986	1,230042	1,343922	1,239509	0,360498	0,69083	0,771711	0,277404	1965
1966	1,004658	-0,49187	0,142039	0,155189	-0,08528	0,169039	-0,19912	0,04099	0,034237	-0,03382	-0,634187	-0,379161	1966
1967	-0,14081	0,422439	-0,15453	-0,36226	-0,22632	-0,34782	-0,13603	-0,69474	-0,673407	0,044359	0,462168	0,633246	1967
1968	1,079172	0,964858	1,025831	0,661112	0,409829	0,450531	0,342827	0,857159	1,680288	1,431435	1,528351	1,358158	1968
1969	0,692392	0,83335	0,693221	1,313576	1,36584	1,416789	1,549627	1,178698	0,047431	-0,40192	-0,8364	-0,326379	1969
1970	-0,42557	0,08355	-0,10267	-0,36284	0,217011	0,158999	-0,15759	-0,12463	0,293281	0,852498	1,20438	1,409513	1970
1971	1,075196	0,398273	1,044507	0,738281	0,324633	0,27369	0,523108	0,603561	0,299316	0,252113	0,311902	0,15267	1971
1972	0,258176	-0,14914	-1,0659	-0,85948	-0,60026	-0,49709	-0,62616	-0,35395	0,16833	0,531623	0,624058	-0,158966	1972
1973	-0,07743	0,318704	0,643351	1,001306	1,026496	1,258582	1,161419	1,11569	0,294412	0,247682	0,63036	1,361818	1973
1974	1,439385	1,116315	1,049307	0,771855	1,121736	0,828019	0,894066	0,865836	1,154593	0,556113	0,420407	0,588975	1974
1975	0,701589	0,930708	0,938012	0,596444	0,490189	0,892263	0,702343	0,316303	-0,179721	0,281069	0,136166	0,129678	1975
1976	0,203047	-0,27086	-0,68549	-0,62615	-0,76925	-0,98429	-0,67888	-0,37031	0,03272	0,249294	0,062874	0,140464	1976
1977	-0,08427	-0,34991	-0,13203	0,240446	-0,22915	-0,44848	-0,70551	-1,21501	-1,346787	-1,45342	-1,090302	-0,941732	1977
1978	-0,57123	0,035444	-0,08337	-0,14957	0,112501	0,093882	0,166836	0,378268	1,06144	1,091767	0,660106	0,259986	1978
1979	0,293012	-0,07821	-0,54587	-0,68596	-0,63136	-0,72595	-0,65025	-0,61524	-1,180566	-1,25948	-1,110983	-1,196547	1979
1980	-0,86538	-0,61861	0,289081	0,242921	0,357904	0,75219	0,795875	0,550998	0,414888	0,330007	0,644874	1,190189	1980
1981	1,306699	1,279988	0,866469	0,394788	0,87694	0,533046	0,666033	0,690121	1,108583	1,202045	0,97586	1,361757	1981
1982	1,156813	1,024569	0,931008	1,668401	1,484709	1,590673	1,729816	1,850717	1,21256	1,009916	0,812863	-0,048634	1982
1983	-0,31466	0,15651	-0,23569	-0,73332	-1,06087	-0,75877	-1,13193	-1,06418	-0,827937	-0,57815	0,131861	0,000443	1983
1984	-0,15847	-0,66509	0,049376	0,545153	0,461577	0,322226	0,727984	0,650658	0,296061	0,026914	-0,4128	-0,593488	1984
1985	-0,56392	-0,38809	-0,88207	-1,48675	-1,42886	-1,42462	-1,80374	-1,87422	-1,619582	-1,15538	-0,94213	-0,85261	1985
1986	-0,6554	-0,57614	-0,83081	-0,79773	-0,82185	-0,51433	-0,56545	-0,68928	-0,836537	-0,9528	-1,38498	-0,597206	1986
1987	-0,25068	-0,98607	-0,15381	0,063612	0,418051	0,288829	0,415079	0,777411	0,704603	0,800871	1,056726	1,074354	1987
1988	-0,13678	0,109691	-0,21833	-0,12036	-0,43983	-0,70445	-0,96511	-1,26356	-1,099427	-1,10379	-0,822333	-1,329184	1988
1989	-1,31138	-1,64052	-1,88477	-2,27246	-2,16862	-2,04373	-1,80519	-1,70146	-1,569897	-0,91533	-1,206982	-1,184355	1989
1990	-1,33181	-0,81003	-0,71743	-0,03076	0,273736	0,130178	0,385956	0,372188	0,748221	0,129756	0,254003	0,543228	1990
1991	0,515246	0,422005	0,325492	0,967813	1,340789	1,405771	1,243523	1,247366	1,339301	1,264457	0,643392	0,320456	1991
1992	0,334556	0,5509	1,019696	0,144046	-0,57565	-0,26496	-0,13588	-0,26819	-0,824724	-0,60527	-0,22021	0,175082	1992
1993	0,42424	0,182184	-0,28115	-0,15212	0,072753	-0,43561	-0,60222	-0,44933	-0,248769	-0,77906	-0,644269	-1,000589	1993
1994	-1,23715	-1,23514	-1,28362	-1,4606	-1,69899	-1,10828	-1,17188	-1,04062	-1,393647	-1,04898	-1,057383	-0,693959	1994
1995	0,103516	-0,45976	0,549722	1,048613	0,795657	0,118852	0,397454	0,268972	0,654135	0,562734	0,654415	0,304768	1995
1996	-0,62563	0,042759	-0,31794	-0,15708	0,005866	0,021769	-0,27561	-0,53216	-0,171773	0,2259	-0,549126	-0,464527	1996
1997	-0,65968	-0,74704	-0,97089	-0,53189	-0,45122	-0,20112	0,163367	0,907689	0,302808	0,854846	1,148604	1,6639	1997
1998	1,732045	1,871095	2,063786	1,135191	1,69139	1,729826	1,574564	0,850025	1,161338	0,927845	1,27265	0,928183	1998
1999	0,91034	1,36557	1,072012	1,084859	0,174581	0,458019	0,156031	0,28314	-0,26883	-0,73777	-0,836096	-1,160125	1999
2000	-0,59764	-1,22378	-0,64656	0,220424	0,48107	0,216467	0,345511	0,305111	0,721054	1,068002	0,699772	0,7894	2000
2001	-0,01331	-0,41313	-1,28743	-1,57956	-1,18441	-1,4516	-1,55666	-1,50988	-1,582735	-2,16676	-1,672995	-0,407395	2001
2002	0,160438	-0,17391	0,293842	0,73228	0,54977	0,723186	1,030377	1,132554	1,347285	1,824194	1,610393	0,425335	2002
2003	0,331517	0,879803	0,580507	0,522207	0,421828	0,197129	-0,0896	-0,44984	-0,423483	-0,28978	-0,326816	0,248725	2003
2004	1,023582	0,698102	0,735807	0,066366	-0,00048	0,382849	0,445481	0,726947	0,049108	-0,61301	-0,457054	-0,813681	2004
2005	-1,04022	-0,99679	-0,80966	-0,8936	-0,75579	-0,82376	-0,40431	-0,73176	-0,093271	0,280612	0,440021	0,810744	2005
2006	0,198195	0,607003	0,34042	0,066932	-0,1728	0,123364	-0,24749	-0,40111	-0,457666	-0,47728	-0,541314	-0,939528	2006
2007	-0,90829	-1,85877	-1,69877	-1,5422	-1,5749	-1,82957	-1,81411	-1,73866	-1,961925	-1,77762	-1,759193	-0,941859	2007
2008	-1,34058	-0,99028	-0,56821	-0,56124	-0,29658	-0,4072	-0,39436	-0,44653	0,517842	0,188931	-0,457552	-1,039065	2008
2009	-0,64002	0,284187	0,379713	0,415838	0,364897	0,213046	0,197486	0,279706	-0,279647	-0,20319	-0,031744	0,221851	2009
2010	0,488594	0,528458	0,418172	0,644198	0,713139	1,633342	1,694628	1,627419	1,486891	2,863554	2,834031	2,564668	2010
2011	2,399627	2,030304	1,872726	2,037675	1,978069	1,401997	1,17595	1,61477	1,500318	-0,49811	-0,450148	-0,599517	2011
2012	-0,14453	0,740352	0,906439	0,865211	1,162951	0,985172	0,921111	0,603323	0,378905	-0,31808	-0,139429	0,420461	2012
2013	0,217681	-0,26648	-0,47413	-0,78678	-1,29516	-0,72327	-0,40758	-0,52004	-0,488449	0,454474	0,654902	-0,198389	2013
2014	-1,0134	-1,55501	-1,58745	-1,09059	-0,3228	0,152171	-0,06982	0,262671	1,146359	0,682145	0,823038	1,696748	2014
2015	2,079598	2,430851	2,685862	2,640462	2,165365	1,926481	1,920664	1,765649	1,363037	1,304103	1,082716	-0,373038	2015
2016	-0,31957	-0,44208	-0,56819	-1,31645	-1,05536	-1,35158	-1,40719	-1,35813	-1,521201	-1,93891	-1,92349	-1,351114	2016
2017	-1,99204	-2,13047	-2,19174	-1,88874	-1,82515	-1,49356	-1,30651	-1,27848	-1,343933	-0,97943	-1,173905	-1,348783	2017
2018	-1,81213	-1,22087	-0,49569	-0,89111	-0,85119	-1,02169	-0,99151	-1,07219	-0,823574	-0,69457	-0,684355	-0,624983	2018
2019	-0,78029	-0,93013	-1,57803	-1,2093	-1,48593	-1,74058	-1,52843	-1,25616	-1,35132	-1,63452	-1,94548	-2,667512	2019

Similar results in studies conducted for other regions in our country, an increase in drought has also been observed. Ilgar (2010) examined the drought situation and trends in Çanakkale Province with the SPI method and observed an increase in the annual drought conditions of the province. Arslan et al. (2016) calculated the SPI values within the Kızılırmak for 1, 3, 6, 9, 12 and 60-month periods

and showed that there were significant increases in the drought periods in the Kızılırmak in terms of 12 and 60 month periods when compared with the droughts in the past years. Karaer et al. (2018) conducted a drought analysis of Bilecik Province with SPI method in 1, 3, 6, 12 and 24 month periods in their study and they found that drought is felt in 6 and 12 month periods and drought is generally seen in summer months. Bonaccorso et al, in their study in Sicily, they stated that periods such as 1, 3, 6, 9, 12 affect the sensitivity of measuring drought. Camalan et al. (2017) calculated the SPEI drought index in 1, 3 and 12-month time periods, examined the temporal and spatial incidence of drought, and evaluated the climatological trend of the drought that may occur in the future.

CONCLUSIONS

In this study, trend analysis and drought analyzes were carried out using temperature and precipitation data from the Bursa Province between 1960 and 2019. When the temperature and precipitation trend analysis results for Bursa Province are examined; an increasing trend was found for all time intervals in temperature data, but a statistically significant trend could not be obtained in precipitation data.

Considering the annual average precipitation results; although there is a general increase, it was observed that there was a decrease in precipitation of -4.19 mm/year for the years 2010 – 2019. These studies are very important in order to predict precipitation and temperature anomalies for the future.

When the results of drought analysis of SPI and SPEI indices in a 12-month period are examined; it was observed that drought was generally at normal levels. According to the results of the annual evaluation for the time period considered; According to the SPI index, 1

year was extremely dry, 2 years severely dry, 3 years moderately dry, but no periodic integrity was found. According to the SPEI index, 1 year was extremely dry, 1 year severely dry, 8 years moderately dry. It could be said that the period between 2016 and 2019 was dry. Extremely dry according to the 1961 SPI index, severe dry according to the SPEI index; 1979, 1988, 1989 were moderately dry for both indices; Severe dryaccording to 1993 SPI index, moderate dry according to SPEI index; While 2019 was severely dry according to the SPI index, it was found to be extremely dry according to the SPEI index. For the years 1999, 2008, 2016 and 2017, the drought value, which was moderately dry in the SPEI index, was observed at normal levels in the SPI index. In this case, it was observed that the SPEI index was more sensitive in detecting drought, but similar values could be obtained for both indices according to the annual evaluation results. By using drought indices, a data bank can be created with the data obtained from temperature and precipitation data for many years and the forecasts made, and necessary precautions can be taken against this natural disaster, which will affect all living things, especially agricultural activities and water resources.

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DETERMINATION OF GERMINATION CHARACTERISTICS OF COVERED *LOLIUM PERENNE* L. SEEDS UNDER DROUGHT STRESS

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Lolium perenne (L.) is one of the most used turfgrass species for the establishment of multipurpose turfgrass both in Turkey and around the world. *Lolium perenne* (L.) is sensitive to environmental abiotic stress conditions such as drought and salinity. This study aims to improve the germination properties of *Lolium perenne* (L.) seeds (turf varieties of Sun and Ringle) under drought stress. The seeds of Sun and Ringle were covered with the seed coating solution, included *Endomycorrhiza, Trichoderma spp., Bacillus subtilis, Bacillus megaterium.* The coating solution was applied 4 liters 1000 kg seeds^{-1}. This germination experiment was conducted in randomized parcel design with 4 replications. A total 6 different drought stress levels (0, -2, -4, -6, -8, -9.8 Mpa) were created in this study and used Polyethylene glycol-6000 (PEG-6000). 20 seeds were laid in each petri dish and placed in the germination cabinet (70% humidity, 20 ^{o}C temperature, 14 hours light-2000 lux, and 10 hours darkness). At the end of the 14th day, the germination rate was calculated and the fresh weight of the shoots and roots, and length of shoots and roots were determined. The results obtained in this study indicated that the coating treatments improved to germination properties of *Lolium perenne* (L.) seeds under drought stress.

Keyword: *Lolium perenne* (L.), seed coating, drought stress, germination

INTRODUCTİON

Lolium perenne also known as “English grass” is the widely used cool climate type in the world. Especially in parks, which have a cool and humid climate, is preferred. In the green areas or from houses to golf courses, L. perenne is the most grown grass type. It is also used for climatization in living areas. Especially L. perenne is used in different kinds of green areas or mixed fields because the seed is cheap and easily available as well as creates a fresh and green appearance in a short time (Gül and Avcıoğlu 1997).

Plants are exposed to important abiotic stress factors such as drought, salinity, the high or low temperature that cause yield losses in agricultural production (Janmohammadi et al., 2008). In general, Drought as a meteorological concept refers to the period without precipitation, which may cause a significant decrease in the development of plants with the water contained in the soil (Örs and Ekinci, 2015). In this dry period, drought occurrence depends on the water holding capacity of the soil in addition to the rate and amount of evapotranspiration by plants (Kalefetoğlu and Ekmekçi, 2005). Drought is affecting approximately 26% of the worldwide area used for agricultural purposes. From this point of view, it can be said that drought is one of the immense environmental stress factors (Blum, 1986; Kalefetoğlu and Ekmekçi, 2005). The problem of water scarcity, extreme temperatures, and low atmospheric humidity causes drought that restraint plant growth and decrease yield (Mostafavi, 2011). Although it varies according to plant species, germination time, early seedling development and flowering periods are most sensitive to drought (Ahmadi et al., 2009).

To combat such stress factors, the development of resistant/tolerant species and varieties with the help of traditional breeding methods, biotechnological applications, and molecular assisted selection technologies is seen as the most top solutions (Samancıoğlu and Yıldırım, 2015). However, since

these methods and applications are costly and take a long time, more practical solutions should be emphasized. Nowadays, seeds are coated with many active substances give tolerance to plants grown under stress conditions and ensuring germination and early seedling development period (Kaufman, 1991). In addition, with the coating method, it is possible to carry the materials that will increase the germination performance of the seed as well as decrease disease and pests susceptibility during the germination and seedling period (Taylor et al., 1998). For this purpose, the transfer of rhizobacteria group bacteria to seeds for biocontrol purposes is one of the appropriate techniques (Deaker et al., 2004; Junges et al., 2013; Vavrina and McGovern, 1990).

MATERIAL AND METHODS

This research was carried out under laboratory conditions at Akdeniz University, Faculty of Agriculture, Department of Agronomy. In the experiment, seeds of Sun and Ringle grass type (turf) Lolium perenne cultivars were used. As the coating material, the commercial product called Panoramix, which is a new generation seed coating preparation and containing Trichoderma harzianum, Bacillus subtilis, and Bacillus megaterium, was preferred. Panoramix was obtained from Koppert Biological Systems.

The coating solution was applied to the seeds as 4 L/1000 kg of seeds. In addition, control trials were created with 0 dose application. The seeds were mixed with the coating solution and covered in a laboratory environment in a way that they would not expose to sunlight.

The experiment was carried out in the "Random Plots Trial Design" with 4 replications. In order to examine the responses of seeds to drought stress during germination periods, 6 different drought levels were determined. To establish these drought stress levels, solutions with PEG-6000 and 0, -2, -4, -6, -8, and -9.8 bar water holding power were used. The osmotic potentials for each drought level were adjusted as suggested by Michel and Kaufmann (1973). Polyethylene glycol (PEG), a high molecular weight substance, regulates water uptake and creates water stress in the environment. However, PEG-6000 is not taken up by plant roots and does not cause any toxic effects to plants. Due to the decrease in the oxygen of the solution prepared with PEG-6000 over time, the papers of the germination medium were changed every 3-4 days, as recommended by Çarpici and Erdel (2015).

A total of 48 petri dishes with a diameter of 15 cm were used in this research. Covered seeds were placed in petri dishes containing double-layer germination paper as 20 seeds per petri dish. The seeds placed between double-layered germination papers were poured with 10 ml of solutions containing various PEG6000 concentrations, and then the petri dishes were wrapped with parafilm to prevent evaporation. The petri dishes were placed in germination cabinets with 10 hours of darkness and 14 hours of light, set at 20±1°C. Petri dishes were kept here for 14 days and necessary observations were taken in the petri dishes opened afterward (Şehirali, 1997; Castroluna et al., 2014).

In the experiment, observations were made at the same time every day, and seeds with a rootlet length exceeding 2 mm were considered germinated according to Soltani et al. (2012). At the end of the 14^{th} day, the germination percentage (%) was determined by counting the total germinated seeds (Scott et al., 1984). In addition, 10 shots were taken as a sample from each petri dish on the 14th day of germination and the lengths of petiole and rootlets and fresh weights were measured in these samples.

As a result of the experiment, Analysis of variance was performed on the data obtained following the experimental designs, and the means were compared according to the Duncan test at the 5% significance level. For this purpose, the SPSS package program was used.

RESULTS AND DISCUSSIONS

As seen in Table 1, drought stress and coating application influenced the germination rate and other early seedling characteristics in this study (Table 1). There are significant interactions among cover application and drought stress levels except for the fresh weight of roots. Drought stress at different levels reduced germination rate, dramatically. The highest germination rate (80.00%) was observed in 0 drought stress-control (Table 1). On the other hand, the lowest ratio was 10.00% in -8 MPa drought stress level. Furthermore, as the level of drought stress increased, seedling values such as length of roots and shoots, fresh weight of roots and shoots decreased. However, coating applications contributed positively to the development of *Lolium perenne* seeds germinating under drought stress. Another important result seen in Table 1 is that the Sun variety showed better germination performance than the Ringle. For example, Sun had the highest length of shoots of 73.87 mm while Ringle had 57.46 mm.

Table 1. Average values obtained in drought stresses and Duncan groups

Turfgrass variety	Drought stresss, MPa	Germination rate, %	Length of roots, mm	Length of shoots, mm	Fresh weight of roots, mg/plant	Fresh weight of shoots, mg/plant
Sun	0	80.00a	53.58a	73.87a	1.44a	6.11a
	-2	62.50b	44.45a	69.65a	1.14ab	6.60a
	-4	58.12b	49.23a	57.81b	0.58bc	3.01b
	-6	46.25c	27.18b	27.55c	0.23c	1.27c
	-8	18.12d	17.56c	9.51d	0.01c	1.16c
Ringle	0	63.12a	48.55a	57.46a	1.54a	6.95a
	-2	53.75b	46.78a	55.76a	0.65b	5.35a
	-4	50.00b	30.70b	37.57b	0.17bc	1.92b
	-6	40.62c	22.26bc	25.83c	0.26bc	1.47b
	-8	10.00d	9.68c	6.92d	0.01c	0.29b
Coating (C)		**	**	**	ns	ns
Drought Stress (DS)		**	**	**	**	**
C*DS		**	**	**	ns	**

**: Means with a different letter(s) in each trait is significantly different at a 1% probability level according to Duncan's multiple range test. ns: not significant

Lolium perenne is commonly known as having poor drought tolerance with a low recuperative ability (Pornaro et al., 2020). Based on this, Pornaro et al. (2020) conducted a drought tolerance experiment with 11 *Lolium perenne* varieties. Although there are differences between the varieties, it has been stated that the recovery of the turfgrasses was slow and at the end of the experiment the variability in green cover between cultivars was greater than at the beginning.

Drought adversely affects the germination of the plants during the germination or early seedling periods, preventing reaching a sufficient number of plants per unit area, causing the plant to not receive the water it needs (Öztürk, 2015). Furthermore, drought stress causes morphological changes of plant parts such as roots and leaves under arid conditions (Yılmaz and Kısakürek, 2021). Similarly, drought stress inhibited to germination ratio and other seedling growth traits in our study.

CONCLUSION

The germination stage is one of the most critical steps for all plant development. Because plants are very sensitive to environmental factors during this period. As a result of the data obtained in this study, it was determined that *Lolium perenne* is sensitive to drought stress during the germination period. However, with coating applications, the severity of drought stress was reduced and a positive contribution was made to germination performance.

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DETERMINATION OF PLANT NUTRIENT CONTENTS OF SOME LOCAL BARLEY (Hordeum vulgare l.) VARIETIES OF EASTERN AND SOUTHEASTERN ANATOLIA REGIONS

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ABSTRACT

Barley is a very important animal feed plant in terms of protein content as well as plant nutrients and vitamins it contains. In this study, it was aimed to determine macro (N, P, K, Ca and Mg) and micro-nutrients (Fe, Mn, Zn and Cu), and protein contents of local barley varieties of Eastern and Southeastern Anatolia Regions. For this purpose, leaf and grain samples were taken from 25 barley varieties. Total N, P, K, Ca, Mg, Fe, Mn, Zn and Cu were analyzed in the leaves and grain samples, and crude protein contents were determined in the grain samples. It was determined that the total N, P, K, Ca, Mg, Fe, Mn, Zn and Cu contents of leaf samples of local barley varieties of Eastern and Southeastern Anatolia Regions were 2.24 - 4.38 %, 0.03 - 0.06 %, 0.75 - 1.79 %, 0.96 - 2.43 %, 0.19 - 0.34 %, 50 - 216 ppm, 35.6-84.2 ppm, 7.9 - 14.2 ppm and 3.4-8.3 ppm, respectively. It was found that the protein, total N, P, K, Ca, Mg, Fe, Mn, Zn and Cu contents of the grain samples of the barley varieties were 11.0 - 15.82.72 %, 1.88 - 2.72 %; 521 - 844 ppm, 3447 - 5276 ppm, 720 - 1581 ppm; 1473 - 2290 ppm; 5 - 96 ppm; 11.8 - 26.9 ppm; 11.9 - 76.4 ppm and 2.1-4.1 ppm, respectively. As a result, it was determined that the leaf and and grain samples of the local barley varieties in Eastern and Southeastern Anatolia Region of Turkey were determined to vary in terms of plant nutrients contents, and that it can be used as animal feed because it was rich in protein content.

Keywords: Local, Barley, Variety, Macro, Micro, Nutrients,

INTRODUCTION

Today, human health and animal feeding are even more prominent the interest in high nutritional products is increasing steadily. Especially in recent years, the need for feed plants has been increasing and the decrease in livestock activities has been an important feed, and at the same time, the need for studies on barley plants, which is an important cereal, is also increasing. Barley contains plant nutrients and vitamins, as well as a very important feed plant in terms of protein content.

Cölkesen et al. (2002) carried out to determine the agricultural and quality characteristics of 25 barley varieties in Kahramanmaraş and Şanlıurfa conditions in 1997-1999, and determined that plant height varied from 79.50 to110.8 cm, spike length from 7.53 to 9.44 cm, 1000 grain weight from 37.14 to 50.49 g and grain yield from 367.2 to734.9 kg/da in Kahramanmaraş. In Şanlıurfa conditions, the plant height was 55.98-80.60 cm, the spike length was 5.59-7.24 cm, the 1000 grain weight was 41.62-52.52 g, the protein ratio was 10.32-11.95% and the grain yield was 419.2-540.8 kg/da.

Kün and Akbay (1980) examined beer characteristics of 6-row and 2-row Tokak 157/37 barley varieties in Ankara conditions, and found that statistically significant differences between varieties in

terms of protein ratio; They reported that the protein ratio and 1000 grain weight of the 2-row Tokak variety were higher than the 6-row varieties.

Karahan and Sabancı (2010) carried out to determine the yield and yield components of some barley varieties in the ecological conditions of Southeastern Anatolia (Diyarbakır, Ceylanpınar); The adaptation of Akhisar-98, Bilgi-91, Bornova-92, Kaya, Sur-93, Süleymanbey-98, Şahin-91, Şerifehanım-98 and Vamıkhoca-98 cultivars to the region were investigated, and found that the earing time of barley varieties was 10 days shorter in Ceylanpınar location compared to Diyarbakır, however, plant height was shorter, hectoliter weight and protein ratio were higher. The grain yield of the cultivars varied from 388-487 kg/da, the lowest grain yield was determined in Bornova-92 variety, the highest grain yield in Vamıkhoca-98 variety, and Ceylanpınar had a high grain protein content. However, they stated that the grain yield decreased by 40%.

Turkey is among the important countries in terms of barley variety. When the literature reviews are examined, it is noteworthy that the studies on the barley plant, which are an important feed plant and utilized in different forms in human nutrition, are evaluated in terms of quality criteria and studies on mineral compositions of barley plants are less.

In this study, it was aimed to determine the contents of crude protein, macro- and micro-nutrients of 25 local barley varieties of Eastern and Southesatern Anatolia Region of Turkey, and to determine the differences among barley varieties. With the data obtained, the fact that these 25 local barley varieties originating in Turkey will be the subject of studies will make it inevitable to conduct studies on local varieties and to evaluate local resources. It is hoped that such a study on these cultivars unique to Turkey will shed light on future studies.

MATERIALS AND METHOD

This research was carried out as a field trial in the field of Akdeniz University Faculty of Agriculture Application Farm in 2015-2016 in winter. Twenty five (25) local barley cultivars from Eastern and Southeastern Anatolia Regions of Turkey, which were obtained from ICARDA (International Center for Agricultural Research in the Dry Areas), were used as trial material (Table 1).

This study was carried out with 3 replications according to the Randomized Block Design at the Research Station of the Faculty of Agriculture, University of Akdeniz, Antalya-Turkey. plants 20 cm row spacing, 3 m row height, planted in 5 rows and the sowing amount was set at 20 kg / da. Planting was taken place on November 28, 2015. Based on the farmer's conditions, fertilizer was applied to the parcels in the form of ammonium nitrate in the order of 8 kg N. Half of nitrogenous fertilizer is planted with sowing, the other half is given before stem elongation period. The harvest of the plants was made by hand when the humidity level in the grain fell below 12-13%.

Prior to the establishment of the experiment, the soil samples were taken to represent the soil characteristics of the experiment area, representing 0-20 cm deep test plots in according to Jackson (1967). In soil samples texture was measured by hydrometer method (Bouyoucos, 1955); soil pH and electrical conductivity (EC) in 1: 2.5 soil: water ratio (Jackson, 1967); $CaCO_3$ by Scheibler calcimeter (Caglar, 1949); organic matter by modified Walkey-Black method (Black, 1965); total nitrogen was determined by the Modified Kjeldahl method (Kacar and Inal, 2008), available phosphorus by $NaHCO_3$ extraction (Olsen and Sommers, 1982); exchangeable K, Ca and Mg by 1 N ammonium acetate (pH = 7) extraction (Kacar, 1995), available iron, manganese, zinc and copper by DTPA extraction methods. The results of soil analysis of the experiment area were given in Table 2.

Table 1. Local barley varieties used in the study

No	Local Varieties	City to which it belongs	Region
1	IG 18768	Tunceli	Eastern Anatolia
2	IG 18773	Batman	Southeastern Anatolia
3	IG 28741	Mardin	Southeastern Anatolia
4	IG 28831	Malatya	Eastern Anatolia
5	IG 113008	Bingöl	Eastern Anatolia
6	IG 115942	Bitlis	Eastern Anatolia
7	IG 128074	Erzurum	Eastern Anatolia
8	IG 128117	Gaziantep	Southeastern Anatolia
9	IG 128118	Şanlıurfa	Southeastern Anatolia
10	IG 128119	Diyarbakır	Southeastern Anatolia
11	IG 128139	Kars	Eastern Anatolia
12	IG 128142	Ağrı	Eastern Anatolia
13	IG 128143	Erzincan	Eastern Anatolia
14	IG 128147	Şırnak	Eastern Anatolia
15	IG 128149	Ağrı	Eastern Anatolia
16	IG 128150	Siirt	Southeastern Anatolia
17	IG 128151	Muş	Eastern Anatolia
18	IG 128152	Elazığ	Eastern Anatolia
19	IG 128154	Van	Eastern Anatolia
20	IG 128164	Kars	Eastern Anatolia
21	IG 128174	Ağrı	Eastern Anatolia
22	IG 128175	Kars	Eastern Anatolia
23	IG 128176	Kars	Eastern Anatolia
24	IG 128178	Erzincan	Eastern Anatolia
25	IG 128191	Diyarbakır	Southeastern Anatolia

Table 2. Physical and chemical properties of the soil of the trial area

Soil Properties		Referencesr
Texture	Clay Loam	Black (1957)
pH	7.33	Kellog (1952)
EC (dS/m)	0.17	Soil Survey Staff (1951)
Organic Matter (%)	2.1	Thun vd (1955)
$CaCO_3$ (%)	53.77	Aereboe ve Falke (Evliya 1964)
Toplam Azot (%)	0.057	Loue (1968)
Available P (ppm)	9.5	Olsen ve Sommers (1982)
ExchangeableK (me/100 g)	0.155	Pizer (1967)
ExchangeableMg(me/100g)	0.715	Loue (1968)
ExchangeableCa(me/100 g)	32.8	Loue (1968)
Available Fe (ppm)	4.15	Lindsay ve Norvell (1978)
Available Zn (ppm)	1.2	Lindsay ve Norvell (1978)
Available Cu (ppm)	1.15	Lindsay ve Norvell (1978)
Available Mn (ppm)	18.35	Lindsay ve Norvell (1978)

As a result of the analyzes made, the texture of the soil of the trial area was clay loam, the soil reaction was neutral, unsalted in terms of EC, little humus, extremely calcareous; It was determined that it is in the class of low in total N, available P, exchangeable K, exchangeable Na and available Zn, good in exchangeable Ca and exchangeable Mg, sufficient in available Fe, Cu and Mn (Table 2).

Leaf and grain samples from each variety were taken, and the concentrations of P, K, Ca, Mg, Fe, Mn, Zn and Cu in the digestates by wet digestion were determined by using ICP-OES according to Kacar and Inal (2008). The total N content of the cultivars was determined by the Modifiye Kjeldahl method (Kacar and Inal, 2008), and the protein content of the grains; the result of multiplying the total N content by a factor of 5.83 is obtained. Analysis results of leaf samples were compared with the optimum limit values which given by Jones et al. (1991).

RESULTS AND DISCUSSION

Macro and micro nutrients contents of leaf samples

In recent years, increases in feed prices and such factors have become a problem for our country's livestock. Animal husbandry activities of a country are extremely important, and the supply of quality feed to the animals raised is of such importance. From this point of view, and when the country's feed needs are evaluated, the nutritional content of the straw obtained from barley is extremely important, and it is very important for animal and therefore human health to produce barley straw of high quality and nutritious value and to feed these nutritious straws to animals.

The total N content of the leaf samples of the local barley cultivars of the Eastern and Southeastern Anatolia Regions were between 2.24-4.38%; P contents ranged from 0.03 to 0.06 %; K contents are 0.75-1.79%; Ca contents ranged from 0.96 to 2.43%; Mg contents ranged from 0.19 to 0.34%. Fe contents ranged from 50.4 to 216 ppm; Mn contents ranged from 35.6 to 84.2 ppm, Zn contents ranged from 7.9 to 14.2 ppm and Cu contents ranged from 3.4 to 8.3 ppm (Table 3).

Demir and Sönmez (2019) determined the nutrients contents of some barley (Hordeum vulgare l.) varieties in the Mediterranean and Aegean Regions. It was stated the total N content of leaf samples of local barley cultivars was between 2.02-3.78%, P content was between 0.19-0.35%, K content was between 0.81-2.40%, Ca content was between 0.9-1.78%, Mg content was between 0.17-0.36%, Fe content was between 70.5-192.9 ppm, Mn contents varied from 35.5–169.5 ppm, Zn contents 6.0–21.8 ppm, and Cu contents between 4.3–26.9 ppm. When the results obtained in our study are compared with the results of Demir and Sönmez (2019), it was seen that all these results were compatible with each other except for P. In our country, it is seen that the farmers generally do not fertilize for other plant nutrients except nitrogen element in barley cultivation. In the study carried out on local barley varieties belonging to the Mediterranean and Aegean Regions, Demir and Sönmez (2019) determined that no fertilization other than nitrogen was applied. This situation suggests that the P utilization efficiency of barley plants grown in Eastern and Southeastern Anatolia Regions is lower. As a matter of fact, when the nutrient contents of local barley varieties in the Eastern and Southeastern Anatolia Regions were compared by Jones et al. (1991); the varieties were found to be in sufficient class in terms of the contents of N, Ca, Mg, Cu, Fe and Mn contents; and the contents of P, K and Zn of the varieties were in the insufficient class.

Table 3. The Mineral contents of leaf samples of local barley varieties

No	Variety	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
1	IG 18768	4.18	0.05	0.86	0.98	0.21	14.2	3.9	106.1	38.3
2	IG 18773	4.38	0.04	0.75	0.96	0.22	12.8	3.4	92.3	39.9
3	IG 28741	2.86	0.04	1.12	1.56	0.31	11.5	5.1	137.9	60.3
4	IG 28831	3.98	0.06	1.63	1.65	0.30	11.8	5.7	91.6	76.0
5	IG 113008	3.30	0.05	1.79	1.29	0.33	12.0	7.0	76.8	82.3
6	IG 115942	3.35	0.04	1.33	1.58	0.32	10.5	6.1	77.3	67.8
7	IG 128074	3.02	0.05	1.70	1.74	0.28	11.3	7.3	216.0	61.8
8	IG 128117	4.18	0.05	1.19	1.37	0.28	10.1	4.5	96.8	63.6
9	IG 128118	3.33	0.04	1.29	1.22	0.31	9.9	5.6	79.6	59.4
10	IG 128119	3.55	0.04	1.14	1.74	0.33	12.1	5.8	69.9	62.3
11	IG 128139	3.93	0.04	1.09	1.38	0.29	9.1	7.5	88.5	51.7
12	IG 128142	3.43	0.04	1.28	1.47	0.25	10.1	6.3	84.5	46.4
13	IG 128143	3.15	0.04	1.23	1.33	0.27	8.8	8.3	78.3	51.8
14	IG 128147	2.24	0.04	1.43	2.43	0.32	12.4	5.6	88.1	84.2
15	IG 128149	3.34	0.04	1.21	1.76	0.34	7.9	4.7	123.6	50.7
16	IG 128150	2.28	0.03	0.92	1.74	0.28	9.6	6.7	101.8	52.7
17	IG 128151	2.65	0.04	0.85	1.79	0.24	8.0	5.3	133.8	50.0
18	IG 128152	3.49	0.04	1.26	1.38	0.23	10.7	4.7	85.4	44.5
19	IG 128154	2.69	0.03	0.92	1.56	0.28	9.5	4.4	80.7	71.2
20	IG 128164	3.36	0.05	1.17	1.42	0.26	10.7	7.3	52.9	43.9
21	IG 128174	3.84	0.04	1.32	1.33	0.26	8.0	5.6	87.7	41.1
22	IG 128175	3.33	0.05	1.31	1.32	0.29	12.3	6.9	50.4	63.2
23	IG 128176	3.35	0.05	1.13	1.50	0.28	12.6	6.0	73.7	41.7
24	IG 128178	3.34	0.04	1.06	1.17	0.19	8.4	5.2	74.4	35.6
25	IG 128191	3.19	0.05	1.07	1.48	0.27	10.6	5.5	98.3	43.8
Minimum		**2.24**	**0.03**	**0.75**	**0.96**	**0.19**	**7.9**	**3.4**	**50.4**	**35.6**
Maksimum		**4.38**	**0.06**	**1.79**	**2.43**	**0.34**	**14.2**	**8.3**	**216**	**84.2**
Average		**3.35**	**0.04**	**1.20**	**1.49**	**0.28**	**10.60**	**5.78**	**93.86**	**55.37**

Protein, macro and micro nutrients contents of grain samples

One of the most used grains in animal nutrition is barley. One of the most important reasons for this is that barley husk contains sufficient amount of husk. Barley is a very tasty feed especially for ruminants, and it is a feed plant mostly used for ruminants. Barley grain production, which is rich in nutritional value and plant nutrients, is extremely important and appears as an important issue in animal nutrition.

The protein contents of local barley variety grain samples in Eastern and Southeastern Anatolia Regions were between 11-15.8%; the total N contents between 1.88-2.72 %; P contents were between 521-844 ppm, K contents were between 3447-5276 ppm, Ca contents were between 720-1581 ppm; Mg contents were between 1473-2290 ppm; Fe contents were between 5-96 ppm; Mn contents were between 11.8-26.9 ppm; Zn contents ranged from 11.9-76.4 ppm and Cu content ranged from 2.1-4.1 ppm (Table 4).

Table 4. The Mineral nutrients contents of grain samples of local barley varieties

No	Variety	N (%)	Protein (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
1	IG 18768	2.40	14.0	619	3805	1311	1881	21.2	2.9	40.6	20.9
2	IG 18773	2.22	13.0	643	4644	1423	1982	20.0	2.1	56.6	26.9
3	IG 28741	1.95	11.4	603	4017	1482	1965	26.7	2.6	28.4	20.0
4	IG 28831	2.33	13.6	613	3447	1130	2070	76.4	3.4	17.9	18.5
5	IG 113008	2.25	13.1	705	3734	1528	2256	17.5	3.7	6.9	19.2
6	IG 115942	1.88	11.0	653	3499	1460	2107	17.1	3.6	32.4	19.0
7	IG 128074	2.32	13.5	783	3670	907	2230	25.1	3.7	14.1	16.5
8	IG 128117	2.72	15.8	779	4225	1251	2270	21.0	3.4	70.6	20.6
9	IG 128118	2.14	12.5	734	4433	1002	2003	35.6	3.4	61.1	13.3
10	IG 128119	2.13	12.4	761	3946	1004	2029	37.6	3.7	13.5	15.2
11	IG 128139	2.47	14.4	844	4901	1202	2290	16.2	2.7	55.8	15.6
12	IG 128142	2.16	12.6	639	4962	990	1714	12.5	3.0	26.2	15.5
13	IG 128143	1.92	11.2	521	3872	1020	1575	11.9	2.2	5.0	11.8
14	IG 128147	1.93	11.2	633	3537	1581	1724	13.7	3.5	53.3	16.1
15	IG 128149	2.66	15.5	727	4479	1358	2148	16.1	4.1	69.7	17.4
16	IG 128150	2.35	13.7	674	3740	720	1565	14.8	3.2	26.3	15.4
17	IG 128151	2.35	13.7	624	4873	1207	1473	20.1	3.3	27.9	19.1
18	IG 128152	2.06	12.0	616	5276	1001	1559	17.2	2.8	28.5	19.0
19	IG 128154	2.18	12.7	651	3943	1171	1822	15.2	3.3	65.9	19.0
20	IG 128164	2.00	11.7	632	3904	1120	1794	16.3	2.6	31.0	14.8
21	IG 128174	2.46	14.3	795	5094	1012	2167	13.8	3.6	57.2	20.6
22	IG 128175	2.57	15.0	819	4488	844	2063	19.2	3.2	5.7	18.3
23	IG 128176	2.66	15.5	823	4281	1156	1978	16.5	3.3	96.0	17.3
24	IG 128178	2.54	14.8	729	4106	907	1665	13.1	3.1	61.0	15.9
25	IG 128191	2.23	13.0	677	4929	1038	1591	13.6	2.9	40.7	17.5
Minimum		**1.88**	**11**	**521**	**3447**	**720**	**1473**	**11.9**	**2.1**	**5**	**11.8**
Maksimum		**2.72**	**15.8**	**844**	**5276**	**1581**	**2290**	**76.4**	**4.1**	**96**	**26.9**
Average		**2.28**	**13.3**	**692**	**4232**	**1153**	**1917**	**21.1**	**3.2**	**40**	**17.7**

In their study, Villacres and Rivadeneira (2005) reported that the P contents of the barley content varied between 2400-4700 ppm, 2200-4800 ppm for K content, 26-72 ppm for Fe content and 30-50 ppm for Zn content.

Altuntaş (2012) determined that the Ca concentration of barley grains varied between 306.7-428.7 ppm, the Mg concentration was between 1214-1439 ppm, the Mn concentration was between 15.4-21.2 ppm, the Zn concentration was between 28.4-39.6 ppm, and the Cu content was between 5.4-8.5 ppm.

Demir and Sönmez (2019) found that the protein content of the grain samples of local barley varieties was between 9.6-14.8%, the total N contents were between 1.65-2.54%, the P contents were between 3476-5993 ppm, the K contents were between 1156-6319 ppm, and the Ca contents were 725-1616 ppm and the Mg contents varied between 1368-2261 ppm. They reported that Fe contents varied between 22.7–75.1 ppm, Mn contents between 12.0–22.1 ppm, Zn contents between 16.9–43.3 ppm and Cu contents between 2.2–4.4 ppm.

When the mineral contents of the grain samples of the Eastern and Southeastern Anatolian local barley varieties were compared with other studies; it was determined that the protein, total N, K, Mg, Cu, Fe and Mn contents were good, the contents of P and Zn were deficient and Ca contents were in the high class.

RESULTS

- In this study, based on the basis of farmer growing conditions; the total N contents of the varieties and thus the protein content were determined at a good level. Also it was found that the majority of barley varieties can be used as fedder barley.
- As a result of the lack of phosphorus fertilization, the phosphorus content of the leaf and grain samples of the varieties was determined to be deficient. Due to the high pH and high Ca content of the soils of Turkey, the phosphorus becomes unavailable form and cannot be taken up by the plants. Considering these factors, it is necessary to fertilize barley at the appropriate rate.
- Leaf samples of the varieties were deficient in terms of K, but it was determined that the K content of the grain samples was generally at good levels, even though potassium fertilization was not applied. Not only the grain but also the straw of the barley plant is used in animal feeding. For this reason, the leaf K content is as important as the grain K content, and it is extremely important to feed the animals with straw.
- The experiment area has a high Ca content. For this reason, For this reason, the Ca content of both leaf samples and grain samples of the varieties was found to be high. At the same time, it was understood from this result that high Ca content can transform the P element into a useless form and limit its availability.
- The Zn content of Turkish soils is generally low. In addition, the lack of zinc fertilization caused the Zn content of both leaf and grain samples of the varieties to be low.
- As a result, leaves and grains of local barley varieties belonging to Eastern and Southeastern Anatolia Regions of Turkey differ from the plant nutrients contents; it has been understood that the grains of local barley varieties are rich in protein content, so it can be used as animal feed. It has been concluded that the leaves and grains of specific local barley varieties to Turkey are generally rich in terms of the mineral contents, however the detailed studies should be carried out in which fertilizer doses are adjusted in order to obtain healthier results.

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AGRICULTURAL USE POTENTIALS OF ACTINOMYCETES

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ABSTRACT

Actinomycetes, belonging to class Actinobacteria and order Actinomycetales, are Gram-positive, aerobic, filamentous bacteria. Because of their morphology, they were formerly considered a fungus, and therefore the word "mykes", which means fungi in Latin, was used in naming. Actinomycetes can be isolated from various environments such as soil, sea, air, insect or marine macro-organisms, plants, and extreme environments. Actinomycetes produce important secondary metabolites. These bacteria are known for their synthesis of bioactive compounds like antibacterial, antiviral, antifungal, anticancer, antidiabetic, anti-inflammatory, herbicide and insecticide. It was proven by studies that some of these bioactive compounds have important potentials in the area of agriculture. Nowadays, high amounts of herbicides or insecticides are used to protect plants from pests, and these chemicals can cause different dangers in the long term. The chemicals used are not only biologically harmful but also dangerous in that the target organisms gain resistance to these chemicals over time. Therefore, there is a need for both new and natural herbicide and insecticidal compounds. Actinomycetes are known to synthesize bioactive compounds that have the potential to satisfy these needs. At the same time, secondary metabolites of actinomycetes can be effective against pathogens such as viruses and fungi that can cause product loss. These bacteria may also be responsible to produce important plant growth promoters such as indole acetic acid (IAA), siderophore production, phosphate dissolution, ammonia production, hydrogen cyanide production. Today, studies about using actinomycetes to both prevent pathogenic organisms from harming plants and to support plant growth, are increasing day by day. For this reason, we aimed to compile studies that reveal the potential of actinomycetes in the field of sustainable agriculture in plant development and protection.

Keywords: Actinomycetes, Agriculture, Bioactive compounds, Sustainable agriculture

INTRODUCTION

Actinomycetes are Gram-positive, spore-like bacteria with a high G+C ratio, filamentous structure and a member of the Actinomycetales order. Actinomycetes are microorganisms that can be found in nature, primarily in soil, in various aquatic ecosystems, deep seas, Antarctica and desert regions (Chaudhary et al., 2013). It has been tried to isolate actinomycetes from many places, including beaches, underground waters, rice fields, orchards. It has been determined by the studies that actinomycetes constitute an important part of the soil microorganism population and they are the bacteria that take part in the recycling of organic materials (Jayasinghe and Parkinson, 2008).

Actinomycetes are biotechnologically important bacteria. Nowadays, more than 60% of known bioactive substances are derived from actinomycetes bacteria (Singh et al., 2018a), so they can be considered as potential producers of important secondary metabolites. The secondary metabolites they produce can be bioactive in various aspects such as antibiotic, antitumor, antifungal, antioxidant, anticancer, antialgal, anthelmintic, anti-inflammatory (Chaudhary et al., 2013). Secondary metabolites produced by actinomycetes can have properties such as drugs, pesticides,

animal and plant growth promoters besides antibiotics (Chaudhary et al., 2013). Due to the different potentials they have, these microorganisms are important in the field of agriculture as well as in the medical and pharmaceutical fields.

In the field of agriculture with actinomycetes bacteria; It is studied to reveal different properties such as supporting plant growth, biocontrol agents, biodegradation or biological improvement of complex organic substances, obtaining new bioactive substances, improving the nutritional value of plants and stimulating various defence pathways (Singh et al., 2018a). This group of microorganisms, which are effective in so many different fields, has promising potentials for sustainable agricultural practices.

In this study, besides the plant growth promoting potential of the microorganisms defined as actinomycetes, various antimicrobial substances on plant pathogens will be mentioned. In addition, in our article, some of the studies on secondary metabolites with herbicidal and insecticidal activity, which are obtained from these bacteria and can be used to reduce the use of harmful chemicals in agriculture, are included.

PLANT GROWTH PROMOTERS ACTINOMYCETES

Bacteria can support the growth of plants with properties such as nitrogen fixation, phytohormone production, specific enzymatic activity, and siderophore production (Ullah et al., 2015). Indole-3-acetic acid (IAA), a phytohormone, is considered to be a natural auxin and supports plant growth by regulating various cellular responses (Gunasinghe and Edirisinghe, 2020). IAA can be obtained by reaction of the supernatant of actinomycetes strains incubated in the appropriate medium with Salkowsky's reagent (Gopalakrishnan et al., 2011). As a result of studies on IAAs produced by microorganisms, it has been determined that the dry weight of the leaves and roots of the plants and the length of the roots increase (Shutsrirung et al., 2013). Siderofor is a kind of plant growth promoter that can be used as an iron source by plants. Siderophore production for actinomycetes can be detected by the presence of orange halos around bacterial colonies using the blue Chrome Azurol S (CAS) dye. This means that the colonies have used iron (Chukwuneme et al., 2020). Hydrocyanic acid (HCN) production plays a role in the suppression of diseases and can be detected by the formation of reddish-brown colour on the colony surface treated with 1% picric acid on filter paper. While protease and chitinase production plays an important role in nutrient mineralization, decomposition of organic matter and promoting plant growth, it can also act on pathogens which has cell walls. Protease production of actinomycetes strains can be detected by halo regions around the bacteria colonies on casein agar. Also, chitinase production can be detected by the formation of chitinase halos around colonies on chitin agar. (Gopalakrishnan et al., 2011).

In a study conducted with cucumber (*Cucumis sativus* L.) some actinomycetes strains were isolated and these are *Actinoplanes campanulatus, Micromonospora chalcea* and *Streptomyces spiralis*. From these bacteria, *S. spiralis* IAA, IPYA, GA3 and iPa; *A. campanulatus* IAA, IPYA and GA3; *M. chalcea* produced plant growth promoters known as IAA and IPYA. *Pythium aphanidermatum* is a type of pathogen bacteria that causes damage to the cucumber plant. In this study, it was determined that the isolates suppressed the pathogenic activity of *P. aphanidermatum* and promoted the growth of the plant (El-Tarabily et al., 2009). In another study, strains belonging to the genus *Streptomyces, Nonomuraea, Actinomadura, Pseudonocardia* and *Nocardia* were isolated from the plant *Aquilaria crassna* Pierre ex Lec. Of these endophytic actinomycetes strains, *Nocardia jiangxiensis* produced 15.13±0.22µg ml^{-1} of IAA and *Streptomyces hainanensis* produced 60µg ml^{-1} of ammonia. Siderophores, which have the feature of promoting plant growth by giving insufficient iron in the soil to the plants, were detected in 8 of the 10 isolated actinomycetes strains. In addition, some of these actinomycetes strains can protect host plants from pathogens by producing lytic enzymes. (Nimnoi et al., 2010).

Some studies show that these bacteria synthesize IAA in a medium with L-tryptophan. IAA is important as it is responsible for increasing roots that help the plant take in nutrients and absorb water. Three actinomycetes strains have been isolated from soils where wheat (*Triticum aestivum*) and tomato (*Solanum lycopersicum*) plants growth and these are *Streptomyces nobilis* WA-3, *S. kunmingenesis* WC-3 and *S. enissocaesilis* TA-3. These bacteria produced 79.5, 79.23 and 69.26 µg/ml IAA at 500 µg/ml L-tryptophan concentration, respectively. In the same study, it was observed that different Streptomyces strains exhibited different growth-promoting activities such as phosphate solubility, HCN production and ACC-deaminase enzyme release. It has also been proven that some of these strains cause an increase in root numbers on wheat seeds. Wheat seeds inoculated with *S. nobilis* WA-3 had a 65% increase in shoot length, while the same bacterium increased the number of leaves by 27% and the number of roots by 30% on the plant. (Anwar et al., 2016). In another study that has been made with wheat plants, especially IAA, siderophore and phosphate solubility properties of actinomycetes were investigated. 15 actinomycetes strain isolated from soil samples collected from the rhizosphere of plants. Of these strains 78% produced IAA and 60% produced siderophores and 5 strains showed phosphate solubility activity. *Streptomyces rochei* IDWR19 strain produced 17.81 ± 2.1 mg l^{-1} IAA, produced 34.17 ± 0.07 mg l^{-1} siderophore and 95.40 ± 5.1 mg l^{-1} phosphate solubility. *Streptomyces thermolilacinus* IDWR81 produced 11.5 ± 2.1 mg l^{-1} of IAA and 26.9 ± 0.07 mg l^{-1} siderophore, at the same time, it was the strain that produced the highest phosphate solubility with 911.6 mg l^{-1}. Among these strains, which were found to have the highest plant support properties, an increase of 12.2% in the shoot length of the plant inoculated with S. rochei IDWR19, and an increase of 24.5% in the shoot length of the plant inoculated with S. thermolilacinus IDWR81 was observed. An increase of 1.8 and 2.3, respectively, was observed in the biomass of the plant. (Jog et al., 2012).

In a study in which bacteria belonging to the genus *Streptomyces, Nocardia, Nocardiopsis, Spirillospora, Microbispora* and *Micromonospora* were isolated from mandarin (*Citrus reticulata* L.), it was determined that these bacteria produced IAA (respectively 13.34, 3.36, 140.38, 12.55, 1.40 and 6.19 µg IAA mL^{-1}). Among these strains, it was determined that the highest IAA production capacity belonged to *Nocardiopsis* isolates. When the growth promoting effects of the selected strains from IAA producing isolates on mandarin were examined, *Nocardia* isolates with the code TGsR 01-012 caused the highest shoot growth with 8.53 cm. *Streptomyces* TGsL 01–001 strain was determined as the second isolate that caused the highest shoot growth with 8.03 cm shoot growth. *Nocardiopsis* strains with the highest IAA producing capacity promoted both root growth and shoots growth compared to the control. TGsR 03-002 *Streptomyces* strain with 0.732 g $plant^{-1}$ and TGsL 02-004 *Nocardiopsis* strain with 0.709 g $plant^{-1}$ were the strains causing the highest fresh root weight (Shutsrirung et al., 2013). In a study in which actinomycetes was isolated from the soil, inner roots and rhizospheres of the rice plant, it was determined that the *Streptomyces kodangensis* isolates could produce high levels of IAA. It has also been measured that this strain produces a high rate of siderophores. In the same study, Streptomyces sp. and Amycolatopsis methanolica strains were found to exhibit high phosphate solubility. In the same study, a *Streptomyces* sp. strain showed high antibacterial activity against the *Xanthomonas oryzae pv. oryzicola* pathogen (Hata et al., 2015).

In a study, Kruasuwan and Thamchaipenet (2016) *Streptomyces* sp. strain was isolated from sugarcane roots. This strain showed IAA, siderophore production, phosphate solubility, and ACC 1-aminocyclopropane-1-carboxylate deaminase production and its activity measured an increase of 11.85 ± 3.83 cm in roots and an increase of 43.98 ± 7.82 cm in shoots of sugarcane. With the 16S rRNA sequencing, it was determined that this strain was 99.36% similar to *S. canus* NRRL B-1989^{T}. Also, it was determined that this isolate showed antimicrobial properties on *Bacillus cereus* ATCC 11778, *Colletotrichum falcatum* 1655 DOAC and *Fusarium moniliforme* DOAC 1224. In a study investigating the effect of actinomycetes strains on maize plant growth in West Africa, it was tested that the isolates had phosphate solubility, chitinase production, cellulase production, ACC-deaminase, nitrogen fixation, IAA and siderophore production abilities. In in vitro experiments, three

isolates with these abilities showed an increase of 8.1, 6.3, 6.8 cm in shoots and 5.1, 8.1, 5.1 cm in roots, respectively. These actinomycetes strains showed antimicrobial activity on *Xanthomonas oryzae* pv. o*ryzae*, *Bacillus pumilus* Od23 and *Alternaria solani* (Dicko et al., 2018).

Wahyudi et al., in their study which was published in 2019, examined the plant growth promoting abilities of actinomycetes strains isolated from soybean (Glycine max L.) rhizosphere. The isolates named ARK 116, ARK 86, ARK 13, ARK 63, ARK 94, ARK 17 and ARK 48 were able to increase hypocotyl length, radicular length and number of lateral roots. Among the selected isolates, ARK 116 was determined as the strongest plant growth promoter. It was determined that these 5 isolates belong to the genus *Streptomyces*. An important example of the effect of symbiont actinomycetes on plant growth is the phosphate dissolving effect of these bacteria. This feature can be detected by the formation of a clear zone around the actinomycetes colonies on a medium containing 2% tri-calcium phosphate (Chukwuneme et al., 2020). Ammonia production can be detected by the brownish colouration of actinomycetes strains which are incubated in peptone water against Nessler reagent and ammonia is considered a growth promoting antimicrobial agent (Devi et al., 2021).

Gopalakrishnan et al. (2011) in their study, eight actinomycetes strain isolated from herbal vermicompost were found to increase 11-34% in shoot weight; found to increase 2-57% in root weight. In addition, it was determined that the isolates showed antimicrobial activity on *Macrophomina phaseolina*, which is the causative agent of charcoal rot infection.

It has been proven by studies that actinomycetes isolated from different environments have plant growth promoting potentials on various plants. In a study in 2014, it was found that *Streptomyces* strains isolated from herbal vermicompost had cellulase, HCN, IAA, siderophore, chitinase, lipase, protease and ß-1,3-glucanase activities that shown increase shoot number, root length, volume and dry weight on rice plant (Gopalakrishnan et al., 2014). In another study, it was determined that *Streptomyces rectiviolaceus* NA8 and *Streptomyces silaceus* CA7 bacteria isolated from the rhizosphere of *Arnebia euchroma* and this bacteria have phosphate solubility, siderophore, HCN, ammonia production, nitrogen fixation ability, and lytic enzyme production ability (Devi et al., 2021). Toumatia et al. showed that the *Streptomyces mutabilis* IA1 strain, which they isolated from Saharan soil in 2016, IAA and gibberellic acid-producing and has shoot length increasing activity on wheat. In another study, the effect of *Streptomyces cyaneofuscatus*, *Streptomyces kanamyceticu*, *Streptomyces rochei* and *Streptomyces flavotricini* bacteria isolated from the rhizosphere on the parasitic fungus *Verticillium wilt* was investigated and it was determined that these strains also produced IAA and siderophores. (Xue et al., 2013).

ACTINOMYCETES WITH HERBICIDAL AND INSECTICIDAL EFFECT

Weeds are agricultural pests that can cause billions of dollars in annual crop loss. To provide the sustainability of agriculture, it is necessary to fight these weeds. Herbicides have been used to control weeds for years, but over time these weed have had a resistance to the herbicides (Délye et al., 2013). Likewise, there is a development of resistance by insects against insecticides used against agricultural pests (Sparks and Nauen, 2015). Resistance acquired by agricultural pests can affect the sustainability of agriculture over time. Therefore, natural herbicidal and insecticidal substances that can fight these agricultural pests, are required for sustainable agriculture and public health.

In a study on actinomycetes isolated from the soils of Saudi Arabia and Egypt regions, it was determined that the secondary metabolites of the isolates showed lethal activity against *Spodoptera littoralis* pupae. In the study, it was found that *Streptomyces* and *Streptoverticillum* genus strains have the strongest effect. (Bream et al., 2001). The herbicidal effect of 500 actinomycetes isolates isolated from different soil samples collected in Honam and Kyounggi regions in South Korea was

investigated. In the study, methoxyhygromycin compound produced by *Streptomyces* sp. 8E-12 strain has been shown to have a herbicidal effect against *Digitaria sanguinalis* and *Echinochloa crusgalli* this effect observed as called bleaching or albino symptom (Lee et al., 2003). In another study, ethyl acetate extract of *Streptomyces* sp. KN-0647 isolate showed high insecticidal activity against *Spodoptera exigua* and *Plutella xylostella* insects, moderate against *Aphis glycines* and *Culex pipiens*, and low against *Heliothesis armigera*. This extract was identified as quinomycin A using the UR, MS, and 1HNMR methods (Liu et al., 2008).

In a study, *Streptomyces* isolate which isolated from the soil examined in terms of herbicidal activity against *Echinochilora crusgalli, Echinochilora colonum, Parthenium sp*., and *Ageratum conizoites* weeds, it was determined that this isolate showed herbicidal activity against *Echinochilora crusgalli* (Dhanasekaran et al., 2010). In another study, butanol extracts of secondary metabolites of 2 *Streptomyces* strains isolated from the soil showed 100% insecticidal activity at a concentration of 5.0 mg/ml against *Aedes aegypti* 2nd instar larvae. It was determined that the insecticidal activities of Streptomyces isolates, named No1 and No2, were dose-dependent. It was observed that the No2 isolate had higher insecticidal activity compared to the no1 (Kekuda et al., 2010).

In a study conducted on actinomycetes isolated from sandy soil in Cairo, chloroform extract of the bacterium called *Streptomyces bikiniensis* A11 showed 100% lethal effect against the 2nd instar larvae of *Spodoptera littoralis* cotton leafworm. With the analysis, it was determined that the extract may be related to aminoglycoside antibiotics. (El-Khawaga et al., 2012). *Streptomyces* sp. KA1-3 was isolated from soils in agricultural areas and was detected N-phenylpropanamidin substance in the secondary metabolite extract of the isolate. This extract was able to inhibit the growth of *Cyperus rotundus* and *Cassia occidentalis* seeds by 80% (Priyadharsini et al., 2013).

Secondary metabolite extract of the isolate S5, identified as Streptomyces lavendulae var. glaucescens, isolated from the roots of the tomato (*Lysopersicon esculentum*) plant, showed herbicidal activity against wheat (*Triticum aestivum L.*), mung bean (*Phaseolus radiatus L.*) and grass (*Paspalum notatum* and *Cynodon dactylon*) (Zhi-Qi et al., 2006). In a study in Pakistan, examining the effects of actinomycetes on red flour beetle, which causes economic losses on cereals, it was determined that extracts obtained from *Streptomyces rochei* SA10BC, *Streptomyces minutiscleroticus* SA-9K and *Streptomyces phaeoluteigriseus* SA-9L has 100% lethal insecticidal activity on *Tribolium castaneum*. In addition, the extracts have a 100% larvicidal effect on the larvae of the mosquito species *Culex quinquefasciatus*, which is the causative agent of elephantiasis, which affects more than 120 million people. (Anwar et al., 2014).

In another study, actinomycetes strains were isolated from 8 different plant species (*Aloe barbadensis, Asparagus racemosus, Barleria prionitis, Catharanthus roseus, Coelogynae ovalis, Coleus blumei, Plumbago zeylanica* ve *Vitex negundo*) and this isolates belonging to genus *Actinomadura, Nocardiopsis, Nocardiodes, Nocardia, Streptomyces, Micromonospora* ve *Saccharopolyspora*. Of these isolates, *Nocardiodes* sp. 1, *Nocardiodes* sp. 2 and *Actinomadura* sp. extracts 60% against *Ageratum conyzoide* seeds; *Nocardiodes* sp. 1 and *Saccharopolyspora* sp. extracts showed 80% herbicidal activity against *Parthenium hysterophorus* seeds. The effects of the extracts against plant seeds were observed in the form of leaf rolling, wilting and burning (Singh et al., 2018b). In a study Kim et al. (2020), it was determined that the KRA17-580 isolate, which is similar to *Streptomyces olivochromogenes*, has herbicidal activity against *Digitaria ciliaris* This isolate has been described to produce compounds cinnoline-4-carboxamide called and cinnoline-4-carboxylic acid. Chen et al., in their study published in 2018, an actinomycetes strain G30 isolated from the *Azadirachta indica* tree had a lethal effect with LC50 value of 1,680 mg/mL and LC95 of 4,370 mg/mL against *Myzus persicae*, also known as green peach aphid, after 48 hours of treatment. As a result of molecular studies, it was determined that the G30 isolate showed 99.6% similarity with *Streptomyces albidoflavus.*

ANTIMICROBIAL PROPERTIES OF ACTINOMYCETES

Viruses, fungi and bacteria cause various plant diseases. Various symptoms such as necrotic spots on parts of the plant, burns, root, fruit and stem rots or death of the plant can be observed in plants infected by these pathogens. Infection of plants results in both crop loss and financial loss. In addition, it can cause hunger, famine, food poisoning and changes in the ecological balance (Agrios, 2009). Therefore, it is important to obtain active compounds against plant pathogens.

In many studies, it has been observed that actinomycetes, which are effective on plant pathogens, live on plants. In a study in which a total of 712 actinomycetes strains were isolated from healthy cotton *(G. hirsutum* L.), chili pepper (*Capsicum annuum* L.), canola (*Brassica napus* L.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus*),ginseng (*Panax ginseng* C. A. Mey) and forage plants in northern and western China, the isolates were found to inhibit Verticillium cotton wilt disease caused by the *Verticillium dahlia* pathogen. As a result of 16S rRNA analysis, these isolates were named as Streptomyces cyaneofuscatus, Streptomyces kanamyceticu, Streptomyces rochei and Streptomyces flavotricini (Xue et al., 2013). In 2003 Taechowisan et al., 330 endophytic actinomycetes were isolated from healthy plant parts, these isolates belonging to mostly *Streptomyces*, *Microbispora* and *Nocardia* genus. Researchers revealed that these bacteria especially Streptomyces sp. isolates have strong antifungal effects on *Colletotrichum musae* and *Fusarium oxysporum*. Endophytic actinomycetes have an important place in both the protection of plants from pathogens and their development. In one of the recent studies on this subject, Ashfield-Crook et al. (2021) revealed that the absence of these bacteria significantly reduces plant growth and plants become particularly vulnerable to fungal infections. In fact, researchers planned to develop bacteriophages targeting the plant pathogen Streptomyces species and eliminate this pathogen before it develops the disease. Experiments on control groups revealed that these phages also destroyed non-pathogenic endophytic actinomycetes, and this situation caused serious problems on the potato plant. In another study, it was determined that *Streptomyces, Nocardia* and *Streptosporangium* strains were isolated from *Azadirachta indica* plant have antagonistic properties against plant root pathogens *Pythium ultimum* and *Pythium oligandrum*. (Verma et al., 2009). In another study, *Saccharopolyspora* O-9 strain isolated from *Ocimum sanctum* strongly inhibited all fungi *Aspergillus niger, Colletotrichum falcatum, Aspergillus flavus, Alternaria brassicicola, Penicillium digitatum, Fusarium oxysporum, Penicillium pinophilum, Phytophthora dresclea, Botrytis cinerea*. O-9 showed 71.4% strong inhibitory activity against *Penicillium digitatum* (Gangwar et al., 2011).

Soil is the main source for actinomycetes isolate and therefore it is the most studied source for antimicrobial effects of actinomycetes. This is also the case in studies on plant pathogens. In a study conducted with actinomycetes isolated from the soil, *Streptomyces anulatus*, *Streptomyces* endosymbiont with *Philanthus venustus*, *Micromonospora flavogrisea*, *S. fimicarius*, *Streptomyces* sp., *Candidatus Streptomyces philanthi* biovar *basilaris*, *S. anulatus*, *M. flavogrisea*, *M. flavogrisea* all actinomycetes strains showed antifungal properties against *Botrytis cinerea, B.cinerea, Fusarium oxysporum f. sp. Albedinis(Foa), Sclerotium rolfsii, Verticillium dahliae* and *Pythium ultimum* fungi (Loqman et al., 2009).

Cuesta et al., in their study conducted in 2012, actinomycetes isolates isolated from soil and compost showed high antifungal activity against *Fusarium oxysporum* f. sp. *melonis*, *Phytophthora cinnamomi*, *Pythium debaryanum*, *Sclerotinia sclerotiorum* ve *Thanatephorus cucumeris* fungi. In addition, S-6, T2-10 and T8-2 isolates have antibacterial properties against Agrobacterium tumefaciens (CECT 4119) bacteria that cause disease in plants. S-1, S-2, S-3 isolates *Streptomyces variegatus*; CO2-16 isolate *S. aureoverticillatus*; S5 isolate *S. griseoruber*; S6 isolate *S. lusitanus*; T8-2 isolate *S. albogriseolus* and T2-10 isolate was identified as *S. coeruleorubidus* by 16S rRNA analysis.

In a study in which 6 different *Streptomyces* strains were isolated from Saudi Arabian soil, it was determined that the secondary metabolites extracts of the isolates showed moderate and high antiviral activity against Tobacco mosaic virus (TMV). Extracts were analyzed by GC-MS and the compounds with the highest percentage of compound peaks in the entire sample were identified as 2-decenal ($C_{10}H_{18}O$), glycerine ($C_3H_8O_3$) ve 4h-pyran-4-one,2,3-dihydro-3.5-dihydroxy-6-methyl ($C_6H_8O_4$). Moreover, extracts of all strains showed antimicrobial activity against *S. aureus, B. subtilis, E. coli, P. aeruginosa, S. suis, S. sonnei* and *C. albicans* pathogens (Ara et al., 2012). In another study, extracts of ATMY-1 isolate isolated from soil and identified as *Streptomyces plicatus* were examined for their antimicrobial potential on plant pathogens *Phytophthora infestans* and *Sclerotium rolfsii*. At 5000 ppm dosage, the metabolite extract inhibited *P. infestans* by 100% and *S. rolfsii* by 94% (Sinha et al., 2014).

In a study conducted on actinomycetes isolated from the soil, it was determined that the extract obtained from an isolate had antifungal activity on *Aspergillus niger, Aspergillus flavus, Alternaria sps, Fusarium sps* and *Rhizopus stolonifer* (Sharma and Parihar, 2010). In a research conducted with 80 different actinomycetes samples isolated from soil samples collected from both urban and rural areas in India, it was determined that the isolate ACITM-1 showed antifungal activity on the pathogens *Macrophomina phaseolina, Collectotrichum truncatum, Fusarium oxysporum* and *Rhizoctonia solani*. By 16S rRNA sequencing, this isolate was identified as *Streptomyces chilikensis* (Singh et al., 2016).

Actinomycetes are bacteria that overcome the environmental barrier thanks to their wide adaptability. Some of the best examples of this are studies using actinomycetes isolated from the marine environment. In 2005, Kathiresan et al. the secondary metabolites of actinomycetes strains were isolated from marine sediment samples were screened for their antifungal activity on the pathogens *Rhizoctania solani, Pyricularia oryzae, Helmintosporium oryzae*, which causes sheath blight, blast and leaf spot in rice, and *Colletotrichum falcatum*, which causes red rot in sugarcane. The isolates showed antifungal activity on 51% *H. oryzae* and *P. oryzae*, 31% on *R. solani* and 12.5% on *C. falcatum*. Actinomycetes strains identified as *Streptomyces fradiae* RHI 1, *S. roseolilacinus* MP1, *S. helvaticus* M III 1, *Actinomyces auriomonopodiales* M I 1, *S. albidoflavus* SEA 5, *S. orientalis* BC 3, *S. roseiscleroticus* BC 1, *S. sclerotialus* SD 3, *S. galtieri* AN 1C, *Streptomyces* sp. AN 1CR showed activity on all 4 pathogens. In another study, it was observed that the *Streptomyces* genus strain isolated from the Arabian Sea synthesized antiviral metabolites effective against cucumber mosaic virus (CMV). The 16S rRNA analysis revealed that the isolate belongs to the *Streptomyces olivaceus* species (Latake et al., 2017).

CONCLUSION

Actinomycetes are microorganisms that can be isolated from different environments and are the main producers of secondary metabolites. Actinomycetes are bacteria that have been investigated in many different areas due to their properties and their potential properties are being researched. Until today, actinomycetes bacteria have been used frequently in the production of antibiotics. However, the potentials of actinomycetes should not just be limited to the pharmaceutical industry. Actinomycetes may hold unexplored potential in obtaining substances with novel and beneficial properties.

Due to the rapid increase in the world population, the need for food is also increasing. Sustainable agriculture is also needed in order to obtain sufficient food products. For sustainable agricultural practices, the benefits that can be obtained from microbial resources should be considered. Actinomycetes, one of these sources, may have the potential to support the growth of plants primarily with the metabolites they produce. In this way, healthy and beneficial plants production can be achieved. Also, actinomycetes have the potential to be used to prevent weeds and agricultural pests that are problematic for agricultural areas. Finally, in this review, it is mentioned

that actinomycetes have antimicrobial properties against pathogens that cause plant disease. Thanks to these antimicrobial properties, thousands of crop losses as a result of plant disease can be prevented. Considering these different potential features, actinomycetes can be used in the field of sustainable agriculture.

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BIOFILM REACTOR DESIGN AND OPTIMIZATION OF BIOFILM DIAGNOSTIC TEST METHOD ON IMPLANT SURFACE

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ABSTRACT

In this study, prototype production of a biofilm reactor that offers benefits such as flow system, controlled delivery of nutritional ingredients, and precise environmental temperature adjustment was realized. In vitro optimization analyses of biofilm diagnostic testing with a standard of microbial cultures for the cause of infection were tested on various material surfaces such as stainless steel, glass, and polystyrene. Besides, to determine microbial adhesion on the surfaces of medical implants and contribute positively to product/surface development, different sizes of implant surfaces and hydroxyapatite (HAP) and silver hydroxyapatite (Ag-HAP) coated titanium surfaces were tested with the strong biofilm producer *S.* Typhimurium strain. Our findings showed that the biofilm mass obtained by discrete systems (classical method) on different implant surfaces was approximately 2 to 8-fold less than the biomass achieved by the flow system.

Keywords: Biofilm, Biofilm Reactor, Implant, Implant, Polystyrene, Glass, Stainless-steel, Coupon

INTRODUCTION

Biofilm structures are microorganism communities in an extracellular matrix that produce and have different genetic regulation and physiological characteristics from the independent (planktonic) forms of the cells that produce the biofilm. Biofilms are their industries as units from those grown at a high-stress level to specific forms, clinical, and food samples. The health problems they cause and the diversity and prevalence of product losses will make the fight against these structures one of the problems of microbiologists (Gao et al., 2017; Loncar et al., 2017). In control strategies developed for this purpose, it is mainly aimed to prevent microorganisms from adhering to surfaces where they will form biofilms. (Donlan and Costerdon 2002; Catto and Cappitelli 2019). For these studies to be carried out correctly, the development of systems closest to the biofilm production conditions in nature is gaining increasing importance. Experimental biofilms created using classical microbiological methods (biofilm structures on polystyrene plates or steel coupons) differ from natural systems, especially in nutrient supply. In addition to the amount and variety of nutrients in the environment, their continuity is critical in biofilm formation. Another critical parameter in this understanding is the ambient temperature. The importance of ambient temperature in the formation of effective biofilms of many pathogens has been defined. The control and optimization of these parameters in batch production systems pose serious difficulties. In addition, semi-batch or continuous biofilm reactors offer the opportunity to provide very efficient biofilm formation and effective preservation on tested surfaces. In reactors controlled by a flow mechanism, the drawbacks seen in conventional batch biofilm production systems and summarized above can be mainly eliminated, primarily because nutritional and environmental conditions can be controlled. Because conditions that make it possible to regulate and observe biofilm production conditions in the said reactors can be created, biofilm coupons of different structures can be used in this direction (Donlan et al., 2004; Goeres et al., 2005; Coenye and Neils 2010). These reactors, which enable biofilms to be formed on different surfaces effectively and efficiently, and examine the effects of different

conditions on biofilm productivity, are also ideal systems for studies aiming at genetic regulation and efficient eradication in removing biofilm structures (Goeres et al., 2005; Pavarina et al. 2011).

This study, it is aimed to produce a prototype of a microbial biofilm reactor, which makes it possible to carry out experiments at different media temperatures with accuracy. It is essential to test the surfaces used in industrial processes (food, medical, etc.) to adhesion pathogenic microorganisms and standardize the surfaces developed to minimize the bacterial adhesion. In addition, the basis of research on biofilms, which are very difficult to remove by disinfection processes, is to know under what conditions microorganisms form these structures on surfaces. The developed biofilm reactor provides facilities such as controlling the distribution of nutritional ingredients and determining optimum biofilm production at different temperatures, with the flow system based on continuous feeding. Using the prototype, in vitro optimization of the biofilm diagnostic test was performed with standard strains of infectious microbial cultures. Testing the surfaces of medical skin permeable materials (implants) for the adsorption capabilities of microorganisms will contribute positively to product/surface development. In addition, this reactor can be used in eradication studies to remove the biofilm. The fact that fast and realistic data can be obtained according to classical methods will form the basis of a new test criterion.

MATERIAL AND METHOD

Device design

The biofilm reactor consists of two parts (Figure 1). It consists of custom-made glass pools with internal drainage taps, surface holders that allow testing of surfaces of different sizes and dimensions, and polyethylene top covers. The polyethylene cover is placed over the glass pool with a gasket. There are stainless steel nutrient inlet, filter, and inoculation ports on this cover. A membrane filter with a pore diameter of 0.45 μM was used. There is a stainless-steel table on which the surfaces will be placed in the pool and a stainless-steel clamp arm coming from the cover center.

Also, coupon surface holders in which surfaces are prepared and attached to a certain extent (Ø 10 mm, 3 mm thickness) similar to the CDC biofilm reactor used in the E2196-12 and E2562-12 ASTM standard methods for measuring Pseudomonas aeruginosa biofilms a separate 1 L reactor pool (ASTM 2012). A magnetic stirrer provides liquid circulation. The system ends when the liquid coming from the nutrient tank with the peristaltic pump reaches the waste tank with the drainage valve. The inner diameter lengths of the silicone tubes used are 3.2 mm and 8 mm, and the two tubes are connected by a 'different end' hose coupling. The liquid coming to the reactor first comes to the specially made glass liquid flow arrester and then enters the reactor pool system.

The outer part consists of a system where the temperature and mixing features are controlled by a single panel and a nutrient tank and waste tanks. The device offers the opportunity to experiment in a stainless-steel chamber. A temperature scale of 0-55 °C was applied in the heating and cooling system, and the temperature accuracy was adjusted to be ± 0.7 °C. Heating, cooling, and mixer equipment are attached to the device. It is designed by calculating the necessary clearances for insulation and the positioning of other equipment to be used, with a capacity of 50 L. The device has a glass window cover, and there are 12 mm diameter inlet and outlet holes for circulation on both sides. Inside, a 600-watt heating device, a 1000-watt cooling unit, and a magnetic stirrer can be adjusted between 300 and 600 revolutions for agitation. Bending dimensions and markings were made by drawing each part separately; the inner chamber was made of 1.5 mm stainless steel; the outer casing and covers were laser cut from a 1.5 mm DKP sheet. Bodypaint (outer case and covers) is painted with electrostatic powder oven paint, which is more resistant to abrasion as a whole. A clamp stand is placed to support the tubing system that provides continuous flow into the cabin. A microfluidic peristaltic pump (with Watson Marlow 120S/D1 pump head, 3.2 mm Pumpsil Tubing) was used externally from the system. The external use of the peristaltic pump allows more accessible intervention for the installation of the continuous flow and tubing system during the sterilization phase. The stepper motor used for magnetic stirring is placed on the base of the body, and the 0-600

rpm mixing feature is adjusted gradually. The temperature value of the device was controlled by the pt 100 sensor and the circulation motor and displayed on the color touch screen. There is a real-time clock (RTC) on the touch panel (65536). Software that records experiment time, temperature, and mixing speed has been added to the control panel.

Sterilization of reactor components and biofilm reactor preparation

Since it is widely preferred in industrial processes, steel coupons and medical implant surfaces have been used. 1 cm diameter stainless steel coupons (Ostim, Ankara), implants, and other metal reactor components were initially soaked in acetone overnight. The surfaces, which were kept in acetone overnight, were then bathed in detergent water for 30 minutes, and during this process, the surfaces were agitated and washed. After this step, the surfaces were washed in tap water, and after this process, they were washed three times with distilled water and left to dry. Finally, it was sterilized at 175 °C for 3 hours. Glass coupons and other reactor components were steam-sterilized at 121 °C for 20 minutes. Before starting to work with bacterial cultures, sterility control trials were carried out with a biofilm reactor. In this test, after the whole system was sterilized, the system was operated for one week with only the medium to screen for any external bacterial contamination (Figure 2).

Investigation of in vitro biofilm formation on polystyrene, stainless steel, and glass

The study examined the biofilm formation properties of bacteria on different surfaces (stainless steel, glass, polystyrene); The strains of *Salmonella* Typhimurium 14028, *Enterococcus faecalis* OG1RF, *Staphylococcus epidermidis* ATCC 14990, *Escherichia coli* O157:H7 ATCC 35150, and *Listeria monocytogenes* were studied. Standard bacterial cultures were obtained from the Ankara University Prokaryotic Genetics Laboratory culture collection. Woodward et al. (2000) and Extremina et al. (2011) were carried out by modifying the microdilution plate method. *S.* Typhimurium 14028 and *E. coli* O157:H7 ATCC 35150 strains at 200 rpm and 37 °C in LB broth, E. faecalis OG1RF, and S. epidermidis ATCC 14990 strains at 37 °C in Trypton soy broth (TSB) medium. The *L. monocytogenes* strain was grown in a TSB medium at 30 °C for 18 hours under static conditions (Woodward et al., 2000; Extremina et al., 2011).

Salmonella cultures were passaged in 5 mL of NaCl-free LB broth (LB^{-NaCl}; Tryptone 10g/L, yeast extract 5g/L) media, grown at 37 °C overnight under shaking conditions, and the cultures were kept in the reactor pool at a final concentration of 550 nm. The biofilm reactor was incubated in LB^{-NaCl} medium and at 20 °C for 24 hours without turning on the peristaltic pump to create a batch culture. It was diluted with LB-NaCl to an OD level of 0.2. The 1 L reactor pool holds ~370 mL of medium when coupon holders and surfaces are attached.

E. faecalis grown in TSB medium for 18 hours was taken from active cultures (10^8 CFU/mL) at a ratio of 1/100 and inoculated into a 1 L reactor pool. The biofilm reactor was incubated in a TSB medium containing 1% glucose and at 37 °C for 24 hours without turning on the peristaltic pump to create a batch culture. According to preliminary studies, glucose is a biofilm-forming stress factor for enterococci, and 1% glucose induces maximum biofilm formation (Diani et al., 2014)

Cultures of *S. epidermidis*, *E. coli*, and *L. monocytogenes* (10^8 CFU/mL) were taken at a ratio of 1/100 and inoculated into a 1 L reactor pool. Stress factors for biofilm formation, respectively; TSB medium containing 1% glucose for *S. epidermidis* strain and LB medium containing 1% glucose for *E. coli* strain and BHI medium for *L. monocytogenes* strain at 37 °C (Seidl et al., 2008) and 30 °C (Stepanovic et al., 2000).

After a 24-hour pre-incubation period for all strains, the magnetic stirrer was performed at 125 rpm to ensure the dispersion of the medium/bacteria. The peristaltic pump was operated at a flow rate of 3 rpm to provide a medium flow of 1.215 mL/min and incubated for 24, 48, 72, 96, 120, and 144 hours. Trials were carried out in two parallel, two repetitions, and separately for all coupons. Medium

flow and pump settings; Flow rate (mL/min)/ [max. flow rate (mL/min)/max. speed(rpm)] =Pump speed (rpm) formula.

After incubation, the surfaces were taken from the reactor pool with the help of sterile forceps, transferred to 24-well plates. After being treated with sterilized saline (0.85% NaCl) (Merck, Germany) 3 times, the coupons were left to dry under laboratory conditions. Then the coupon surfaces were left to dry for 1 hour at 60 °C. Then fixation was done with 95% methanol for 15 minutes. 1 mL of 0.1% crystal violet was used to the microtiter plate wells to cover the coupon surfaces completely, and these media were kept under laboratory conditions for 30 minutes. Then the coupons were washed twice with sterilized distilled water. In order to remove the dye adhered to the biofilm structure, 1 mL of 33% glacial acetic acid was used, and these media were kept under laboratory conditions for 45 minutes. At the end of this period, the wells transferred to 96-well plates were measured in an Elisa reader at OD_{595} nm.

In these trials, the specified cut-off value conversions were used to determine the biofilm production characteristics of the strains ("weak producer," "intermediate producer," "strong producer" without biofilm producer) (Stepanovic et al., 2004). Threshold values represent the statistical deviation determined in the biofilm quantification of the negative test groups used in the study. The values obtained by these processes; OD≤ODc; non-biofilm producer, ODc<OD≤2xODc; Weak biofilm producer, 2xODc<OD≤4xODc; Intermediate biofilm producer, 4xODc<OD; It is determined as a strong biofilm producer. ODc was determined by summing the mean of the measurements for the negative control with three times the standard error.

Investigation of in vitro biofilm formation on different implant surfaces of S. Typhimurium 14028 strain

To test the bacterial biofilm formation on medical surfaces, implants obtained from different companies were tested using S. Typhimurium 14028 bacteria, a strong biofilm producer, in conditions where the bacteria formed biofilm at the optimum level. Cultures added with OD_{550}=0.2 to 1 and 2 L reactor pools with implants of different sizes were incubated in the flow system for 96 hours after 24 hours of batch culture. As a negative control, LB^{-NaCl} medium and implant surface were incubated without culture. Simultaneously, the implant surfaces were studied in static conditions (flow system-batch system without medium input), thus comparing our system with the existing classical test systems. The method used to determine the amount of biofilm formation on surfaces is as indicated in 2.3. Implant surfaces obtained from different companies and used in the study are shown in Table 1 (Figure 3).

Table 1. Implants and surfaces used in the experiment

#	Surface
1	Dental implant
2	Screw (Ti6AI4V-ELI Grade 23- NORMED)
3	Stabilization Plyaxial Screw Body (MSFX-MICRON SPINAL)
4	Stabilization Plyaxial Screw (MSFX-MICRON SPINAL)
5	Radius Volar Plate
6	Titanium surfaces coated with hydroxyapatite (HAP) and silver hydroxyapatite (Ag-HAP)

Statistical analysis

The evaluation of the data obtained from dissolving the crystal violet dye attached to the biofilm matrices of microorganisms attached to stainless steel, glass, polystyrene, and implant surfaces and reading them in the ELISA reader was carried out using GraphPad Prism 8 software. Based on the "F" value of the results obtained from the experiments, a one-way ANOVA test was applied to determine whether the differences between the groups were statistically significant. Tukey's accuracy test was used to evaluate the variation between groups. A statistically significant P value was taken as <0.05 in data analysis.

RESULTS AND DISCUSSION

Biofilm reactor production

Biofilm reactors have advantages over conventional microbial tests. Microbiological analysis methods take a long time. There is the possibility of experiment contamination that could occur through user error. This possibility is minimized thanks to the biofilm reactor, which is a closed system. The sterile medium in the waste tank after the sterility control trials carried out in the study proved that the system was working in a sterile manner. The sterility control test is essential for the working principle of the prototype since any external bacterial contamination affects all test data.

Investigation of in vitro biofilm formation on polystyrene, stainless steel, and glass

As test bacteria, *S.* Typhimurium 14028, *E. faecalis* OF1RF, *S. epidermidis* ATCC 14990, *E. coli* O157:H7 ATCC 35150, and *L. monocytogenes* were studied. The amount of biofilm formed on stainless steel, glass, and polystyrene surfaces on different days in the experiment carried out to determine the maximum biofilm formation capacities is shown in Figure 1. As a result of the statistical analysis of the data obtained in the trials, *S.* Typhimurium 14028 strain was determined as a "strong biofilm producer," with the highest biofilm production at 20 °C after 96 hours on all surfaces ($P<0.05$). Although it formed a biofilm in all incubation times tried, after 96 hours, biofilm production decreased as the incubation time continued. *S.* Typhimurium 14028 adhered the most to the stainless-steel surface and the least to the glass surface. Maximum biofilm production of *E. faecalis* OG1RF strain at 37 °C was observed after 72 hours on all surfaces. At the end of 72 hours, it was determined as "weak biofilm producer" on a glass surface, "moderate biofilm producer" on polystyrene surface, and "strong biofilm producer" on stainless steel surface ($P<0.05$). Maximum biofilm production of *L. monocytogenes* strain at 30 °C was determined on all surfaces after 24 hours ($P<0.05$). It produces moderate biofilm on stainless steel and polystyrene surfaces and weak biofilm on glass surfaces. As the incubation period increased on all tested surfaces, the biofilm production amount decreased due to the breakdown of the biofilm matrix structure. After 48 hours, it does not produce biofilm on the glass surface. It was determined that the *S. epidermidis* ATCC 14990 strain produced maximum biofilm on all surfaces at 37 °C after 144 hours ($P<0.05$). It was determined as an "intermediate biofilm producer" on the stainless-steel surface and a "weak biofilm producer" on polystyrene and glass surface. Similarly, it was determined that *E. coli* O157:H7 ATCC 35150 strain produced maximum biofilm on all surfaces at 37 °C after 144 hours ($P<0.05$). After 144 hours, it was determined as a "medium biofilm producer" on stainless steel and polystyrene surfaces and a "weak biofilm producer" on glass surfaces.

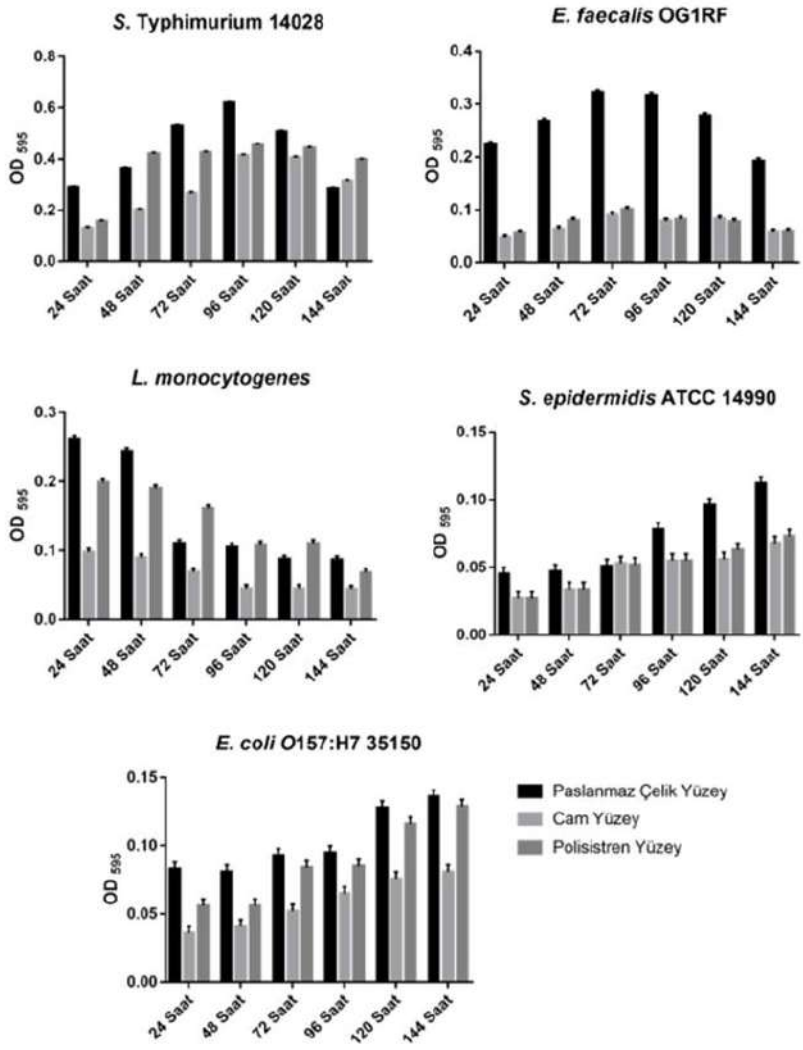

Figure 1. Biofilm production by strains at different incubation times and surfaces

In vitro biofilm formation on different implant surfaces of *S.* Typhimurium 14028 strain

S. typhimurium 14028 strain, a strong biofilm producer, was studied to perform bacterial biofilm adhesion tests on medical implant surfaces. In the biofilm formation experiments performed on different implant surfaces, biofilm formation on the implant surfaces was tested in a controlled manner with the continuous flow system and the batch system applied in classical microbiological test methods. The data obtained confirm the hypothesis of obtaining objective data. A difference was observed between the biofilm mass flow system obtained with batch systems (classical method) and the biomass obtained. Examples showing the biofilm structures on the surfaces compared with the batch system in the experiments are shown in Figure 2.

Figure 2. Biofilm structures on a dental implant. The crystal violet stained (purple) regions on the surface show the biofilm structures. 1: The implant surface tested with the biofilm reactor, 2: The implant surface tested with the batch system, 3: The implant surface in the batch system and the pellicle structure formed in the liquid-air interface.

The data obtained from the trials show that bacterial adhesion to the surface occurs more than in conventional test methods in the reactor system with a continuous flow system. This results from continuous nutrient input and confirms our hypothesis of obtaining realistic data since it partially simulates a realistic environment. Due to the use of implant surfaces of different sizes and dimensions in the test method, a standard cannot be applied in the volume of solvent used in the dissolution of the dyes adhered to the surface. This is explained by the designation of the data obtained from the optical reader as '1 Biofilm Reactor Unit' (1x BR) and its multiples compared to the control used in the test method. The values obtained by dissolving the dye bound to the biofilm matrix on the tested implant surfaces after 96 hours are given in Table 2. It was determined that the biofilm structure formed on the surface with the reactor was approximately 1.5 to 8 BR times more than the batch system (Table 2).

Biofilm structures formed on hydroxyapatite (HAP) and silver hydroxyapatite (Ag-HAP) coated titanium surfaces are shown in Figure 3. It was determined that *S.* Typhimurium 14028 bacteria produced OD_{595}=0.265 biofilm on HAP coated surface and OD_{595}=0.283 biofilm on Ag-HAP coated surface after 96 hours. Of these qualitatively determined biofilm structures; The biofilm structure should be broken down and live cell counts should be made and examined under a Scanning Electron Microscope, and further studies should be done.

Table 2. Averages of 595 nm OD values

Tested implant	**Batch system (OD)**	**Biofilm Reactor (OD)**	**Batch system (BR)**	**Biofilm Reactor (BR)**
Dental implant	0.040	0.153	1 X	3.8 X
Screw	0.005	0.038	1 X	7.6 X
Stabilization Plyaxial Screw Body	0.007	0.024	1 X	3.4 X
Stabilization Plyaxial Screw	0.011	0.019	1 X	1.7 X
Radius Volar Plate	0.005	0.017	1 X	3.4 X

Figure 3. Biofilm structures formed on HAP and Ag-HAP coated titanium surfaces

CONCLUSIONS

In this study, developing a prototype of an efficient biofilm production reactor will allow biofilm structures of major food pathogens. Thus, their examination in experimental processes has been carried out. Industrial biofilms cannot be mainly removed in food production, even if classical sanitation techniques are applied perfectly. Therefore, they can persist by creating a permanent source of contamination (Raffaella et al., 2017). This situation shows a high level of similarity in the clinical course of biofilms. The most striking evidence is that biofilms are responsible for 80% of medical infections (Davies 2003; Jamal et al., 2018). These literature data indicate that biofilm eradication strategies should be determined by defining specific biofilm formation models for each microorganism. Whether for food production or medical purposes, it is critical to define the optimum biofilm production parameters of the target microorganism or microorganisms when determining the method to be chosen for biofilm eradication.

For this reason, the maximum biofilm production capacities of strains on industrial surfaces were determined in this study. In biofilm productions carried out in conventional batch systems, standard and efficient biofilm production cannot be realized because there are no continuous flow systems required for microorganisms to produce biofilm structures. Because the biofilm thickness is smaller than 50 μm in these methods, biofilm structures larger than 50 μm can be obtained in reactors with continuous flow systems (Macia et al., 2014). With the application of antibiotics to the reactor in flow systems, it is also possible to obtain a more accurate result in the effectiveness of the antibiotic with continuous circulation. In addition, flow systems for antimicrobial susceptibility testing provide better control of growth parameters and dynamics (Lourenço et al., 2014). In classical test systems, data close to reality cannot be obtained due to the accumulation of wastes resulting from microorganism activity, the competition of microorganisms for nutrients, and the disintegration of the formed biofilm structures. However, it is possible to continuously transfer sterile and fresh medium using a multi-channel peristaltic pump in a reactor providing a flow system. It is also known that flow systems are the best approach for modeling biofilm formation in confocal laser scanning microscopes (Kim et al., 2008).

L. monocytogenes, *E. coli*, and *Salmonella* can form biofilms by adhering to many food contacts surfaces such as stainless steel, polystyrene, and glass used in the food industry. The ability to produce

biofilms, which is a common characteristic of many pathogenic microorganisms, increases the virulence and persistence of these microorganisms in host systems to very high levels, thus making them a constant source of contamination (Vestby et al., 2009).

On the other hand, compared to the planktonic forms of pathogenic microorganisms capable of producing biofilms; They have been found to contain a much higher resistance to antibiotics, disinfectants, and sterilizing agents (Wilks et al., 2005; Ryu and Beuchat 2005). This poses a serious problem for food safety, as it continues the source of contamination. It has been determined that these persistent contaminations caused by biofilm structures can produce infections from a single source for years (Ferreira et al., 2014). However, *L. monocytogenes* is known to produce biofilms at low temperatures used during food processing and storage, leading to cross-contaminations; there is currently no direct evidence that *L. monocytogenes* associated biofilms play a role in food contamination or foodborne outbreaks. This is probably because biofilm isolation and identification are not part of an outbreak investigation or because the diagnosis of the biofilm is not precise. If *L. monocytogenes* is known to colonize surfaces, contamination patterns should be studied in how biofilm formation is affected by the environment within food processing plants. The findings of our study are of the nature to eliminate such drawbacks in biofilm research.

In the field of orthopedic surgery, prostheses and trauma plates are used for various purposes. The disadvantage of these biomaterials is that they provide a suitable site for bacterial colonization in implant-associated infection. When bacteria attach to the biomaterial surface and multiply, they secrete exopolysaccharides and form a biofilm. The biofilm structure surrounding the bacteria protects them from the immune system and antibacterial agents, so the treatment of implant-related infections is challenging. In addition to long-term antibiotic therapy, many cases require surgery to remove and amputate the implant and/or to implant an antibiotic-containing filler to cure the infection. Therefore, investigating the problem of bacterial adhesion to biomaterials is clinically critical. Solid biomaterials used for clinical purposes are tightly controlled by standards such as the International Organization for Standardization (ISO) and the American Society for Testing and Materials. Biomaterials can be made from only a few types of standard materials depending on their application, including titanium, stainless steel, cobalt-chromium-molybdenum alloy (Co-Cr-Mo), and ultra-high molecular weight polyethylene. Although such biomaterials are microbiologically tested, they are not tested for bacterial adhesion/biofilm formation. There is little research on the adherence of S. epidermidis to other medicinal materials used in clinical practice, and the results are primarily inconsistent (Shida et al., 2013).

For this reason, the necessity of creating model systems based on test criteria arises. Similarly, surfaces coated with antimicrobial agents should be tested with biofilm structures compared to the planktonic forms of bacteria. Researches have left to biofilm studies instead of studies with planktonic forms of bacteria. Our data confirm the necessity of working with biofilm structures to fight against diseases, and the test gives accurate data. In this direction, the trials carried out to diagnose bacterial biofilm structures on implant surfaces form the basis of a test criterion used in an industrial process.

The biofilm reactor developed within the scope of this study, which enables the control of medium flow rate, mixing, and ambient temperature parameters. It will add a new horizon and impetus to biofilm studies by eliminating the drawbacks explained in detail above. It enables optimum biofilm production on both glass, stainless steel, and polystyrene surfaces and commercial implants by using different pathogens.

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TRANSMISSION OF COVID-19 IN CURRENT FOOD SYSTEMS AND FOOD SAFETY

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ABSTRACT

In 2021, the reality of exponential technology innovations being operative in almost every aspects of life put both the innovation and appropriation on an exponential curve. The COVID-19, broadly referred to as "coronavirus", a global pandemic, while thousands of infections and deaths are reported daily. The proper implementation of innovative technologies would help to enhance education and communication for not only fighting local and global medical emergencies but also food sector management. How this pandemic may impact the future of food science technology, innovations and the workplace for future generations? This ensures insights regarding the characteristics of bioactive ingredients from foods, nutraceuticals and pharmaceuticals for the human immune system support against infections prior to discussing the possibility of COVID-19 transmission through the food chain.

Keywords: Covid-19, Pandemic, Transmission, Food Safety, Risk Management

Introduction

Coronavirus disease (COVID-19, caused by the novel coronavirus SARS-CoV-2) is the latest in a continuing series of infectious disease epidemics in the history of the human race. The COVID-19 is an easily transmissible disease which was identified within December 2019 (WHO,2020a). The World Health Organization (WHO) declared, on January 30, 2020, that the outbreak of the disease caused by the new coronavirus (COVID-19) constitutes a Public Health Emergency of International Importance - the Organization's highest alert level, as provided for in the International Health Regulations- and declared a pandemic by WHO on 11 March 2020 (WHO,2020a).

COVID-19, newly emerged virus SARS-CoV-2 strain, dramatically disrupted every day social and economic pattern of societies around the world. COVID-19 has resulted in a reappraisal of global economic prospects. The World Trade Organization (WTO) forecasts substantial declines in both the real value of global gross domestic product (GDP) and volume of trade in 2020, with economic recovery in 2021 being dependent on uncertainty about the duration of the pandemic and measures utilized to contain it.

It is well known that the food sector and its stakeholders are in the spotlight, as food is necessary for human survival and cannot be lockdown. It is critical that the protection of the consumer

health and immune system through healthy nutrition strategies, availability of bioactive ingredients of food and beverages and by functional food products or value-added food products may be at the forefront. In this context, the demand for the mentioned products is increasing; food safety is a prominent issue in order to avoid the spreading of the corona virus between producers, retailers, and consumers as last destination point.

Considering that a serious epidemic causing a reduction of more than 25% in the workforce availability may cause significant food shortages worldwide; the research communities and authoritative officers should quickly identify the most critical threats to the food systems during a COVID-19 pandemic in order to exert mitigation measures.

The food procuration systems will also need to consider various supply possibilities that are being stockpiled by restocking as quickly and safely. This replenishing indicates the need on the mobilization of non-governmental organizations, food banks, and also community-based groups during lockdown periods. Recent food systems are not sustainable and efforts are continuing on the decreasing of postharvest losses of products. Meanwhile the implementation of non-thermal innovative food processing technologies provided food safety, the regain of bioactive constituents from food processing by-products, their reevaluation in the food chain and also their reutilization in health, environment and energy recovery era. The sustainability of the food systems in the pandemic duration is another issue that this sector should address in order to restrain the possible forthcoming pandemic times. Figure 1 shows the food science era and food systems in COVID-19 pandemic duration (Figure 1). We shown that there is strongly need to detect the current food systems including food safety, food security sytems; nutraceuticals, pharmaceutical manufacturing systems; food toxicology control systems; the manufacturing the functional and superfoods with bioactive compounds; the manufacturing systems on antioxidants/anticarcinogenics/ antimicrobials/probiotics/prebiotics ; food by-products and wastes based bioactive manufacturing systems; value-added agrifood product systems; value-added goods and renewable energy systems, nanotechnological packaging systems; sustainability of the food processing systems/ new analytical detections systems; the controlling of the non-thermal food processing systems, innovative food processing systems, the control of shelf life stability ; the detection of public health systems; procuring food-cancer studies and providing nutritional quality in COVID-19 pandemic duration (Figure 1).

Regarding Coronavirus (COVID-19; Novel Coronavirus SARS-CoV-2): Its Definition, Chemical Structure, Clinical Properties

Seven human coronaviruses (HCoVs) have already been identified: HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-COV (which causes severe acute respiratory syndrome), MERS-COV (which causes respiratory Middle East) and the most recent new coronavirus (which at first was temporarily named 2019-nCoV and, on February 11, 2020, received the name SARS-CoV-2). This new coronavirus is responsible for causing COVID-19 disease (WHO, 2020a). CA novel coronavirus, designated as 2019-nCoV, emerged in Wuhan, China, at the end of 2019. The first infections were linked (with some, but not firm evidence) to the Huanan Seafood Market (Wuhan, China) (WHO,2020a, Unhale et.al.,2020).

By November 24, 2021, worldwide 258,164,425 confirmed cases of COVID-19 including 5,166,192 deaths were confirmed, reported via WHO (WHO,2021). The main incidences are in America, followed by Europe, South-East Asia, Eastern Mediterranean, Africa and West Pacific. As of 21 November 2021, a total of 7,408,870,760 vaccine doses have been administered (WHO,2021).

The WHO reports that COVID-19 transmission comprises through respiratory droplets and contact routes. Droplet transmission eventuates via directly exposition to infective respiratory droplets of the person who is within 6-ft (1.8-m) of someone with respiratory symptoms including coughing and sneezing. Person-to-person spread is the main mode of COVID-19 transmission. The flu-like symptoms of COVID-19 usually appear 5–6 days after infection and contain fever (37.5 degrees Celsius or above), coughing, breathing difficulties, sore throat, fatigue, muscle and body aches, and even loss of smell or taste in some cases (Anonymous 2020ab: Bourouiba, 2020).

It is stated that if a CoV finds its way to a food surface, some data suggest that the virus has a limited survival rate, unless there is optimum moisture, secure porous anchor site, and a virus-friendly temperature (Pressman et.al.,2020).

The virus can progress even further and can infect the alveolar type II pneumocyte cells, similar to SARS-CoV. It has been shown that COVID-19 are released in large numbers from infected type II pneumocytes and cause cell apoptosis. Type II pneumocyte cells normally comprise 10-15% of total lung cells (Mason,2020). It invades the lung parenchyma, resulting in severe interstitial inflammation of the lungs; this is evident on computed tomography (CT) images as ground-glass opacity in the lungs at initially; then this lesion expands to multiple lung lobes (Unhale et.al.,2020; Silva et.al.,2018). The histological assessment of lung biopsy samples received from COVID-19-infected patients elicited diffuse alveolar damage, hyaline membrane formation, cellular fibromyxoid exudates, and desquamation of pneumocytes, indicative of acute respiratory distress syndrome.

Meanwhile, it was found that the COVID-19 infected patients often have lymphocytopenia with or without leukocyte abnormalities (Carsana et.al.,2020).

It is stated that viruses facilitate their replication cycle using 3 basic steps: 1) attachment to the host cell, 2) genomic material injection into the host cell, 3) replication through the host cell genome mechanisms to form new virions (Pellett et.al.2014). The virus has 4 structural proteins: spike (S), envelope (E), membrane (M), and nucleic acid (N). Among these protein constituents, S protein plays the critical role in viral attachment, fusion, and entry (Du et.al.,2009). Using the spike-like protein on its surface, the Coronavirus binds to ACE2 prior to entry and infection of cells (as a key being inserted into a lock). Due to the fact that, ACE2 acts as a cellular doorway (acts as a receptor) for the virus that causes COVID-19. ACE2 is present in various cell types and tissues containing the lungs, blood vessels, heart, kidneys, liver and gastrointestinal tract. Besides, ACE2 is present in epithelial cells, that line certain tissues and create protective barriers (Insel,2020).

Transmission of COVID-19 in Food Sectors

The transmission of COVID-19 to food system should be considered. Infected food handlers and employees infected by coronavirus, can contaminate the food direct breathing in the viral particle containing environment may cause other to get infected unwittingly. Highly precautionary measures to control the spread of the virus via person-to-person transmission at food industry, food service and retail sector should be applied (Shahbaz et al., 2020).

Employers should encourage workers to use an employer approved face mask or cloth face covering at all times while in the workplace as precautionary measures ; employers should ensure that workers can practice social distancing or employ engineering solutions if that is not possible; employers should make available facilities and materials for worker hygiene so workers can practice recommended handwashing; employers should clean and disinfect workplaces/stations at frequent intervals (FDA, 2020).

In U.S., the food supply remains safe for both people and animals. There is no evidence of food or food packaging being associated with transmission of COVID-19 regardless of the status of the worker in a plant. FDA does not anticipate that food products will need to be recalled or be withdrawn from the market should a person that works on a farm or in a food facility test positive for COVID-19 (FDA, 2020).

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VALUE-ADDED PRODUCTS IN FOOD SECIENCE IN TERMS OF INDUSTRY 4.0 APPLICATIONS FOR COVID-19 PANDEMIC DURATION

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ABSTRACT

Food and beverage industries encounter manifold challenges from recalls regarding serving customer demands. It is predicted that Industry 4.0 will turn manufacturers into predictors instead of reactors. The sustainability of the food systems in the pandemic duration is another issue that this sector should address in order to restrain the possible forthcoming pandemic times. In COVID-19 duration, there is need the sustainability of bioactive constituent applications for ``value-added food and supplement products`` and ``functional foods`` and need the pursuing and monitoring of food plant design studies, production strategies through Industry 4.0. implementations. In this proceeding content, value-added food products in food science in terms of Industry 4.0 procedures in COVID-19 duration

Keywords: Value-added products, food science, Industry 4.0, COVID-19

Introduction

Innovation focuses on improving quality for existing processes, procedures, products, services or for creating new ones. It is expressed that accomplished value-added ideas focus on restricted, highly technical, geographically large markets where competition is sparse. In this context, innovative value-added implementations improved on farms or at agricultural experiment stations are sources of national growth through alterations either in the kind of product or in the technology of production.

Food and beverage industries encounter manifold challenges from recalls regarding serving customer demands. It is predicted that Industry 4.0 will turn manufacturers into predictors instead of reactors; this fact will give easier collecting of data, time-saving / money-saving for investors and who utilize and apply in the food science and technologies.

Industry 4.0 Systems and transition equipment preparations require a considerable investment in new equipment and employees. Various manufacturers feel extempore for replacing

their equipments that currently working well, or for replacing less-talented labour with technical professionals. In numerous places, there is a deficiency of talented labour with the necessary qualifications. As another efficiency defect and its solving prior to real manufacturing, production matching must be controlled. When manufacturing model does not match demand, overproduction occurs; owing to the fact that food and beverage manufacturers must gather data regarding the demand for their products prior to altering production levels.

The sustainability of the food systems in the pandemic duration is another issue that this sector should address in order to restrain the possible forthcoming pandemic times. On the other hand, it is stated that there are three category engage in value-added productions but their differences are substantial in attempting to figure out their decisions.

The first category (1) food sector is concerning demand driven; companies are located in exceedingly populated areas and metropolitan locations aim for developing innovative and value-added products. The second category (2) of food industry is concerning agriculturally related sectors owing to principal food ingredients are bulky or perishable products and also farmers are their customers ; these food companies are located in prominent agricultural production and husbandry zones. The third category (3) of food industry named as footloose industry can generally satisfy demand from a wide geographic era with one plant; these type of sector typically produce foods and beverages with high values such as functional foods enhanced with biaoctive components, prepared flour mixes, frozen foods, canned specialties, functional beverages enhanced vitamines and minerals.

The food systems act an utmost important role in acquiring United Nations (UN) Sustainable Development Goals (SDGs), to end hunger by achieving food security and improved nutrition (SDG2) and to provide sustainable consumption, sustainable expenditure and production strategies (SDG12). Besides, it is stated that, it is indispensable for halving the per capita global food waste at the retail and consumer standards, for lessening food losses throughout supply chains (SDG 12.3), for providing life quality, and for encouraging well-being of people and public health. Moreover, it is expressed that the environmental and economic actions of food waste constitute at least 15% of the impacts of the overall food value chain (Scherhaufer, et.al,2018; Anonymous 2020; Tokusoglu,2018).

United Nations (UN) Sustainable Development Goals (SDGs) also beggar the optimum utilization of food ingredients and all raw materials through food and beverage systems in food manufacturing era.

In COVID-19 duration, for decreasing of post harvest product losses, ensuring food safety, improving food quality and quantity and for extended shelf life without food additives, there are need

the implementations of non-thermal processing technologies and emerging technologies and need the sustainability of mentioned applications (Tokusoglu,2020; Tokusoglu and Swanson,2015).

The recapturing of bioactive compounds from food by-product based functional food powders and their reincluded to food and beverage systems and also reutilization for food supplement industry and also sustainability of bioactive regaining actions have been significant in COVID-19 duration (Tokusoglu,2020). It is expressed that the current food systems not show sustainability ; approximately 14% of food is lost in some manufacturing / production steps prior to the retail position including agriculture stage, harvest step, slaughter situation, and catch step). Besides, on account of one-third of food is mondially wasted (approximately 1.3 billion tonnes per year, that is equivalent to 3300 metric tonnes per year of CO emissions/year).

In the food industry, the diverse types of by-products can be evaluated by various branches of industry due to their selected desired properties of food by-products. The pulps, dregs and wastes in food processing depends on the quality of by-product management, while ensuring the environmental protection and sustainability (Tokusoglu,2018). Food by-product in the food industry is characterized by a high ratio of product specific waste not only does this mean that the generation of this waste is unavoidable, but also that the level and the kind of by-product which consists primarily of the organic residue of processed raw materials, can scarcely be changed if the finished product quality is to remain consistent (Tokusoglu,2018).

In food science, recent trends are ``value-added`` foods and ``value-added`` supplements and ``functional foods``; especially bioactives from fruits, vegetables, from spices, from by-products, also from alternative sources (like algaes) are hot topic. Epidemiological studies have pointed out that fruits and vegetable consumption imparts health benefits including certain types of cancer, reduced risk of coronary heart diseases. The health benefits of fruits and vegetables are majorly attributed to bioactive nutrients as phytochemicals, phenolic compounds, carotenoids, vitamines (ascorbic acid, tocopherol etc.), also to dietary fiber of these products (Tokusoglu and Hall,2011; Tokusoglu,2018)

Flavonoids in the cycle may protect against cardiovascular inconveniences through their interaction with low-density lipoprotein (LDL). Biochemical and clinical studies in both humans and experimental animals have proposed that oxidized low-density lipoprotein (oLDL) has its atherogenic action through the lipid hydroperoxide formation and the another products derived therefrom. Various phytochemicals and especially Vitamin E (tocopherol) have antioxidant activity *in vitro*, that has led to the utilize of the general term “antioxidants” (Tokuşoğlu and Hall,2011).

Food tablets and food effervescents as dietary supplements and/or fortificated foods, food byproduct based food powders may be great value-added products for getting healthy bioactive

components. Nutraceutical food tablets have been prepared by direct compression method through selected tablet machines and have been manufactured according to established prescription methods. The functional constituents of the foods or some preferable functional foods must be standardized as the nutraceutical product and generate under Good Manufacturing Practices (GMPs) (Tokusoglu,2018).

In COVID-19 duration, there is need the sustainability of bioactive constituent applications for ``value-added food and supplement products`` and ``functional foods`` and need the pursuing and monitoring of food plant design studies, production strategies through Industry 4.0. implementations. Meanwhile, a nutraceutical or selected food /food supplements must be detected for non-toxic components and possible toxic constituents by advanced toxicity analyses and further food safety approaches in terms of Industry 4.0 enforcements including disease treatment and/or prevention.

One of another recent trends in food sector is alternative protein sources contain the dietary shift from beef to poultry and seafoods owing to the reducing red meat consumption; in this point, microalgae, seaweed, novel plant-based sources like quinoa and also lab-grown artificial meat concepts in aseptic conditions can be performed. Another current issues are essential oils and essential fatty acids (as omega-3; omega-6, DHA, EPA) as potential anti-inflammatory, relaxing, and stimulating substances, and modern exploitation in clinical medicine. Essential fatty acids (EFA) (especially linoleic and α-linolenic acids) must come from human diet. EFAs are major constituents of cell membrane matrix, modulate gene transcription, function as cytokine precursors, and serve as energy sources and influence the vital body functions including cardiovascular systems and mental health. In COVID-19 pandemic duration, there is also need the sustainability of protein and lipid sources monitoring from natural and alternative sources for manufacturing strategies with using Industry 4.0. implementations.

Seafood based ``Value-Added`` constituents are also crucial and can be utilised in cancer cure and mFish waste can also be utilized for production of various value-added products including proteins, amino acids, oil, minerals, enzymes, bioactive peptides, collagen and gelatin. Chitosan is commercially obtained mainly from chitin isolated from shell waste of crabs, shrimp and krill. It is reported that shrimp cuticle exhibits the higher amount of chitin, i.e. 30–40%, followed by crab, i.e.15–30%; in both cases the remaining fraction is made of proteins and minerals. Chitin and Chitosan are linear polysaccharides, comprised of two monomeric units namely N-acetyl-2-amino-2-deoxy-d-glucose (N-acetylated groups) and 2-amino-2-deoxy-D-glucose residues (N-deacetylated groups, amino groups). It is stated that chitosan production from crustacean is economically feasible, especially if it also includes recovery of pigments such as carotenoids. Chitosan has multifaceted

applications in cancer therapy including assisting in gene delivery, chemotherapeutic delivery, and as an immunoadjuvant for vaccines. For producing 1 kg of 70% deacetylated chitosan from shrimp shells, 6.3 kg of HCl and 1.8 kg of NaOH are needed, additionally to nitrogen and water.(Tokusoglu,2018).

Astaxanthin (3,3-dihydroxy-β,β-carotene-4,4-dione) is a ketocarotenoid oxidized from β-carotene, that plays biological roles and can be utilized in medical applications owing to its natural ketocarotenoid content, nontoxic property, high versatilite, hydro and liposolubility characteristics. Astaxanthin (AX) has attractive pink color and provide as vitamin A precursor and superior antioxidant characteristics. AX is active in protecting against chemically induced cancers and is protective effective on age-related macular degeneration (AMD) in eyes, and also in enhancing the immune system and in preventing damages rising from ultraviolet radiation. It is reported that AX represents 74-98% of the total pigments in crustacean shells, which contains 2.3-33.1 g/100 g of carotenoids. Due to the high levels of astaxanthin, crustacean shells are used not only for recovery of chitin but also for recovery of carotenoids (Tokuşoğlu et.al.,2018)

In COVID-19 duration, the sustainability of above-mentioned seafood bioactive constituent practices for ``value-added food and supplement products``,``cancer chemotherapy agents``, and ``natural pharmaceticals`` is indispensable and there is a necessity on monitoring of food and drug plant strategies in terns of Industry 4.0. implementations.

As sample shows biogas production (upper part) and biodiesel production (below part) from food by-products and wastes as ``value-added ``goods. It is stated that fish waste into three fractions: fish oil, protein and water and mentioned oil will be utilized for methyl ester (biodiesel) production while protein can be fed into an aerated composting drum together with support material to produce compost soil. Moreover, the waste water from the process is treated in a waste water treatment plant (WWTP) (Tokusoglu,2018). In tomato growing and processing industry, the major problem faced is the accumulation, handling and disposal of processing wastes. Recently, tomato by-products are either utilized as fertilizer or disposed at cropfields or dump with its inherent carbon footprint and commercial costs. In novel researches, tomato production waste is evaluating in the production of biogas via mesophilic anaerobic digestion (AD); 50-70% of the chemical energy maintained in the tomato organic matter can be converted into methane gas.

In this point, standardized procedures to measure BMP of lignocellulosic biomass need to be addressed for avoiding underestimation of the methane production. Methane gas is one of the causes of global warming and essentially it is a prominent constituent of the natural biogas we utilize in daily life (Tokusoglu,2018).

For sustainability assesment, general flow can be given as inputs, supply chain step and

outputs parts (Yakovleva and Flynn, 2004). It is expressed that the advanced set of sustainability indicators should be contemplated in conjunction with the inputs and outputs model of the food consumption and production system outlined (Tokusoglu,2020; Yakovleva and Flynn, 2004). The proposed indicators mplicate environmental criteria that could include impacts on the system, such as energy utilising as well as outputs such as waste.

The sustainability assessment is broadened from a potentially narrow economic focus on the supply chain to a broader analysis. Even though a sectoral-based assessment to sustainability appraisal is advantageous for improving sustainability indicators for the food supply chain, it can be utilized only for the processing stage. In this context, in COVID-19, there is a requirement on pursuing of input /output strategies of other steps in supply chain in terns of Industry 4.0 implementations.

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PUBLIC FINANCE APPROACHES AND ECONOMICS IN COVID-19 DURATION

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ABSTRACT

Food and related sectors including agriculture and aquaculture are prominent sources of national income for progressive countries. COVID-19 disrupted nations around the world in 2019-20 and 2021. It continues to cause death and economic damage in many countries. COVID-19 has resulted in a reappraisal of global economic prospects. As COVID-19 prevention sytematical, the mainstream economic theory ensures a framework for public financial analysis. For instance, rational theory can be utilized to appraise public finance in the era of application of microeconomics within a normative framework of public finance which implicates economic welfare. e, as well as economic actions, can be achieved to meet the strategic demands. This review content is related to public finance approaches and economics in COVID-19 duration.

KeyWords: Public finance, Food Science, Economic, COVID-19

Introduction

Alongside policies to decrease the spread of COVID-19, the government should relocate public budgets in the norm of fiscal and non-fiscal stimulus to meet the requirements of the community by taking into account the primary necessities and strategies.

The report released by the IMF (2020) declares that the COVID-19 pandemic not only indicates a public health emergency throughout worldwide but has also become an international economic emergency that, in its negative influence, can surpass the 2008-2009 global financial crisis. In this context, the large impact of COVID-19 could lead to a global recession in 2020 (IMF,2020).

Food and related sectors including agriculture and aquaculture are prominent sources of national income for progressive countries. The processed food industries are also valued globally at over $2 trillion dollars and deployed in more than 400,000 businesses.

The food processing industry is a mature area that is experiencing a turbulent period owing to the expanding global requisitions for food safety, increasing food insecurity and based on consumer

discretionary for higher quality and sustainability. There is a considerable economic efficacy of getting food safety wrong if contemporary food supply chains are incorrectly assessed and risk mitigation is deficient. Even a minor impact on a supply chain can have a major economic impact. Food processing is substantially impacted through multiple external factors containing economic trends, climatological alterations, demographic shifts, world population growth predictions, emerging power markets, and possible trade partnerships.

With the prolonged lockdown around the world and after the preliminary panic on food purchasing, the questions are about long-term effect will the virus have on market trends, and how the companies will adapt to this new situation and is a change in consumer interests are inevitable. The social restrictions, self-isolation, limitations have caused a decline in labor in food industry as well as in all sectors of the economy and cause numerous jobs to be lost, so the emergence of crises and economic recessions will come out.

The studies performed by WHO (2020), Wilson et al. (2020), and Wilder-Smith et.al. (2020) advocate that COVID-19 cosubstantiate two fatal characteristics: three to thirty fold more deadly than seasonal influenza, based on the fatality rate of abusive cases, and at least ten fold more infectious than SARS. Therefore, the potential damaging on the health of people throughout the world is enormous, both in developed and developing countries (WHO,2020; Wilson et.al.,2020; Wilder-Smith et.al.,2020).

It is noted earlier by World Bank (2013) that noted earlier that "global stagnancy, in general, might disproportionately damage to low and middle-income countries, owing to they tend to lack resources and capacity to deal with these shocks (World Bank,2013). In this point, it is seen that COVID-19 has transformed into a pandemic, with small transmission chains in various countries and large chains resulting in the extensive distribution in various countries, such as USA, Italy, Iran and South Korea.

COVID-19 disrupted nations around the world in 2019-20 and 2021. It continues to cause death and economic damage in many countries. COVID-19 has resulted in a reappraisal of global economic prospects. The changes in lifestyle and measures to reduce the spread of COVID-19 have significantly altered economic activity, employment, food consumption, and workplace environments. Occurring broad macro-economic impacts and the agricultural finance issues that arise during and related to COVID-19 significantly influenced the food and agricultural sector.

The World Trade Organization (WTO) forecasts considerable declines in both the real value of global GDP and volume of trade in 2020, with economic recovery in 2021 being dependent upon uncertainty regarding the pandemic duration and measures utilized to implicate it (Sheldon and Grant,2020).

WTO declares three scenarios concerning recovery: V-shaped, U-shaped, and L-shaped, based upon how long containment precautions remain in place (WTO, 2020). WTO reported that relative to the prepandemic baseline, real global GDP is forecast to decline in 2020 by -4.8%, 9.2%, and -11.1%, respectively, for the above-mentioned three scenarios. It is estimated that, forecast rates of recovery in 2021 are 4.2%, 8.1%, and 2.8%, respectively (WTO, 2020).

The recession in economic activity is anticipated to be accompanied by a substantial decline in trade that could exceed that recorded during the 2008-2009 financial crisis. According to these three WTO scenarios, the real value of exports in 2020 is forecast to drop by -8.1%, -16.5%, and -20.4% (WTO, 2020). Even though, agricultural commerce is not conjectured to be hit as hard as other sectors, the WTO foretells a substantial decreasing in the real level of exports by -6.5%, -11.2%, and -12.7% across the scenarios.

Research exhibited by Bénassy-Quéré et.al. (2020), Gopinath (2020), and Furman (2020) put forwarded that COVID-19 caused the extremely contagious global shock that synchronously comprised negative supply shocks and negative demand shocks. The mentioned shock results in decreasing the capability of people to work and companies to produce, and it reduces incentives and possibilities regarding spending of people, and investment of companies (Bénassy-Quéré et.al.,2020; Gopinath,2020).

Susceptible-infected-recovered (SIR) model can assure a theoretical framework and forecasts that can be utilized by government authorities for controlling the spread of COVID-19. Atkeson (2020) performed a specific study that presented a susceptible-infected-recovered (SIR) model to economists regarding the COVID-19 development in the United States over the next 12-18 months. The SIR model has greatly evidenced epidemiology despite its apparent simplicity and is a Markov model of the spread of epidemics in a population where the total population is separated into categories as susceptible to disease (S), as actively infected with the disease (I), and as recovering (or dying) and as no longer contagious (R). It is reported that this study can predict the outbreaks level, the state of the disease through social distance, and its progression on the population, as well as the state of the health system, staff shortages which have an influence on the primary finances, economical infrastructure, and cumulative burden of the epidemic for longer than 18 months (Atkeson,2020).

The macroeconomic influence of the COVID-19 pandemic with this extreme speed involves indicators which must be accomplished by policymakers through measures with real-time forward-looking ambiguity including stock market volatility, economic ambiguity and subjective uncertainty in business expectations questionnaire (Baker et al., 2020).

COVID-19 has tremendous economic and social impacts containing marketings and financial institutions, that can be observed from the impact of other past incidents that are analogous with COVID-19, so those future investigations can be carried out (Goodell, 2020). It is expressed that the function of government underwent alters in various activities including direct supervision, social expenditure of public goods, the steadiness of state finances and monetary policy, government procurement, and welfare spending (Samuelson, 2008).

It has been explained that focusing on economic welfare conditions where the allocation of economic resources reaches Pareto efficiency by Rossen & Gayer (2008). In this context, Pareto efficiency is expressed as "allocation so that the only way to make one person better is to make others worse". Pareto efficiency seems like to be a moderate normative criterion, if Pareto inefficient resource allocation, it is "prodigal" in the sense that it is possible to ensure someone better without hurting others (Rossen & Gayer,2008). If two assumptions compensate as a results of welfare economy, an economy will attain Pareto's efficient allocation of resources without government intervention. The Pareto performance can definitely be tested by government policies in preventing and ceasing the spread of COVID-19 with a series of economic policies regarding foremost public health precautions, the providing of medical devices, direct assistance to public and as tax subsidies.

In COVID-19 duration, the government must also have to take steps to improve the indispensable economic downturn. The accurate and reasonable prevention and countermeasures are required to restrict the COVID-19 spread and save lives and to keep the death rate as low as possible, to maintain impacts on the economy at a manageable level, and leveling the epidemic curve to await vaccine development and manufacturing in antiviral drug scale / therapy.

As COVID-19 prevention sytematical, the mainstream economic theory ensures a framework for public financial analysis. For instance, rational theory can be utilized to appraise public finance in the era of application of microeconomics within a normative framework of public finance which implicates economic welfare.

Based upon the economic stimulus, the government must offer the taxation policies to the manufacturing and manufacturing industries affected through the COVID-19 pandemic. Besides, sub-sectors connected to the food manufacturing sector such as supply sector, material/ingredient sector, mechanical sector, food plant construction sector, mechathronic, agencies, shipping/transporting sector, distribution, tourism sector, accommodation sector should also be within the scope of utilization.

For the utmost utilities, both public health and the mentioned sectors must especially use all aspects of the Internet of Things (IT) from supplies to consumer purchases in terms of Industry 4.0. These implications include collection, transferring, analytics and data concealment, so provides aids

in the fight against COVID-19. Like other technologies, smart manufacturing and / or smart food plant (smart factory) comes along with hidden costs. These implications not only require an investment up front, but also need steady renovation and higher-paid employees to operate well. Bringing Industry 4.0 into a factory could take the focus off the significant things which come with running a food manufacturing facility. In accordance with the 80/20 rule, solving 20 percent of the troubles results in financial gains of 80 percent.

The prominent government policies should involve in the implementation of the national economic recovery program to promote the country's financial policies for handling the COVID-19 pandemic. The economic stimulus must primarily focused on supplying the basic necessities (such as a possible shortage situation). For compensation of this situation, the government can exert a budget performance policy from all ministries and institutions, as well as an expenditure budget from other activities (like infrastructure development) that can be postponed in the next fiscal year. The stimulation can be accomplished through creating the full-time employee /part-time categories and non-employees for all industrial sectors influenced by the corona pandemic. In this context, there are two policy emphasis bearing economic stimulus for all industrial sectors that has the consequence of incorporation to state revenues or prioritizing protection to the people.

Conclusion

The crisis of the COVID-19 pandemic has composed the new period. The speedy spread of the COVID-19 pandemic could have an economic action that could lead to a stagnation and a global economic crisis. The manufacturers, academic part constituents and food industry experts will have to face various considerable alterations including food safety providing, food security and publicity of Industry 4.0 constituents to decrease quality losses and food waste management, as well as assigning and monitoring the bioactive food properties which meet the nutritional expectance of public.

In COVID-19 duration, there is need the sustainability of bioactive constituent applications for ``value-added food/good and supplement products`` and ``functional foods`` and necessity the monitoring and monitoring of food plant design studies, production strategies through Industry 4.0. implementations. Moreover, a nutraceutical or selected food /food supplements must be detected by advanced toxicity analyses and further food safety approaches in terms of Industry 4.0 implementations including disease treatment and/or prevention.

We should introduce innovations quickly enough with the imminent economic crisis in the COVID-19 pandemic period; in Industry 4.0 scope, should offer value-added industrial goods and economically competitive nutraceutical products, pharmaceticals and supportive novel foods fortified with bioactive constituents/ antioxidants/ anticarcinogenics/ antimicrobials which promote health and support of consumers` immune system.

The Governments should issued policies in handling this pandemic through the public finance management that are diverted in the form of compensate health infrastructure and economic stimulus which can assist the society to survive, as well as economic actions, can be achieved to meet the strategic demands.

Alongside policies to decrease the spread of COVID-19, the government should relocate public budgets in the norm of fiscal and non-fiscal stimulus to meet the requirements of the community by taking into account the primary necessities and strategies.

The main goal on the relevant policies is not only to manage this current outbreak well, but also to meet the needs of survivors after the pandemic, and to introduce sustainable measures for health, nutrition, development and innovation strategies.

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NETWORK FORMATION AND INTERFACE BEHAVIOR OF PU COATINGS WITH DUAL HYDROPHILIC/HYDROPHOBIC DANGLING CHAINS*

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ABSTRACT

In this study, we present the results of coarse-grained (CG) simulation study on a PU coating consisting dual hydrophobic/hydrophilic dangling chains. We employed Dissipative Particle Dynamics (DPD) simulation to create the network structures through a two-step reaction route. The dual nature of dangling chains gives a smart behavior to the coatings in response to the environmental polarity. The effect of solvent evaporation on the final structure of the coatings and the smart response of the coatings to water (polar) and air/oil (apolar) environments has been extensively studied. This study paves the way of formulating stimuli-responsive PU coatings for anti-biofouling applications.

INTRODUCTION

Polyurethane (PU) smart coatings are industrially relevant polymeric materials that are widely used in aviation, automotive, and medical device industries. These coatings are generally designed for many different purposes such as self-cleaning, anti-corrosion, anti-bacterial, anti-fouling, low friction, biocompatibility [1]. PU coatings with dual functionality, namely involving hydrophobic and hydrophilic functionalities, can serve as materials with superior properties compared to conventional ones. To that purpose, smart amphiphilic PU coatings can be designed by introducing hydrophilic and hydrophobic dangling chains to the network during polymerization. By incorporating the hydrophilic and hydrophobic chains, the material becomes responsive to the change of the environment by the segregation of the dangling chains towards the interface [2].

In this work, we synthesized the network formation of a particular PU coating containing 1-octadecanol (oDEC) and methoxy polyethylene glycol (mPEG) dangling chains and characterized its smart behavior in response to the environment by employing CG molecular dynamic simulation method, DPD. This method is quite useful for modeling and studying cross-linked polymer network structures [2,3]. We model the network creation process of the PU systems contained the dual hydrophobic/hydrophilic dangling chains by initially forming the cross-link reactions in the system in the presence of the solvent. Later, we evaporate the solvent from the system by gradually deleting the solvent molecules, which is followed by a further relaxation at the constant pressure condition. We employed a solvent evaporation procedure which to mimic the real reaction conditions and to form a surface.

In the current work, two types of monofunctional dangling chains, one hydrophilic (mPEG) and the other hydrophobic (oDEC), were initially introduced to the system forming covalent bonds with tris(isocyanatohexyl)biuret (HDI-BT) in simulation. Afterwards, poly(hexamethylene carbonate)diol (PC) was introduced to the reactor with the additional cross-linker to create the final polymer network structure. We studied the cross-linking processes and the smart and responsive behavior of the coatings under polar (water) and apolar (air and oil) environments. We found out that solvent evaporation from the surface leads to the migration of mPEG dangling chains towards the surface. We also proved the smart and responsive behavior of the coatings by simulating the polymer/water polymer/air and polymer/oil interfaces.

MATERIALS

The chemical structure and coarse graining procedure are schematically shown in Fig. 1.

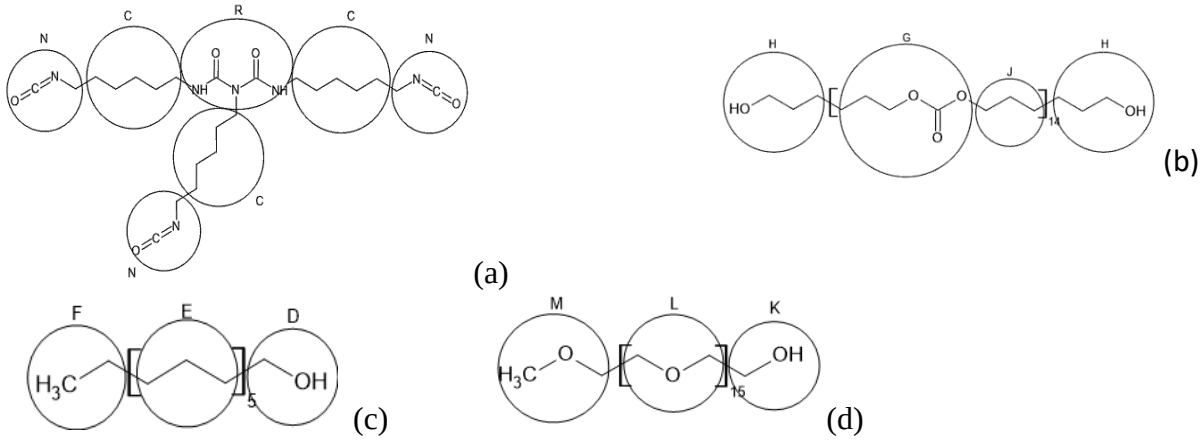


Figure 1: Schematic representation of the coarse-graining and the beads of (a) HDI-BT, (b) PC with n = 14, (c) 1-octadecanol with n = 5 and (d) mPEG with n = 15.

PC (M_w = 2134 g/mol) as the macrodiol, mPEG (M_w = 736 g/mol) as the hydrophilic dangling chain, oDEC (M_w = 270 g/mol) as the hydrophobic dangling chain, HDI-BT as the cross-linker, n-butyl acetate (nBAc) and methyl ethyl ketone (MEK) as the solvent are used as materials in this work. The mass ratio in experimental synthesis and the total number of molecules used in our simulations are shown in Table 1.

Table 1: Materials and number of molecules used in the study.

System details							
Case		**HDI-BT**	**PC**	**mPEG**	**1-octadecanol**	**nBAC**	**MEK**
Mix 15%	**Step 1**	1040	–	470	350	31406	9923
	Step 2	–	1150	–	–	6168	70237

METHODS

The simulation methods we employed in our study is coarse-grained DPD. The idea of coarse-graining is dividing the molecules into molecular sub-units, which are called as the coarse-grained beads [4]. In DPD, the non-bonded interactions between these beads are completely repulsive. Performing this coarse-graining and identifying the repulsive interactions between beads allows achieving longer time and length scales compared to the atomistic scale molecular simulations. This is especially important for the cross-linked polymer systems, which have significantly high relaxation times. In our simulations, beads have variable volumes. Therefore, instead of conventional parameterization, we use the alternative DPD parameterization, where the volumes of beads are dictated by their pure-liquid densities [5]. We carried out simulations in three steps: polymerization at the *NVE* condition, polymer relaxation and solvent evaporation at the *NPT* condition.

RESULTS

Throughout the cross-link simulations, the beads supposed to cross-link turn into new bead types as a result of polymerization at the coarse-grained level. The cross-link conversion is obtained by dividing the number of cross-links that a particular bead makes to the total number of cross-links that HDI-BT molecule is supposed to make. In the simulations, where two types of dangling chains are present, the total conversion equals to 94.77%. The individual cross-link conversions of mPEG, 1-octadecanol and PC with HDI-BT read as 15%, 11.18% and 68.59%, respectively. This means that 0.28% mPEG, 0.42% 1-octadecanol and 6.95% PC beads remain unreacted.

To comment on the molecular structure of the coating, we plot the radial distribution function (RDF) between the reactive ends prior to and after cross-linking takes place. The RDFs are shown in Fig. 2.

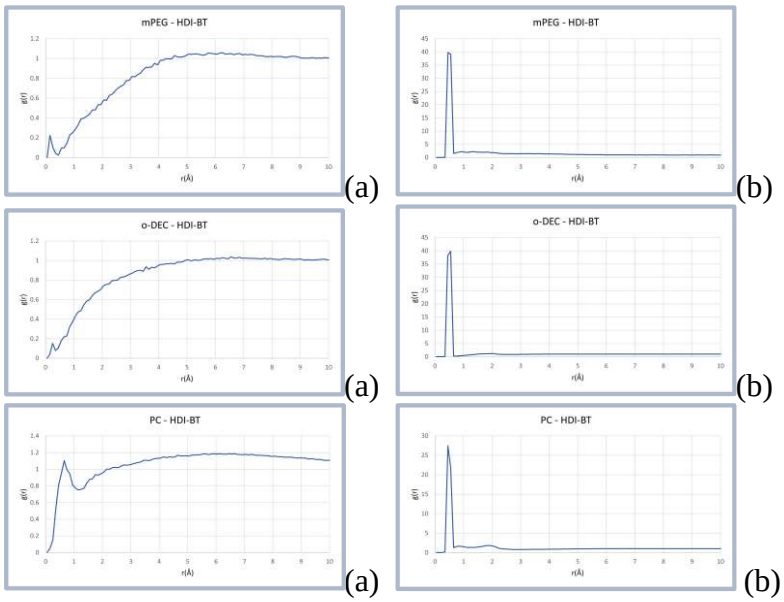
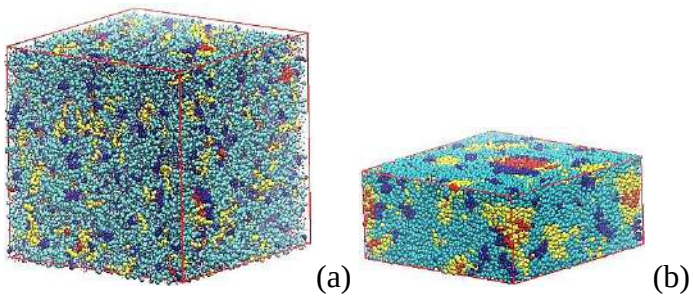


Figure 2: RDF plots between reactive ends before (a), and after (b) the cross-linking.

Following the cross-link and polymer relaxation simulations (Fig. 3a), we perform the solvent evaporation simulation as shrinking in only *z*-dimension (Fig. 3b). The idea in shrinking the simulation box only in *z*-dimension is to observe the effect of the dimensionality in the polymer film formation.

The final structure of the coating was placed in contact with a polar (water) and apolar (air and oil) environments. A responsive behavior of the coating with dangling chains is illustrated in Fig. 3c,d,e respectively.



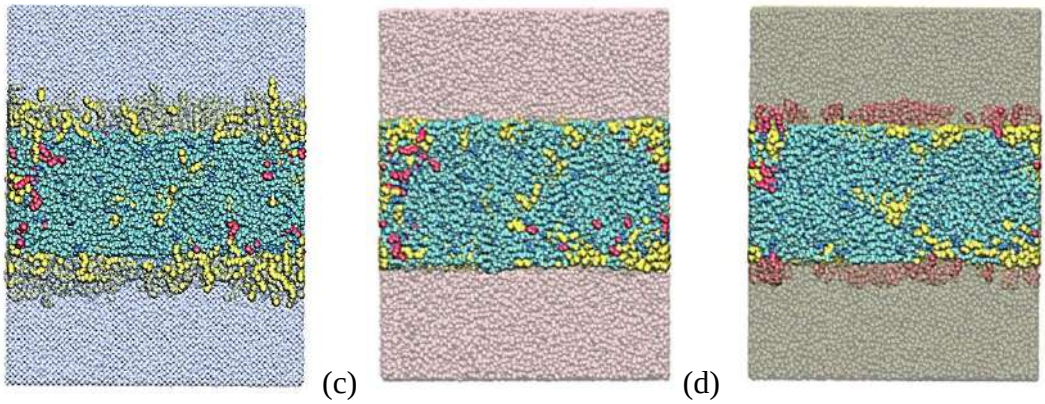


Figure 3: Simulation snapshots of the solvent evaporation to form the surface before (a), and after (b) evaporation takes place, and the polymer/water (c), polymer/air (d), polymer/oil (e) interfaces. Colors navy blue, yellow, red, cyan and dark green are assigned to cross-linked HDI-BT, mPEG, 1-octadecanol, PC, nBAc and MEK, respectively.

CONCLUSIONS

In this work, we modeled and simulated a hydrophobic/hydrophilic smart coating for studying its network formation and molecular structure as well as its interface with polar (water) and apolar (air and oil) environments. We reached a high cross-link conversion is obtained at the end and we observed that solvent evaporation can lead to a dangling-chains-rich interfacial layer when a mixture of hydrophilic/hydrophobic dangling chain strategy is applied. We evaluated the surface-stimuli response of the coatings to the environment, in which a smart surface rearrangement was observed. The next steps in our work involve incorporating different ratios of dangling chains and estimating the material properties *via* atomistic molecular dynamics simulations with the reverse-mapped coordinates.

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RECENT DISSIPATIVE PARTICLE DYNAMICS PARAMETERIZATION MIMICS EXPERIMENTAL STRUCTURE AND PROPERTIES OF WATER AND ALCOHOLS

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ABSTRACT

Mimicking experimental properties of water and simple hydrogen bonding liquids (methanol, ethanol and 1-propanol) *via* simulations has been one of the major challenges in the modeling community. So far, atomistic molecular dynamics simulations have been employed to study the structure and thermodynamic properties of such systems at their pure-liquid states or in liquid mixtures. Recently, attention has been given at the coarse-grained scale to characterize these properties due to the presence of water in complex environments such as physiological conditions and interacting with polymers. One of the challenges in parameterizing the coarse-grained interactions is the absence of atomistic detail, which leads to poor definition of interactions. Moreover, poorly defining interactions would possibly lead to unrealistic mesoscopic structure and properties. With this motivation, we aim at building a parameterization scheme for a coarse-grained method, namely dissipative particle dynamics (DPD) to achieve a realistic modeling of hydrogen bonding liquids. Our parameterization scheme involves the contribution of hydrogen bond interactions as computed from a statistical mechanic's approach combined with a fine-tuning of interactions based on experimental radial distribution functions (RDF). The results describe the experimental RDF and some physical properties of water and low molecular weight alcohols (i.e., methanol, ethanol and 1-propanol) such as viscosity, angle distribution and isothermal compressibility reasonably well. With the proposed parameterization, we hope to extend the current parameterization practice of DPD to cover a wider range of applications, where hydrogen bonding interactions are dominant.

Key words: Mimicks, parameterization, Dissipative Particle Dynamics (DPD) method

INTRODUCTION

We aim at developing a DPD parameterization scheme to examine the anomalies in the structural and physical properties of water, alcohol and their mixtures in a simulation environment with the Dissipative Particle Dynamics (DPD) method, which is a coarse-grained simulation method. The reason for using DPD is that coarse-grained methods are convenient tools to obtain the structural properties of complex molecular systems, which characteristically require long simulation times. In other words, long-term structural evolution of polymers or biological molecules are not suitable for studying with classical molecular dynamics methods (Kacar and Peters 2013). Since the DPD method covers a wide range of length and time scales and accurately reveals the hydrodynamic properties of liquids, it has become frequently used in the modeling of complex systems (Groot and Warren 1997). DPD interactions consist of only repulsive interactions, they are insufficient to model attractive

interactions such as hydrogen bonds. For this reason, in order to model attractive hydrogen bonds, attractive interactions formed as Morse potential were defined in addition to the repulsive interactions defined in the classical DPD method. In order to obtain the numerical values of the attractive interactions, the computational procedure were carried out for the adaptation of the partition function of water, which was previously developed as the Mercedes-Benz water model (Urbic and Dill 2018). In our approximation to model pure water and alcohols, the Morse potential is used as the attractive contribution, which is added to the DPD potential.

The results that are obtained during this work will add to the current literature by; defining attractive interactions for water-water interactions in DPD, developing statistical mechanics techniques to compute interactions to be used in DPD simulations, study of structural anomalies via DPD simulations, analysis of currently available materials or developing procedures to design new materials that are relevant to biological systems (DNA, RNA, cell membrane) and nanomaterials and biotechnological applications (hydrophilic polymers, hydrogels, drug delivery nanostructures).

MATERIALS

The chemical structure and coarse graining procedure are schematically shown in Fig. 1.

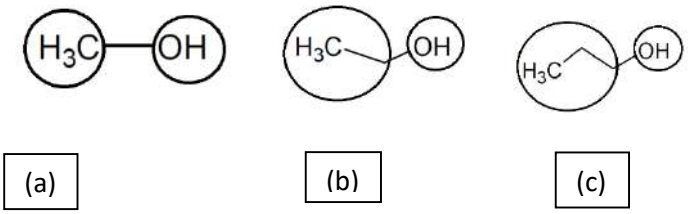

Figure 1: Schematic representation of coarse modeling of molecules a: methanol, b: ethanol, c: 1-propanol

The Morse potential was calculated for the water-water interaction. The reason is that our coarse-graining of alohols include an -OH group, which is practically a water molecule In the proper bead representation. Since the beads formed after the coarse-graining contain hydrogen bonds, the alcohols are modeled as two particles such as methanol (methane + water), ethanol (ethane + water) and 1-propanol (propane + water). The composition of the simulated systems are given in Table 1.

Table 1: Number of beads used in the study

	System details			
Case	Water	Methanol	Ethanol	1-Prophanol
Pure 100%	24000	24000	24000	24000

METHODS

In our studies, DPD potential is used for repulsive interactions and Morse potential was used for the attractive interactions. Equilibrium and cut-off distances of Morse potential were estimated from the OH-OH RDF graph as a result of the molecular dynamics simulations, hich we performed as a separate study. Since the systems we use are very small molecules, the parameters we will calculate

are very sensitive to the proper selection of the bond length and bond stiffness. Molecular dynamics simulations were used for the bond length calculations in DPD. Our alcohols consist of two particles and the bond length between the centers of mass (CoM) of the two particles is used. The results are given in Table 2. With the Morse potential, the bond length can vary greatly in small systems, which can cause the system to freeze and solidify. To prevent this, the Shake algorithm is used in the DPD simulations, which resulted in a fluid the system. The comparison of the simulations with and without the Shake algorithm is shown in Figure 2.

Table 2: Bond lengths connecting two beads and the scale factor to convert DPD units to the physical units.

System	Bond length	r_{DPD} [Å]
Methanol	0.30	4.483
Ethanol	0.35	5.063
1-Propanol	0.45	5.519

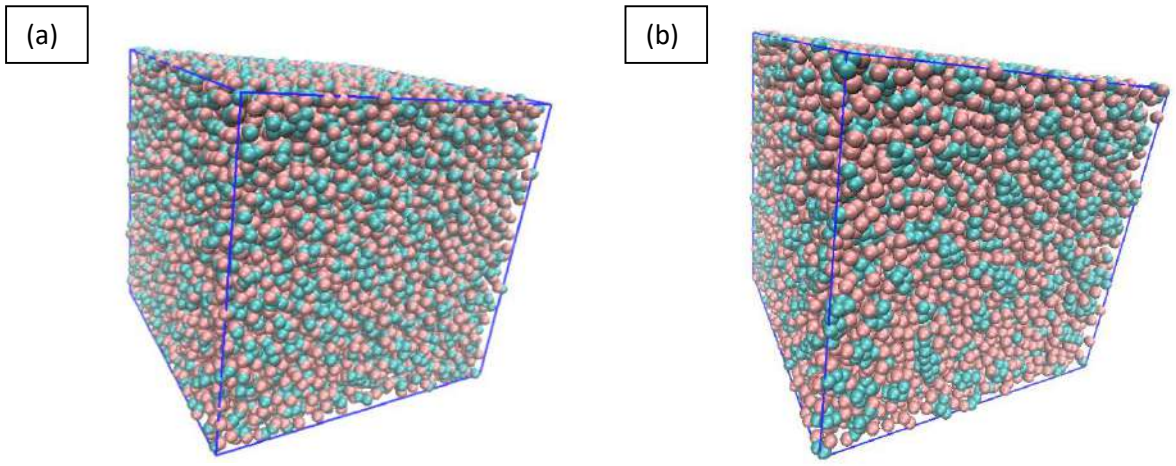

Figure 2. *a* and *b* show the simulation results with and without the shake algorithm, respectively.

In our parameterization, the DPD repulsive interaction is computed as

$$a_{ij} = \hat{a}_{ij} + \frac{p}{0.0454(a_{ii}\rho_{i,pure}+a_{jj}\rho_{j,pure}} k_B T \qquad (1)$$

where, χ_{ij} is a function of solubility parameters for each molecule and varies with temperature. To calculate the attractive strength of the hydrogen bond, the partition function obtained by Urbic *et* al. (Urbic and Dill 2018) is used as shown in equation 2.

$$\Delta_{HB} = 4\pi^2 c(T) v_{ef}^{HB} \exp\left(\frac{\epsilon_{HB}+\epsilon_{LJ}-pv_{HB}/2}{k_B T}\right) \sqrt{\frac{4k_B T\pi}{k_s}} \operatorname{erf}\left(\sqrt{\frac{k_s}{4k_B T}}\right. \qquad (2)$$

In this equation, $c(T)$ is the kinetic energy contribution, v is the molecular volume, T and p are the temperature and pressure, respectively.

The viscosity is computed at *NVE* conditions by using the Green-Kubo relation (Viscardy, Servantie et al. 2007). The results are presented given both in DPD units and in real units in Table 3. The viscosity η is,

$$\eta = \frac{V}{k_B T} \int_0^\infty dt \langle P_{xy}(0)\, P_{xy}(t) \rangle \tag{3}$$

In this expression, V is the volume of the system, T is the temperature, k_B is the Boltzman constant, $\langle \ldots \rangle$ is the ensemble mean, and P_{xy} is the off-diagonal element of the pressure tensor. τ indicates the unit DPD time in real units. To compute τ, the expression in the work of Groot and Rabone (Groot and Warren 1997) is used:

$$\tau = \frac{N_m D_{sim}\, r_{DPD}^2}{D_{su}} \tag{4}$$

In Eq. 4, N_m is the coarse-grained particle number density, D_{sim} is the diffusion coefficient obtained from the DPD simulations, r_{DPD} is the DPD unit length in Ångström, and D_{water} is the experimental diffusion coefficient for pure water. Eq. 5 is used to calculate the D_{sim} parameter as in (Partington, Hudson et al. 1952).

$$D = \lim_{t \to \infty} \frac{\langle [r(t) - r(0)]^2 \rangle}{6t} \tag{5}$$

To convert the viscosity result obtained from DPD units to real units,

$$H = \eta \tau k_B T \,/\, r_c^3 \tag{6}$$

is used as in (Boromand, Jamali 2015). H and η are viscosity in real units and in DPD units, respectively.

SIMULATION DETAILS

DPD simulations are performed for 24000 beads and 20^3 r^3_{DPD} box sizes. The systems are first equilibrated at 10000 steps after energy minimization, and then simulated for 200000 steps under *NVE* conditions. The time step used for each system is 0.001 t_{DPD}. Data to analyze are collected in the last 50000 steps of the simulations and used for calculations. Bond lengths in alcohols are fixed with shake algorithm and used in radial distribution plots. Simulations for viscosity calculations are run for another 10^6 steps under *NVE* conditions. In MD simulations, firstly, 20000 step energy minimization is performed with the steepest-descent algorithm, and then the systems are simulated for 1 nanoseconds at *NPT* conditions to maintain the experimental density. Finally, 1 nanosecond *NVE* simulations are performed and data are collected in the last 200 picoseconds. Berendsen thermostat is used for pressure and temperature control. The force field used in the simulations is OPLS-AA and TIP4P and SPCE water models are used for water. The cut-off distance was determined as 1.1 nm for electrostatic and van der Walls interactions in alcohols and 0.9 nm for water.

RESULTS

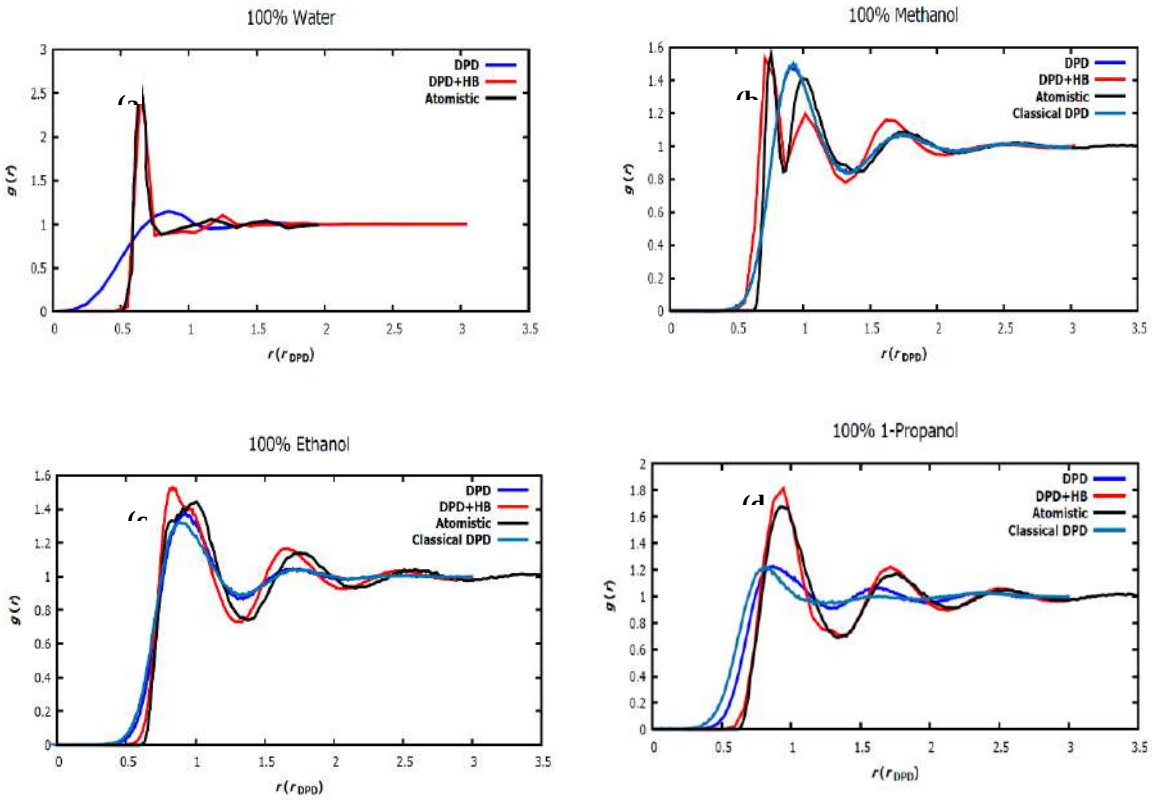

Figure 3. The Computed CoM RDF results for pure systems. **DPD** shows the classical DPD technique, **DPD+HB** is the DPD with the Morse potential, **Atomistic** is the Molecular Dynamics simulation. *a*, *b*, *c*, *d* are water, methanol, ethanol and 1-propanol, respectively.

It can be observed in Figure 3 that the classical DPD is insufficient to model structures that is a result of the hydrogen bond interactions. In other words, the Morse potential and the hydrogen bonds lead to more realistic results.

Table 3. The viscosity values calculated for pure alcohol and water at 298.15 K.

System	*Viscosity (t_{DPD})*	*Viscosity(cP)*	*Experimental (cP)*
Su	3,13	0,865	0,888 (Lide 2002)
Metanol	5,27	0,50	0,544 (Pal and Gaba 2008)
Etanol	6,68	0,98	1,040 (Center. 2007)
Propanol	15,40	1,20	1,959 (Pal and Gaba 2008)

Viscosity results obtained as a result of DPD simulations are very close to the experimental results. To comment more on the structure, we compute the threebody angular distribution calculations. This property will lead to an estimation of the tetrahedral structure of molecules, that is present in the experiments. For the calculations, an in house written script is used and angles are calculated between beads that are present within a cut-off distance. The results for both DPD and MD

simulations and compared. In line with the results obtained, the DPD simulation for pure water successfully models the known tetrahedral lattice structure formed by water as shown in Figure 4.

The atomic data was obtained from the MD simulationare performed in Figure 4, 5, 6 and 7. Again The center of mass (CoM) of the DPD simulation used Figure 4, 5, 6 and 7. In Figures 5-7 the presence of tetrahedral structure is noted. In all, we achieve the proper atomistic structural representation of water and alcohols *via* DPD simulations.

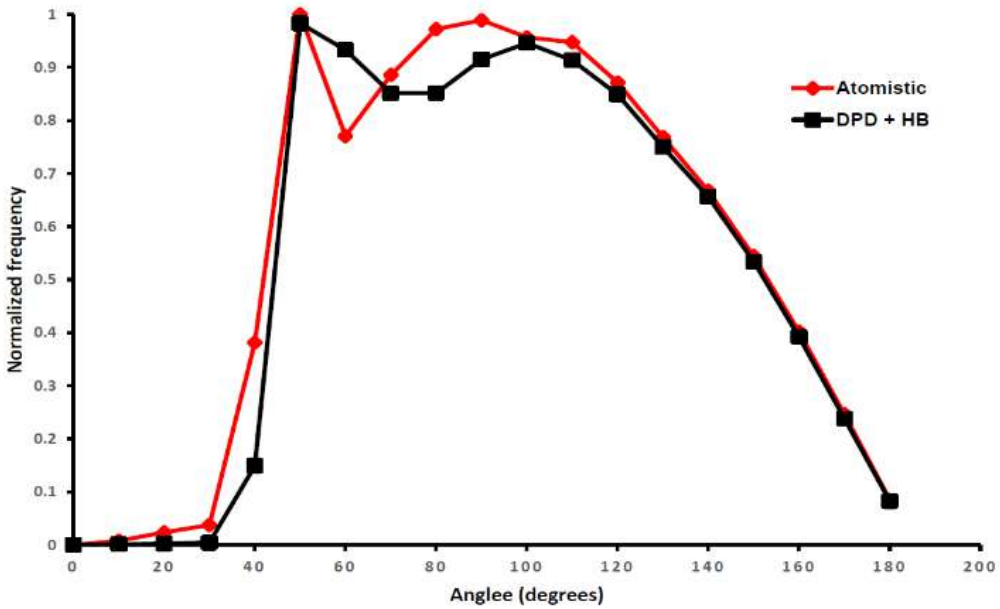

Figure 4. Angle frequency plot for pure water.

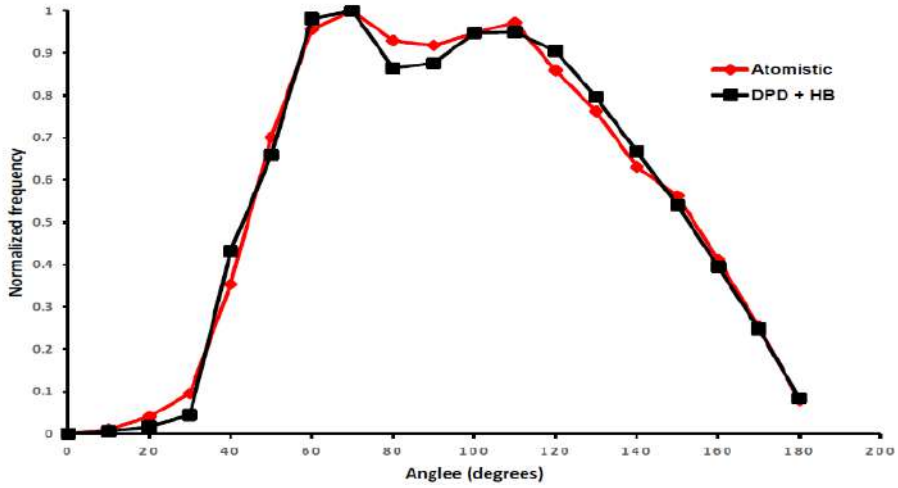

Figure 5. Pure methanol angle frequency plot

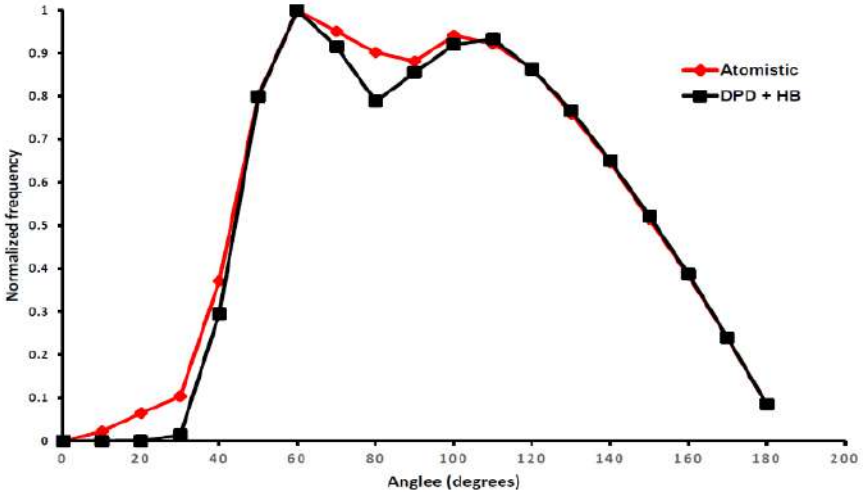

Figure 6. Pure ethanol angle frequency plot.

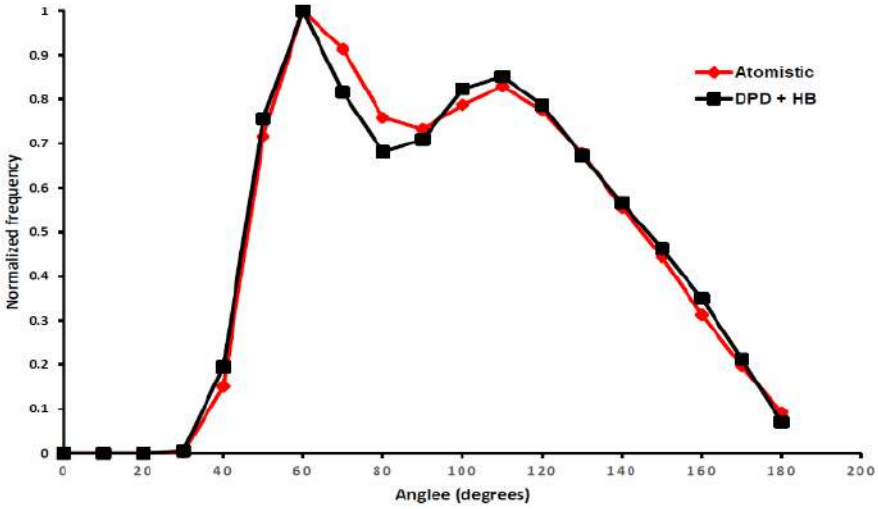

Figure 7. Graph of pure 1-propanol angle frequency

CONCLUSION

Hydrogen bond modeling in DPD simulations can properly model attractive interactions that are lacking in DPD. The hydrogen bond attractor interaction calculated by the partition function can be adapted to other systems and more complex structures can be simulated in DPD with more accurate results. Since coarse grained method gives faster results than MD in simulating large systems such as DNA, protein, polymer systems and drug transport systems, this study can be integrated into future studies.

ACKNOWLEDGEMENTS

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COMMUNICATION AND NAVIGATION OF AN AMPHIBIOUS DRONE

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ABSTRACT

In our lives, the role of drones is increasing day by day. The reason for this is that they are faster, environmentally friendly and ergonomic than other vehicles when doing the same job. With the development of communication and navigation technologies, we will not only see drones in the air. Thanks to the drones that can work in two different areas such as air-water and air-land, their usage areas will expand and they will be more beneficial for us human beings. In this study, it is mentioned how the communication and navigation system of an amphibious drone that can operate both in the air and in the water can work. A buoy system that can send and receive radio and acoustic signals will be used to operate the communication and navigation system of the amphibious drone. In this way, the drone will be able to complete its operation under water with the help of a buoy.

Key words: Amphibious drone, underwater communication systems, underwater navigation, UAV

INTRODUCTION

Drones, another name for unmanned aerial vehicles (UAVs), have become quite common today. It has been commonly used in many areas such as cargo transportation, photography, video shooting, mapping, irrigation and spraying in agriculture, search and rescue missions and firefighting. They provide superiority over other vehicles in terms of size, cost, design, environmental friendliness, convenience and configuration. These advantages are the reasons why drones are this popular.

With the development of technology, studies have been carried out to use these drones in other areas as well, which are designed to operate only in the air. In this way, the usage areas of drones will not be limited to air only. Drones that can change shape or have the necessary equipment are designed to be able to move in different environments.

For example, a drone will have the opportunity to land on the ground in bad weather conditions and continue its movement thanks to the wheel equipment on it. Likewise, it will have the opportunity to move in the water by moving its propellers from its horizontal position to its vertical position. With the production of drones with this capability, we will have the opportunity to help human beings more in daily life and science. Therefore, in this project, it is planned to work on an amphibious drone that can switch from air to water and fulfill the required task in both areas. In this study, ideas have been produced about how to design an amphibious drone which has a system that can provide communication and navigation under water.

The designs of amphibious drones should be made knowing that they have different parameters such as density, viscosity, pressure in two different areas (in the air and under water). These parameters are different even in fresh and salt water. It is necessary to produce systems that can move smoothly within the limits of these different parameters, communicate with the user, and operate the navigation system successfully.

This is a very difficult process and may encounter some problems. An example of one of these problems is that communication is different in fresh and salt water. While the conductivity in fresh water is 0.04 s/m (siemens/meter), it is 400 s/m in salt water.

Another difference between water and air regarding communication is that the power consumption in underwater communication is higher than in terrestrial radio communication, which means that an operation that can be done in the air must be done faster when entering the sea (Domingo, 2011). Communication in underwater vehicles generally takes place with acoustic signals. The reason why other signals are not preferred is that their effects are reduced in the aquatic environment compared to the air environment.

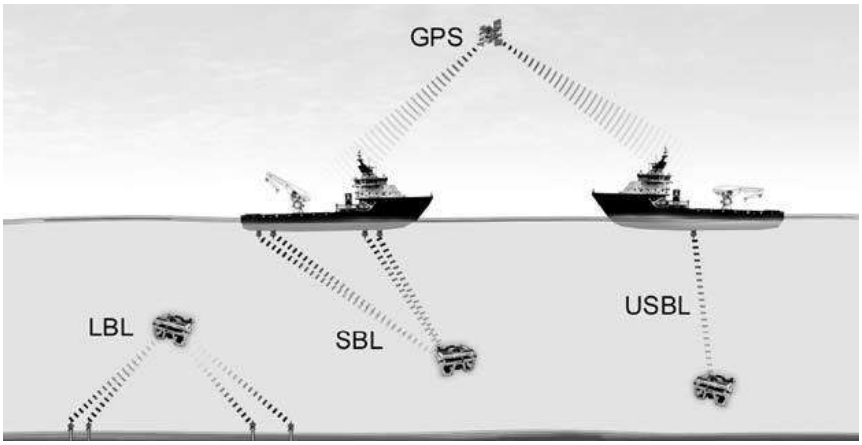

Figure 1: Positioning techniques. (Mallios et al. 2009)

UV (Underwater Vehicles), an acoustic channel with GPS (Global Positioning System)-enabled surface platforms, can achieve accurate positioning using long or short baseline (LBL/SBL) techniques (Su, Ullah, Liu, & Choi, 2020, Vaganay et al., 2006).

CONCEPTUAL APPROACH ON THE MODEL

According to the literature research and the information obtained, a portable surface buoy will be used for communication and navigation. There will be electronic systems that can make acoustic and radio conversion in the buoy. At the same time, there will be a system that will convert acoustic signals and radio signals to each other.

The buoy will have solar panels equipped for power generation in long-term operations. The buoy system can be deployed on the water in two ways, with the help of a ship or by carrying it with carrier equipment under the drone.

COMMUNICATION

Communication will be made on the water via radio signals from a receiver, as in all drones. Data from the ground station will be transferred to the flight control system with the help of receivers inside the drone. When the drone goes under water, it is aimed to communicate with acoustic signals that will be sent from the acoustic transducer from the buoy. Likewise any data or information from the drone will be sent to buoy through a hydrophone which produces acoustic signals. The electronic system inside the buoy will convert the acoustic signal to radio signal and transfer it to the ground station.

Figure 2: Communication with drone on air. (Raza et al. 2016)

Figure 3: Communication with drone underwater. (Raza et al. 2016)

NAVIGATION

The drones position will be calculated by GPS, IMU and barometer while in the air. These are the necessary systems of an air vehicle for navigation. The GPS provides three-dimensional velocity and position, the IMU provides three-dimensional linear accelerations and angular rates, the barometer provides the altitude of the vehicle based on the pressure.

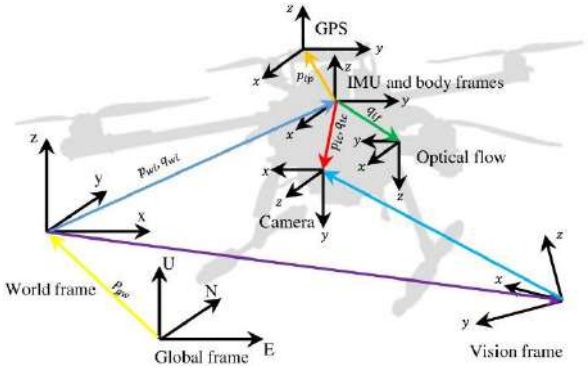

Figure 4: 3D localization of an UAV. (Bulunseechart 2018)

Under the water, drones navigation will be supplied by 4 systems. These systems are GPS, IMU, DVL and Sensors. Since the usage of GPS is limited underwater due the signals can't penetrate water well, the system can only supply up to certain sea level.

The inertial measurement unit (IMU) will contain gyroscope, accelerometer and compass. These will be used for measuring angular and linear motion information both in air and under water.

A Doppler velocity log (DVL) is a fixed frequency ultrasonic wave that the system sends to the relative node. Because the object and the node are in relative motion, the frequency of the received signal is different from that of the transmitted signal where this difference is the Doppler shift [26]. That's why, the relative radial velocity can be obtained with the Doppler shift. Using DVL and IMU will provide high-precision positioning. In this method the node will be the buoy and the object will be amphibious drone. In order to measure the height of the underwater drone from the water surface coordinate, pressure sensors or ultrasonic altimeters will be used.

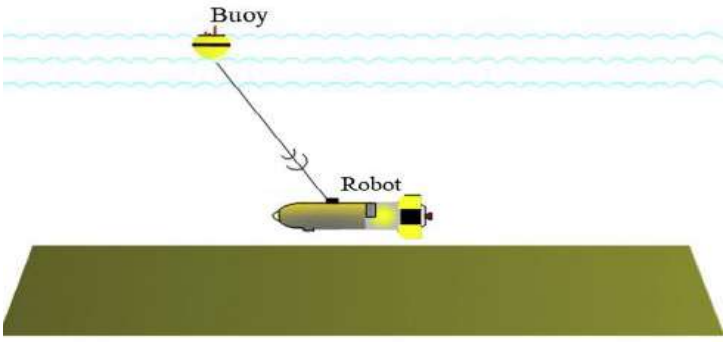

Figure 5: Single-buoy positioning principle diagram (Wu et al. 2019)

CONCLUSIONS

Single buoy positioning technology may be enough to solve the positioning and navigation problem in underwater navigation of amphibious drones. With the buoy system, communication can be provided by both acoustic and radio signals. With this method, amphibious drones can do remote positioning, multiple underwater applications and following the path.

Using a single navigation method may not meet accuracy or precision requirements, so a controller or additional navigation system can be needed. After checking the accuracy of the system at the next stage, it will be decided whether to add this part or not.

Another disadvantage may be that amphibious drone have to come to the surface to correct their navigation information in order to avoid any disruption in their underwater position. Like the previous problem, this can be corrected with a controller and supplementary navigation system

Since the buoy will not be fixed on the surface, it can move away from its position due to the waves, which can create problems in communication and navigation with the drone. Such as position shift and weakened aqustic signals.

FUTURE WORK

In the next research, it will be tested whether this system works or not. According to the data obtained, the system will be integrated and the communication and navigation part of the amphibious drone will be completed. If various malfunctions and problems appear in the system, alternative methods will be produced.

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VIRTUAL SCREENING FOR IDENTIFICATION OF PHARMACOLOGICAL CHAPERONES FOR ACID BETA-GLUCOSIDASE

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ABSTRACT

Several sporadic and genetic diseases are caused by protein misfolding. Gaucher Disease (GD) is a lysosomal storage disease caused by mutations in the gene GBA encoding acid β-glucosidase (GCase) that cause the protein not to fold into the stable form. The disease manifests itself with symptoms like enlarged spleen and liver, liver failure, skeletal and bone disorders, anemia and in severe cases central nervous system (CNS) involvement. Mutations in GCase disrupt the degradation of glucosylceramide into glucose and ceramide resulting in accumulation of glucosylceramide in the lysosomes, and thus causing Gaucher Disease. Even though there are over 250 mutations related to GBA, the disease provoking mutations are a few prominent ones. Small molecules that bind to misfolded proteins and guide them to correct folding by stabilizing the native state of these mutant proteins are called "pharmacological chaperons" and they have been proposed as new methods for treatment of GD and other protein-misfolding diseases. In case of GCase, these molecules are competitive inhibitors of the protein and bind to the mutant GCase in the ER, allowing it to avoid ERAD and to be trafficked to the lysosome where the original substrate replaces the chaperone. Molecules like N-nonyl-deoxynojirimycin, N-octyl-β-valienamine, the iminosugar isofagomine and ambroxol have been shown to increase lysosomal GCase activity. The concept of pharmacological chaperoning makes it possible that orally administered small molecules may take over intravenous enzyme replacement therapy as the standard treatment for GD and other lysosomal storage diseases. Virtual screening is a method to scan and prioritize large and chemically diverse compound libraries computationally to see whether they bind to the protein and function in the desired manner and to identify a subset of compounds for experimental testing. Depending on the knowledge about the target protein and/or known binders, ligand-based or receptor-based techniques can be employed for virtual screening studies. Receptor-based approaches are responsible for evaluating the complementarities and predicting the possible binding modes and affinities between small molecules and their macromolecular receptors. Having a well-defined binding site and known active binders makes GCase a fitting target for virtual screening. In this study, a virtual screening procedure that integrates ligand-based and receptor-based methodologies was applied to scan a library of around two million molecules. 3D flexible pharmacophore filters derived from both the target protein and the known ligand were applied to the small molecule library, giving the procedure both ligand-based and receptor-based properties. Whereas, high-throughput molecular docking experiments of the filtered library correspond to a receptor-based procedure. In this study, large-scale molecular dynamics simulations were also carried out to calculate and analyze binding free energies with linear interaction energy (LIE) method for the docked molecules selected with consensus scoring. A computational workflow for virtual screening was developed and applied to find candidate ligands for GCase. The workflow includes i) pharmacophore creation from either the target

protein or the known active ligand or from both, ii) pharmacophore filtering to reduce the size of the library, iii) molecular docking of the filtered compounds to the target's binding site, iv) scoring the bound poses with different scoring functions, and v) running two sets of molecular dynamics simulations on a smaller selected subset of compounds to predict their binding free energy to the target protein by LIE method. To enhance the hit variety, two different pharmacophore filters were created—one with the information from both the binding site and the known inhibitor and one with the information only from the known inhibitor—and 3 different docking runs were applied—two with AutoDock Vina and one with Surflex-Dock (Version 2.4)—to the hitlists generated by these pharmacophore filters.

Keywords: computer-aided drug design, docking, pharmacophore, bioinformatics, Gaucher Disease.

INTRODUCTION

Several sporadic and genetic diseases are caused by protein misfolding.[1] Gaucher Disease (GD) is a lysosomal storage disease caused by mutations in the gene (*GBA*) encoding acid β-glucosidase (GCase) that cause the protein not to fold into the stable form.[2,3,4] The disease manifests itself with symptoms like enlarged spleen and liver, liver failure, skeletal and bone disorders, anemia and in severe cases central nervous system (CNS) involvement. Mutations in GCase disrupt the degradation of glucosylceramide into glucose and ceramide resulting in accumulation of glucosylceramide in the lysosomes, and thus causing Gaucher Disease. Even though there are over 250 mutations related to *GBA*, the disease provoking mutations are a few prominent ones.[5]

GD related mutations either reduce the catalytic activity of GCase or cause a loss of protein stability during synthesis.[6,7] However; of all these GCase mutations, the ones that cause reduced protein stability, and therefore misfolding inside the endoplasmic reticulum are the main reasons for GD.[8] Mutant GCase is mostly broken down by endoplasmic reticulum associated degradation (ERAD) due to misfolding and can not be even trafficked to lysosome even though the remaining fractional activity of mutant GCase is still enough to hydrolyze glucosylceramide.[8] It has been shown that mutant GCase is almost as stable as the wild type in acidic environment of lysosome while it displays reduced stability in the neutral environment of the ER.[9]

There are two different types of treatment available for GD. The first one is enzyme replacement therapy[10,11] (ERT) with recombinant human GCase or substrate reduction therapy[12] (SRT) with *N*-butyldeoxynojirimycin[13]. Both therapies aim to reduce the glucosylceramide stock in the lysosome, thus treating the maladies caused by its accumulation.[14] However, costly and life-long treatment with ERT and the abundance of side effects of SRT make it necessary to seek new therapeutic approaches.[14]

Small molecules that bind to misfolded proteins and guide them to correct folding by stabilizing the native state of these mutant proteins are called “pharmacological chaperons” and they have been proposed as new methods for treatment of GD and other protein-misfolding diseases.[15] In case of GCase, these molecules are competitive inhibitors of the protein and bind to the mutant GCase in the ER, allowing it to avoid ERAD and to be trafficked to the lysosome where the original substrate replaces the chaperone.[16] Molecules like N-nonyl-deoxynojirimycin[16], N-octyl-β-valienamine[17], the iminosugar isofagomine[18,19] and ambroxol[20] have been shown to increase lysosomal GCase activity.

High-throughput screening of small molecule libraries is the most common experimental method used to identify lead compounds. However, synthesis and testing of all ligand candidates for one specific protein is still not easy. Therefore, a complementary method to reduce the large space of small molecules to an optimal size would solve the problem for the experimentalists.[21,22]

Virtual screening is a method to scan and prioritize large and chemically diverse compound libraries computationally to see whether they bind to the protein and function in the desired manner and to identify a subset of compounds for experimental testing.[23] Depending on the knowledge about the target protein and/or known binders, ligand-based[24,25] or receptor-based[26] techniques can be employed for virtual screening studies. Receptor-based approaches are responsible for evaluating the complementarities and predicting the possible binding modes and affinities between small molecules and their macromolecular receptors.

Molecular docking is one of the highly used receptor-based virtual screening techniques and it involves aligning each compound of the small molecule library in the protein binding site, predicting the possible binding modes and assigning quality measures to these binding modes.[27,28] Pharmacophore based techniques, on the other hand, can be applied in both ligand and receptor-based virtual screening studies. Pharmacophore modeling can be done either by analyzing the structure of the target protein and the binding site if known or by extracting the common chemical features from a set of known binders of the target.[29,30] The pharmacophore model created is then used for screening the small molecule library and searching for hits.

However, even though different docking algorithms are able to produce experimentally observed binding modes of ligands to a protein, it is still a challenge to recognize and pick them in huge libraries and assign accurate scores to rank them.[31] The efficiency of a computational drug design procedure relies on accurate prediction of binding affinities.[32] Scoring a docked ligand-protein complex with different scoring functions and assigning an overall score, namely consensus scoring is one of the ways to enhance the reliability of predicted binding free energies.[33,34] Although scoring functions and consensus scoring approaches are not as sophisticated as other more computation-time demanding methods, they are applicable to larger numbers of ligand-protein complexes in short times. A more advanced but more demanding approach is to use linear interaction energy (LIE)[35,36] models for prediction of binding free energies. LIE suggests that binding free energy of a ligand to a protein can be estimated by the difference between the bound and free states of a ligand. LIE method has been applied to predict binding affinities of ligands for a wide variety of targets and gave very good correlations with experimental results.[37,38,39,40,41] Therefore it has been accredited that LIE can be a powerful method that can be used on a large scale in computer-aided drug design.[42]

Having a well-defined binding site and known active binders makes GCase a fitting target for virtual screening. We developed a computational workflow for virtual screening and applied it to find candidate ligands for GCase. The workflow includes i) pharmacophore creation from either the target protein or the known active ligand or from both, ii) pharmacophore filtering to reduce the size of the library, iii) molecular docking of the filtered compounds to the target's binding site, iv) scoring the bound poses with different scoring functions, and v) running two sets of molecular dynamics simulations on a smaller selected subset of compounds to predict their binding free energy to the target protein by LIE method.

To enhance the hit variety, we created two different pharmacophore filters—one with the information from both the binding site and the known inhibitor and one with the information only from the known

inhibitor—and we applied 3 different docking runs—two with AutoDock Vina[43] and one with Surflex-Dock[44] (Version 2.4)—to the hitlists generated by these pharmacophore filters.

METHODS

Small molecule library and the target protein preparation:

The small molecule library:

The small-molecule library used in this work is a subset of a compilation of compounds commercially available from several vendors. For compounds in the raw library, molecular configurations (states) and three-dimensional conformations have been generated with LigPrep[45], enumerating tautomers, ionization states and, when the chirality is not specified, enantiomers. Finally, the molecules were minimized using the OPLS force-field[46]. To this raw library, filters such as the Lipinski Rules[47], Veber Rules[48], not having reactive moieties and more than 4 states (tautomeric, ionization and enantiomeric) were applied to select a subset of compounds for virtual screening. This filtered virtual screening library contains 2157575 compounds and it was stored in a single multi-SD file. From this single SD file; UNITY[49] databases for pharmacophore search, PDBQT[50] files (with MGLTools 1.5.4[51]) for docking with AutoDock Vina, and MOL2 files[52] for docking with Surflex-Dock and consensus scoring with CSCORE[34,49] were created.

The target protein:

The GCase structure used for virtual screening has the PDB id 2NSX and it has the competitive inhibitor isofagomine (IFG) bound.[53] The protein structure was treated with Biopolymer Structure Preparation tool of SYBYL-X[49] by Tripos before docking; IFG and water molecules were removed, hydrogens and charges (AMBER7_F99 charge set) were added. A small minimization using the AMBER7_F99 force field with Powell method and termination after 500 iterations was done after the addition of hydrogens. These pre-processed structures were used as input for pharmacophore modeling with UNITY, docking with AutoDock Vina and Surflex-Dock, and consensus scoring with CSCORE. However, for AutoDock Vina's file format PDBQT, the charges were replaced with Gasteiger charges[54] and non-polar hydrogens were merged.

Pharmacophore filter creation and pharmacophore search:

In this study, UNITY tool from SYBYL-X was used to create pharmacophore queries and 3D flexible database search for these queries. The PDB structure 2NSX has the competitive inhibitor IFG bound in the active site with an extensive network of hydrogen bonding. The imino group of IFG is stabilized by Glu235 and Glu340 while Asp127, Trp179, Trp381 and Asn396 interact with the hydroxyl groups of IFG.[53]

The first pharmacophore filter (**pharma1**) was designed from the hydrogen bond between Glu340 and imino group of the pyranose-like ring of IFG, and from the hydrogen bond between Asp127 and hydroxyl group of IFG as shown in Figure 1. A hydrogen bond acceptor site feature (tolerance=0.3 Å) was placed on the carbonyl oxygen atom of the carboxyl group of Glu340, requiring a hydrogen bond donor atom feature (tolerance=0.3 Å) in the pharmacophore query for the candidate ligand. This feature was defined from the protein binding site. On the other hand, the pharmacophoric feature for

a hydrogen bond donor atom (tolerance=1 Å) on the candidate ligand was derived from the hydroxyl group of IFG, hydrogen bonding with hydrogen bond acceptor atom feature (tolerance=1 Å) on the carbonyl oxygen of the carboxyl group of Asp127. Afterwards, **pharma1** was completed by adding all binding site residues as excluded volume with Van der Waals atom radii scaled by 0.25.

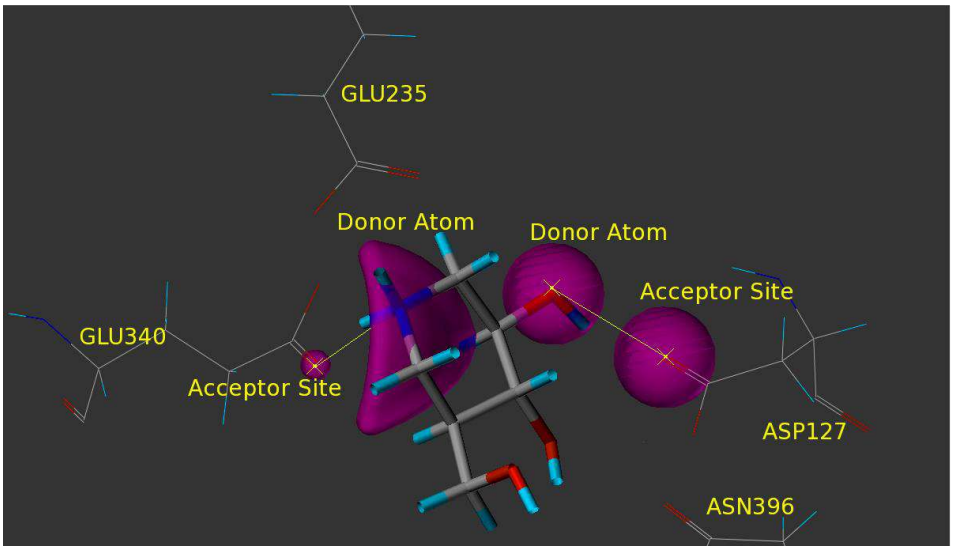

Figure 1: The first pharmacophore filter (**pharma1**) designed for GCase. A hydrogen bond acceptor site feature was placed on Glu340, requiring a hydrogen bond donor atom feature on the candidate ligand. A hydrogen bond donor atom feature on the candidate ligand was derived from the hydroxyl group of IFG, hydrogen bonding with hydrogen bond acceptor atom feature on Asp127 (excluded volumes not shown).

The features of the second phramacophore (**pharma2**) were mainly deduced from the the known ligand, IFG. The hydrogen bonds between the imino group of IFG and Glu235 and Glu340, stabilizing the ring of IFG composes the first part of **pharma2**. A hydrogen bond donor atom feature (tolerance=1 Å), making hydrogen bonds with hydrogen bond acceptor site features on Glu235 and Glu340 (tolerances=1 Å), was placed on the imino group of pyranose-like ring. The second part of **pharma2** was derived from the hydrogen bond between a hydroxyl oxygen of IFG and carbonyl oxygen of the carboxyl group of Asp127. A hydrogen bond donor atom feature (tolerance=1 Å) requiring a hydrogen bond acceptor site feature (tolerance=1 Å) on Asp127 was located on the corresponding hydroxyl oxygen of IFG. **pharma2** was also finalized with the addition of excluded volume features as explained in **pharma1**.

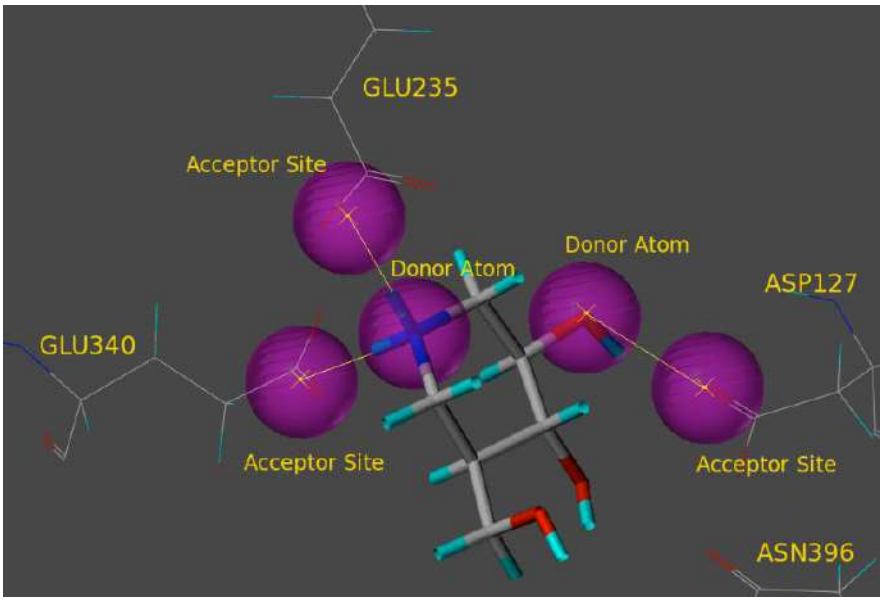

Figure 2: The second pharmacophore filter (**pharma2**) designed for GCase. A hydrogen bond donor atom feature making hydrogen bonds with hydrogen bond acceptor site features on Glu235 and Glu340 constructed the first part of the query. The second part of **pharma2** was derived from the hydrogen bond between IFG and Asp127 (excluded volumes not shown).

While the **pharma1**-filtered library was docked to GCase using AutoDock Vina and Surflex-Dock, only AutoDock Vina was employed for docking of the **pharma2**-filtered library.

High Throughput Docking of the pharmacophore-filtered libraries to the targets:

Molecular docking was done to predict the optimum non-covalent binding poses of the ligands in the receptor binding sites and their corresponding binding affinities. For this purpose, AutoDock Vina and Surflex-Dock were employed for 3 high-throughput docking experiments in total. All docking experiments were done with rigid target and flexible ligands. Docking experiment **dock1** was done with AutoDock Vina and **pharma1**-filtered library, **dock2** was done with AutoDock Vina and **pharma2**-filtered library and **dock3** was done with Surflex-Dock and **pharma1**-filtered library.

Since the binding site is a small cavity with well defined residues, determining the center and the dimensions of the grid for the docking experiments **dock1** and **dock2** with Autodock Vina was straightforward. The molecules were docked to the GCase structure using a grid with dimensions 20x16x16 Å and 1 Å spacing, which was placed on the bound ligand, IFG. Vina docking experiments with an exhaustiveness parameter of 8 yielded 9 different poses per compound and the pose with the lowest calculated binding free energy was kept for each compound for the next step of consensus scoring. For docking experiment **dock3** with Surflex-Dock, the computational representation of the intended binding site, protomol, was created from the binding site residues (Asp127, Trp179, Glu235, Glu340, Trp381 and Asn396) with the default values. The docking experiment was done with the default parameters for the "screen" docking mode (parameters can be found in the Surflex-Dock manual[55]), with the only exception being the number of final poses set to 10 instead of the default value of 3. Out of 10 poses for each ligand, the pose with the best binding affinity calculated by Surflex-Dock's scoring function was kept for consensus scoring.

Consensus Scoring

In this study, a given ligand-receptor complex produced by AutoDock Vina or Surflex-Dock was rescored using D-Score[56], G-Score[57], PMFScore[58] and ChemScore[59] with the CSCORE program of SYBYL-X. From the PDBQT files output by AutoDock Vina, we created proper MOL2 files with correct atom types, bonds and bond types, and all hydrogen atoms added to be input for CSCORE. Since Surflex-Dock outputs MOL2 files with all hydrogen atoms already added, no conversion was done on Surflex-Dock outputs. The four scores produced by CSCORE were combined with the score obtained with AutoDock Vina for docking experiments **dock1** and **dock2** while only the scoring functions employed by CSCORE were used for **dock3**. However, instead of CSCORE's rank-by-vote[60] consensus scoring approach, a modified rank-by-number[60] approach was designed to combine results of different scoring functions.

Our modified rank-by-number approach normalizes all scores to values between 0 and 1 (0 representing the most favorable compound, 1 representing the least favorable) for each scoring function. This normalization is done to put the scores from different scoring functions on the same scale and to make them comparable.

Two different normalization procedures were implemented: the first one being with *cut-off*=100% and the second one being with *cut-off*=99.5%. Setting the *cut-off* to 100% denotes no truncation and to 99.5% denotes 0.5% truncation. Therefore, the first normalization procedure was applied to all scores of all docked ligands while the second normalization was done after truncating the 0.5% most poorly scoring molecules for each scoring function. The truncation was done simply by directly assigning 1 as the normalized scores for the most poorly scoring 0.5%. In other words, 99.5% of the scores were normalized after the truncation of the poorly scoring 0.5% part.

The generalized formula for the normalized score of compound *i* with scoring function

F, $S_{cut\text{-}off,F}(i)$, is given as,

$$S_{cut-off,F}(i) = \min\left(1, \frac{E_F(i) - E_{\min,F}}{E_{cut-off,F} - E_{\min,F}}\right) \qquad \textbf{Equation 1}$$

where $E_{\min,F}$ is the lowest (most favorable) score obtained with the scoring function *F*, while $E_{cut\text{-}off,F}$ denotes the smallest value that is higher than *cut-off* % of the scores obtained with function *F*. This equation defines both normalization procedures, with or without the truncation.

Finally, to obtain an overall consensus score over all scoring functions, all normalized scores for a given compound were summed, yielding the "normalized consensus score", $NCS_{cut\text{-}off}$.

The normalized consensus score of compound *i*, $NCS_{cut\text{-}off}(i)$, is thus defined by

$$NCS_{cut-off}(i) = \sum_F S_{cut-off,F}(i) \qquad \textbf{Equation 2}$$

where $S_{cut\text{-}off,F}(i)$ is the normalized score of compound *i* with scoring function *F* after the normalization procedure with a *cut-off* truncation. The compound with the smallest normalized consensus score is supposed to be the most favorable. For the results of **dock1** and **dock2**, $NCS_{cut\text{-}off}$ values of ligands change between 0 and 5 (5 scoring functions), however the molecules docked with **dock3** experiment have $NCS_{cut\text{-}off}$ values changing between 0 and 4 (4 scoring functions).
The docked poses from each docking experiment were ranked according to their $NCS_{99.5}$ scores. The best-ranking 600 molecules from each docking experiment, 1800 molecules in total, were selected for binding free energy estimation with LIE. There were some molecules that ranked in the top-600

in more than one docking experiment. These molecules were not reduced to only one conformation. All docking results of the same molecule, if there is more than one, were treated as separate inputs for LIE.

Estimation of binding free energies by LIE

An implementation of the LIE method[61] was employed to calculate binding free energies of the ligands. This approach basically approximates binding free energy of a ligand from the difference in the ligand-surrounding interaction energies between the protein-bound and the free states.

The energies that enter into the LIE equation are averaged potential energies of the ligand with its surroundings obtained from separate MD simulations of the ligand in water or bound to the solvated protein system (with initial coordinates of the protein-ligand coordinates obtained from docking experiments). All MD simulations have been performed with the program Q[62] and the OPLS force field implemented therein.[63] Since many of the parameters as well as topologies needed for the ligands were not present in the original version of the force field, an automated parameterization protocol was designed. First, the MOL2 files used for CSCORE were converted to Schrodinger's Maestro format (.mae) using the mol2convert utility of the Schrodinger suite. Next, each ligand was energy-minimized with the bmin program of Macromodel[64] and the OPLS parameters and topology information generated by that program were translated into the syntax required by the program Q, using a set of *ad hoc* scripts.

MD simulations in Q were performed using spherical boundary conditions, thus a definition of a solvation sphere around the ligand was required. In our two cases, the centers of the spheres were determined manually, making small adjustments to the docking grid centers used. The sphere radii were calculated according to the diameter of largest compound as docked into the protein binding site, according to equation 4:

$$r_S = \left[1/2\, L_{max(ligand)} + c\right] \qquad \textbf{Equation 3}$$

where r_S denotes radius of the sphere, $L_{\max(ligand)}$ is the diameter of the largest docked compound and c is a constant which was set to 14 Å for this study. This ensures a margin of at least 14 Å of water around every atom of a ligand centered in the sphere. The same size of the solvation sphere was used for the protein-bound and the protein-free simulations. The water surface of this sphere was subjected to radial and polarization restraints[65] in order to mimic bulk water at the sphere boundary. Non-bonded interaction energies were calculated up to a 10 Å cutoff, except for the ligand atoms for which no cut-off was used. Beyond the cut-off, long-range electrostatics were treated with the local reaction field (LRF) multipole expansion method.[66] Protein atoms outside the simulation sphere were restrained to their initial positions, and only interacted with the system through bonds, angles and torsions. The ionization states of titratable residues inside the simulation sphere were manually assessed, in order to obtain neutral simulation systems in the protein simulation, which is needed to compare the ligand-surrounding energies between bound and free states. Any titratable residues closer than 3-5 Å to the boundary of the solvation sphere, as well as those outside the solvent sphere, were modeled as neutral because of the lack of dielectric screening. Even though only movement within the sphere is allowed, amino acids with all their atoms further than r_S + 2 Å away from the sphere center were removed.

For both the protein-bound and the protein-free simulations, a heating and equilibration MD simulation was carried out before the data collection phase, starting with a very short time step of 0.1 fs, a strong coupling to a temperature bath of 1 K and positional restraints of 25 kcal/(mol·Å^2) on all

non-hydrogen protein and ligand atoms in the case of the protein-ligand complex simulation. The system was then gradually heated up to 310 K during 95.5 and 65.25 ps for the protein-bound and the protein-free simulations, respectively, in which the bath coupling was relaxed to a final value of 100 fs, the timestep was increased to 1 fs and the force constant of the positional restraints was gradually lowered to 0.

A production-run molecular dynamics simulation then followed for 500 ps at 310 K (100 fs coupling time) with a time step of 1 fs. In the case of the protein-free simulation, the center of the ligand was restrained to the center of the solvation sphere with a force constant of 5 kcal mol^{-1} $Å^{-2}$. Energies were collected at regular intervals of 25 fs. Energy averaging was performed on this collection period, and stability was addressed by an estimation of the convergence errors of the potential energies of the ligand with its surroundings.

For the estimation of the binding free energies according to **Hata! Başvuru kaynağı bulunamadı.**, o nly the period 100-500 ps of the production-run simulations were considered. Convergence of the simulations was assessed by calculating the difference between the LIE energy calculated over the periods 100-300 ps and 300–500 ps of the production-run simulations. We refer to this difference as the LIE error.

RESULTS AND DISCUSSION

The 3D flexible pharmacophore search with **pharma1** reduced the library size to 136252 and **pharma2** reduced to 206428 from 2157575. Both filters managed to decrease the number of molecules to be docked by a factor of around 10. Even though no primary restrictions about the sizes of ligands were designed in either of the pharmacophore filters, large molecules (molecules with more than 20 A for the longest distance between two atoms) were mostly filtered out by both filters. Out of the 19678 large molecules in the small molecule library, only 769 could pass the filter **pharma1** and 2518 could pass **pharma2**. The sizes of the ligands to be docked are important because it is known that, regardless of the scoring function used, larger molecules tend to produce better scores than smaller molecules simply because of the abundance of hypothetical interactions in the binding sites.[27,67]

After the docking experiments **dock1**, **dock2** and **dock3**, the poses with the lowest binding free energies calculated by the corresponding scoring function (AutoDock Vina scoring function for **dock1** and **dock2**, Surflex-Dock scoring function for **dock3**) were selected for rescoring with the four scoring functions implemented in CSCORE for consensus scoring. Consequently, all scores given by each scoring function were normalized to values between 0 and 1.

Table 1 shows Pearson's correlations coefficients between different scoring functions for the docking experiment **dock1** for the **pharma1** filtered library. For docking experiment **dock1**, normalization of the scores output by different scoring functions to values between 0 and 1 with a truncation *cut-off* of 100% generated similarly shaped sigmoidal curves for all scoring functions (Figure 3a). However, especially in the case of G-Score, the individual scoring functions showed trails of high values to largely different extents. For the scoring functions except for D-Score and ChemScore, the values at the poor scoring ends are quite different than the rest, making the distinction between the fairly well docked ligands and the fairly poor docked ones quite difficult. These values at the poorly scoring end are also problematic when combining the different scores to reach a consensus. Using a rank-by-vote strategy for consensus scoring based on scores being within the top *n%* of the obtained score range,

with a frequently-used "vote cut-off" of 0.5 (dashed grey line in Figure 3a), as in CSCORE, results in G-Score voting for more than 99% of the molecules, and PMFScore and Vina score for around 75% of the molecules, rendering these scoring functions nearly redundant.

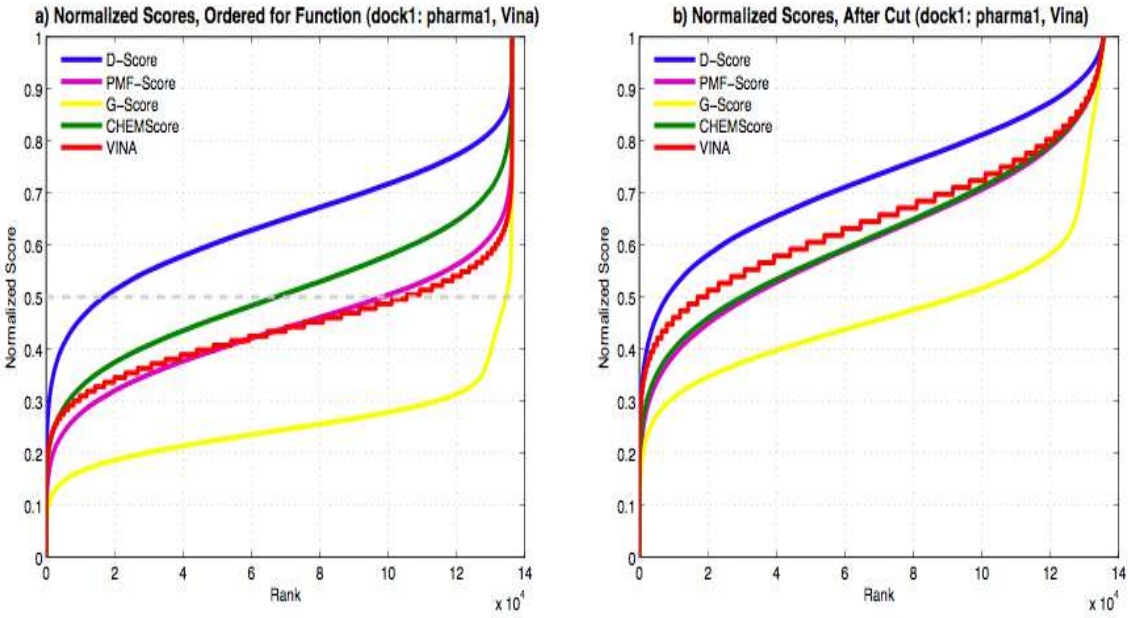

Figure 3: a) Normalized scores calculated without truncation with the five scoring functions Vina, PMFScore, G-Score, D-Score and ChemScore for **dock1** experiment of GCase. **b)** Normalized scores calculated with the second normalization procedure against compound rank (after the worst-scoring 0.5% were excluded) for **dock1** experiment of GCase.

Using a smaller "vote cut-off" value (around 0.3) would still not be enough because this time, all scoring functions except G-Score would be very specific and decisive and vote for a very small amount of molecules, while G-Score would still give a passing vote to more than 90% of the molecules. Summing the normalized scores, NCS_{100}, would be problematic as well because of the discrepancies between the decisiveness of individual scoring functions. For example, a compound ranked around 10,000 by D-Score receives a score that is twice that of another molecule ranked around 1,000 by D-Score, making D-Score highly decisive and sensitive against fairly poor and fairly well docked molecules. However, G-Score scores almost 100,000 of the molecules with almost the same value, showing no sensitivity except for very poorly docked molecules. Therefore, a solution to close the gap between the decisiveness and sensitivity of different scoring functions was to exclude the very poorly scoring end of each scoring function and to calculate the *NCS* score after the truncation.

Normalization with a truncation *cut-off* of 99.5% decreased the slope of G-Score at the poorly scoring end, increasing the overall sensitivity (Figure 3b). The curves of Vina score, ChemScore and PMFScore became more similar to the curve of D-Score. Even though G-Score's sensitivity is increased, it is still not close to the rest. To make G-Score converge with the rest would require the truncation of at least 5% of the molecules instead of 0.5%. However we decided not to diminish the number of molecules and thus it was decided to stop at 0.5% truncation and calculate the $NCS_{99.5}$ values for each compound.

$NCS_{99.5}$ values for **dock1** show large correlation values with all individual scoring functions even though the correlations among individual scoring functions are mostly moderate. However,

PMFScore shows weak correlations with G-Score and ChemScore. Therefore, it can be concluded that $NCS_{99.5}$ combined all scoring functions equally and good enough to be the representative ranking method for each of them despite the fact that some scoring functions are hardly correlated with each other.

Table 1: Pearson's Correlation coefficients between different scoring functions and $NCS_{99.5}$ for docking experiment dock1

	NCS	D-Score	PMFScore	G-Score	ChemScore	VinaScore
NCS	1	0.76	0.62	0.65	0.76	0.75
D-Score	0.76	1	0.48	0.42	0.49	0.33
PMFScore	0.62	0.48	1	0.17	0.13	0.36
G-Score	0.65	0.42	0.17	1	0.43	0.35
ChemScore	0.76	0.49	0.13	0.43	1	0.63
VinaScore	0.75	0.33	0.36	0.35	0.63	1

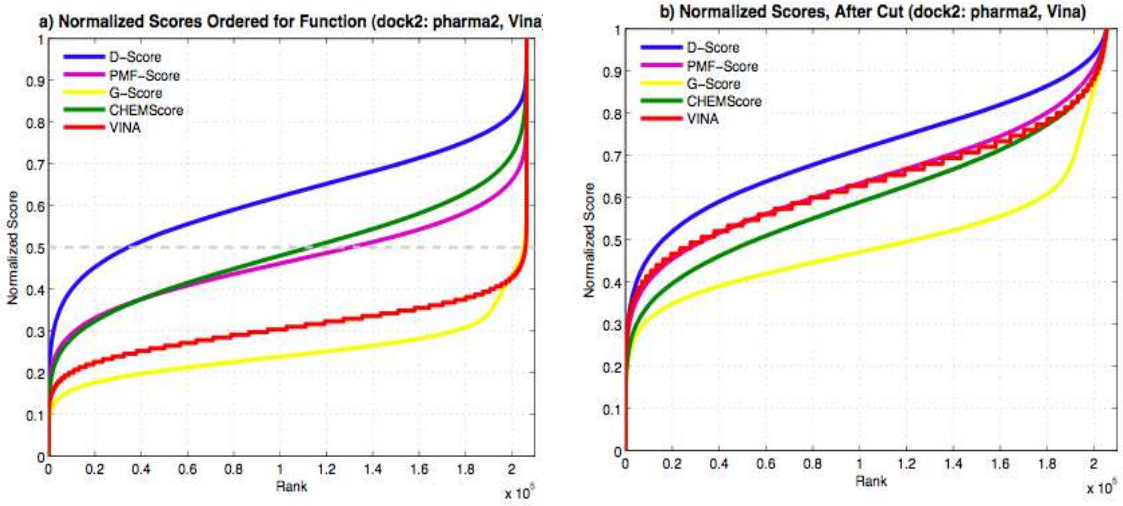

Figure 4: a) Normalized scores calculated without truncation with the five scoring functions Vina, PMFScore, G-Score, D-Score and ChemScore for **dock2** experiment of GCase. **b)** Normalized scores calculated with the second normalization procedure against compound rank (after the worst-scoring 0.5% were excluded) for **dock2** experiment of GCase.

Even though the molecules docked in the experiment **dock2** were filtered by a different pharmacophore filter, the curves of scoring functions are not very different from those of **dock1**, with the curve for Vina score being the exception. Normalization with 100% *cut-off* (Figure 4a) shows that Vina score and G-Score gave very high penalties to a few very poorly docked molecules, making the area between very well and very poor scoring ends quite flattened. Ranking the molecules according to their NCS_{100} would be problematic for the experiment **dock2** as well, since D-Score is sensitive to poorly docked molecules while almost 99% of the molecules would be classified as well-docked and only a small amount of molecules would receive a high normalized score by G-Score and Vina score.

Using a truncation *cut-off* of 99.5% for normalization brought the curves of different scoring functions close to each other as in the case of **dock1** (Figure 4b). While cutting the poor-scoring end brought Vina score on the same order as D-Score, ChemScore and PMFScore, G-Score's improvement was not as remarkable. G-Score would need more molecules to be excluded to converge, and thus would reduce the number of molecules even further. However, even with a truncation cut-off of 99.5%, it would still vote for 50% of the molecules with a "vote cut-off" of 0.5, therefore it was decided to stop truncation at 0.5% and calculate the $NCS_{99.5}$ values to proceed to rank the molecules. Even though the number of molecules docked in experiment **dock2** was twice as large, all correlation values of **dock2** are very similar to those of **dock1** (Table 2).

Table 2: Pearson's Correlation coefficients between different scoring functions and $NCS_{99.5}$ for docking experiment **dock2**

	NCS	**D-Score**	**PMFScore**	**G-Score**	**ChemScore**	**VinaScore**
NCS	1	0.77	0.64	0.67	0.77	0.75
D-Score	0.77	1	0.51	0.43	0.49	0.33
PMFScore	0.64	0.51	1	0.23	0.19	0.38
G-Score	0.67	0.43	0.23	1	0.42	0.35
ChemScore	0.77	0.49	0.19	0.42	1	0.65
VinaScore	0.75	0.33	0.38	0.35	0.65	1

When normalized without any truncation, the four scoring functions show two patterns for the distributions of normalized values for the docking experiment **dock3** (Figure 5a). While D-Score and ChemScore were equally decisive and would vote for 45-50% of the molecules with a vote cut-off of 0.5 (dashed grey line in Figure 5a), both G-Score and PMF-Score scored few molecules with very high penalties, decreasing the overall sensitivity. These scoring functions would vote in favor of almost all of the molecules with 0.5 vote cut-off, making no contribution to the overall ranking.

On the other hand, with a small truncation of 0.5% of the molecules, the curves of all four scoring functions could be brought to similar sensitivity levels. G-Score again, like in the case of **dock1** and **dock2**, couldn't converge as good as the rest of the curves (Figure 5b).

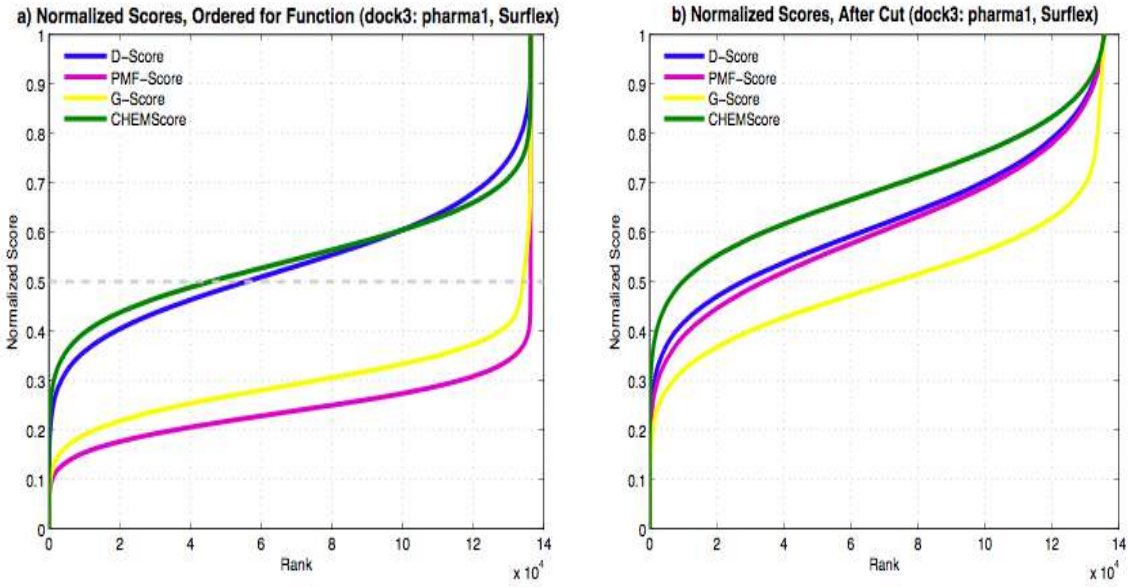

Figure 5: a) Normalized scores calculated without truncation with the four scoring functions PMFScore, G-Score, D-Score and ChemScore for **dock3** experiment of GCase. **b)** Normalized scores calculated with the second normalization procedure against compounds rank (after the worst-scoring 0.5% were excluded) for **dock3** experiment of GCase.

Correlation values of **dock3** are overall better than those of **dock1** and **dock2** (Table 3). PMFScore is better correlated with the other scoring functions, while better correlation values between $NCS_{99.5}$ and the scoring functions is also observed. Since **dock1** and **dock3** experiments were done with the same set of molecules, the differences between the correlation values enables us to make comparisons between **dock1** done with AutoDock Vina and **dock3** done with Surflex-Dock. In **dock3**, individual scoring functions, especially PMFScore, show improved correlations with each other and also with $NCS_{99.5}$. This shows that Surflex-Dock was able to find binding modes that were concurred more consistently and coherently by the additional scoring functions.

Table 3: Pearson's Correlation coefficients between different scoring functions and $NCS_{99.5}$ for docking experiment dock3

	NCS	**D-Score**	**PMFScore**	**G-Score**	**ChemScore**
NCS	1	0.9	0.71	0.76	0.79
D-Score	0.9	1	0.46	0.73	0.66
PMFScore	0.71	0.46	1	0.28	0.45
G-Score	0.76	0.73	0.28	1	0.44
ChemScore	0.79	0.66	0.45	0.44	1

After the calculation of $NCS_{99.5}$ values for each compound from each docking experiment, the compounds were ranked according to their $NCS_{99.5.}$ From the three docking experiments, the

compounds ranking in the top 600 were selected for the following step of binding free energy estimation with LIE simulations, adding up to 1800 compounds in total. There were some intersecting molecules, 178 molecules were in the top 600 of any two docking experiments and 28 were in the top 600 of all three docking experiments. However, repeating molecules were not reduced to only one conformation, all docked conformations of the repeating molecules from different docking experiments were included in the simulations. Each conformation was kept as input for the molecular dynamics simulations because each conformation corresponds to a different starting point and different starting points may affect the outcome of molecular dynamics simulations dramatically. Therefore, the number of unique molecules was 1566; 1360

molecules with a single conformation, 178 molecules with two conformations and 28 molecules with three conformations, adding up to 1800 conformations in total (Figure 6).

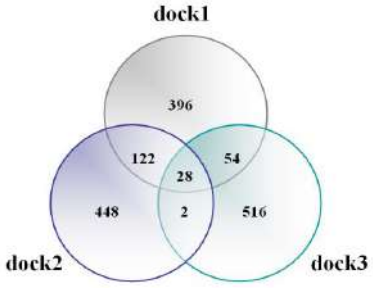

Figure 6: Numbers of intersecting molecules chosen by more than one docking

The step of binding free energy estimation through LIE simulations mainly consists of five tasks: deriving force field parameters and topologies for the compounds for the molecular dynamics simulations, defining the simulation parameters, deciding the size and location of the solvation sphere in which the simulations take place, defining the protonation states of ionizable amino acids of the target protein, and finally performing the binding free energy calculation of each compound-protein pair. The first two steps were done in an automated way. However, deriving parameters regarding the solvation sphere and the target protein needed visual inspection and manual fine-tuning. The same solvation sphere was used for the simulations of all selected ligands from the three docking experiments. Since the defining factor for the size of the solvation sphere is the sizes of compounds, the largest size of the final set of molecules in their docked conformations was 24 Å , and therefore the radius of the solvation sphere was set to 27 Å.

To have a neutral environment for both the free and bound states, the following titratable residues were left charged: Arg120, Asp127, Arg131, Glu152, Asp153, Lys157, Lys186, Lys194, Glu235, Asp282, Asp283, Arg285, His311, Asp315, Glu340, Lys346, Glu349, Arg353, Arg359, Glu388, Arg395 and Asp399. The remaining charged residues were neutralized because they were out of the solvation sphere. 1566 molecules with 1800 different conformations were simulated for binding free energy calculation with LIE method.

The 600 selected molecules from the docking experiment **dock1** have been evaluated for the correlations between the values assigned by individual scoring functions and the binding free energies calculated with the LIE method (Table 4). As expected (from the analysis with the larger set of molecules given in Table 1), all individual scoring functions gave low correlation values to the normalized consensus score, *NCS*. However, the correlations among individual scoring functions were not significant for the selected 600 molecules from **dock1** experiment. The only scoring function that showed significant correlation with LIE energies for experiment **dock1** was D-Score.

Table 4: Pearson's Correlations of scoring functions with each other and with LIE energies for the selected molecules from dock1 experiment

	NCS	**D-Score**	**PMF Score**	**G-Score**	**Chem Score**	**Vina**	**LIE**
NCS	1	0.33	0.36	0.23	0.24	0.26	0.17
D-Score	0.33	1	0.08	0.12	-0.27	-0.55	0.47
PMFScore	0.36	0.08	1	-0.27	-0.39	-0.03	0.01
G-Score	0.23	0.12	-0.27	1	-0.1	-0.28	0.26
ChemScore	0.24	-0.27	-0.39	-0.1	1	0.26	-0.27
Vina	0.26	-0.55	-0.03	-0.28	0.26	1	-0.27
LIE	0.17	0.47	0.01	0.26	-0.27	-0.27	1

In the case of experiment **dock2**, which employed a different pharmacophore filter from **dock1**, the results were not very different (Table 5). Again D-Score was the only scoring function with a significant correlation of 0.48 with LIE values.

Table 5: : Pearson's Correlations of scoring functions with each other and with LIE energies for the selected molecules from dock2 experiment

	NCS	**D-Score**	**PMF Score**	**G-Score**	**Chem Score**	**Vina**	**LIE**
NCS	1	0.27	0.38	0.2	0.15	0.22	0.12
D-Score	0.27	1	0.07	0.06	-0.33	-0.56	0.48
PMFScore	0.38	0.07	1	-0.3	-0.46	0.05	-0.04
G-Score	0.2	0.06	-0.3	1	0.03	-0.35	0.18
ChemScore	0.15	-0.33	-0.46	0.03	1	0.09	-0.25
Vina	0.22	-0.56	0.05	-0.35	0.09	1	-0.26
LIE	0.12	0.48	-0.04	0.18	-0.25	-0.26	1

Table 6: Pearson's Correlations of scoring functions with each other and with LIE energies for the selected molecules from dock3 experiment

	NCS	**D-Score**	**PMF Score**	**G-Score**	**Chem Score**	**LIE**
NCS	1	0.53	0.23	0.38	0.15	0.06
D-Score	0.53	1	-0.35	0.4	-0.33	0.06
PMFScore	0.23	-0.35	1	-0.43	-0.46	0.05
G-Score	0.38	0.4	-0.43	1	0.03	0.11
ChemScore	0.51	0.07	-0.22	-0.04	1	-0.1
LIE	0.06	0.06	0.05	0.11	-0.25	1

However, for the docking experiment **dock3**, a different story can be told (Table 6). First, individual scoring functions show better overall correlation with the normalized consensus scores, *NCS*. Second, except the correlation between D-Score and G-Score, individual scoring functions don't seem to be associated with each other. Especially PMFScore is significantly negatively-correlated with all remaining scoring functions. And lastly, none of the individual scoring functions are correlated with LIE energies.

Comparing between docking experiments **dock1** and **dock2** means comparing the two different pharmacophore filters used in this study. Even though the molecules that passed the pharmacophore filter **pharma2** were more than twice the number of molecules that passed **pharma1**, the docking experiments **dock1** and **dock2** didn't yield very different results. There were 150 intersecting molecules in the selected sets of **dock1** and **dock2**.

Table 7: Experimentally observed activities of the selected molecules

Compound Number	% activity 20 mg/mL	% activity 10 mg/mL	Compound Number	% activity 20 mg/mL	% activity 10 mg/mL
1	96.8 ± 2.5	100.1 ± 3.8	14	97.9 ± 0.9	98.7 ± 2.3
2	102.4 ± 2.9	104.9 ± 3.0	15	93.8 ± 1.3	95.6 ± 1.1
3	49.0± 2.4	73.6 ± 2.1	19	81.3 ± 1.0	82.5 ± 0.9
4	51.3 ± 2.8	69.1 ± 2.5	20	111.1 ± 1.8	106.7 ± 1.0
5	95.3 ± 1.4	103.5 ± 3.6	21	68.7 ± 4.3	80.8 ± 1.3
6	97.1 ± 3.7	105.1 ± 3.5	22	100.3 ± 2.7	91.1 ± 5.8
7	104.4 ± 2.1	97.1 ± 5.5	23	104.4 ± 3.0	98.1 ± 6.8
8	114.2 ± 2.6	104.7 ± 3.5	24	97.5 ± 3.0	95.9 ± 0.9
9	97.9 ± 0.6	105.2 ± 3.3	25	102.0 ± 3.0	100.9 ± 3.0
10	105.1 ± 1.4	101.8 ± 5.4	26	126.5 ± 3.6	120.0 ± 2.0
11	96.5 ± 4.6	99.7 ± 4.8	27	103.5 ± 1.3	100.6 ± 1.2
12	102.2 ± 0.9	103.2 ± 4.5	28	102.6 ± 4.5	102.5 ± 3.2
13	40.2 ± 1.2	58.8 ± 1.1	29	95.8 ± 0.8	95.1 ± 2.7

On the other hand, comparing **dock1** and **dock3** enables the comparison of docking programs AutoDock Vina and Surflex-Dock since both experiments employed the same pharmacophore filter, **pharma1**. Since correlation values between the *NCS* and the individual functions are higher in the case of **dock3**, it can be concluded that Surflex-Dock was able to create binding modes that were overall more favorable by the individual scoring functions.

To choose for the candidate molecules that would continue to experimental testing, we applied three criteria and the molecules that fulfill at least two of these criteria were chosen for experimental testing. The first criterion was passing the visual inspection step. All 1800 conformations were inspected visually using MOE[32]. At the end of visual inspection, 100 molecules were chosen, regarding their abilities to make hydrogen bonds with the binding site residues, their exposure to the solvent and the existence of π-π or cation-π interactions with the protein. The second determining factor was to be in the intersection set of molecules chosen in the top 600 by all three docking experiments. There were 28 molecules in total that fulfilled this requirement. Finally, the last test was to be in the top 100 after ranking according to binding free energies calculated with LIE. For this last test, repeating conformations of the same molecule were not treated as separate cases, the conformation with the lowest binding free energy was chosen, and thus 100 different molecules were taken. To define the list of molecules that would be tested experimentally, we selected 22 molecules that fulfilled at least two of the three requirements. In addition to this, to increase the number of molecules, 7 more molecules ranking in the top 30 according to LIE results, but failing the other two criteria were also added. Therefore, 29 molecules in total were selected for experimental testing (Figure 6). While selecting the molecules, we also considered the rmsd values between docked and simulation conformations, and tried to choose molecules that show similar binding modes in both docking and simulation results.

Activities for the selected molecules at two concentrations are listed in Table 7. Even though the control molecule NN-DNJ performed clearly better than all tested molecules, compounds **3**, **4**, **13**, **19** and **21** affect enzyme activity, while the remaining compounds hardly have any effect. Therefore, it can be concluded that the computational workflow managed to identify five possible hits.

Docked conformations of the five hit molecules show that while most of the hydrogen bonding interactions occur with binding site residues (Asp127, Trp179, Glu235, Glu340, Trp381 and Asn396), Compounds **3**, **4**, **19** and **21** also occupy the hydrophobic groove between loop L1 (Phe347 and Trp348) and loop L3 (Trp312, Leu314 and Phe316). This suggests that Compounds **3**, **4**, **19** and **21** can establish hydrophobic interactions with the side chains of loops L1 and L3 (Figure 7).

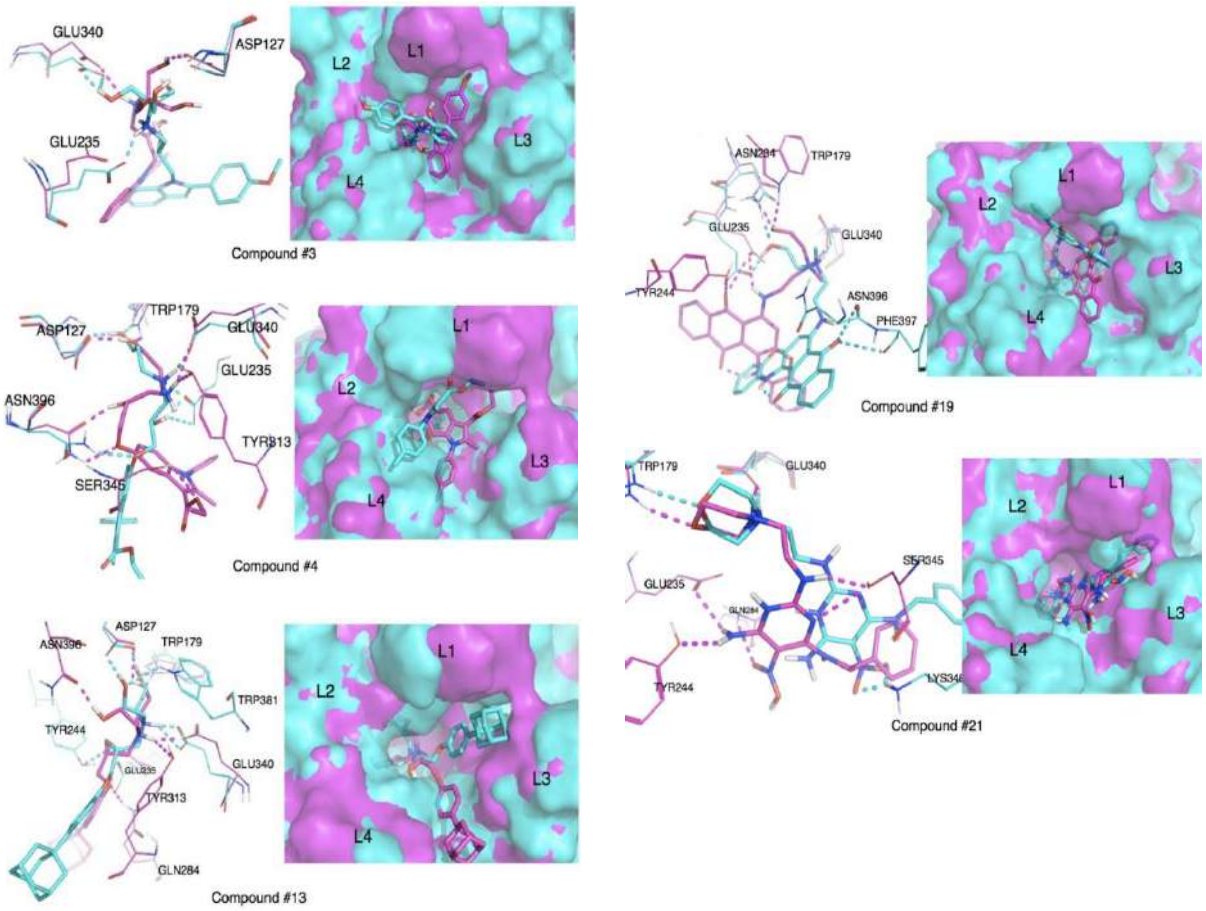

Figure 7: Docked and simulation binding modes of compounds. Magenta colored binding mode represents the docking result and cyan colored binding mode represents the LIE simulation result.

CONCLUSIONS

In this study, we proposed a virtual screening procedure that combines pharmacophore design, high-throughput molecular docking, consensus scoring and evaluation of binding free energy by the LIE method. A large library of small molecules have been screened to find potential active binders to human acid beta-glucosidase, which is a key protein involved in Gaucher's Disease. For the screening experiment, pharmacophore filtering and molecular docking was employed to reduce the library size and to find possible hits. The pharmacophore filters used did not contain many different features to enable hit variety. The flexible ligand - rigid protein approximation was used for the docking experiments in this study for efficiency. However, this approximation restricts allowed poses for molecules and may cause primarily false negatives but also false positives.

The evaluation of binding modes of molecules docked in the protein binding site was done with a modified rank-by-number consensus scoring method. Consensus scoring was used to find out molecules that scored well with all five scoring functions. The compounds with the best normalized consensus scores were subjected to two molecular dynamics simulations for binding free energy estimation by LIE. LIE based methods are known to perform better than existing scoring functions, mainly because the proteins are not rigid as in docking and the simulations take place in a solvated environment. Therefore, flexibility to both ligand and protein was introduced during the simulations. Predicted binding free energies were not significantly correlated with either the individual scoring

functions or the normalized consensus score for the three screening experiments.

In the continuously developing world of computer-aided drug design, hybrid approaches are needed to compensate for the weaknesses of individual standard methodologies. Increasing computation power enables exploration of rigorous but expensive methods. Fully automated treatment of small molecules for different applications makes the workflow explained in this study a very versatile approach for virtual screening of different targets.

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THE COMPARISON OF GRAIN YIELDS AND YIELD TRAITS OF SOME BARLEY CULTIVARS IN TRAKYA REGION

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ABSTRACT

Barley has higher adaptation capability even in so dry areas then could be grown in many parts of the world. Barley which mostly produced for animal feed and malt is one of the most produced cereals and main rotated crops especially rainfed areas in Turkey. Trakya region which is European part of Turkey is one of the most cereal produced areas and growing the most bred animals. Therefore, barley produces as the main cereal crop especially in dry areas other than wheat like other part of Turkey and exists as the main crop in the rotation for mostly animal feed. To evaluate of some barley cultivar performances in Trakya Region, the study was performed in 2010-2020 growing season in Edirne and Tekirdag Malkara locations. The candidate barley lines and standard commercial cultivars which are the most planted ones in the Turkish market existed in seed yield trials in the study. Based on study results, three barley candidate lines exhibited higher seed yield performances over all standard cultivars. The candidates also were better than standard cultivars for quality traits. As conclusions, TRAGEN Ltd candidate barley lines exhibited promising results then the best ones will be selected for registration in next years.

Key words: Barley, Trakya Region, Adaptation, Seed yield, Yield traits,

INTRODUCTION

Barley (*Hordeum vulgare* L.) is grown mainly for animal feed and is preferred especially in dry conditions and poor and salty soil areas by farmers. Therefore, breeders should consider new developed lines to adapt to wide range of diversified environments in their barley breeding programs. Furthermore, these new cultivars should affect from stress conditions in dry areas with rainfed regimes due to global warming (Aktaş, 2017; Aydoğan et al., 2017; Kara et al., 2019; Dyulgerov and Dyulgerova, 2021). However, barley is grown also in rich soil areas and more humidity regions to get higher yielding but lodging is the biggest problem in these conditions especially after heavy rains and strong winds. Therefore, lodging is one of the most important morphological yield traits for concerning by barley breeders (Yılkan et al., 2020).

Barley production and planted areas have reduced in recently in Turkey comparing with ten and twenty years before (Kizilgeci et al., 2019). However, the plant areas increased a bit but the production is so variable due to drought effects in last five years. Barley is consuming mostly by animal husbandry (85 %), industry (3 %) and human food % 1) in Turkey. In Turkey, two rows barley are producing mostly malt production and also animal feed but 6 rows barley is totally for animal feeding (Sirat and Sezer, 2017; Sönmez et al., 2020 and 2021). Turkey exists in top ten barley producer countries in the world and it has 5 % rate of world barley production (about 150 million

MT) and has 7,5 % rate from world barley planted areas (51 million ha) in 2020-21 season (TUIK, 2021 and USDA, 2021).

Turkey barley planted areas is around 3 million ha and the production is 7-8 million MT in recent years. The seed yield is around 2600-2800 kg/ha. However, due to heavy droughts as well as late frosts in spring season especially in middle Anatolia the estimated production reduced to 6 million MT in 2030-2021season (TUIK, 2021 and USDA, 2021). Turkish domestic barley production is not enough so Turkey imported about one million tons from abroad in 2020-2021 season. Certified barley seed uses have been increased in recent years in Turkey. While certified barley seed production was 315.000 MT in 2010, it reached to 484.000 MT in 2019. Furthermore, private seed sector rate was doubled comparing with ten years before.

The most produced provinces in Turkey are Konya (15,3 %), Ankara (9,8 %), Kırşehir (4,5 %), Sivas (3,9 %) and Diyarbakır (3,9 %), respectively. Trakya Region exists also among the most barley produced regions about 70,000 ha and seed yiels is over 5.0 t ha-1 (TUIK, 2021 and USDA, 2021). In the region, the average annual rainfall is about 560mm, but rainfall is not well distributed regularly during the growing season (Öztürk et al., 2016). Due to irregularity of rainfall, biotic and abiotic stress have been some common issues almost in all recent years. Among biotic stresses, net blotch (*Pyrenophora teres*), powdery mildew (*Blumeria graminis f. sp. hordei*) and some root rot are the main reducing factors affecting seed yield in the region as well as some pathogens (including bacteria, fungi, viruses and nematodes) and some pests (İmamoğlu et al., 2016; Aktaş, 2017; Öztürk et al., 2019; Öztürk, 2020). The study was performed for determining seed yield potential of some candidate barley lines in Trakya Region (Tekirdağ – Malkara and Edirne province) in 2019-2020 growing seasons.

MATERIAL AND METHOD

The study was conducted in Edirne province and Tekirdag Malkara county in Trakya Region in 2019-2020 growing seasons to determine yield performances of some barley candidate lines. There were 3 lines belonging Tragen R& D Ltd Co, Trakya University Technopark, Edirne in the trials with 10 controls which planted mostly in both in Trakya region which is European part of Turkey also other parts of Turkey.

The experimental design was a RCBD with four replications in the study. The four rows plots were 6-m long with the six rows as 17 cm plant spacing and 450 grains per m^2, density, so total plot area was 6 m^2 at planting, 5 m^2 at harvesting. The previous plant in the rotation is sunflower both years.

The compose fertilizers (20-20-0, Zn) applied 200 kg/ha dose at planting. At tillering stage in February, 200 kg/ha Urea (%46), in April 200 kg/ha Amonium Nitrat (%26) were used as nitrogen fertilizers. Weed control was applied with Glean 75 DF herbicide (Sulfonyl Urea) with 10 g/ha dose after planting then Mustang SE herbicide (452.42 g 2,4-D EHE + 6.25 g Florasulam) (500 lt/ha dose) at February in the study.

Planting time is 07.11.2019 at Edirne & 05.11.2019 at Tekirdag Malkara location. Harvesting time is 26.06.20202 at Edirne and 06.07.2020 at Tekirdag Malkara location. There was no irrigation in the study only rainfeed planting. After harvesting the plots, they were threshed and weighed and plot results were adapted 10% humidity then quality analysis were performed from each plot of varieties.

The cold tolerance of lines was determined based on 1-9 tolerance scales: 1- Less cold damage; 9 - More cold damage. For disease resistance evaluation; the percentage % rate of infected plants were counted during between tillering and the spiking date at the same row. The intensity rate is calculated by the infected plants are divided to total plants based on 1-9 scale: 1: Totally Resistant- Not any disease observation. 2,3: Resistant- Few diseases observed (% 1-20 rate at plant vegetative parts). 4,5: Mid Resistant - % 20-50 of plant vegetative parts infected. 6,7: Sensitive- % 50-75 of plant vegetative parts were covered by diseases. 8,9: Very Sensitive - % 75-100 of plant vegetative parts infected.

RESULTS AND DISCUSSION

The candidate lines performed higher performances than almost all control cultivars in two locations based on study results (Table 1). The highest seed yield was obtained from YK-5 line as two rows in both locations. YK-8 and YK-19 as candidate lines and Hazar and Nektar followed these lines in both locations in the experiments (Table 1 and 2).

Table 1. 2019-2020 barley yield trials field observations in Tekirdağ Malkara location

Varieties	Row Type	Plant Height (cm)	Spiking Date (m/day)	Cold Damage (1-9)	Lodging (% / Degree)	HG (%)	RS (0-99)	EG (1-9)	YR	Seed Yield (kg/da)
YK-5	2	106	02.05	1	30/40	2	2	1	20MR	793,9
YK-8	2	88	01.05	1	35/40	2	3	1	30MR	703,0
SLADORAN	2	101	30.04	1	25/30	2	3	1	30MS	555,4
HAZAR	6	90	29.04	1	20/30	2	3	5	30MS	654,2
BOZLAK	2	124	12.05	1	90/90	2	3	5	40MS	468,6
ZEYNELAĞA	2	111	30.04	1	50/90	2	3	1	30MS	621,8
ÇETİN 2000	6	118	10.05	1	50/90	2	4	1	30MR	594,0
AVCI 2002	6	109	12.05	1	50/90	2	3	1	10MR	469,3
TOSUNPAŞA	2	122	10.05	1	100/90	1	4	3	30MS	565,1
ANKA06	2	118	11.05	1	100/90	1	3	3	40S	515,5
AKAR	2	125	10.05	1	100/90	1	3	3	40MS	514,9
YK-19	2	97	02.05	1	30/40	1	3	3	40MS	678,9
NEKTAR	2	96	30.04	1	5/10	1	3	3	40MR	641,6

HG: Helminthosporium gramineum
RS: Rhynchosporium secalis
EG: Erysiphe graminis f.sp. Hordei
YR: Puccinia hordei

The candidate lines have two rows and had less lodging and higher tolerance to some barley diseases were observed in the trials (Table 1 and 2). Plant heights of lines in the experiments changed between 88 - 122 cm. The shortest one was YK8 line and Hazar followed that one (Table 1 and 2). The candidate barley lines had lower lodging capability comparing the control cultivars. The most tolerant one was Nektar and the most sensitive cultivars to lodging were Tosunpaşa, Akar and Anka06 among cultivars in the trials conducted in both two locations.

Based on diseases tolerance, the candidate lines existed in the top almost all diseases observations (Table 1 and 2). The most tolerant to yellow rust one was YK-5 and the most sensitive cultivar was Anka06 among cultivars in the trials conducted in both two locations.

Table 2. 2019-2020 barley yield trials field observations in Edirne location

Varieties	Row Type	Plant Height (cm)	Spiking Date (m/day)	Cold Damage (1-9)	Lodging (% / Degree)	HG (%)	RS (0-99)	EG (1-9)	YR	Seed Yield (kg/da)
YK-5	2	106	31.04	1	30/40	1	2	1	20MR	816,0
YK-8	2	88	31.04	1	35/40	2	1	1	40MR	823,1
SLADORAN	2	101	24.04	1	25/30	2	2	1	40MR	574,0
HAZAR	6	90	28.04	1	20/30	2	2	5	35MR	766,0
BOZLAK	2	124	05.05	1	90/90	2	1	5	50MR	708,0
ZEYNELAĞA	2	111	28.04	1	50/90	2	2	2	40MR	716,0
ÇETİN 2000	6	118	30.04	1	50/90	2	2	2	30MR	567,5
AVCI 2002	6	109	31.04	1	50/90	2	2	2	20MS	500,8
TOSUNPAŞA	2	122	03.05	1	100/90	2	2	3	40MS	646,0
ANKA06	2	118	11.05	1	100/90	2	2	3	50S	642,0
AKAR	2	125	30.04	1	100/90	2	2	3	45MS	724,0
YK-19	2	97	28.04	1	30/40	1	1	3	40MS	747,0
NEKTAR	2	96	31.04	1	5/10	1	1	3	40MR	756,7
NEKTAR	2	96	31.04	1	5/10	1	1	3	40MR	756,7

HG: Helminthosporium gramineum
RS: Rhynchosporium secalis
EG: Erysiphe graminis f.sp. Hordei
YR: Puccinia hordei

Table 3. 2019-2020 barley average yield results in two locations in Trakya region

Varieties	Row Type	Plant Height (cm)	Spiking Date (m/day)	Malkara Seed Yield (Kg/da)	Edirne Seed Yield (Kg/da)	Average Seed Yield (kg/da)
YK-5	2	106	02.05	793,9	816,0	**805,0**
YK-8	2	88	01.05	703,0	823,1	**763,1**
YK-19	2	97	02.05	678,9	747,0	**713,0**
HAZAR	6	90	29.04	654,2	766,0	710,1
NEKTAR	2	96	30.04	641,6	756,7	699,2
ZEYNELAĞA	2	111	30.04	621,8	716,0	668,9
AKAR	2	125	10.05	514,9	724,0	619,5
TOSUNPAŞA	2	122	10.05	565,1	646,0	605,6
BOZLAK	2	124	12.05	468,6	708,0	588,3
ÇETİN 2000	6	118	10.05	594,0	567,5	580,8
ANKA06	2	118	11.05	515,5	642,0	578,8
SLADORAN	2	101	30.04	555,4	574,0	564,7
AVERAGE		108,0		**608,9**	**707,2**	**658,1**

The candidate lines existed in the top three based on average results of two locations (Table 3). YK-5 had the highest seed yield in both locations and followed by YK-8 and YK-19 as candidate lines and Hazar and Nektar as control lines.

The candidate lines existed higher performance in the quality traits in the experiments also in both two locations (Table 4 and 5). YK-5 had highest hectoliter and seed weights among candidate lines. However, The highest 1000 seed weight was obtained from Bozlak cultivar in Edirne location and the highest hectoliter weight was obtained from Tosunpasa cultivar in Edirne location.

Table 4. Barley quality results in 2019-2020 trials at Tekirdag Malkara location

Varieties	1000 Seed Weight (gr)	Hectoliter Weight (kg)	Protein (%)	2.8+2.5 over Sieve	2.2 under Sieve
YK-5	65,9	46,6	14,5	62,5	3,0
YK-8	64,5	41,8	14,0	84,0	6,5
SLADORAN	63,1	43,2	15,7	48,5	3,0
HAZAR	62,5	29,8	13,6	84,4	14,6
BOZLAK	61,0	39,8	15,6	74,5	2,2
ZEYNELAĞA	62,9	43,0	16,2	69,5	4,5
ÇETİN 2000	57,5	36,8	14,5	63,2	12,1
AVCI 2002	55,8	29,0	13,4	84,5	4,6
TOSUNPAŞA	62,7	45,0	13,3	91,7	2,8
ANKA06	60,6	44,2	12,4	86,2	14,8
AKAR	58,9	38,0	13,0	84,2	2,4
YK-19	61,1	44,0	12,6	78,1	3,5
NEKTAR	62,5	44,4	14,5	82,5	2,5

Table 7. Barley quality results in 2019-2020 trials at Edirne location

Varieties	1000 Seed Weight (gr)	Hectoliter Weight (kg)	Protein (%)	2.8+2.5 over Sieve	2.2 under Sieve
YK-5	64,4	40,8	17,6	61,0	3,0
YK-8	63,8	36,4	17,6	34,5	6,5
SLADORAN	64,6	43,2	17,3	78,2	2,0
HAZAR	63,3	27,2	16,0	45,2	14,2
BOZLAK	67,3	40,2	17,5	63,9	2,2
ZEYNELAĞA	64,7	42,4	17,8	74,5	4,5
ÇETİN 2000	60,1	34,4	17,4	67,5	14,2
AVCI 2002	55,2	31,0	17,8	63,0	5,1
TOSUNPAŞA	66,8	49,2	17,7	84,5	4,8
ANKA06	64,2	46,4	17,7	60,8	16,4
AKAR	64,4	40,8	17,6	61,0	3,0
YK-19	65,6	38,0	16,7	78,0	3,5
NEKTAR	63,7	39,2	16,6	79,2	2,5

The highest protein rate was obtained from Avcı 2002 (17,8 %) cultivar in Edirne location (Table 4 and 5). Tosunpasa cultivar had also longer and wider seeds as having over siege rates based on study observations in both locations.

CONCLUSIONS

The study results indicated that three candidate barley lines exhibited higher seed yield performances over control cultivars and the average of seed yield of all cultivars was calculated as 658,1 kg/da. TRAGEN YK-5 was existed in top as average seed yield as 805 kg/da and TRAGEN-YK-8 and YK-19 followed this candidate. The candidates also were evaluated and compared for seed quality results so they have also satisfaction results as well as lodging. As a result, this TRAGEN Ltd barley candidate lines exhibited promising yielding performances and these best ones will select based on future yield trials and will send to registration then to give to farmers for selling in Turkish seed market.

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THE DETERMINATION SEED YIELDS AND SOME YIELD TRAITS IN THE BREAD WHEAT IN TRAKYA REGION

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ABSTRACT

Bread wheat is a main and widely grown crop throughout Turkey. Because of having higher adaptation capability, growing in almost all kind of soil and consuming larger by people, it makes to wheat is the most important case in the agriculture and the most important issue in the human life in Turkey. Trakya region which is European part of Turkey is one of the main wheat grown areas in Turkey. The study was performed to measure some wheat lines and cultivar performances in Trakya Region in 2019-2020 growing season. Yield trials were planted at two locations as Edirne and Lüleburgaz Kırklareli and the candidate bread wheat lines existed in yield trials with five control wheat cultivars as Pehlivan, Gelibolu Rumeli, Esperia and Flamura-85 cultivars which are mostly planted and preferred by farmers and industry in Trakya Region. The experiment results indicated that some candidate lines exhibited over yield performances than control cultivars. TRAGEN YK-6 was existed in top rank in Edirne location while Klima was in Lüleburgaz location. The candidate lines were also evaluated and compared with control cultivars based seed and flour quality results. As results, TRAGEN Ltd wheat cultivars had favorable results based this study and promising ones will be tested again future trials and selected ones will send to registration trials to produce and then exist in Turkish seed market.

Key words: Bread Wheat, Trakya Region, Seed yield, Adaptation, Yield traits

INTRODUCTION

Cereals are the most important crops in Turkey both for production and planted areas (70% of total cultivated lands). Wheat is the most planted cereal could grow in every part of Turkey mostly for bread purposes but also for pasta and biscuits (Mihova, 2020). Bread wheat (*Triticum aestivum* L.) is the most planted crop and mostly growing in dry conditions in Turkey (Karaman and Aktas, H. 2020). Turkey produces about 20-million-ton wheat in recent years in mostly in Middle Anatolia and Marmara Region and Southeast Anatolia Region. Trakya Region provinces Tekirdağ, Edirne and Kırklareli exist in the top ten producing provinces ((Öztürk et al., 2021; TEPGE, 2021).

Both bread and durum wheat is a key issue in Turkey both for production and also trade in Turkish economy because Turkey is one of the most produced and consumed bread per capita countries in the world. Turkeys play a key role also in the world both wheat trade for seed, flour and pasta an also for export and import because Turkey is one of the major countries international wheat trade (Mut et al., 2017; TEPGE, 2021).

Diseases especially fungal ones are the most serious reducing factor in wheat production. Wheat rusts are leaf rust (also known as brown rust or orange rust), stripe rust (commonly known as yellow rust), and stem rust (commonly known as black rust or stem rust) leaf, stripe, and stem rust caused by *Puccinia recondita f. sp. tritici*, *Puccinia striiformis* f. sp. *tritici*, and *Puccinia graminis* f.

sp. *tritici*, respectively Among fungal diseases mostly affecting leaves and roots Brown leaf rust is the most important ones leading significant yield loses and seed quality in the Thrace Region. However, yellow rust also has observed in recent years, but it does not lead an important epidemic (Baser et al., 2020 and 2021).

Quality of bread wheat is also so important issue in Turkey and all produced seeds and cultivars appraise and prefer by buyers and industry based on important quality traits in the Turkish market. Gluten amount and index, protein content, zeleny sedimentation rate are the most important quality traits in Turkey currently and followed by alveograph value, flour yield, thousand seed weight, hectoliter weight and grain hardness respectively (Aktaş et al., 2017; Mut et al., 2017; Öztürk et al., 2017; Öztürk, 2021).

On the other hand, the adaptation capability and higher yielding stability of new cultivars to different environments is so important issue. There were huge yield losses in especially in wheat production appeared in Turkey and other part of the world due to both for severe droughts because of global warming and also especially late frosts in the spring (Albayrak et al., 2021). Therefore, wheat breeders should consider new cultivars for higher adaptations and yielding capacity in yield trials various climatic conditions as well as controlling their quality and tolerance to diseases in their wheat breeding programs (Öztürk et al., 2017; Mihova, 2020; Öztürk, 2021; Smutná et al., 2021). The study was conducted to evaluate to determine of yield performances some bread wheat cultivars and candidate lines in the Trakya region.

MATERIAL AND METHOD

The yield trials were conducted in Edirne and Kırklareli – Luleburgaz locations in Trakya Region in 2019-2020 growing seasons to determine yield performances of bread wheat candidate lines. The trials planted on time with planting machine in dry conditions and germination of plants were well in the experiments.

The climatic data during the wheat vegetative period in Edirne province was given in Table 1. Based on data there were very lower rainfalls in the winter seasons which is so important time for higher yielding in wheat production comparing longer periods. Both the winter rainfall and also total one in the wheat vegetation period was so lower comparing longer years. This lower amounts reduced wheat yields in Turkey. Furthermore, there was so lower temperatures at the April and beginning of May then the lowest yields have been observed for 50-60 years.

Table 1. Edirne province 2019-2020 wheat growing season climatic data

Months	Longer years average rainfall (mm)	Rainfall (mm)	Temperature ^{o}C		
			Min.	Max.	Average
September 2019	34,0	12,2	5,6	34,4	21,1
October 2019	52,9	22,8	3,7	32,1	15,8
November 2019	72,4	10,8	4,8	25,9	13,7
December 2019	61,7	28,0	-2,4	25,9	10,2
January 2020	48,1	10,2	-8,1	18,1	3,2
February 2020	46,9	34,8	-5,9	20,2	6,8
March 2020	52,2	41,8	-4,5	23,6	9,9
April 2020	51,0	94,8	-1,5	26,5	11,7
Mayıs 2020	56,0	92,8	4,6	33,3	18,2
June 20202	41,5	34,6	9,1	35,0	22,6
Total	516,7	383,6			

There were 20 lines in the trials with 5 controls which planted mostly in Trakya region which is European part of Turkey (Flamura-85, Pehlivan, Rumeli, Gelibolu and Esperia). The previous crop was sunflower in the rotation in the fields and there was no irrigation in in both locations. The experimental design was a Randomized Completely Block Design (RCBD) with four replications in the study.

The four rows plots were 6-m long with the six rows as 17 cm plant spacing and 500 grains per m^2, density, so total plot area was 9 m^2 at planting, 8 m^2 at harvesting. Trials were planted by planter in 06 November in 2019 in Lüleburgaz location and in 07 November in 2019 in Edirne location. The experiments were harvested by harvest combine in 26 June in 2020 in Edirne location and in 29 June in 2020 in Lüleburgaz location. After harvesting the plots, they were treshed and weighed and plot results were adapted 10% humidity then quality analysis were performed from each plot of varieties based on requested by government rules.

The compose fertilizers (20-20-0, Zn) applied 250 kg/ha dose at planting. At tillering stage in February, 80 kg/ha Urea (%46), in March 145 kg/ha Urea (%46), in April 300 kg/ha Amonium Nitrat (%26). Weed control was applied with Laren Plus herbicide (Sulfonyl Urea) dose after planting in the study.

RESULTS AND DISCUSSION

Based on study results; some candidate lines belonging to Tragen R & D company had promising results and had higher performances than almost all control cultivars in Edirne location. There were lower yields were obtained in the lines in Edirne location comparing with Luleburgaz location. Among candidate lines, higher seed yields were obtained from YK-6 and Klima lines in Edirne location (Table 2 and 5). Klima had the highest seed yield in Lüleburgaz location too (Table 2 and 3). Among controls, Gelibolu had highest yield in both locations in the trials. Plant heights were 73 - 104 cm. The shortest control cultivar was Esperia (Table 3 and 5).

The highest hectoliter weight, protein and gluten amount, seleny sedim and Alveograph value were obtained from Albertos cultivar as called the highest quality line among cultivars in the experiment but it has the lowest seed yield in the experiment (Table 2 and 6). GB-66 and ARTEMİDA were another lines having higher quality among lines existed in the trials. Lüleburgaz location had lower quality results such as protein, wet gluten, zeleny sedim, amounts, etc. comparing with Edirne location.

Based on experiment results, some candidate lines exhibited higher seed yield performances over control cultivars and TRAGEN YK-6 was existed in top rank based on average seed yield and TRAGEN YK- 10 followed this candidate in Edirne location (Table 2 and 5). The candidate also evaluated and compared with control cultivars based seed and flour quality results and TRAGEN Ltd wheat cultivars had favourable results based this study.

Table 2. 2019-2020 wheat yield trial results in two locations and the average

#	Cultivars	Location		Average Seed Yield (kg/da)
		Edirne	Lüleburgaz	
1	RUMELİ	635 BCDE	882 DEF	758,5
2	ESPERİA	678 ABC	912 CDEF	795
3	GELİBOLU	678 ABC	992 ABCD	835
4	GLOSA	-	1012 ABC	-
5	FLAMURA-85	593 CDEF	861 F	727
6	KLİMA	707 AB	1075 A	891
7	ASTORTA	643 BCDE	991 ABCD	817
8	NOVASİMALENKA	612 BCDEF	935 BCDEF	773,5
9	BUJANKA	621 BCDEF	1036 AB	828,5
10	SOLOMİYA	650 ABCD	986 ABCDE	818
11	NATALKA	547 EF	875 EF	711
12	PEHLİVAN	558 DEF	-	-
13	TRAGEN 103	582 CDEF	-	-
14	YK-6	743 A	-	-
15	YK-10	654 ABCD	-	-
16	ALBERTOS	527 F	-	-
17	GB-1	581 CDEF	-	-
18	GB-2	620 BCDEF	-	-
19	GB-6	627 BCDE	-	-
20	GB-66	596 CDEF	-	-
21	ARTEMİDA	566 DEF	-	-
Average		621		
(LSD: 0.05) (kg/da)		98,75	112,6	
C.V (%)		11	8	
F		**	**	

Table 3. Trakya Region 2019-2020 Bread Wheat yield trials field observations in Lüleburgaz location

#	Cultivars	Spike Type	Plant Height (cm)	Spiking date (day /month)	Cold damage (1-9)	Lodging level %	Yellow Rust	Stem Rust	Brown Rust	Powdery Mildew (0-99)	Septoria tritici (0-99)	Yield (kg/da)
1	RUMELİ	+B	104	25.04	3		1	-	-	55	33	882 DEF
2	ESPERİA	+B	81	25.04	5		1	-	-	55	55	912 CDEF
3	GELİBOLU	+B	94	28.04	3		1	-	-	55	33	992 ABCD
4	GLOSA	+B	92	26.04	3		1	-	-	33	33	1012 ABC
5	FLAMURA-85	+B	89	05.05	3		3	-	-	33	33	861 F
6	KLİMA	+B	82	05.05	5		1	-	-	55	55	1075 A
7	ASTORTA	-B	85	05.05	5		3	-	-	55	55	991 ABCD
8	NOVASİMALENKA	+B	94	03.05	3		3	-	-	33	33	935 BCDEF
9	BUJANKA	+B	90	03.05	5		5	-	-	33	33	1036 AB
10	SOLOMİYA	+B	90	05.05	5		1	-	-	33	55	986 ABCDE
11	NATALKA	+B	95	05.05	5		1	-	-	33	33	875 EF

Notes: Spike type: +: Awn, -: Non awn, B: White color, K: Red Color

Table 4. Bread Wheat Trials 2019-2020 Luleburgaz location quality results

#	Cultivars	1000 Seed Weght (g)	Hectoliter Weight (kg)	Humidity (%)	Protein (%)	Wet Gluten (%)	Sedim. (Zel) ml	Starch	Alveograph W	Grain Hardeness
1	RUMELİ	48	82,3	12,7	15,5	35,9	62	66,7	355	91,4
2	ESPERİA	40	79,4	11,4	15,5	36,0	65	66,2	443	96,8
3	GELİBOLU	38	81,9	11,7	13,6	31,5	48	68,1	311	84,9
4	GLOSA	40	81,0	10,9	14,4	33,4	53	67,6	360	91,4
5	FLAMURA-85	42	89,0	12,1	14,6	34,0	55	66,4	364	89,0
6	KLİMA	42	77,2	11,3	15,2	35,2	60	66,9	387	95,9
7	ASTORTA	34	71,0	12,7	14,4	33,4	54	67,3	327	84,1
8	NOVASİMALENKA	26	78,0	12,6	15,3	35,4	60	67,4	360	83,0
9	BUJANKA	22	67,6	12,2	15,1	35,1	59	66,4	347	104,8
10	SOLOMİYA	42	79,6	12,4	13,8	31,9	49	67,7	328	87,4
11	NATALKA	30	74,4	12,5	15,7	36,4	65	66,1	290	86,5

Table 5. Trakya Region 2019-2020 Bread Wheat yield trials field observations in Edirne location

#	Cultivars	Spike type	Plant Height (cm)	Spiking date (day/month)	Cold damage (1-9)	Lodging level %	Yellow Rust	Stem Rust	Brown Rust	Powdery Mildew (0-99)	Septoria tritici (0-99)	Seed Yield (kg/da)
1	RUMELİ	+B	86	20.04	3	-	3	-		22	55	635 BCDE
2	PEHLİVAN	-B	96	20.04	3	-	2	-		33	33	558 DEF
3	ESPERİA	+B	78	21.04	3	-	0	-		55	33	678 ABC
4	FLAMURA- 85	+B	91	21.04	3	-	0	-		33	55	593 CDEF
5	GELİBOLU	+B	86	12.04	1	-	2	-		33	33	678 ABC
6	TRAGEN103	-B	85	23.04	3	-	3	-		22	33	582 CDEF
7	YK-6	+B	84	23.04	1	-	0	-		33	22	743 A
8	YK-10	+B	88	25.04	1	-	2	-		22	33	654 ABCD
9	ALBERTOS	+B	99	30.04	5	-	3	-		11	33	527 F
10	GB-1	+B	80	22.04	3	-	-	-		22	22	581 CDEF
11	GB-2	+B	82	22.04	3	-	1	-		22	22	620 BCDEF
12	GB-6	+B	88	24.04	5	-	2	-		55	33	627 BCDE
13	GB-66	+B	85	26.04	5	-	-	-		55	33	596 CDEF
14	ARTEMİDA	+B	92	28.04	2	-	3	-		33	33	566 DEF
15	ASTORTA	-B	90	23.04	3	-	3	-		55	22	643 BCDE
16	NOVASMELANKA	+B	85	21.04	3	-	3	-		33	33	612 BCDEF
17	BUJANKA	+B	84	21.04	3	-	5	-		33	55	621 BCDEF
18	SOLOMİYA	+B	84	23.04	5	-	5	-		44	55	650 ABCD
19	NATALKA	+B	95	23.04	3	-	3	-		33	33	547 EF
20	KLİMA	+B	73	23.04	3	-	-	-		22	22	707 AB

Notes: Spike type: +: Awn, -: Non awn, B: White color, K: Red Color

Table 6. Bread Wheat Trials 2017-2018 Edirne location quality results

#	Cultivars	1000 Seed Weght (g)	Hectoliter Weight (kg)	Humidity (%)	Protein (%)	Wet Gluten (%)	Sedim. (Zel) ml	Starch	Alveograph W	Grain Hardeness
1	RUMELİ	42	80,7	12,3	17,3	40,1	75	66,4	444	82,4
2	PEHLİVAN	42	79,0	12,1	14,9	34,6	56	68,1	365	77,5
3	ESPERİA	40	80,0	12,4	15,8	36,6	67	66,8	401	76,3
4	FLAMURA 85	44	80,8	12,8	16,0	37,2	67	66,9	358	77,2
5	GELİBOLU	38	79,6	12,7	14,5	33,7	55	67,8	314	76,2
6	TRAGEN103	34	77,7	12,6	16,9	39,2	73	66,5	425	83,4
7	YK-6	42	76,6	12,5	15,1	35,0	57	68,1	329	68,4
8	YK-10	38	78,0	12,4	15,9	36,9	67	66,7	394	85,5
9	ALBERTOS	34	81,9	12,7	19,2	44,7	94	64,4	496	82,4
10	GB-1	40	72,7	12,5	14,8	34,4	57	67,3	352	86,0
11	GB-2	40	71,8	12,4	17,0	39,4	74	66,2	440	76,7
12	GB-6	34	78,6	12,3	17,2	40,0	77	65,5	439	89,3
13	GB-66	26	75,3	12,5	18,2	42,2	87	64,4	491	98,4
14	ARTEMİDA	38	77,5	12,0	18,5	42,9	90	64,1	482	88,5
15	ASTORTA	38	77,4	12,7	15,8	36,6	66	66,6	348	83,0
16	NOVASMELANKA	32	78,0	12,6	15,3	35,4	60	67,4	360	83,0
17	BUJANKA	32	78,1	12,6	15,6	36,3	63	67,2	389	89,0
18	SOLOMİYA	34	79,0	12,1	15,4	35,8	62	66,8	353	83,9
19	NATALKA	32	78,2	12,5	17,6	40,8	78	65,5	402	86,8
20	KLİMA	36	74,0	11,8	17,0	39,4	74	66,1	470	88,7

Note: Hectoliter weight, 1000 seed weight and protein rate were measured based on 0 % dry matter, but sedimentation rate was performed according to % 14.

CONCLUSIONS

As results, TRAGEN Ltd wheat cultivars which had favorable results and promising ones based on this study will be tested again future trials and selected ones having desired traits such as lower lodging even had higher plant height, higher protein, larger and longer grains and some disease tolerances will send to registration trials to produce and then exist in Turkish seed market.

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EVALUATION OF 2 YEARS AGRONOMIC CHARACTERISTICS OF 33 *CAMELINA SATIVA* GENOTYPES OF DIFFERENT ORIGINS IN MEDITERRANEAN CLIMATE CONDITIONS

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ABSTRACT

This research was carried out in the agricultural fields of Ege University Faculty of Agriculture, Department of Field Crops between 2019-2021. The research was planned according to the randomized blocks experimental design. In this study, 33 different *Camelina sativa* genotypes were used and these genotypes were imported from the US Department of Agriculture. The origins of these genotypes are based on 8 different countries. The features examined in the research are; plant height, branch height, number of branches, number of capsules per plant, number of seeds per capsule, thousand grain weight and yield. The main purpose of the research is to obtain vegetable oil from the seeds of camelina genotypes, whose agronomic properties have been examined for 2 years, by cold pressing method and to determine the quality characteristics of these vegetable oils. The next step of the research is to determine the amounts of oleic acid, linoleic acid, erucic acid, palmitic acid in camelina oils.

Keywords: *Camelina sativa*, Mediterranean

INTRODUCTION

With the rapid increase in the world population, vegetable oil consumption has also increased. As a result of this situation, oilseed plants have become strategically important in the world and in Turkey. (Kurt et al., 2015). In order to close our vegetable oil deficit and to reduce our energy need, it is necessary to include oil plants that can meet the same purposes wholly or partially in the production program in addition to the oil crops cultivated today (Göre ve Kurt, 2017).

Kamelina oil is a rich source of linoleic (omega-6) and alpha-linolenic (omega-3) fatty acids. These fatty acids are known to reduce the level of LDL-cholesterol in the blood and are beneficial for the health of the heart and heart vessels. Kamelina oil contains many natural antioxidants such as tocopherols, which make the oil stable and usable as an edible oil. The amount of tocopherols in the oil is 700 mg/kg. In addition, 100 g of camelina oil contains 10 mg of vitamin E. (Zubr, 1997). Kamelina oil has traditionally been used in human nutrition. It is also used in cooking mixed with rapeseed oil. Kamelina oil can be used in salads, meals, frying excluding cakes and chips. (Kurt ve Seyis, 2008). The plant's cultivation started in the Neolithic age and was used as an oil plant throughout the Iron Age. It is reported that it was grown in a wide area up to Southeast Europe and Southwest Asian steppes during the Roman Empire (Putnam et al., 1993).

Kamelina oil is used in the production of bath and soap bubbles for skin care purposes, in the production of liquid biodiesel raw materials, in the cosmetic industry due to the dermatological effects of polyunsaturated fatty acids, in the production of lipopeptides and lipoamino acids, in the substitution of fish oil due to having similar fatty acids, as candle oil in traditional lighting,

It is used in candle making, pure cooking oil and salad oil. (Peredi, 1969; Korsrud et al., 1978; Sang ve Salisbury, 1987; Robinson, 1987; Zubr, 1997). The amount of erucic acid in cooking oils has been set to certain standards by the World Health Organization (WHO). Most kamelina cultivars contain 2-4% erucic acid (C22:1n-9), which is higher than the 2% maximum accepted for quality edible oil in rapeseed. However, kamelina varieties containing 0% erucic acid have also been developed in recent years. (Kurt ve Seyis, 2008).

In this study, which was subsidized by the Aegean University Scientific Research Projects Committee, a total of 33 *Camelina sativa* genotypes from 7 different origins were examined in terms of their agronomic characteristics.

MATERIAL AND METHOD

The research was carried out in the province of Izmir in 2019 and 2020. Sowing was done in mid-November and harvesting in mid-June. 33 *Camelina sativa* genotypes of different origins were used in the study. The study was carried out in 3 replications according to the randomized blocks experimental design. The camelina genotypes used in the study were imported from the United States Department of Agriculture (USDA) in 2017. These genotypes and their origins;

Table 1. Genotypes and Origins of *Camelina sativa* used in the Research

	Plant Name	Country of Origin
1	GE.2011-01	Georgia
2	GE.2011-05	Georgia
3	Voronezh 349	Soviet Union, Former
4	No. 403	Sweden
5	No. 406	Sweden
6	Borowska	Poland
7	Przybrodzka	Poland
8	163-2073-72	Denmark
9	CR 492/94a	Germany
10	Giessen Nr. 3	Germany
11	Came	Germany
12	NU 52279	United States, Minnesota
13	CS-163-2073-72	Denmark
14	Boha	Denmark
15	BRSCHW 28347	Germany, Mecklenburg-W.P.
16	BRSCHW 30021	Sweden
17	Came	Sweden
18	Giessen #3	Germany, Mecklenburg-W.P.
19	Giessen #4	Germany, Mecklenburg-W.P.
20	Hoga	Denmark
21	Svalof	Sweden
22	CPS-CAM23	Germany
23	CPS-CAM10	Soviet Union, Former
24	CSS-CAM25	Soviet Union, Former
25	CSS-CAM27	Poland
26	CSS-CAM29	Soviet Union, Former
27	CSS-CAM34	Soviet Union, Former
28	CSS-CAM35	Soviet Union, Former

29	CSS-CAM36	Poland
30	CSS-CAM37	Soviet Union, Former
31	CSS-CAM7	Soviet Union, Former
32	Index Seminum 144	Poland, Przemysl
33	NE2006-1	United States, Nebraska

Table 2. 2019 climate data of Izmir province

	Jan.	Feb.	March	Apr.	May	June
Lowest Temperature (°C)	-2°C	2°C	4°C	5°C	10°C	14°C
Highest Temperature (°C)	16°C	20°C	22°C	25°C	32°C	36°C

Table 3. 2020 climate data of Izmir province

	Jan.	Feb.	March	Apr.	May	June
Lowest Temperature (°C)	-4°C	-5°C	1°C	4°C	3°C	10°C
Highest Temperature (°C)	17°C	20°C	23°C	26°C	40°C	38°C

RESULT AND DISCUSSION

The variance analysis tables of the parameters obtained from the research and affecting the yield are given below.

Table 4. Thousand grain weight (g)

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	0.005	0.003	2.652 ns
Year	1	0.017	0.017	16.09 **
Genotype	32	6.953	0.217	211.8 **
Year x Genotype	32	0.762	0.024	23.22 **
Error	130	0,133	0,001	
General	197	7.871		

CV: %3.16

Table 5. Plant height (cm)

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	42.69	21.34	1.637 ns
Year	1	1337.7	1337.7	102.6 **
Genotype	32	14051.9	439.1	33.6 **
Year x Genotype	32	2746.4	85.82	6.584 **
Error	130	1694.6	13.03	
General	197	19873.4		

CV: %4.47

According to the results obtained from the research; thousand grain weights showed statistically significant differences between genotypes. Likewise, the Year x Genotype interaction was also observed to be significant. However, it was observed that the difference between replications was statistically insignificant. The genotypes with the highest thousand-grain weight are Swalof (1.54 g) and Hoga (1.48 g), respectively.

According to the results obtained from the research; plant height showed statistically significant differences between genotypes. Likewise, the Year x Genotype interaction was also observed to be significant. However, it was observed that the difference between replications was statistically insignificant. The genotypes with the highest plant height are Giessen #3 (91.23 cm) and CSS-CAM25 (88.86 cm) genotypes, respectively.

Table 6. Number of capsules

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	832	416	6.912 **
Year	1	105494.6	105494.6	1752.6 **
Genotype	32	417759.2	13054.9	216.8 **
Year x Genotype	32	120719.7	3772.4	62.67 **
Error	130	7824.9	60.19	
General	197	652630.5		

CV: %4.68

According to the results obtained from the research; when examined in terms of the number of capsules, it was observed that there were statistically significant differences between genotypes. Likewise, it was observed that the difference between the Year x Genotype interaction and the recurrences was statistically significant. The genotypes with the highest number of capsules are GE.2011-01 (241.6), 163-2073-72 (239.1) and CPS-CAM10 (238.4), respectively.

Table 7. Number of grains in the capsule

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	1.657	0.829	11.41 **
Year	1	60.55	60.55	834.3 **
Genotype	32	358.6	11.2	154.4 **
Year x Genotype	32	100.2	3.133	43.15 **
Error	130	9.43	0.073	
General	197	530.5		

CV: %2.22

According to the results obtained from the research; the number of grains in the capsule showed statistically significant differences between genotypes. Likewise, it was observed that the difference between the Year x Genotype interaction and the recurrences was statistically significant. The genotypes with the highest number of grains in the capsule are Boha (14.73) and Came (14.12), respectively.

Table 8. LSD grouping of thousand grain weight, plant height, number of capsules and number of grains per capsule

Genotype No	T.G.Weight (g)	Plant Heigt (cm)	Nr. of Capsule (nr)	Nr. of Grains in the Capsule (nr)

1	1.112	E	80.67	DEFGHIJKL	**241.6**	**A**	11.21	KLM
2	0.882	LMN	87.40	ABCD	183.7	FGH	12.40	FGH
3	0.953	HIJK	84.77	ABCDEFGHI	141.5	LM	13.27	DE
4	0.927	IJKLMN	70.83	MN	170.8	HIJ	10.60	NO
5	1.075	EF	87.03	ABCDE	193.4	EFG	11.53	JKL
6	1.230	D	76.37	KLM	196.6	EF	12.52	FG
7	1.050	FG	43.70	O	61.1	P	11.15	LM
8	0.872	NO	82.27	BCDEFGHIJK	**239.1**	**AB**	12.67	F
9	0.972	HIJ	75.62	LM	217.4	C	12.08	GHI
10	0.897	KLMN	86.05	ABCDEFG	163.7	IJK	13.55	CD
11	0.867	NO	78.03	IJKL	138.6	LM	11.93	HIJ
12	0.818	O	80.43	DEFGHIJKL	188.1	EFG	12.57	FG
13	0.898	KLMN	80.30	EFGHIJKL	192.8	EFG	13.30	CDE
14	0.917	JKLMN	84.07	BCDEFGHI	164.7	IJK	**14.73**	**A**
15	0.935	IJKLM	78.33	HIJKL	202.8	DE	11.71	IJK
16	0.967	HIJ	77.23	JKLM	146.2	L	13.30	DE
17	1.343	C	79.37	FGHIJKL	133.2	MN	**14.12**	**B**
18	1.055	EFG	**91.23**	**A**	139.3	LM	12.60	FG
19	0.865	NO	83.53	BCDEFGHIJ	183.1	FGH	11.52	JKL
20	**1.480**	**B**	86.00	ABCDEFG	123.7	N	12.38	FGH
21	**1.540**	**A**	81.80	CDEFGHIJKL	94.9	O	11.05	LMN
22	0.568	P	85.40	ABCDEFGH	120.8	N	11.23	KLM
23	1.080	EF	81.90	CDEFGHIJKL	**238.4**	**AB**	13.82	BC
24	0.983	HI	**88.86**	**AB**	163.8	IJK	11.10	LMN
25	1.038	FG	84.73	ABCDEFGHI	178.3	GHI	10.23	OP
26	0.884	LMN	88.26	ABC	149.7	KL	13.60	BCD
27	0.938	IJKL	78.87	GHIJKL	86.6	O	**14.67**	**A**
28	1.087	EF	80.79	DEFGHIJKL	212.3	CD	10.00	P
29	1.260	D	83.47	BCDEFGHIJK	162.5	JK	9.90	P
30	1.002	GH	81.30	DEFGHIJKL	164.5	IJK	10.97	MN
31	1.092	EF	85.40	ABCDEFGH	187.9	EFG	9.40	Q
32	0.887	LMN	86.20	ABCDEF	223.6	BC	11.97	HIJ
33	1.003	GH	63.83	N	67.5	P	12.85	EF

Table 9. Number of lateral brunches

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	3.632	1.816	3.868*
Year	1	662.3	662.3	1410.9**
Genotype	32	473.7	14.80	31.53 **
Year x Genotype	32	329	10.28	21.9 **
Error	130	61	0.469	
General	197	1529.8		

CV: %8.18

According to the results obtained from the research; when the number of lateral brunches was examined, it was observed that there were statistically significant differences between genotypes. Likewise, it was observed that the difference between the Year x Genotype interaction and the recurrences was statistically significant. The genotypes with the highest

number of lateral brunches are Voronezh 349 (11.38) and Index Seminum 144 (11.07), respectively.

Table 10. Lateral brunches height (cm)

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	69.04	34.52	5.338 **
Year	1	5490.3	5490.3	848.8 **
Genotype	32	9172.9	286.6	44.31 **
Year x Genotype	32	5413.5	169.1	26.15 **
Error	130	840.8	6.468	
General	197	20986.7		

CV: %6.07

According to the results obtained from the research; When the lateral branch height was examined, it was observed that there were statistically significant differences between the genotypes. Likewise, it was observed that the difference between the Year x Genotype interaction and the recurrences was statistically significant. The genotypes with the highest lateral branch height are Svalof 349 (53.9 cm) and Hoga (52.4 cm), respectively.

Table 11. Yield (kg/da)

Source of Var.	Deg. of Freedom	Sum of Squares	Mean of Squares	F Value (%)
Recurrence	2	506.5	253.2	1.426 ns
Year	1	56886.3	56886.3	320.1 **
Genotype	32	514252.7	16070.4	90.44 **
Year x Genotype	32	96654.7	3020.4	16.99 **
Error	130	23099.5	177.6	
General	197	691399.9		

CV: %8.02

When the results obtained from the study were examined in terms of grain yield, it was observed that there were statistically significant differences between genotypes. Likewise, it was observed that the Year x Genotype interaction was also statistically significant. However, the difference between replications was not statistically significant. The genotypes with the highest grain yield are CPS-CAM10 (289.6 kg/da) and GE.2011-01 (251.9 kg/da) respectively.

Table 12. LSD grouping of number of lateral brunches, lateral brunches height and yield

Genotype No	Number of Lateral Brunches (nr)	Lateral Brunches Height (cm)	Yield (kg/da)
1	8.70 CDEFGHIJ	35.1 LM	**251.9 B**

2	8.37	EFGHIJK	47.7	BCDE	172.8	HIJKL
3	**11.38**	**A**	40.0	HIJK	150.3	LMN
4	6.70	MNOP	35.4	LM	134.6	MNO
5	7.80	IJKLM	45.9	DEFG	184.4	GHIJ
6	7.67	IJKLMN	37.8	JKLM	238.9	BC
7	5.60	PQ	19.2	O	66.1	Q
8	9.93	BCD	46.4	DEF	225.5	CD
9	9.90	BCD	37.5	JKLM	206.9	DEFG
10	6.92	LMNO	51.9	ABC	151.5	LM
11	8.47	EFGHIJK	48.6	BCD	127.8	NO
12	9.69	CDE	43.2	EFGHI	160.6	IJKLM
13	9.50	CDEF	45.2	DEFG	210.2	DEF
14	9.77	BCD	41.2	GHIJ	195.4	EFGH
15	9.47	CDEF	38.6	IJKLM	193.4	EFGH
16	8.37	FGHIJK	41.3	GHIJ	155.9	KLM
17	7.87	IJKLM	46.2	DEF	224.1	CD
18	9.70	CDE	48.5	BCD	136.7	MNO
19	8.18	GHIJKL	47.2	CDE	142.7	MNO
20	6.40	NOP	**52.4**	**AB**	178.1	HIJK
21	5.73	OPQ	**53.9**	**A**	122.6	O
22	6.77	MNOP	39.7	IJKL	59.2	Q
23	9.27	CDEFG	35.3	KLM	**289.6**	**A**
24	8.13	HIJKL	48.1	BCDE	154.6	KLM
25	9.87	BCD	44.9	DEFGH	160.7	IJKLM
26	8.00	IJKLM	45.4	DEFG	155.5	KLM
27	8.73	DEFGHI	38.5	IJKLM	95.8	P
28	10.00	BC	42.2	FGHIJ	184.7	FGHI
29	9.27	CDEFGH	39.3	IJKLM	172.7	HIJKL
30	7.43	JKLMN	38.8	IJKLM	150.00	LMN
31	7.25	KLMN	38.6	IJKLM	145.4	MNO
32	**11.07**	**AB**	38.0	JKLM	213.6	CDE
33	4.67	Q	30.0	N	72.3	PQ

CONCLUSION

In the results of the 2-year research conducted in Bornova-İzmir ecological conditions, only agronomic features are the features that are considered. In this research, which is still ongoing, quality analyzes of the seed oils of these 33 genotypes will be made as well as agronomic observations. As a result of the research, superior genotypes in terms of both yield and oil quality will be determined and breeder material will be created for future studies. As a result of the first stage, agronomic observations, CPS-CAM10 (289.6 kg/da) and GE.2011-01 (251.9 kg/da) genotypes come to the fore in terms of yield. If the same genotypes are superior in terms of oil quality, they will be registered as promising genotypes.

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AWARENESS OF OLIVE GROWERS TO WEEDS IN OLIVE ORCHARDS IN EDREMIT

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ABSTRACT

Olive has been considered a divine tree in the Mediterranean region since ancient times. The tree is vulnerable to weed presence on the early growth stage, and they cause many adverse impacts on the olive tree, including wildfire, but it was ignored by growers during the youth infertility in many times. Edremit district provides the most suitable growing conditions to olive tree; therefore, with 11 million olive trees the district is a prominent location in Turkey. This study was conducted to determine the attitude of olive growers to the weeds in the Edremit district of the Balıkesir Province, Turkey. For this aim, a questionnaire form consisted of 29 questions was prepared and applied to 20 olive orchard growers in Edremit. The results showed that age of producers were 30 to 73. Interestingly, one-third of the growers was the only considered themselves farmers, and most farmers (%65) had less than 2 ha olive orchards. The weeds were *Cynodon dactylon, Sorghum halepense, Tifolium pratense, Xanthium spinosum, Tribulus terrestris, Cyperus rotundus*. The growers mainly controlled them with ploughing, cutting, and herbicides. One-quarter of them only used glyphosate in the spring months to control weeds, but most of these growers applied the herbicide using knapsack sprayer. Even if the growers have general information about the loss of herbicide efficacy, half of them had any info or no action if loss of herbicide efficacy occurred. There was no relationship between age, experience, occupation, education, and size of field and methods applied for olive cultivation. It was concluded that research, training and outreach activities are necessary to increase yield and quality of olive and olive oil.

Keywords: Growers' profile, orchard size, tillage, cutting, herbicide, glyphosate.

Introduction

Olive, one of the oldest crops of the world as well as popular health crop recently, originated from Syria and spread north of Mediterranean through Anatolia then Aegean islands to Spain and south of Mediterranean through Egypt to Morocco (Koca, 2004; Ayaz and Varol, 2015; Duran, 2006; Bayramer, 2015; Hantekin, 2019). It is grown in temperate zone and Mediterranean type climates, i.e., between 30th and 45th north and south meridians in 37 countries (29 of them in northern hemisphere) (Bedestenci and Vuruş, 2000; Ilgar, 2016; Ayaz and Varol, 2015). In 2019, 19.4l million tones olive were produced from 900 million trees covering 10.57 million hectares' area (FAO, 2021) and processed into olive oil 90 % (Erdal and Vural, 2017).

Turkey is one of the foremost olive producing countries due to its location and climate (Soyyiğit and Yavuzaslan, 2018). Olive and olive products have importance also Turkey's industry, international trade, national trade, and employment (Erdal and Vural, 2017; Boyraz et al., 2010; Adıgüzel and Kızılaslan, 2019; Karslı, 2006). Oil processed olive crops' percentage is 73% as average of the last decade (Günç Ergönül and Dinçer, 2020) and 224,500 metric tonne olive oil is produced in 2019 (TEPGE, 2021). There are 28 registered olive varieties out of 84 local varieties in Turkey which is 4% of total 2000 varieties worldwide (Boyraz et al., 2010; Özer, 2020). Area of olive groves in Turkey keeps 3.4% of total agricultural land (TUİK, 2021), which corresponds 8.3% of world olive areas (FAO, 2021). Almost all parts of Turkey have olive trees (Özkaya et al., 2008; Ilgar, 2016; Koca, 2004; Ayaz and Varol, 2015; Özer, 2020). But 68% is in three seaside regions in the South and West and 14% in the Southeast Anatolia region (TÜİK, 2021; Özer, 2020). Most of the olive groves in Turkey is in hilly areas and only 8% is in irrigated fields (Öztürk and Yalçın¸ 2014).

Edremit locality is among important olive growing and processing areas of Turkey where its climate, geomorphology, bedrock, soil, and ground and underground waters determine specific quality of its olive and olive oil. Olive agriculture and related industries in Edremit are the main economic activities because 84% of agricultural land, 23553 ha, is covered by olive trees (EdremitBel, 2020). Average olive yield per tree is 38 kg but fluctuates year by year due to periodicity (EdremitBel, 2020). Olive yield drops up to18 kg per tree in Edremit where mostly irregular olive orchard establishments on infertile slopy areas (Efe et al., 2013) where olive trees, in addition, help to prevention of erosion (Köksal, 2009).

Plant protection including weed control is one of the main applications to produce olives and olive oil in acceptable yield and quality (Hepdurgun et al., 2003; Uludag et al., 2003). It was mentioned that weeds can cause yield loss in olives as it happened in other crops (Da'u and Al-Saghir, 1986; Civantos, 1988). Weed control is more important in establishment years of orchards because weeds affect negatively root development of olives (Bini and Ghisolfi, 1986). Weeds in olive orchards also have role on increasing disease incidence such as verticillium (Thanassoulopoulos et al., 1981) and being alternative host for insects (Deghiche-Diab et al., 2021), furthermore being host of an insect (*P. Spumarius*) in some stages of life cycle that is vector of a pathogen, *Xylella fastidiosa* (Capusso, 2021). In Turkey, 81 and 92 plant species from 30 and 29 plant families were found in teo weed surveys in the Bursa and Hatay Provinces, respectively in olive orchards.

The aim of this study was to determine profile of olive farmers and their views on weeds and other related subjects in olive orchards in Edremit where is a foremost olive growing district of the Balıkesir Province of Turkey.

Materials and Methods

Twenty olive growers from 12 settlements/villages were surveyed with face-to-face discussions in the Edremit District of the Balıkesir Province of Turkey in 2014 using a questionary with 29 questions including personal information. The data were analyzed using SPSS 22 (Statistical Packet for Social Sciences) for Windows. Frequency and percentage tables were calculated for each categorical variable.

Results and discussion

The youngest producer was 30 years old and the oldest one 73 with an average age 49.6. Most of the farmers were in age between 41 and 60, while a quarter them was younger than 40 (Table 1). Olive farmers from the Köprübaşı District of the Manisa Province were older range that vast amount of them were 40 to 69 (Uyar and Uludag, 2021). Half of the growers is secondary school graduate, a quarter graduated from a high school and remaining has university degrees. The educational profile of olive producers from Köprübaşı was slightly different with university degrees' holders followed to primary school graduates (Uyar and Uludag, 2021).

Table 1. Age of growers

Age Interval (years)<>	Percentage
≤40	25
41-50	25
51-60	40
≥60	10

Table 2. Olive growing experience of growers

Experience (years)	Percentage
≤10	15
10-19	20
20-29	40
30-39	10
≥40	15

Farmers have long experience in olive growing between 5-50 years, which is in average 22.15 years that is as much as almost half of their ages (Table 2). Olive farming experience was less than 30 years in the Köprübaşı district of Manisa (Uyar and Uludağ, 2021). However, only 30% of them mentioned their main occupation is farming (Table 3), which was very low comparing to Köprübaşı olive producers 80 of them mentioned olive husbandry is their main activity (Uyar and Uludağ, 2021). Teachers and retired people constitute 50%, which is probably due to high number of early retirements and longer free times of teachers after school. In other reason might be spending shorter times for olive husbandry as well as owning small sized olive grows (Table 4). Most of the farmers (65%) have acreage less than 2 ha and only 5% has over 5 ha. But, it was reported in earlier literature that average size of fields in Edremit is 1.25 ha and 25% of orchards are larger than 5 ha (Efe et al., 2013), which is not parallel with our data especially in larger fields percentage.

Table 3. Main occupation of growers

Profession/Job	Percentage
Farmer	30
Teacher	25
Retired	20
Shopkeeper	10
Others	15

Table 4. Olive grove acreages of producers

Area (ha)	Percentage
<1.0	15
1.0-1.9	50
2.0-4.9	30
>4.9	5

It is found that grafted wild olive trees with 80% consist of the biggest part of olive tree source. Only 25% of olive orchards in plain part the remaining on hills and higher elevations. Irrigation also not a common practice with 20% of fields irrigated only, of which 15% of farmers pointed out as problem (Table 5). Plant protection including weeds and lower olive prices are two main problems of growers. But producing olive is mentioned as the foremost problem half of the farmers. Weeds are considered less problematic comparing to the diseases and insect pests as it was mentioned vineyards in the Sarıgöl district (Çinkılıç et al., 2016).

Table 5. Growers' problems related to olive agriculture and sector

Problems	Percentage
Lower olive prices	30
Olive leaf spot	15
Irrigation	15
Olive fruit fly	15
Supports and publicity	10
Picking	5
Weeds	5

Farmers, prefer local varieties mainly, mentioned Edremit variety and Edremit-oil variety are used 40 and 60%, respectively, which implies that main process is olive oil production in the district. Most growers apply chemical fertilizers (65%) without any analysis. Almost all farmers apply manure (Table 6).

Table 6. Amount of manure applied

Manure kg per tree	Percentage
0	10
2-4	5
20	15
25	10
30	15
40	20
50	25

There was no correlation with age, education, size of owned field and experience with any method applied in olive husbandry techniques although smaller size of interviewees might be a reason as well. Half of the farmers prune trees once a year, mainly in February-March (Table 7). In addition, pruning every other year is also a common practice.

Table 7. Pruning months and intervals

Interval, Months	Percentage
Every other year, March-April	10
Every other year, February-March	30
Every year, March-April	10
Every year, February-March	40
Twice in a year February-March and October	5
Twice in a year February-March and November	5

Weeds considered the most important or the most common by growers were *C. dactylon, S. halepense, T. pratense* and *X. spinosum* (Table 8), which was very similar by Köprübaşı farmers (Uyar and Uludağ, 2021). It was found in olive orchards in other provinces of Turkey that similar weeds are among common weeds although there was some difference due to probably regional differences (Uremis, 2005; Tuğrul 2013). One quarter of the farmers were not able to recognize weeds properly. Although few weed species were mentioned by farmers, it could be more species in the fields because it had been reported that 109 species were detected in olive sapling nurseries in Kemalpaşa (İzmir) and Edremit. The richness of species had been attributed to use of manure in high amounts (Erten and Nemli, 1993). However, 20 weed species were considered by 373 grape producers (Uludag et al., 2016).

Table 8. Weeds growers consider important

Weed Name	Percentage
Cynodon dactylon	5
C. dactylon, Sorghum halepense	10
C. dactylon, Trifolium pratense	5
C. dactylon, S. halepense, T. pratense	15
S. halepense, T. pratense	5
T. pratense, Xanthium spinosum	10
Tribulus terrestris, T. pratense	5
X. spinosum	15
X. spinosum, Cyperus rotundus	5
No idea	10
Many kind	15

The most common method for weed control was considered tillage, which was applied alone or combination with herbicides or cutting (Table 9) similar to Köprübaşı olive farmers who used mechanical and chemical weed control techniques (Uyar and Uludağ, 2012). On the contrary to grape farmers from the Sarıgöl district who had preferred chemical weed control mainly out of 95% were applied a weed control technique (Uludag et al., 2016). Similar to grape farmers, 10% of olive farmers mentioned that no weed control was applied. Actually, tillage seems not a good method especially in groves in hilly areas due to risk of erosion (Civantos and Torres, 1981).

Table 9. Weed control methods applied

Methods	Percentage
Cutting	10
Herbicides	10
Nothing	10
Tillage	50
Tillage+Cutting	5
Tillage+Herbicides	15

Most growers till the orchards once a year in March mainly, on the other hand 10% of farmers said that no tillage was applied (Table 10). Tilling twice is done in October first and the second tillage implemented February, March or April. In the Mardin Province farmers tilled soil three times (% 57) followed by once (%21), twice (%15) and none (%6) (Bayyiğit, 2018). However, mulching is reported as sustainable option in weed control in olive groves via decreasing soil erosion and increasing soil organic carbon (Soriano et al., 2014) or the best yielding option (Huqi et al., 2009) comparing to tillage; but; no farmer mentioned that they apply any mulching. Olive harvesting occurs in September-October and November-December almost equally, but the latter was the preferred slightly.

Table 10. Tillage months and intervals

Interval, Months	Percentage
Once, March	40
Once, April	20
Once, February	5
Twice, October and March	10
Twice, October and April	5
Twice, October and February	10
None	10

Herbicide, which was glyphosate mixed with surfactant were applied in May, June, or less extent in April using backpack sprayer mainly due to smaller size of fields. Glyphosate was also common in vineyards and almost a unique herbicide, which can cause inevitable herbicide resistance problems (Çinkılıç et al., 2015). However, some Köprübaşı olive producers preferred ACCase herbicides as well as glyphosate (Uyar and Uludağ, 2021). It is understandable why farmers prefer glyphosate as its effectiveness on weed control in olive orchards over other herbicides has been seen in literature (Huqi et al., 2009; Kanatas et al., 2021) in spite of evolving weeds resistant to glyphosate (İnci et al., 2019).

Farmers use field sprayer, backpack sprayer, or atomizer, respectively 25%, 20%, or 40% to control diseases and pests. 15% of farmers mentioned that they do not apply chemicals to control diseases or pests. Growers were careful about cleaning spraying tools following use. This is parallel with farmers producing grapes that two third of them were aware of environmental effect of pesticides (Uludag et al., 2016).

Growers responded the question if they have noticed loss of effectiveness in any pesticides as yes 55% and 35% no, very similar to Köprübaşı olive farmers (Uyar and Uludağ, 2021). In a case of loss of efficiency, they mainly changed herbicide (35%), increased rate of pesticide in the application (15%), and repeated pesticide application (20%).

This small study shows that there is huge need to improve olive production in Turkey via enlarging olive areas, increasing yield and quality as mentioned by Gökçe (2003). It is interesting more than a decade later the problems are almost the same. Age, education level, experience or size of field did not affect farmers behavior related to olive cultivation. As mentioned Uludag et al (2003) research, education and outreach activities should be intensified.

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AN EVALUATION OF YOUNG WOMEN IN THE PERIOD OF PANDEMIC WITH THE BREAST FEEDING KNOWLEDGE QUESTIONNAIRE; EDIRNE PROVINCE

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ABSTRACT

For women's health, breastfeeding has extraordinary benefits. Breast milk has a high impact on the healthy nutrition and development of children. It provides the baby with the necessary vitamins and minerals. Our study aimed to examine the knowledge and attitudes of young mothers towards breastfeeding. During the Covid-19 pandemic process, breastfeeding and barriers to breastfeeding were examined. Participants consisted of women between the ages of 18 and 38. Those with at least 1 living child who applied to the Trakya University hospital gynecology outpatient clinic were included in the study. Those who were pregnant or suspected of pregnancy were excluded from the study. Face-to-face interviews were conducted with mothers who agreed to participate in the study. The Breast Feeding Knowledge Questionnaire is a scale developed based on WHO and UNICEF breastfeeding recommendations for optimal infant feeding as well as previous research with similar aims used. The study showed that; participants had a good knowledge of the benefits of breastfeeding for infant and maternal health. The outcome of this study was twofold; Breastfeeding attitudes and knowledge among young women were evaluated and the barriers to breastfeeding during the pandemic were tried to be determined. The most women; She had a positive attitude towards breastfeeding. However, there were many obstacles to breastfeeding during the pandemic period. Health professionals must actively work with women to overcome these barriers. It is important to run an awareness campaign to make women aware of the importance of breastfeeding. Studies should be developed on how to overcome breastfeeding and barriers to breastfeeding during the pandemic.

Keywords; Breastfeeding information questionnaire, Women's health, Lactation, Breast milk, Breastfeeding knowledge level, Breastfeeding attitude.

INTRODUCTION

For the protection and promotion of women's health, breastfeeding has extraordinary benefits. Breast milk has a high impact on the healthy diet and development of children (Organization, 2020). The World Health Organization (WHO) recommends exclusive breastfeeding for up to 6 months. After 6 months, of course, complementary feeding should be started. However, WHO recommends: Even after babies are 6 months old, breast milk should be given until they are 2 years old or older. Unfortunately, many countries do not adequately follow these recommendations. (Organization, 2020; Suárez-Cotelo, Movilla-Fernández, Pita-García, Arias, & Novío, 2019).

WHO (2011) reports that "breastfeeding is a unique way of providing ideal food for healthy growth and development. In addition to having important effects for the development of babies, it is an integral part of the healthy life process. It supports the child's immune system. The act of breastfeeding stimulates the proper growth of the baby. Breastfeeding leads to positive reflections for both mother and baby in terms of behavior, speech, and well-being. Studies have shown that; It reduces the risk of chronic diseases such as obesity, high cholesterol, diabetes mellitus, childhood asthma in children.

The breast milk; In addition to having important effects on the development of babies, it is known that children are an integral part of the healthy life process. It provides the necessary vitamins and minerals to the baby in a hygienic way.

The act of breastfeeding stimulates the proper growth and development of the baby (Organization, 2020). Breast-feeding; It leads to positive reflections for both the mother and the baby in terms of motor skills, speech, and well-being (Victora et al., 2015). The studies in the literature show that; breastfeeding not only improves intelligence into adulthood, it also increases children's educational attainment. Being breastfed is a lifetime advantage for these children. Breastfeeding, which increases the cognitive ability of children; It appears to have an impact on both the individual and the societal level (Organization, 2020; Victora et al., 2015).

Our study aimed to examine the knowledge and attitudes of young mothers towards breastfeeding. During the Covid - 19 pandemic process, breastfeeding and possible barriers to breastfeeding were examined. In our study; The "Breastfeeding Knowledge Scale" was used to determine the attitude and knowledge level of young mothers.

MATERIAL AND METHOD

Volunteers who applied to Trakya University Hospital's gynecology outpatient clinic between October 2020 and July 2021 and had at least 1 living child were included in the study. Participants consisted of women between the ages of 18 and 38. Those who had multiple pregnancies, who did not give birth to a healthy baby, who did not breastfeed due to a chronic illness, who used cigarettes and/or alcohol during breastfeeding were excluded from the study.

To collect data in the study; A scale and a sociodemographic form were used. Those who were pregnant or suspected of pregnancy were excluded from the study. Face-to-face interviews were conducted with mothers who agreed to participate in the study. In our study; The "Breastfeeding Knowledge Scale" was used.

To all participants; Questions were asked about the use of breast pumps, bottle feeding, whether they had mastitis, their sleeping habits, whether they felt depressed and their social life, including housework, breastfeeding habits, breastfeeding symptoms, and breast care. Questions about the birth of their baby; History of mastitis in previous lactation periods was asked. Sociodemographic information of all participants was also evaluated and recorded.

Breastfeeding Knowledge Scale:

The scale is used to measure mothers' attitudes towards breastfeeding. The Breastfeeding Knowledge Questionnaire was used, a scale based on WHO and UNICEF's breastfeeding recommendations for optimal infant feeding, as well as previous research with similar objectives (Brodribb, Fallon, Jackson, & Hegney, 2008; Sobti, Mathur, & Gupta, 2002)(Brodribb ve diğerleri 2008) (Brodribb et al., 2008). The validity and reliability of the scale has been proven (Saied, Mohamed, Suliman, & Al Anazi, 2013).

The Breast Feeding Knowledge Questionnaire consisted of 15 items on the benefits of breastfeeding for both babies and mothers. Responses to knowledge questions were categorized as true or false. 1 point was awarded for each correct answer. The total score was calculated by summing the individual scores of 15 knowledge questions ranging from 1 to 15, the higher the score, the higher the knowledge.

Total attitude scores can vary and high scores; It reflects that women have a more positive attitude towards breastfeeding. This scale has been used by many researchers in different international locations (Ahmed, Bantz, & Richardson, 2011; Brodribb et al., 2008; Organization, 2020; Victora et al., 2015).

For the study, necessary permissions were obtained from the Scientific Research Board of the Ministry of Health. The study was approved by Trakya University Faculty of Medicine Scientific Research Ethics Committee.

RESULTS

In our study, the mean age of women was 25.22 (19-40). Participants were asked about their level of knowledge about breast milk and breastfeeding. Our work; It was held between October 2020 and July 2021. In the participants; The condition of having given at least one live birth was sought. Women younger than 19 and older than 41 were not included in the study. Those who were pregnant or breastfeeding at the time of the study were not included in the study.

Sociodemographic information was structured with questions about age, marital status, education level, number of births, occupation, place of residence and health institutions where the pregnancy continued. It was asked if there were any changes in their socio-cultural life during the pandemic period. They were also asked about their previous breastfeeding experiences (in cases of multiple births). The women were asked what they thought about having a baby in the future and breastfeeding in the first 6 months.

There was no significant difference between the knowledge level of the participants who had given multiple births and breastfed before, and the knowledge levels of the women who had only one live birth. Our study has shown that; Participants had a good knowledge of the benefits of breastfeeding for infant and maternal health. The vast majority of women (89.8%) stated that they wanted to breastfeed their babies for up to 6 months.

The result of our study was twofold. First, breastfeeding attitudes and knowledge among young women were evaluated. Second, we tried to identify barriers to breastfeeding during the pandemic period.

196 women were included in the study, but 191 participants completed answering all questions. They were asked if they were highly motivated to breastfeed the baby. Most of the participants gave a positive response (71.36%).

Only 32% of the participants had accurate knowledge of good breastfeeding technique. And 16.8% of the participants had insufficient knowledge. Approximately 24% of the women thought that breastfeeding alone would not be sufficient until their baby was 6 months old. Those who thought that breast milk would protect as a birth control method were 24.8%. The

rate of knowing the correct breastfeeding posture and holding the baby correctly remained at 32.12%. The rate of those who thought to stop breastfeeding because they did not have sufficient knowledge about breastfeeding was 29.23%.

Most women had a positive attitude towards breastfeeding. However, there were many obstacles to breastfeeding during the pandemic period. First of all, when there is a problem with breastfeeding (when there is a physical or psychological pathology); They were hesitant to go to hospitals for examination. In addition, since they do not stay outside for a long time and their participation in social activities is minimized; their motivation to breastfeed was also low.

During the pandemic period; participants stated that their fluid consumption decreased as their mobilization decreased. They stated that they also reduced their nutrition because they were worried about weight gain during their stay at home. During the pandemic period; if the baby does not want to breastfeed because of the pump or the baby's health problems; He was asked if he would express his milk regularly. The approach to pumping was often positive. Those who had occasional pain during lactation did not cause them to stop breastfeeding, 93.21%. If they breastfeed; The rate of those who stopped breastfeeding due to the fear of malformed breast shape was low (5.32%).

Prenatal and postnatal support of their families in breastfeeding their babies was asked. Especially those who gave birth after their first pregnancy stated that they received encouragement from their families for breastfeeding. When there is insufficient milk production; They were asked if they would stop breastfeeding. Those who said that they stopped breastfeeding when they thought that breast milk was not enough for their baby were compared with those who stated that they continued to breastfeed even if their breast milk was not enough. There was no significant difference between the two groups.

DISCUSSION

Breastfeeding reduces under-five mortality and morbidity from infectious diseases. In addition, breast milk is one of the most effective public health measures to reduce chronic diseases such as diabetes mellitus and obesity. Furthermore, there is growing evidence to support long-term benefits such as cognitive ability, intelligence, and educational attainment (Victora et al., 2015). In the literature; Prospective studies with breastfeeding women have also reported a low incidence of discontinuation of breastfeeding before infants are 6 months old (Babakazo, Donnen, Akilimali, Ali, & Okitolonda, 2015).

In our study, it was observed that increased stress and worries about the future, generally during the pandemic period, were risk factors for discontinuing breastfeeding in women. The scale scores also tended to decrease as the income level of the participants decreased. Another issue was nutrition. During the pandemic period; stated that their fluid consumption decreased as their mobilization decreased. As they were worried about weight gain during their stay at home, they also limited their nutrition. Studies on breastfeeding, in women during puerperium; Fatigue, stress, changes in the number of feedings, family infection, and poor diet were negatively correlated (Organization, 2020; Tamim et al., 2016).

However, there were many obstacles to breastfeeding during the pandemic period. The first of these is when there is a problem with breastfeeding, when they have mastitis or when they want to get milk increasing support; They were afraid to go to the hospitals. Apart from this, their motivation for breastfeeding was low, since they stayed outside for a long time and their participation in social activities was minimized. In the literature; Evidence of a positive effect of breastfeeding on the child is often given. The results of the studies conducted in recent years show that; There is a direct positive relationship with the motor and sensory development of breastfed children (Tandon et al., 2016; Victora et al., 2015).

In adulthood, the impact on income is mostly mediated by IQ. Provides additional evidence for a causal link between breastfeeding and intelligence (Luby, Belden, Whalen, Harms, & Barch, 2016; Tandon et al., 2016). In children breastfed for 12 months or longer, the increase in income increases with duration of breast-feeding in comparisons between children breastfed for less than one month.(Victora et al., 2015). Health professionals should actively counsel and train women of reproductive age to overcome barriers to breastfeeding.

CONCLUSIONS

To women, especially during the pandemic; It is important to run an awareness campaign to raise awareness about breastfeeding in a positive way. Studies should be developed on how to overcome breastfeeding and barriers to breastfeeding during the pandemic. In our study, in stopping breastfeeding; Risk factors fall into two general categories. These; It is a state of decreased immunity secondary to poor breastfeeding technique and stress and sleep deprivation. During the pandemic, breastfeeding education should be increased for women of childbearing age, and even intensified during pregnancy.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and
approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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THE GENETIC CHARACTERIZATION OF WILD SUNFLOWER SPECIES (HELIANTHUS SPP.) AND INTERSPECIFIC HYBRIDS BASED ON BROOMRAPE RESISTANCE

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ABSTRACT

Sunflower is grown in many parts of the world and in the Marmara (especially Thrace) and Central Anatolia regions of our country as a snack and oil. It is mostly grown in the Central Anatolia Region as dried nuts as a snack and as bird feed for birds. It is a very popular agricultural plant in the Thrace Region, and since it is seen as an important oil plant in this region, it is mostly cultivated as oil. The sunflower was seen wild in nature in the 1000s BC and attracted attention due to its appearance. Over time, it has been improved by many sunflower growers and researchers to obtain the oil in its content and to benefit from its seeds and has taken its place as a very important commercial agricultural plant in the world. It is still being bred for many improvement studies and is the main subject of many molecular studies. The cultivated sunflower germplasm contains and preserves 50% of the genetic diversity found in its wild relatives. The strength of coupling quality requires the use of genetic diversity present in cultured hybrids and wild gene sources to develop pre-cultivation lines and material that provides the elite standard. Sunflower hybrid breeding includes the development of appropriate breeding lines for diseases, abiotic stress and herbicide resistance. These lines are created by crossing wild species with cultured sunflowers, crossing wild species with other wild species, or hybrid cultivating with other species as a result of crossbreeding. The biggest problem in cytoplasmic male infertility, diseases, abiotic and especially sunflower is *Orobanche spp.* wide crosses were made to find solutions to their resistance. These goals are fulfilled through repeated choices to improve the population. But choosing phenotypically durable lines is time consuming and not very reliable. Thanks to the molecular markers, durable breeds are detected in a short time and the time to obtain hybrid varieties is shortened and accurate, reliable results are obtained. Thus, a high increase in efficiency will be observed by providing a permanent endurance.

Keywords: Sunflower, Wild Species, *Helianthus*, *Orobanche* parasite, Broomrape, Resistance,

INTRODUCTION

Sunflower (*Helianthus annuus* L.), whose gene center is North America, is cultivated and consumed as commercial oilseed plant, nuts and bird feed, and has taken its place among the four most preferred oilseed crops in the world, along with soybean, rapeseed and palm. The cultivated sunflower: It belongs to the genus *Helianthus* of the order Asterales, family Asteraceae. It is an annual angiosperm with a chromosome number of 2n=34. In general, the genus *Helianthus* has 51 species and 19 subspecies, of which 14 varieties have annual growth habits and 37 varieties have perennial growth habits, and all wild annual species from these varieties have the same chromosome number as the cultivated sunflower, that is, they are diplopid (2n=2x=34). Perennial wild species usually have 2n = 4x = 68 (tetraploid) and 2n = 6x

= 102 (hexaploid) chromosome numbers. However, some species contain more than one ploidy level. Examples of these are *H. ciliaris* L. and *H. decapetalus* L. *H. ciliaris* L. species has tetraploid and hexaploid ploidy levels, while *H.* decapetalus L. species has diploid and tetraploid forms. (Meral, 2019, Atlagić, 2004).

Sunflower is among the most important oil crops grown for oil production in many parts of the world and in our country, and it is the oilseed plant that has the largest cultivation area and is produced in Turkey. The demand for sunflower production is increasing every year. High-quality sunflower oil production is a major reason for sunflower cultivation, as the high content of saturated and unsaturated fatty acids in its seeds ensures that the amount of oil obtained from the unit area is high and the quality of sunflower oil is high (Meral, 2019). As a different factor, population growth and the lack of affordable alternative oils trigger the production and consumption of sunflower oil at a high rate. Planting sunflowers for oil; It is made to obtain oil, to form pulp for animal feed and to produce biodiesel. About 90% of the produced sunflower seeds are processed for sunflower oil and the rest is consumed as snacks. World sunflower production has been around 50 million tons in recent years, and Turkey is among the top ten countries in sunflower agriculture. Turkey realizes approximately 33% of the world's sunflower imports. Other prominent country names in exports are Russia and EU countries. In our country, oil sunflower production is generally done in Thrace-Marmara Region. While it is produced as snack is mostly done in Central and Eastern Anatolia Region.

Despite the progress in production, there are some factors affecting the normal sunflower development. Considering growing sunflower in the world, one of the difficult issues is to ensure the correct development of the plant to provide a good yield without threatening abiotic or biotic factors. The most important threat limiting the growth and production of sunflower is a sunflower broomrape (*Orobanche cumana Wallr.*) parasitic plant, which could result huge yield losses in sunflower (González, 2021, Yonet et al., 2018). In addition, other disease factors such as sunflower rust, sunflower downy mildew are among the factors limiting sunflower development and yield. Sunflower broomrape is a parasitic plant that causes yield losses of up to 100% in sunflower in our country, European and Balkan countries. thus, it causes rapid epidemics in sunflower production areas (Kaya, 2003). Ensuring effective and sustainable broomrape resistance is the goal of sunflower breeding and molecular-based studies (Fernandez-Martinez et al., 2010; Kaya et al., 2012b). Sunflower breeders therefore began to constantly search for a resistance gene that contains resistance to broomrape races. Thanks to molecular marker techniques, it has become quite possible to achieve these breeding goals, to determine the source of broomrape resistance, the gene region, and whether they are dominant or recessive (Škorić et al., 2010).

Orobanche cumana Wallr.

Despite the progress in production, there are factors that affect the normal development of sunflower. Considering that sunflower is grown around the world, one of the difficult issues is to ensure the correct development of the plant to provide a good yield without threatening abiotic or biotic factors. The most important threat limiting the growth and production of sunflower is a parasitic plant called sunflower broomrape (*Orobanche cumana Wallr.*), which can cause huge yield losses in sunflower crops in areas where the parasite is present (González, 2021). In addition, other disease factors such as sunflower rust, sunflower downy mildew are among the factors limiting sunflower development and yield. Sunflower broomrape is a parasitic plant that causes up to 100% decreases in sunflower yield in our country, European and Balkan countries. Thus, it causes rapid epidemics in sunflower production areas (Kaya, 2003). Ensuring effective and sustainable broomrape resistance is one of the most important goals of sunflower breeding programs (Fernandez-Martinez et al., 2010; Kaya et al., 2012b).

Sunflower grows therefore began to constantly search for a resistance gene that contains resistance to broomrape races. Thanks to molecular marker techniques, it has become quite possible to achieve these breeding goals, to determine the source of broomrape resistance, the gene region, and whether they are dominant or recessive (Škorić et al., 2010).

Sunflower broomrape is a diploid holoparasitic plant with 2n=38 chromosomes, which is completely dependent on the host plant to meet water and nutrients and is an obligate root parasite because they cannot photosynthesize. Broomrape seeds are easily spread by wind, water, insects or other agents and produce many small seeds, while their flowers form thousands of seeds and these seeds can remain in the soil for years in order to germinate again, which poses the greatest threat to producers (Prider et al., 2013, Castejon et al., 1991). It clings to the roots of the plant it hosts and meets the water, minerals and nutrients necessary for its life thanks to it. Therefore, significant growth and yield decreases are experienced in the host plant. Despite the development of sunflower resistant to broomrape in case of differences in climatic conditions and environmental changes, the formation of new physiological races poses a major problem. (Aksoy and Pekcan 2014).

Yield loss caused by broomrape in sunflower is not only a decrease in grain yield; in addition, it negatively affects many areas such as the oil and protein ratio in the grain, the weight of a thousand grains, the plant height, the size of the head, the yield and quality ratio per plant. Sunflower cultivation in our country started in the Balkans and later on, it was started to be cultivated throughout the country. The problem of broomrape, which is experienced with yield and quality losses in sunflower production in our country, was first seen in 1956 (Demirbaş, 2006). The average yield, which was 86-100 kg/da between 1951-1955, decreased to about 60 kg in 1956. Although it is known that the breeds currently found in our country are F, G and H races, the first races could not be identified (Kaya et al. 2004, Kaya et al. 2012, Kaya, 2016, Molinera-Ruiz et al. 2014). Evaluation of the germplasm of *Helianthus* species to create resistance against different races of broomrape showed that in wild sunflowers, broomrape is the main source of resistance genes that provide resistance to new virulence races. In addition, very valuable sources of resistance were observed in the cultured sunflower germplasms. In Romania, Vranceanu et al. (1980), it was revealed that there are five broomrape races, dominant genes including broomrape resistance against these known races were determined, and a "race differential set" was created for Or1, Or2, Or3, Or4 and Or5 genes to facilitate the identification of races. Broomrape was not considered a major problem for sunflower cultivation until the F race was introduced in Spain. In 1995, the F race, which Or resistance genes could not control, was detected and its spread occurred very rapidly (Alonso et al., 1996; Dominguez, 1999). In studies to control the F race, the presence of genes containing resistance to the F race was detected in the germplasm of the cultured sunflower and wild sunflower populations (Sukno et al., 1999; Fernández-Martínez et al., 2000; RodríguezOjeda et al., 2001). A new race, race G, has begun to emerge, killing off race resistant to race F controlled by Or genes for more than 20 years (Molinero-Ruiz et al., 2015). Recently, it has been stated that a new race, H, has emerged in Romania (PacureanuJoita et al., 2009), Russia (Antonova, 2014) and Turkey (Kaya et al., 2009). In addition, in recent studies, it has been observed that the race seen in Şahinköyü region in the Thrace region exhibits a different infestation pattern from other races and this race is called the I race (Yonet et al., 2018).

Table 1. *Orobanche cumana* races known in some countries (Molinero-Ruiz et al. 2015).

Country	Determined *O. cumana* races		References
	Past	Current races	
Bulgaria	A,B,C,D,E	E, F, G	Shindrova 2006, Batchvarova 2014
Chinese	A	A,B,C,D,E,F,G	Ma and Jan 2014, Shi et al. 2015
France	Not available	Unknown	Jestin 2012, Jestin et al. 2014
Hungary	A, B, C, D	E, F	Zoltan 2001, Hargitay 2014, Molinero-Ruis et al. 2014
Kazakhistan	Unknown	C, G	Antonova 2014
Moldova	B, C	E, F	Gisca et al. 2013, Duca 2014
Romania	A, B, C, D, E	F, G	Vrancanu et al. 1980, Pacuranu- Joita et al. 2008, Pacuranu 2014
Russia	A, B, C, D	D, E, F, G, H	Tolmachyov 1990, Antonova et al. 2009; 2013, Antonova 2014
Serbia	B, E	E	Mihaljcevic 1996, Miladinovic et al. 2014
Spain	B, C, D, E	E, F	Gonzales- Torres et al. 1982, Melero- Vara et al. 1989, Saavedra Del Rio et al. 1994, Alonso et al. 1996, Molinera- Ruiz et al. 2006, Fernandez- Escobar et al. 2008, Molinera- Ruiz and Dominguez 2014
Turkey	D, E	F, G, H, I	Kaya et al. 2004, Kaya et al. 2012, Kaya 2016, Molinera- Ruiz et al. 2014, Yonet et al., 2018
Ukraine	A, B, C, D	E, F, G, H	Tolmachyov 1990, Pototskyi 2014, Maklik et al., 2018

Vranceanu et al. (1980); S-1358, Kruglik-A-41, Jdanov 8281, P-1380 and Record indicated that Or1 and Or5 genes were dominantly resistant to A-E broomrape races in their studies with sunflower plants. Sukno et al., 1999 and Perez-Vich et al., 2004 stated that the dominant Or5 gene contains resistance to race E. It was stated by Rodríguez-Ojeda et al. in 2001 and, Akhtouch et al. in 2002 that the two dominant genes, Or6 and Or7, provided resistance to the E race. Perez-Vich et al. (2002), Pacureanu et al. (2004) and stated that it is controlled by the dominant allele of the Or6 gene (Guchetl et al., 2019). In studies with species with different sources of resistance, Velasco et al. (2007) said that these two genes are partially dominant; Rodríguez-Ojeda et al. (2001) stated that these genes are dominant. Examination of sunflower germplasm for the determination of resistance genes to broomrape races reveals that wild sunflower species constitute an important source of resistance to newly formed virulence races of broomrape.

Wild Sunflower Gene Resources and Broomrape

Wild sunflowers are perennial and annual plants that occur wild in the northern regions of Canada, the western United States, and Mexico. Their spread is realized by natural events or animals. They are highly competitive with other self-grown wildflowers for the water, sunlight and nutrients in the soil necessary for their growth and development. In general, wild sunflowers; It has a flower head of varying widths with yellow petals and a red-brown central disc. Their leaves may vary along their stems and may have a rough, green, egg-shaped or heart-shaped, itchy texture, depending on the species. Hairiness can be seen on the leaves or stems of these plants. Wild sunflower stems are slightly tilted and flower heads follow the sun throughout the day. Its flowers are known to consist of small seeds 2 to 3 inches wide. Wild sunflowers have been grown since at least 1000 BC. In the western part of the USA, Native

Americans used sunflowers as a direct food, in addition to using sunflowers as a food source, crudely extracting the oil and bark as a source of dye, the leaves for herbal medicine, and the pollen for religious ceremonies (Putt, 1997 and Seiler, 2017). When Europeans came to settle in that area, they started to plant wild sunflowers for their oil and appearance; They have increased the popularity of sunflower oil and for this purpose, it has started to be cultivated. Due to the inability to meet the adequate food needs with the increase in population, breeding efforts have increased and efforts have been made for better seeds and better plants. It is thought that wild gene sources should be used as well as various scientific methods for diseases and pests that increase over time. For this purpose, the wild sunflower gene bank collection was created in Bushland, Texas in 1976. Moved in 1985 and now NCRPIS (North Central Regional Factory Entry Station) is located in Ames, Iowa. The wild species collection includes all 51 species and subspecies, 14 annual and 37 perennials species (Schilling, 2006; Stebbins et al., 2013). Classification of annual and perennial *Helianthus* species in the tables below and USDA-ARS for each species; The number of participations in the NPGS sunflower gene bank collection is given.

"The first report of broomrape resistance was obtained from cultivars Progress and Novinka developed using the Group Immunity breeding approach with germplasm derived from wild perennial *H. tuberosus*." (Pustovoit and Gubin, 1974). Several researchers showed that sunflower germplasm studies for resistance to *Helianthus spp*. constitute an important gene reservoir providing resistance to new virulence races (Fernández-Martínez et al., 2000, 2010; Nikolova et al., 2000; Terzic et al., 2010; Bervillé, 2002; Škorić and Pacureanu-Joita, 2011; Antonova et al., 2011; Christov, 2013). Resistance to E, F, G races were found in wild sunflower species. In 1995, a new fast-spreading broomrape race, race F, which spread rapidly in Spain, overcomes genes previously known to contain resistance (Alonso et al., 1996). However, Sukno et al. (1998) reported that perennial *H. resinous, H. giganteus, H. pauciflorus* and *H. laevigatus* species showed resistance to this new race in Spain. The perennial *Helianthus* species *H. maximiliani, H. divaricatus* and *H. grosseserratus* crosses with cultivated sunflowers and improved populations have greatly contributed to the development of sunflower varieties through interspecies hybridization. In *H. tuberosus*, the presence of Or5 and Or6 gene regions was determined. (Škorić et al., 2010). The transmission of resistance created by the dominant gene against the F race was investigated by using two perennial species, *H. grosseserratus* and *H. divaricatus,* and cultured *H. annuus* (Pérez-Vich et al., 2002). Petcu and Pacureanu (2011) mentioned that the hybrids obtained with *H. argophyllus* contain resistance to E and F races found in Romania.

Christov (2013) mentioned "that resistant to broomrape races A to G of 17 species of wild perennial sunflower, *H. tuberosus, H. pauciflorus (= rigidus), H. eggertii, H. × laetiflorus, H. decapetalus, H. hirsutus, H. divaricatus, H. giganteus, H. maximiliani, H. nuttallii ssp. rydbergii, H. salicifolius and H. smithii and* annual *H. annuus* (wild), *H. argophyllus, H. debilis, H. petiolaris and H. praecox*. Perennial diploid species *H. divaricatus, H. giganteus, H. glaucophyllus, H. grosseserratus, H. mollis, H. nuttallii* and *H. smithii* and their interspecies hybrids have been reported to be resistant to broomrape" (Nikolova et al., 1998). Thus, they achieved extraordinary results with interspecies hybridizations. However, the G race, which was seen as a more lethal strain by overcoming the F race resistant cultivars, has been identified (Škorić et al., 2010). Recently, it has been observed that there is resistance to race G studies with *H. divaricatus*. (Velasco et al., 2012, Cvejic et al., 2012). The following table lists wild annuals that are resistant to broomrape races. (Seiler, 2015).

Table 2. The classification of annual *Helianthus* species and accessions in the USDA-ARS, NPGS sunflower gene bank collection **(**Schilling ve Heiser, 1981; Schilling, 2006).

Chromosome Number	Species	Common Name	Number of Attendance
Helianthus			
(2n=34)	*H. annuus L.*	Annual, Prairie	929
(2n=34)	*H. anomalus Blake*	Anomalous	6
(2n=34)	*H. argophyllus T.&G.*	Silver-leaf	51
(2n=34)	*H. bolanderi A. Gray*	Bolander's, Serpentine	14
(2n=34)	*H. debilis ssp. debilis Nutt.*	Beach	12
(2n=34)	*H. debilis ssp. cucumerifolius (T.&G.) Heiser*	Cucumber-leaf	11
(2n=34)	*H. debilis ssp. sylvestris Heiser*	Forest	22
(2n=34)	*H. debilis ssp. tardiflorus Heiser*	Slow-flowering	9
(2n=34)	*H. debilis ssp. vestitus (Watson) Heiser*	Clothed	3
(2n=34)	*H. deserticola Heiser*	Desert	21
(2n=34)	*H. exilis A. Gray*	Serpentine	30
(2n=34)	*H. neglectus Heiser*	Neglected	22
(2n=34)	*H. niveus ssp. niveus (Benth.)*	Snowy	1
(2n=34)	*H. niveus ssp. tephrodes (Gray) Heiser*	Ash-Colored, Dune	11
(2n=34)	*H. paradoxus Heiser*	Pecos, Puzzle, Paradox	12
(2n=34)	*H. petiolaris ssp. canescens (A. Gray) E.E. Schilling*	Gray	20
(2n=34)	*H. petiolaris ssp. fallax Heiser*	Deceptive	31
(2n=34)	*H. petiolaris ssp. petiolaris Nutt.*	Prairie	103
(2n=34)	*H. praecox ssp. hirtus Heiser*	Texas	7
(2n=34)	*H. praecox ssp. praecox Engelm. & A. Gray*	Texas	8
(2n=34)	*H. praecox ssp. runyonii Heiser*	Runyon's	26
Agrestis			
(2n=34)	*H. agrestis Pollard*	Rural, Southeastern	10
Porteri			
(2n=34)	*H. porteri (A. Gray) J. F. Pruski*	Confederate Daisy, Porter's	9

Table 3. The classification of perennial *Helianthus* species and accessions in the USDA-ARS, NPGS sunflower gene bank collection (Schilling, 2006; Stebbins et al., 2013).

Chromosome Number	Groups	Species	Common Name	Number of Attendance

Ciliares	**Ciliares**			
(2n= 34)		*H. arizonensis R. Jackson*	Arizona	2
(2n= 68, 102)		*H. ciliaris DC.*	Texas blueweed	32
(2n= 34)		*H. laciniatus A. Gray*	Alkali	7
Ciliares	**Pumili**			
(2n= 34)		*H. cusickii A. Gray*	Cusick's	23
(2n= 34)		*H. gracilentus A Gray*	Slender	14
(2n= 34)		*H. pumilus Nutt.*	Dwarfish	59
Atrorubens	**Coronasolis**			
(2n= 102)		*H. californicus DC.*	California	22
(2n=34,68)		*H. decapetalus L.*	Ten-petal	30
(2n= 34)		*H. divaricatus L.*	Divergent	28
(2n=102)		*H. eggertii Small*	Eggert's	13
(2n=34)		*H. giganteus L.*	Giant	26
(2n=34)		*H. grosseserratus* Martens	Sawtooth	48
(2n=68)		*H. hirsutus Raf.*	Hairy	12
(2n=34)		*H. maximiliani Schrader*	Maximilian	68
(2n=34)		*H. mollis Lam.*	Soft, Ashy	28
(2n=34)		*H. nuttallii ssp. nuttallii T.&*	Nuttall's	25
(2n=34)		*H. nuttallii ssp. rydbergii (Britt.) Long*	Rydberg's	12
(2n=102)		*H. resinosus Small*	Resinous	23
(2n=34)		*H. salicifolius Dietr.*	Willow leaf	19
(2n=102)		*H. schweinitzii T.&G.*	Schweinitz's	1
(2n=68, 102)		*H. strumosus L.*	Swollen, Woodland	33
(2n=102)		*H. tuberosus L.*	Jerusalem artichoke	92
Atrorubens	**Microcephaly**			
(2n=34)		*H. glaucophyllus Smith*	White leaf	12
(2n=34)		*H. microcephalus T.&G.*	Small-headed	14
(2n=34, 68)		*H. smithii Heiser*	Smith's	7
Atrorubens	**Atrorubentes**			
(2n=34)		*H. atrorubens L*	Purple-disk	14
(2n=34)		*H. occidentalis ssp. occidentalis Riddell*	Few leaf, Western	5

(2n=34)		*H. occidentalis ssp. plantagineus (T. &G.) Heiser*	Branching, Western	12
(2n=102)		*H. laetiflorus Pers.*	Mountain	11
(2n=102)		*H. pauciflorus Nutt. ssp. pauciflorus*	Stiff	21
(2n=102)		*H. pauciflorus ssp. subrhomboides (Rydb.) O. Spring*	Stiff	17
(2n=34)		*H. silphioides Nutt.*	Odorous	15
Atrorubens	**Angustifolii**			
(2n=34)		*H. angustifolius L.*	Narrow leaf, Swamp	28
(2n=34)		*H. carnosus Small*	Fleshy	5
(2n=34)		*H. floridanus A. Gray ex Chapman*	Florida	9
(2n=34)		*H. heterophyllus Nutt.*	Variable leaf	17
(2n=34)		*H. longifolius Pursh*	Longleaf	3
(2n=34)		*H. radula (Pursh) T.&G.*	Scraper, Rayless	40
(2n=34)		*H. simulans E. E. Wats*	Muck, Imitative	7
(2n=34)		*H. verticillatus Small*	Whorled	5
(2n=34)		*H. winteri Stebbins*	Winter's	5

Table.4. Resistance to Wild Perennial Species and Broomrape Races

Species	Races
H. annuus	E
H. anomalus	E-F
H. argophyllus	F
H. debilis ssp. tardiflorus	G
H. deserticola	E-F
H. exilis	E-F
H. petiolaris	A-G
H. praecox	A-G

Molecular Markers and Genetic Characterization

Morphological broomrape tests do not produce always reliable results due to the effect of environmental factors, insufficient amount of broomrape seeds in the soil, difficulty in collecting broomrape seeds, and seed germination problems, etc. Constant changes in the composition of broomrape races have forced sunflower breeders to constantly search for resistance genes for new broomrape races. In order for sunflower breeders to identify the

sources of broomrape resistance and whether these gene sources are dominant or not, it is necessary to identify the broomrape race and to provide the germplasm and differential lines that need to be known, as well as to select the appropriate grafting method and molecular marker technique (Škorić et al., 2010). One of the important challenges in sunflower breeding is to identify the races containing new resistance sources and develop molecular markers to identify these resistance sources and to identify the 'Or' genes. Recent studies in MAS, in particular, have proven that new molecular techniques can be very valuable in sunflower breeding programs to speed up and facilitate resistance gene identification, broomrape resistance breeding (Kaya, 2014).

The development of cultivated sunflower is possible by using a large number of molecular markers and generating multiple linkage maps of varying intensities (Knapp et al., 2001). The most reliable method and easily practice to screen breeding materials for broomrape resistance utilize from molecular markers. RFLP (Restriction fragment length polymorphism), QTL (Quantitative trait locus), TRAP (Target Region Amplification Polymorphism), RAPD (Random Amplified Polymorphic DNA) and SSR (Simple Sequence Repeats) markers use for this aim so far (Imerovski et al., 2013; Pérez-Vich et al., 2013). Markers are generally used to identify specific genomic regions. Three types of markers are used in genome analysis and genetic studies: morphological, biochemical and DNA markers. After the discovery of the polymerase chain reaction (PCR), PCR-based markers started to be preferred more in genetic studies. Genetic characterization studies are very important for studies such as determining the level of genetic diversity within and among populations, development of conservation programs, determining of domestication.

Mitochondrial DNA, different biochemical marker systems, Y chromosome specific markers and alloenzymes are used in genetic characterization studies. Polymorphic microsatellite markers (DNA marker) constitute the most preferred marker system in PCR applications. Random repeat sequences occurring in a row at the locus in the genome called STR (Short Tandem Repeat). Markers consisting of 1–6 bp repeats of STRs are called microsatellite markers or simple sequence repeats (SSR-"Simple Sequence Repeat") (Weber and May, 1989; Liu, 1998). The number of repeats of microsatellites is usually less than 100 (Liu, 1998). Microsatellites show their presence in any region of the eukaryotic and prokaryotic genome. Although microsatellite markers generally consist of 2-nucleotide repeats [(CA)n], there are also types with higher repeat numbers (AC, AT, AAC, AAT, CCG, etc.) (Orti et al., 1997; Ellegren et al., 1997; Bruford et al., 2003). STR markers have become the most preferred markers in genetic studies because they are carried out using PCR technology (Weber and May, 1989; Liu, 1998; Metta et al., 2004).

Microsatellites are preferred in many molecular biology studies because they are widely found in the genome, have a high rate of polymorphism and are easy to use. Recently developed new molecular biology techniques allow the analysis of single nucleotide polymorphisms faster and more economically utilizing from microsatellites (Özşensoy and Kurar, 2012). Molecular studies for race characterization and mapping have been carried out before. For example, Pacureanu et al. (2009) stated that resistance to F race is determined by two recessive genes or a dominant gene in inbred lines depending on their origin. This case show that the markers are specific to the developed genetic materials (Imerovski et al., 2011). Berry et al. (1995, 1996) and Gentzbittel et al. (1995, 1999) created RFLP maps for the first time in sunflower. A linker group containing the Or5 gene that utilizes RFLP markers for the characterization of broomrape races was combined with the GIE Cartisol RFLP map. In addition to working on the development of markers for Or5, it is desirable to map a new gene that confers resistance to the more virulence broomrape races than the F race (Cvejić et al., 2012). Molecular studies have been conducted to map the genes that confer resistance to the E and F races. The Or5 gene,

known to contain race E resistance, has been mapped to (LG) 3, a telomeric linkage group of the sunflower genetic map (Lu et al., 2000; Tang et al., 2003; Pérez-Vich et al., 2004b). The linkage between the markers on this linkage group and the three different broomrape resistance genes indicates that the Or genes are closely related and on the same gene family. Resistant and susceptible genotypes were compared molecularly, and a polymorphism was detected in LG3 of the SSR map. This result shows that new resistance genes are on this gene family (LG3) (Imerovski et al., 2012).

Further testing on the mapping population will enable the development of the specific molecular marker that will determine the precise position of the gene and accelerate the development of resistance to new sunflower lines, commercial sunflower lines. Tang et al. (2002) created the first linkage map using SSR markers in sunflower, followed by Yu et al. (2003) used a new population of recombinant inbred lines to increase the density of the map created with SSR markers. Joel et al. (2004) stated the necessity of molecular markers for the study of sunflower broomrape. Imerovski et al. (2013) examined twenty sunflower genotypes resistant to SSR markers and different races of broomrape (A-F) and found a significant relationship between the ORS1036_240 and ORS1114_265 alleles of the Or6 gene by electrophoresis analysis. Pineda-Martos et al. (2014) studied 50 populations of broomrape in Spain using 15 microsatellite markers and noted the existence of two distant gene pools, one in the province of Cuenca and the other in the Valley of Guadalquivir. They stated that inter-gene and inter-population variability is low within gene pools and genetic recombination between distant gene pools is important in terms of creating a new variation and having an effect on race development. Again, Pineda-Martos et al. (2014) used 4200 simple sequence repeat (SSR) markers to characterize broomrape and 217 SSR primer pairs were used for validation. Of them, 87 SSR primers showed polymorphism among 18 populations of broomrape taken from different points, that is, they showed reproducibility at the desired size, producing high quality amplicons. Rieseberg et al. (1995) studied the effects of chromosomal structural changes with the use of RAPD markers, Burke et al. (2004) created the SSR/RAPD genetic linkage map, and some researchers (Gedil et al., 2003; Langar et al., 2003) created other genetic maps using AFLP markers.

The virulence effects of broomrape populations in different parts of the world are different, and the most virulent parasitic races are in the Black Sea, which has more than 50% of the rapidly spreading sunflower plantations. Sunflower breeders and researchers have found resistance genes from some wild *Helianthus* races and then transferred these genes to cultivated sunflower cultivars to develop broomrape resistant hybrids. However, an international project is urgently needed to investigate and screen both the natural and artificial conditions of wild sunflower species against the rapid changes in the racial composition of the broomrape races, and the new wild broomrape and molecular screening races (Kaya, 2014).

RESULTS AND DISCUSSION

Sunflower broomrape (*Orobanche cumana Wallr*.) is a parasitic plant that causes up to 100% decreases in sunflower yield in our country, European and Balkan countries, and creates many races. Because it contains very small and many seeds, it spreads easily and thus causes rapid epidemics in sunflower production areas (Kaya, 2003). Ensuring effective and sustainable broomrape resistance is one of the most important goals of sunflower breeding programs (Fernandez-Martinez et al., 2010; Kaya et al., 2012b). Molecular markers are the most reliable and accurate address, as it takes a long time to phenotypically detect resistant varieties

containing resistance to broomrape, it is not reliable and the detection of resistant gene regions is not morphologically possible. Molecular marker studies to identify Or gene regions and QTLs (Quantitative Trait Locus) associated with broomrape resistance genes provide knowledge of how genetic control of broomrape resistance is achieved in sunflower plants, facilitate the pyramid of different resistance genes, and accelerate the development of resistant inbred lines through marker assisted selection. (Fernández-Martínez, Domínguez, Pérez-Vich, and Velasco, 2010).

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LEVEL OF KNOWLEDGE AND AWARENESS OF UNIVERSITY STUDENTS ABOUT THE CORONAVIRUS PANDEMIC (COVID-19); AN ASSESSMENT FROM EDİRNE PROVINCE

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ABSTRACT

During the pandemic, stress is caused by the imbalance between the individual's perception and external environmental demands. Studies have shown that psychological stress is closely related to anxiety, depression, and physical conditions such as cardiovascular diseases and cancer. Students at universities face many stressors, including the volatile environment, lifestyle changes, academic burdens and interpersonal relationships, all of which can lead to significant psychological dysfunction. In particular, they are vulnerable to the stress that most university students in developing countries have to cope with, for themselves and their families, to fight the Covid-19 infection. The sample of this descriptive cross-sectional study consisted of 141 undergraduate health science students from Trakya University in the north west region of Turkey. The study was conducted April 2021 to July 2021. The sociodemographic characteristics of the university students were evaluated. Health sciences students, from the perspective of the Turkish cultural context; we identified the stress levels that have been elevated due to Covid-19. In addition, from the high perceived stress levels due to Covid-19, which is associated with anxiety and depression; We have seen that school success is negatively affected in university students. Many issues related to health science students have been discussed many times in the literature. However, our current research in a goal-oriented context; "students who will work with infections and may even encounter new pandemics"; self-regulation and an approach that jointly develops the solution.

Keywords: developing country, Coronovirus pandemic (COVID-19), university students

INTRODUCTION

Since December 2019, the world is facing a new epidemic of infectious disease known as the Coronavirus disease (COVID-19), which has spread rapidly globally and has been declared a pandemic by the World Health Organization (Spinelli & Pellino, 2020).

Faced with this large-scale contagious threat, young people are under increasing psychological pressure. The literatur show that the younger generation experiences significant fear and panic, resulting in a significant negative psychological impact (Fofana et al., 2020; Tsamakis et al., 2020). Various studies conducted during the initial phase of the COVID-19 epidemic found that about one-third of respondents reported moderate to severe anxiety, with

more than half the psychological impact; showed that it rated moderate-to-severe (Tsamakis et al., 2020).

As it is known, while mental illnesses can show symptoms in the body, physical illnesses also have a direct effect on the mental state (Kaya et al., 2021; Baltacı, 2020). More than just a physical illness, COVID-19 is also an epidemic that has a major impact on the biopsychosocial health of young people.

Psychological stress reflects a subjective assessment of one's ability to cope with demands. People experience stress when they perceive that their resources are insufficient to cope with a situation.

Students at universities; face many stressors, including a volatile environment, lifestyle changes, academic burdens, and interpersonal relationships, all of which can lead to significant psychological dysfunction. In particular, they are vulnerable to the stress that most college students in developing countries have to deal with for themselves and their families to fight the Covid-19 infection. The aim of our study; The aim was to provide a contribution to the psychosocial assessments of health science students regarding the Covid-19 pandemic.

MATERIAL AND METHOD

The sample of this descriptive cross-sectional study consisted of 141 undergraduate health sciences students from Trakya University in the northwest region of Turkey. Permission for the study was obtained from the Scientific Research Platform of the Ministry of Health of the Republic of Turkey. The study was carried out between April 2021 and July 2021. For our study, first of all, online one-to-one interviews were conducted via Microsoft Teams. Then by the participants; Microsoft online forms based on scale were filled.

The study was done on a voluntary basis. The age range of the students was 18-28. University students were selected for this study because they constitute a well-educated segment of the society. It is easier for educated individuals to accept or understand the importance of Covid-19. First of all, the sociodemographic characteristics of the participating volunteer students were evaluated. Those with psychological and neurological diseases were not included in the study. Care was taken not to include actively infected patients or those with isolated contacts.

Scale for Evaluating the Mental and Psychosomatic Effects of the COVID-19 Pandemic

The Scale for Evaluating the Mental and Psychosomatic Effects of the Covid-19 Pandemic was used (KAYA, KIRLIOĞLU, & TOPTAŞ, 2021). Considering the psychosomatic effects of COVID-19; The "Scale for Evaluating the Mental and Psychosomatic Effects of the COVID-19 Pandemic" takes its place in the literature as a valid and reliable measurement tool.

The Scale of Evaluation of Mental and Psychosomatic Effects of COVID-19 Pandemic; It contains 18 items. It is a 5-point Likert-type scale consisting of two sub-dimensions named "Moral Effects" and "Psychosomatic Effects" (Kaya et al., 2021). A minimum of 18 points and a maximum of 90 points can be obtained from the scale. The participant's high score from the whole scale and its sub-dimensions; This means that the negative mental and psychosomatic effects of the Covid-19 on the participant are high. There is no reverse coded item in the scale.

Why was this scale used in the study?

In the national and international literature, studies have been carried out to evaluate the organic and psychological effects of Covid-19 in many individual and social areas (KAYA et al., 2021). Various scales have been developed to measure the participants in the dimensions of fear, anxiety, and impulse in the psychological context. Unlike other scales, this scale provides the opportunity to evaluate the psychosomatic dimension in the field of mental and nervous health, specifically for Covid-19.

RESULTS

University students were selected for this study because they constitute a well-educated sector of society. Acceptance or understanding the importance of Covid-19 is easier for educated individuals. The age range of the students was 18-28 years.

In the study; the number of female participants was 92; male participants were 49. The mean score of the girls for the Scale for Evaluating the Mental and Psychosomatic Effects of the Covid-19 Pandemic was 75.50 (± 5.84). No statistical difference was found for the scale in terms of gender (p= 0.761).

When the participants are evaluated according to their hometowns; There was no statistically significant difference between scale scores (p= 0.365). When evaluated in terms of class levels, there was no significant difference between classes for the scores obtained from the scale (p= 0.624).

It was analyzed according to socioeconomic level. The mean scale score of 20 people with a low family income was 68.02 (± 7.85); 23 people who were quite good had a scale score of 44.32 (± 5.62); the mean score of the middle-income scale (n=98); It was 57.43 (± 8.46). No statistical significance was found (p=0.827). No significant relationship was found between the increase in the number of siblings and the scale scores of the participants (p=0.862).

In health sciences university students, within the framework of the cultural context of young people; We have detected the stress levels rising due to Covid-19. There were also high levels of perceived stress due to Covid-19, which is associated with anxiety and depression. University students stated that they think that their school success is affected negatively as a general judgement. However, our current research in a target-oriented context; "a generation that may encounter pandemics with infections and different temporal pandemics"; evaluation was made. It was observed that an approach that jointly develops self-regulation and solutions should be supported by young people.

It should not be forgotten that the soul and the body are one. The psychosomatic aspect of the Covid-19 Pandemic should be taken into account in the whole society, especially in the youth. The second important result of this study is that young people who will have a job in the context of a target-oriented change are prepared for acute negativities in society.

Our study evaluated the perceived stress against Covid-19, especially in Turkish health sciences university students. Our study revealed that university students in northwest Turkey have high levels of perceived stress, and this correlates with anxiety and depression. It is necessary to integrate solution-oriented thinking into basic education in the education of young people. This model will prevent students from spending too much time on problem analysis. They need to be careful about building a solution-oriented thinking ability.

DISCUSSION

During the Covid-19 pandemic, those at risk of psychological distress spread to the general population. However, experience shows that the psychological pressure on health workers who find themselves at the forefront of efforts to suppress the epidemic is significant (Spinelli and Pellino, 2020). Studies have shown that Covid-19 is significantly to moderately associated with anxiety and depression in participants.

Indeed, studies in developing countries; It has been shown that university students have high levels of stress and depression due to the Corona (Covid-19) pandemic.

Health professionals, in pandemic periods; they feel the pressure to act on time and successfully diagnose, isolate and treat cases (Yu, Lou, & Zhang, 2020). In terms of health workers; it becomes more difficult for them to do their job, especially by being caught between the increasing public pressure and media criticism day by day. In addition, front-line healthcare workers are under long-term psychological pressure, as the risk of exposure to the virus is greater than that of the population. Health professionals are concerned about bringing the virus to their families and carrying it home (Simione & Gnagnarella, 2020; Yu et al., 2020).

Health professionals also deal with patients and their relatives who do not help in coordination or do not follow the instructions of infection committees; indicate an increase in stress levels. In the context of limited intensive care beds and health care resources, feelings of helplessness arise. Indeed, past research has shown that problem-focused coaching approaches are less effective than solution-focused approaches in promoting well-being and facilitating goal progression. (Grant et al., 2012; Simione and Gnagnarella, 2020).

The disease is spreading rapidly, especially in countries where there is insufficient infrastructure to identify the virus using real-time PCR diagnostic tools and public health infrastructure to implement and quarantine (Kelvin and Rubino, 2020). In our study, in parallel with the literature; health science students; shows that in line with the pandemic reports, willingness to work is not really affected (Johnson et al., 2020).

University students may also have different perceived psychological pressures depending on their education level. However, in our study, the participants; After high school, they received similar education at similar ages. Second, construct validity was limited to reported measure comparisons. Therefore, these questions should be addressed in various populations in our country and around the world, and more objective measures should be applied to improve the psychometric quality of the results.

Studies have shown that Covid-19 was significantly and moderately associated with anxiety and depression in participants (Kelvin & Rubino, 2020). Our study, as far as we know, is the first to evaluate the perceived stress against Covid-19 in Turkish health sciences university students. The study revealed high levels of perceived stress among university students in northwest Turkey, which correlated with anxiety and depression.

Past research has shown that problem-focused coaching approaches; It has shown to be less effective than solution-focused approaches in increasing well-being and facilitating goal progress (Grant et al., 2012). The second important result of the study; The importance of

support in the development of young people who will become healthcare professionals in the context of targeted change against acute situations. It is necessary to integrate solution-oriented thinking into basic education in the education of young people. This model suggests that students be mindful of building a solution-oriented thinking ability rather than spending too much time on problem analysis.

Many issues related to health science students have been discussed many times in the literature. However, our current research reminds us that students who will work with infections and even encounter new pandemics in a targeted context should adopt an approach that fosters self-regulation, self-regulation, and resolution collaboratively.

In order to facilitate the development of university students in the context of change in national and international health events, solution-oriented thinking should be supported.

CONCLUSIONS

Developing countries may be the weakest part of the chain to stop the spread of current and future epidemics. As much as possible should be invested in education in the field of health to prevent millions of people from dying from pandemics. We suggest that for satisfactory psychometric results of Turkish university students, it should be widely applied to health sciences university students in other parts of the country as well.

However, due to differences in university levels and social, economic and cultural differences between regions, additional studies should be conducted in these regions to confirm the generalizability of our results. Although all of our study participants were health sciences university students, different undergraduate students may have different levels and types of stress, which may require different assessments. For example, middle school, high school or graduate students may face higher psychological stress due to the accumulating Covid-19 burden and media pressures.

Therefore, these questions should be addressed in various populations in our country and around the world, and objective, wide-ranging studies should be applied to increase the psychometric quality of the results.

Scientific confirmation

Approval for the study was obtained from the Scientific Research Board of the Ministry of Health of the Republic of Turkey before the study.

Ethical approval

Ethical approval for the study was obtained from the Turkish Republic Trakya University Scientific Research Ethics Committee before the study.

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MOLECULAR PHLOGENETIC ANALYSIS AND GENETIC CHARACTERIZATION OF WILD SPECIES IN SUNFLOWER (*HELIANTHUS SPP.*)

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ABSTRACT

Sunflower (*Helianthus spp*.) is a family of *Asteraceae* family. Most wild species are of North American origin. This plant, which has a very beautiful appearance, was known as an ornamental plant in Europe before, but it was started to be used to increase the oil rate in Russia in the 18th century. There are many wild varieties of this plant, which originated in ancient times. The most common and important is *Helianthus annuus*. Wild Sunflower species each have their own characteristics; they are adapted to a wide variety of habitats. They have genetic

diversity that can be a sufficient source of allele for the continuous improvement of cultivated sunflower. They are very useful in terms of increasing hybrid plant production today. Wild *Helianthus* species are potential sources of genetic variability. They have provided many genetic resources in production for increased quality and yield, such as drought resistance, disease resistance, soil salinity resistance, resistance to biotic and abiotic stress sources, adaptation to weak soils. These beneficial genes from wild species have expanded the narrow genetic basis of the cultivated sunflower, to provide a permanent source of the desired agronomic properties, to develop the cultivated sunflower. Useful genes that play a role in the higher efficiency and better quality performance of hybrids obtained by expanding the genetic material capacity, increasing the heterology, durability and cross-species hybridization should definitely be utilized. They are very necessary in line with increasing needs. To develop new sunflowers, germplasms must be preserved and preserved. Because they are accepted as a model organism for cultivated sunflower. A phylogeny dissolved in the genus is essential for your studies in this area. Molecular phylogenetic studies have contributed significantly to our understanding of *Helianthus*' phylogenetic relationships.

Keywords: *Helianthus*, wild species, phylogenetically, marker

INTRODUCTION

Sunflower (*Helianthus annuus L*) has high oil content (45-50%) and oil quality, it is an economically important plant among the industrial plants in the world and in our country. Sunflower is a species rich plant that shows great variation in many morphological features of *Helianthus* species from the *Asteraceae* (*Compositae*) family, and it is a plant that has perennial forms as well as annual forms (Fick and Miller, 1997). It is known that the origin of the sunflower is North America. But it also grows wild in many different regions. It was brought to Europe by the Spanish. Sunflower was primarily used for making flour and bread. It has also been used as a source of medicine, dye and oil (Fick and Miller, 1997).

There are 51 wild species of *Helianthus*, of which 14 are single and 37 are perennial. Annual *Helianthus* species are divided into 3 sections and consist of 14 species. *Helianthus* section consists of 12 species and Agrestes section consists of 14 species. Perennial *Helianthus* species consists of 2 sections, 6 series and 37 species. Of these sections, Ciliares consists of 2 series and includes a total of 6 species. Ciliares and Pumili series belonging to this section each contain 3 species. Atrorubens section consists of 4 series and 31 species. Among the series belonging to this section, Corona-solis series consists of 15 species, Microcephali series consists of 4 species, Atrorubentes series consists of 4 species and Angustifolii series consists of 8 species (Önemli, 2014). Chromosome number is n=17 in cultivated sunflower. In the genus *Helianthus*, there are diploid species as well as tetraploid and hexaploid species (Meral, 2019). All annual species are diploid. Among the perennial species, 27 are diploid, 4 tetraploid, 6 hexaploid and 4 mixed ploid.

Each of the types has different advantageous features. Each of these features is very important for the development of cultivated sunflower. These inter-species relations need to be evaluated and interpreted. For this, intraspecies molecular phylogenetic analysis is required. Today, with the development of molecular techniques, many conveniences are provided in studies. Especially molecular markers are powerful tools used in all organisms. In this way, plant

molecular systematics is also highly developed. They are more advantageous and preferred than other markers (morphological and biochemical), especially since they are applicable for any tissue, developmental stage, require very little material, and theoretically represent every point of the genome, regardless of environmental conditions (Tingey, 1993).

Microsatellite (SSR) Markers

SSR markers were used in this study capture. These markers are reproducible, fast, easy, and show high polymorphism. SSR markers are used for population analysis, creation of genetic maps and selection with the help of markers in plant species (Gupta and Varshney, 2000). Short tandem repeats, also known as microsatellites, are DNA sequences of 1-7 bases repeated in tandem that are short enough to reach approximately 150 nucleotides in length. The high number of alleles with different repetitions of these sequences in the population is one of the features that makes the DNA of living things polymorphic (Akbar, 2018). If the sequences of the regions surrounding the microsatellites (flanking region) are known, primers suitable for those regions can be contrive (usually 20–25 bp long) and amplified by PCR. Additionally, interspecies SSR primers can be used in different organisms. Sequence replication, mismatching and unequal crossing over events that occur during DNA replication are the main events that cause differences in the number of microsatellites and are expressed by gel electrophoresis.(Matsuoka et al., 2002)

Molecular Phylogenetics

It is a biological science that tries to make sense of the relationships and similarities at the molecular level between living things using genomic information. These relationships are called phylogeny. This word is of Greek origin and is derived from the terms elephant or filon and geneticos meaning "tribe, race". Phylogenetics refers to the evolutionary relationships between lineages of organisms or between parts of them, such as gene regions. There is a fundamental synergy between molecular systematics and molecular phylogenetics. It uses genetic markers to achieve molecular systematic results. Compares databases for specific gene regions. Molecular phylogenetics, on the other hand, uses this data by adding time and evaluates molecular change rates (Freeman and Herron, 1999).

Genomes evolve and differentiate through many mutations. At the end of this process, nucleotide sequence differences in the genomes of organisms occur. This nucleotide sequence difference may reflect the timing of the separation of the two genomes. By comparing different genomes, one can learn about the evolutionary relationship between them (Mount, 2001).

Phylogenetic classifications are also widely used to obtain genetic information necessary for more professional use of genetic resources in important crop plants, to obtain information about the status of changes in the genomic structure of living things over a long period of time and the point reached by these changes. There is no single and definitive result in phylogenetics, so many different methods are used. The kinship relations established between living things and phylogenetic data give us an idea about the development of life, kinship trees, that is, the path that life follows. The changes seen in this process help us understand the evolutionary process.

Molecular methods have also been used in genetic characterization to investigate the phylogeny of wild sunflower species and hybrids of sunflower.(Burke et al., 2004; Mwangi et al., 2019; Rieseberg and Doan, 1990; Schilling, 1997; Suresha et al., 2017) However, both morphological characterization and molecular phylogenetic analysis, including all species of wild sunflower,

have not been performed in the world, and the database to be obtained as a result of this study will form the basis for many future studies. By looking at the DNA sequence differences between sunflower varieties, the relationships between species or between species can be examined and phylogenetic trees can be formedA highly resolved Phylogenetic tree for sunflower was constructed for the first time using the nuclear 16S, 26S rDNA regions (Timme et al., 2007) Sunflower is a plant belonging to the Asteracae family. In a study conducted in China in 2016, a phylogenetic tree belonging to this family was extracted using three cpDNA regions (ndhF, matK, rbcL). (Fu et al., 2016) A study was conducted based on variation of nuclear genome size. An extensive database was created for 49 *Helianthus* and variability in the genus was examined. And a comparative phylogenetic analysis is presented (Qiu et al., 2019). Another study was conducted using SNPs with cultivated and common sunflower seeds obtained from the USDA North Center Regional Plant Promotion Station. (Park and Burke, 2020) There is also a study conducted with RFLP analysis. However, there is not yet a comprehensive phylogenetic study with the SSR marker including all *Helianthus* species.

Phylogenetic Trees

The graphical representation of the phylogenetic relationship detected between species is through phylogenetic trees. These phylogenies, which show the evolutionary relationships between organisms, are also known as the "evolutionary tree" or the "tree of life". Phylogenetic trees are also used in gene interaction, drug designs, pathogen strain diversity in vaccine studies, epidemiology of genetic diseases and infectious diseases, determination of the functions of new genes, and microbial ecology studies (Mount, 2001). Branching in a phylogenetic tree describes the patterns and times of events. It gives information about the kinship relationships of species, which species are closely related and which species are distant relatives, and the speciation period. The phylogenetic tree consists of two basic elements. The root (node) and branches (branch) are the two basic parts of the phylogenetic tree. Branches show changes over time in the ancestral population. Roots indicate the point at which one species splits into different species (Saitou and Imanishi, 1989). The task of determining the phylogenetic relationship of various organisms is difficult, as there is an incredible diversity of species in the world. This diversity can be not only phenotypically, but also structural, biochemical and molecular.

CONCLUSIONS

Species are the product of many processes throughout evolutionary history. Therefore, the structuring and interpretation of genres is very important. Knowledge the past ancestors of current varieties is essential for their development. Therefore, intraspecific phylogeny is required. It has not been fully resolved until now, the biggest reason for this is the difficulty in detecting and structuring both polyploid and diploid large hybrid species. Wild *Helianthus* species are potential sources of genetic variability. They have provided many gene sources in production for quality and yield increase such as resistance to drought, resistance to diseases, resistance to soil salinity, resistance to biotic and abiotic stress sources, adaptation to poor soils. These useful genes from wild species have broadened the narrow genetic basis of the cultivated sunflower, providing a permanent source of desirable agronomic traits to improve the cultivated sunflower. Beneficial genes that play a role in higher yield and better quality performance of hybrids obtained by expanding genetic material capacity, increasing heterosis, resistance and interspecies crossing must be utilized in sunflower production (Kaya et al., 2012).

As a result of a series of processes, phylogenetic analysis of species has become easier. In addition to the use of traditional methods such as morphological or biochemical methods in the analysis of phylogenetic relationships, the use of molecular methods is inevitable today. Phylogenetic analyzes are made and phylogenetic trees are created by using various markers and software. Then, these trees are evaluated and information about the evolutionary process is obtained. In this study, SSR marker was used because of its advantageous features such as high polymorphism, frequent presence in the genome and reproducibility. It is aimed to extract phylogenetic trees and genetic characterization for desired traits by using molecular methods in 54 different wild sunflower species. All wild species belonging to the genus *Helianthus* are accepted as a model organism for the cultivated sunflower *H. annuus*. Considering the economic and cultural importance of *H.annuus*, there is great interest in the breed from which it evolved. In order to advance in this area, a resolved phylogeny of the genus is required.

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MAIN PSYCHOSOCIAL FEATURES IN THE COVID-19 PANDEMIC FROM THE PERSPECTIVE OF PREMENOPOSAL AND POSTMENOPOSAL WOMEN

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ABSTRACT

The peri and postmenopausal period has long been an important requirement in the health science for the protection and promotion of women's health. Menopause is a transitional period characterized by fluctuating physiological changes that affect the quality of life of many women in the short term, along with long-term changes such as vasomotor symptoms, sleep and mood disturbances, as well as genitourinary symptoms and decreased bone density. The pandemic period carries potential concerns. The aim of the study is the Covid-19 pandemic process; evaluation of its effects on women in this period; It was desired to evaluate the necessity of conducting studies in clinical areas to develop basic public health support skills. Our study was carried out with women aged 50 – 65 years who came to Trakya University Hospital Gynecology outpatient clinic between August 2020 and July 2021 for routine control. After evaluating their sociodemographic characteristics; Solution-oriented inventory (DOE) was used. This scale was developed by Grant, Cavanagh, Kleitman, Spence, Lakota, and Yu (2012). In our study, it was observed that perimenopausal women avoided coming to health institutions. Vasomotor symptoms were among the complaints that most decreased their quality of life for women, but it was observed that they took a place in the back ranks among the reasons for applying to the hospital. During the pandemic period, it was observed that they had more difficulties in coping with menopausal problems. As the age progressed, the spectrum of finding solutions widened. Health care of perimenopausal women should be kept in mind, as menopause can pose a long-term risk to health. In addition, the experience of experts with great clinical experience should be examined. On the other hand, it should not be forgotten that perimenopausal and menopausal symptomatic women may delay seeking health care during the pandemic period. It is important to emphasize that this can lead to worsening of pre-existing diseases. Strategies to minimize these problems should be adopted and women should be provided with appropriate guidance to better manage their health.

Keywords: Menopause, perimenopause, women's health, elderly health, Covid-19 pandemic

INTRODUCTION

Menopause is characterized by long-term changes such as vasomotor symptoms, sleep and mood disturbances, as well as genitourinary symptoms and decreased bone density. Menopause, for women; It is a transition period that manifests itself with fluctuating physiological changes that can affect the quality of life of many women in the short term.

Women older than 45 years of age who experience vasomotor symptoms with irregular menses are presumed to be in perimenopause, and those who have not menstruated for more than twelve consecutive months are menopausal. The perimenopausal period includes challenging experiences for women. The pandemic period carries potential concerns in perimenopausal women (Dietz et al., 2020; Mehrotra et al., 2020).

All of the world is currently under the influence of the ongoing outbreak of coronavirus disease-2019 (COVID-19), which is caused by a novel coronavirus called severe acute respiratory syndrome coronavirus (SARS-CoV-2). COVID-19 has become a public health emergency of international concern. Current studies, advanced age and concomitant diseases; demonstrated to be associated with poor prognosis. Peri and postmenopausal periods, especially during the pandemic period, have been an important requirement in the protection and development of women's health in the health sector (Dietz et al., 2020; Kim et al., 2020).

Considering the COVID-19 epidemic all over the World and to reduce the risk of transmission of the virus, healthcare professionals; with many communication tools; They stated that it is not a good time to go to health institutions during the pandemic process. In women participating in obstetric and gynecological outpatient clinic visits; There was a decrease of approximately 46%. Among the older women (65-74 years old) who went to menopause clinics, there was a decrease of nearly 60% (Hipolito et al., 2020).

However, since menopause can pose a long-term and sometimes devastating risk to women's health, the professional support that should be given to the health care of these women should be kept in mind. Therefore, healthcare professionals should also ask themselves how to create better care for the health of this important segment of communities amid the pandemic.

Purpose of the study; Covid-19 pandemic process; The aim of this study was to evaluate its effects in women in the perimenopausal period. In addition, it was desired to evaluate the necessity of conducting studies in clinical areas in order to develop basic women's health support skills.

MATERIAL AND METHOD

Our study was carried out with volunteers aged between 48 and 65 years, who came to Trakya University Hospital Gynecology outpatient clinic between August 2020 and July 2021 for routine control. After evaluating their sociodemographic characteristics; Solution-oriented inventory (DOE) was used.

The Solution-Focused Inventory (SFI);

The Solution-Focused Inventory (SFI) was developed by Grant, Cavanagh, Kleitman, Spence, Lakota and Yu (Grant et al., 2012). They found that this 12-item scale was negatively correlated with The SFI is a scale consisting of 12 items and filled by the client himself. It is a 6-point Likert type scale (1= strongly disagree, 6= strongly agree).

The sub-dimensions of Solution-Focused Inventory are: First, separation from the problem: 1.,2.,4.,5. substances, The second is goal orientation: 9th,10th,11th,12th. substances, Third, mobilize resources: 3.,6.,7. and 8 items. 1,2,4,5. Items are reverse scored.

SFI has been found to be a reliable and valid measure of solution-oriented thinking and is associated with perspective-taking capacity, flexibility, and psychological well-being (Grant et al., 2012). Examples of items include “setbacks are a real opportunity to turn failure into success”, “There are always enough resources to fix a problem if you know where to look” and “Every problem always has a solution.” have such definitions. The scale consists of 12 items and is filled by the client herself. It has been found that the scale is a reliable and valid measure of solution-oriented thinking and is associated with perspective-taking capacity, flexibility, and psychological well-being.

Necessary permissions were obtained from the Scientific Research Board of the Ministry of Health of the Republic of Turkey for the study. Approval for the study was obtained from Trakya University Scientific Research Ethics Committee.

RESULTS

In our study, it was observed that perimenopausal women avoided coming to health institutions. Vasomotor symptoms were among the complaints that most decreased their quality of life for women, but it was observed that they took a place in the back ranks among the reasons

for applying to the hospital. During the pandemic period, it was observed that women had more difficulties in coping with perimenopausal problems.

Those with uncontrolled Diabetes Mellitus, uncontrolled Hypertension, Acute/Chronic kidney failure, Acute/Chronic liver failure, Neurological deficit, Psychiatric disease were not included in the study. The study was conducted on a voluntary basis, through face-to-face interviews with the participants.

When the participants are evaluated; As the age progressed, the spectrum of finding solutions widened. Health care of perimenopausal women should be kept in mind, as menopause can pose a long-term risk to health. Therefore, health professionals, especially those specializing in women's health, should pay attention to how to create better care for the health of this important part of our population during the pandemic. In total, 102 female participants who completed all questions were included in the analysis. The mean age was 55.3 . (n=102). More than 54% of the participants stated that there was a serious decrease in the quality of social life due to Covid-19. After the scale score evaluation, the results indicated that 23.7% of the perimenopausal women hesitated to apply to the hospital even if they had serious illness.

The study showed that a positive view of getting vaccinated was significantly higher in postmenopausal women than in premenopausal women (64.7% vs. 64.7%), with an improvement rate of 86.1% (p= 0.041). In this study, during the COVID-19 pandemic, five out of ten women in the climacteric stage reported emotional (49.0%), social (52.2%) and general (51.5%) loneliness.

Differences in cultural factors and living conditions according to their hometowns are important in explaining the differences in the prevalence of loneliness. This trend is in women from the West and Aegean regions; It tended to be higher than the East and Southeast region. At the same time, there were differences between the Western and Eastern regions. Feelings of loneliness were increasing during the pandemic period as we went towards those who were in the hometown of the western region. The relationship between urinary incontinence and frequency of exercise during the pandemic period of the participants was evaluated (Table 1). No statistical significance was found. There was a slight correlation between the time spent by the participants watching television and the scale scores. Statistical significance was observed (Table 2).

Table 1. The relationship between the participants' urinary incontinence and the frequency of exercise during the pandemic period (The Solution-Focused Inventory (SFI))

Urinary incontinence	**The frequency of exercise**	**The SFI**	**n**	**p**
1 time or less / in a week	More than once a day (Every day)	47.06	33	.703
	1 or less per day	36.22	29	
2 times or less / in a week	More than once a day (Every day)	27.26	57	
	1 or less per day	16.62	22	

Table 2. The relationship between the participants' time spent watching television daily and the scale scores (The Solution-Focused Inventory (SFI))

The time spent watching television	**For one day**	**The SFI**	**n**	**p**
The period before the pandemic	1 hour and less / 1 day	--	55	.0480*
	More than 1 hour / 1 day	--	86	
The period since the pandemic	1 hour and less / 1 day	32.02	25	
	More than 1 hour / 1 day	49.52	116	

DISCUSSION

Postmenopausal women are at higher risk of succumbing to nCoV19 than men. Mortality in the postmenopausal age group is higher than mortality in younger women, highlighting the protection that estrogen can provide (Garg et al., 2020). Postmenopausal women have a higher risk of serious illness. Mortality rate due to Covid-19 in men; more than women, further reinforcing the role of estrogens. Estrogenic compounds and antiandrogenic drugs are also being tested in various research trials for the treatment of coronavirus (Garg et al., 2020).

Especially in elderly patients; cancer care and screening, conditions that may have a direct impact on oncological treatments; Disproportionately negatively impacted by the COVID-19' pandemic (Lai et al., 2020). New data estimates a 20 percent increase in human deaths from cancer due to the pandemic (Hipolito Rodrigues & Carneiro, 2020). In the increase of some of the deaths due to oncology; delayed diagnosis is shown as the reason. Further; It is

important to evaluate that there will be an increase in cancer-related mortality because radiotherapy, chemotherapy, and surgical treatments have to be done late (Lai et al., 2020). Especially for perimenopausal women, new projects should be implemented to continue cancer screening programs during the pandemic period.

During the coronavirus pandemic, healthcare workers; They are focused on treating patients affected by Covid-19 and protecting others from infection. It is emphasized that the number of chronic internal and surgical patients in intensive care centers has increased significantly because they delay seeking medical help or are afraid to go to the hospital to use the given drugs or to consult their doctors. In addition, patients who really need palliative care; They were hesitant to seek medical help because of the pandemic.

During pandemic periods; For good women's health, there is a need for in-depth studies in the field of "protecting women's health". In addition, the experiences of women's health and diseases specialists, who have great clinical experience, should be examined. On the other hand, it should not be forgotten that perimenopausal and menopausal symptomatic women delay seeking health care during the pandemic period. It is important to emphasize that this may lead to worsening of pre-existing asymptomatic diseases. Strategies to minimize these problems should be adopted and women should be provided with appropriate guidance to better manage their health. For good public health, both in-depth studies in the field of medicine are needed.

It is important that the menopause transition is of high quality and that adequate counseling is provided. Thus, preventive strategies can be identified that can be applied as a tool to assess cardiovascular, metabolic and oncological risk in women and to mitigate the effects of social isolation on women. It offers women the opportunity to receive counseling on adopting healthy aging. The Researchers; reports on preventive strategies, lifestyle improvements, and management of the most common Cardiovascular Disease risk factors for optimizing cardiovascular health in women during the pandemic: hypertension, glucose intolerance, and dyslipidemia (Seiffert et al., 2020). In the pandemic, the opportunity to stay at home and eat more home-cooked meals has provided a unique opportunity to embrace healthy eating. Recommendations for lifestyle preventative strategies include smoking cessation, regular physical activity, weight management, and eating a healthy diet.

CONCLUSIONS

Health professionals should encourage women to adopt a healthier lifestyle; Regular physical activity at home may be recommended to emphasize the intake of vegetables, fruits and whole grains. Diet should include low-fat dairy products, poultry, fish and legumes. For the reasons, healthcare professionals concerned should pay attention to how to create better care for the health of this important part of our population in the midst of the pandemic.

Women's health service providers should reach women in the perimenopausal period. For cervical and breast cancer screening, pandemic-specific recommendations should be expanded in accordance with relevant women's health protocols. These screening protocols should be maintained to protect female patients and caregivers from COVID-19 infection despite the pandemic.

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COMPARING OF SEED YIELD AND QUALITY TRAITS OF IMI HERBICIDE RESISTANT BREAD WHEAT LINES

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ABSTRACT

Wheat is the most planted crop as well as the most important food and the main crop in the rotations in Turkey. Wheat grows in all parts of the Turkey and Trakya region which is European part of Turkey exist at the 3rd region after Middle and Southeastern Anatolia. Clearfield System which is developed by BASF with combination of IMI (Imidazolinone) herbicide and resistant varieties started firstly in corn, but currently IMI sunflower, canola, rice, wheat, etc. were cultivating by farmers widely in the world. In Turkey, Clearfield system exist only in sunflower and rice not in the wheat yet. IMI wheat could be good solution for proper weed control in especially some areas which were invaded by some perennial grassy weeds. TRAGEN Research Company which is in Trakya Technopark, Edirne, Turkey started first time in Turkey IMI resistant wheat cultivar breeding program. The study was conducted to test performance of some IMI type wheat cultivars in Trakya Region conditions in 2019-2020 growing season in Edirne and Tekirdağ Malkara locations. The experiments were conducted with four replications with five control wheat cultivars which are the most planted and preferred wheat cultivars in Trakya Region. The study results indicated that some IMI cultivars exhibited higher seed yield performances over control cultivars. The promising candidate IMI lines after evaluating quality and yield performances will be sent to registration trials then to produce in Turkish seed market.

Keywords: Bread Wheat, Trakya Region, Seed yield, Yield traits, Herbicide Resistant, Clearfield System, IMI (Imidazolinone) herbicide,

INTRODUCTION

Imidazolinone herbicide group covering of Imazapir, imazapic, imazethapir, imazamox, imazaquin and imazamethabenz control weeds commonly in many crops with inhibiting acetohydroxyacid synthase (AHAS), or acetolactate synthase (ALS) (Frihauf et al., 2005;

Rojano-Delgado et al., 2014; Jimenez et al., 2015 and 2016). IMI herbicide group, which are applied to the soil after and before emergence, could control a wide range of narrow and broadleaf weeds even at very low application doses absorbing by leaves and roots with reaching growth spots by phloem and xylem (Rojano-Delgado et al., 2014). Clearfield system developed by BASF as using Imidazolinone (IMI)-resistant crops with IMI herbicides successfully control common weeds as applying post emergence applications. In the Clearfield system the IMI resistant AHAS genes are not GMO because they were mutated and selected in different crops such as corn, paddy rice, canola, sunflower and wheat utilizing via conventional breeding method (Hanson et al., 2006; Rojano-Delgado et al., 2014; Nakka et al., 2019; Breccia et al., 2020).

Clearfield field system has been firstly used in sunflower production both controlling broomrape and also broad leaf weeds since 1992 (Kaya, 2015). Following sunflower, Clearfield technology has been started in the rice for 5-6 years in Turkey then the first IMI herbicide was registered in wheat in 2020 (Sürek et al., 2016; Beşer et al., 2019). In addition to BASF Co the IMI herbicides and generic IMI drugs developed by domestic companies are selling commonly in Turkish market (Kaya, 2015). Although IMI wheats have not been registered in Turkey yet, IMI wheat varieties developed by traditional breeding containing mutated IMI-resistant genes are selling highly in many countries in the world (Pozniak and Hucl, 2004; Dominguez-Mendez et al., 2017; Carter et al., 2021; Anastasini et al., 2021).

Clearfield system in the wheat expected to exist successfully in Turkey because the control of the weeds such as brooms, grasses, wild barley, wheat, rye and oat are the main problem in some parts of Turkey wheat production areas (Öztürk et al., 2017). Therefore, Tragen Ltd research company started to develop firstly Clearfield wheat cultivars (Beşer et al., 2019). The study was performed to determine of the performances of some IMI resistant bread wheat varieties in Trakya region in 2019-2020 vegetation period.

MATERIAL AND METHOD

The yield trials were conducted in Edirne and Tekirdağ Malkara locations in Trakya Region in 2019-2020 growing seasons to determine yield performances of bread wheat candidate lines. The trials planted on time with planting machine in dry conditions and germination of plants were well in the experiments.

There were 20 lines in the trials with 5 controls which planted mostly in Trakya region which is European part of Turkey (Flamura-85, Pehlivan, Rumeli, Gelibolu, Tragen 103). The previous crop was sunflower in the rotation in the fields and there was no irrigation in in both locations. The experimental design was a Randomized Completely Block Design (RCBD) with four replications in the study.

The four rows plots were 6-m long with the six rows as 17 cm plant spacing and 500 grains per m^2, density, so total plot area was 9 m^2 at planting, 8 m^2 at harvesting. Trials were planted by planter in 06 November in 2019 in Tekirdağ Malkara location and in 07 November in 2019 in Edirne location. The experiments were harvested by harvest combine in 26 June in 2020 in Edirne location and in 29 June in 2020 in Tekirdağ Malkara location. After harvesting the plots, they were treshed and weighed and plot results were adapted 10% humidity then quality analysis were performed from each plot of varieties based on requested by government rules.

The compose fertilizers (20-20-0, Zn) applied 250 kg/ha dose at planting. At tillering stage in February, 80 kg/ha Urea (%46), in March 145 kg/ha Urea (%46), in April 300 kg/ha Amonium Nitrat (%26). Weed control was applied with Laren Plus herbicide (Sulfonyl Urea) dose after planting in the study. Phytotoxity trials were performed with Intervix (40 g / lt Imazamox) IMI herbicide of BASF as double doses 2500 ml / ha. Statistical analysis was performed with JUMP statistical program.

RESULTS AND DISCUSSION

There was so lower seed yields were observed in the experiments comparing previous years due to late frost in late April 2020. The lowest seeds yields have been obtained in the bread wheat since 70 years. However, the study results indicated that some IMI candidate lines had promising results exhibiting higher performances than controls cultivars in both two locations (Table 1). The higher yields were observed in the candidate lines Edirne location comparing with Tekirdağ Malkara location. Among candidate lines, the higher seed yields were obtained from TRAGEN IMI-3 candidate line in both locations and existed in first groups and it was 2^{nd} after Rumeli control cultivar at the average values. Rumeli control hybrid had highest yield among all cultivars in the average values but Esperia had highest seed yield in Edirne location (Table 1).

Table 1. 2019-2020 Wheat Yield trial results

#	Cultivars	Location		Average Seed Yield (kg/da)
		Edirne	Tekirdağ Malkara	
1	RUMELİ	396,8 ABC	482,9 A	439,9 A
2	PEHLİVAN	348,9 BCDEFG	370,4 D	359,7 DEF
3	ESPERİA	423,6 A	-	423,6 ABC
4	FLAMURA-85	370,9 ABCDE	385,6 CD	385,6 ABCDE
5	GELİBOLU	423,8 A	388,6 CD	406,2 ABCD
6	TRAGEN103	363,6 BCDE	365,5 D	365,5 CDEFG
7	IMI-3	406,6 A	453,4 AB	430,0 AB
8	IMI GB-64	385,6 ABCD	385,4 CD	385,5 BCDE
9	IMI-22	327,6 CDEFG	430,3 ABC	379,0 CDE
10	IMI-GB-12	367,6 ABCDE	411,1 BCD	389,4 BCDE
11	IMI-GB-62	399,0 ABC	397,3 BCD	398,2 ABCDE
12	IMI-GB-55	355,9 BCDEFG	373,1 D	364,5 DE
13	IMI-GB-46	301,7 FG	-	301,7 FG
14	IMI-GB-56	298,8 G	-	298,8 G
15	IMI-GB-43	360,2 BCDEF	-	360,2 CDEFG
16	IMI-44	314,8 EFG	403,0 BCD	358,9 DEF
17	IMI-GB-51	342,4 CDEFG	-	342,4 EFG
Average		364,0	403,9	374,9
(LSD: 0.05) (kg/da)		59,1	56,9	67,2
C.V (%)		11,4	9,8	12,6
F		**	**	**

There were highly promising candidate lines for quality results in IMI wheat lines in the study. Among all lines tested in the study, IMI-GB-46 candidate line had highest quality results with having highest protein, gluten, sedim and Alveograph values but it has lower seed yield comparing other candidates (Table 2). Based on experiment results, some IMI cultivars exhibited higher seed yield performances over control cultivars and some of them exhibited higher seed and flour quality results than controls. However, on evaluating seed yield and quality together, IMI-3 candidate line showed that it has higher yielding as well as higher quality results comparing with bread wheat cultivars which are the best ones preferring by farmers and industry together existed in the study.

Based on phytotoxity results for applying IMI herbicides conducted experiment in the study, IMI bread wheat candidate lines were observed as tolerant until to double doses (Table 4). However, some deformations were observed in over doses such as triple and four times in the study.

Table 2. Bread Wheat Trials 2017-2018 Edirne location quality results

#	Cultivars	Hectolt Weight (kg)	Humd (%)	Protein (%)	Wet Gluten (%)	Sedim. (Zel) ml	Starch	Alveo graph W	Grain Hardeness
1	RUMELİ	80,7	12,3	17,3	40,1	75	66,4	444	82,4
2	PEHLİVAN	79,0	12,1	14,9	34,6	56	68,1	365	77,5
3	ESPERİA	80,0	12,4	15,8	36,6	67	66,8	401	76,3
4	FLAMURA-85	80,8	12,8	16,0	37,2	67	66,9	358	77,2
5	GELİBOLU	79,6	12,7	14,5	33,7	55	67,8	314	76,2
6	TRAGEN103	77,7	12,6	16,9	39,2	73	66,5	425	83,4
7	IMI-3	82,2	12,2	16,5	38,2	74	65,5	470	86,9
8	IMI GB-64	76,4	12,3	16,6	38,5	73	65,9	433	86,7
9	IMI-22	77,3	12,7	16,6	38,5	74	65,2	385	80,5
10	IMI-GB-12	79,3	12,9	17,6	40,9	83	63,9	443	99,1
11	IMI-GB-62	79,7	12,9	17,7	41,2	80	65,5	458	84,5
12	IMI-GB-55	78,2	12,3	17,9	41,7	86	64,5	406	70,7
13	IMI-GB-46	73,8	12	20,8	48,5	110	62,6	625	78,3
14	IMI-GB-56	74,9	12,4	18,8	43,8	92	64,0	468	77,4
15	IMI-GB-43	78,3	12,3	20,1	46,6	99	64,1	504	83,9
16	IMI-44	81	12,5	18,1	42	86	64,7	433	76,9
17	IMI-GB-51	77,2	12,4	18,2	42,2	86	64,5	406	87,1

Table 3. Bread Wheat Trials 2019-2020 Tekirdağ Malkara location quality results

#	Cultivars	Hectoliter Weight (kg)	Humd (%)	Protein (%)	Wet Gluten (%)	Sedim. (Zel) m	Starch	Alveo graph W	Grain Hardeness
1	RUMELİ	82,3	12,7	15,5	35,9	62	66,7	355	91,4
2	PEHLIVAN	81,0	10,9	14,4	33,4	53	67,6	360	91,4
3	FLAMURA-85	89,0	12,1	14,6	34,0	55	66,4	364	89,0
4	GELİBOLU	81,9	11,7	13,6	31,5	48	68,1	311	84,9
5	TRAGEN103	77,7	12,6	16,9	39,2	73	66,5	425	83,4
6	IMI-3	79,1	12,4	14,7	34,1	60	66,4	403	85,1
7	IMI GB-62	80,9	12,6	15,9	36,9	66	66,7	397	82,1
8	IMI-GB-12	79,4	12,7	14,4	33,4	58	66,2	355	89,5
9	IMI-GB-64	75,1	12,5	14,7	34,1	58	66,7	340	91,3
10	IMI-44	81,6	12,4	16,8	39	77	65,6	399	69,8
11	IMI-22	78,4	12,5	15,4	35,9	65	66,2	342	70,7
12	IMI-GB-55	77,9	12,4	16,7	38,8	76	65	385	82,4

Table 4. 2017-2018 Phytotoxity trial results in Edirne location

	Cultivars	125 gr/da Intervix	250 gr/da Intervix	375 gr/da Intervix	500 gr/da Intervix
1	TRAGEN 103	9	-	-	-
2	Pehlivan	9	-	-	-
3	Rumeli	9	-	-	-
4	Esperia	9	-	-	-
5	Gelibolu	9	-	-	-
6	Flamura-85	9	-	-	-
7	IMI-3	1	2	6	7-Steril and deformed plants
8	IMI GB-64	1	2	6	7-Steril and deformed plants
9	IMI-22	1	2	6	7-Steril and deformed plants
10	IMI-GB-12	1	2	6	7-Steril and deformed plants
11	IMI-GB-62	1	2	6	7-Steril and deformed plants
12	IMI-GB-55	1	2	6	7-Steril and deformed plants
13	IMI-GB-46	1	2	6	7-Steril and deformed plants
14	IMI-GB-56	1	2	6	7-Steril and deformed plants
15	IMI-GB-43	1	2	6	7-Steril and deformed plants
16	IMI-44	1	2	6	7-Steril and deformed plants
17	IMI-GB-51	1	2	6	7-Steril and deformed plants

1 Resistant, 9 sensitive

CONCLUSIONS

Clearfield wheat both for bread and durum type is not producing in Turkey, the registration of herbicide process was completed in 2021 so it could be possible to register wheat cultivars after that. Due to urgent needs of the killing grassy weeds not controlling by current herbicides especially in some parts of the Anatolia, TRAGEN Ltd Co started to develop IMI bread wheat lines in its breeding program because Clearfield technology will give alternative wed control option to wheat farmers. However, it need that these new IMI bread wheat lines should have both for high yielding and also quality to exist longer in the Turkish bread wheat market and be preferred by both producers and also flour industry. TRAGEN- IMI-3 bread wheat candidate lines existed in top rank for seed yield in the experiments and it has also higher seed and flour quality but this candidate need to evaluate on more year to be sent to registration There were other IMI promising candidate lines based seed and flour quality results when compared with control cultivars. As results, TRAGEN Ltd IMI bread wheat cultivars keep promising potential then after evaluation all aspects then they will send to registration trials to produce and exist in Turkish wheat seed market firstly.

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THE EVALUATION OF DROUGHT STRESS TOLERANCE IN SUNFLOWER INBRED LINES

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ABSTRACT

Sunflower (*Helianthus annuus* L.) sustains frequently into severe droughts in recent years due to global warming as a spring crop. These abiotic stress affects seed yield highly resulting important yield loses as well as in oil yield in sunflower because especially the hot temperatures during grain filling period firstly reduce water quickly in the seed with resulting empty kernels. Therefore, sunflower breeders screen genotypes for searching drought tolerant genes in their sunflower breeding programs frequently to develop drought tolerant hybrids because only tolerant ones which have higher adaption capability to dry areas could be planted widely in the next years. The study was conducted in under controlled environmental conditions in Trakya Agricultural Research Institute, Edirne Turkey where is in Trakya Region which is European part of Turkey has about 50% of Turkish sunflower production areas growing in rain fed conditions. Some phenological and yield traits as well as total chlorophyll contents of some sunflower male inbred lines at R3, R5-1 and R6 growing periods as three drought stress applications were examined in the study. Correlation and regression analysis were performed to determine the relationships between seed yield and other yield traits. Based on correlation study results, the significant relationships were observed between seed yield per head and other yield traits (plant height, head diameter, leaf number and area, oil content and yield and thousand seed weights) in sunflower male lines both in control and three stress conditions except total Chlorophyll content in stress conditions. There are significant linear and quadratic relationships between seed yield and other yield traits based on regression analysis results in the study. The relationships of yield traits in this drought tolerance research will associate in sunflower breeding program then it will help starting to develop drought tolerant genotypes for future to escape away from drought stress in global warming conditions.

Keywords: Drought tolerance, Sunflower, Yield traits, Regression and Correlation Analysis

INTRODUCTION

Oil type sunflower grows mainly in the rainfed conditions so environmental conditions affect seed and oil yield severely in some years. Then sunflower could not compete other main crops such as wheat in the rotation and sunflower production push into the marginal regions. Both global warming and also starting of sunflower production in poor soils, water stress plays important role for determining of seed and oil yield in sunflower production in recent years. Therefore, sunflower breeders put drought tolerance in top priority in their breeding programs

for developing new stabile sunflower cultivars in addition to high yielding and biotic stress tolerance (Kaya, 2016; Harsányi et al., 2021).

Sunflower normally could categorize as drought tolerant plant because it has deep roots and could utilize from water in deep areas in the soil. Furthermore, wild sunflowers have huge genera and survive in almost all stress conditions as well as in the deserts and they could grow all parts of US (Pekcan et al., 2016). Therefore, sunflower breeders have demonstrated huge efforts both screening of wild species to determine of these drought tolerance genes and also to transfer these ones to cultural types via interspecific hybridization etc. However, drought tolerance is so complex trait and this quantitative trait could not manage easily via conventional plant breeding. Therefore, drought tolerance studies could conduct via molecular markers and QTL analysis as well as conducting experiments under controlled conditions generally to understand of drought tolerance mechanism (Pekcan et al., 2016).

Many studies were performed to understand of plant responses to drought stresses in sunflower during the vegetation period in hot summer seasons analyzing of anatomical, cellular or molecular processes and levels as well as indicators of drought tolerance (Rauf and Sadaqat, 2008; Geetha et al., 2012; Hasan et al., 2020;). The researchers indicated that water stress leaded to reduce photosynthesis rate and efficiency via chlorophyll content and leaf area index as well as decreasing total plant dry weight and growth rate, net assimilation rate of leaves, stem, and root in sunflower (Kaya et al., 2016; Çiçek et al., 2019Arslan et al., 2020; Hilli and Immadi, 2021; Pekcan et al., 2021). However, the period and time of drought stress is so important issue to determine how it affects plant development in sunflower. While drought stress lessens quickly number and size of leaves, leaf area index as well as reducing plant height and head in earlier vegetative period (4-8 leaves), in later growth periods, it mostly leads reducing of seed number and weight and also lower oil content in sunflower (Soorninia et al., 2012; Mubshar et al. 2018).

Regression and correlation studies were preferred mainly to understand yield relationships and how to affect each other especially in stress conditions (Yankov and Tahsin, 2015; Varalakshmi et al., 2019; Zeinalzadeh-Tabrizi et al., 2019). Therefore, our study was conducted to determine the relationships among yield traits under drought stress conditions in controlled environments by using sunflower male inbred lines developed in National Sunflower Project conducted by Trakya Agricultural Research Institute (TARI) in Edirne, Turkey.

MATERIAL AND METHOD

The study was carried out in TARI research fields with fifty male inbred lines originated different genetic sources in 2014. Tunca commercial hybrid belonging Limagrain Co were used as control selected as one of the most stabile sunflower hybrids in different environments in Turkey. Trials were conducted with RCBD with one row and three replications. In each row, there were five plants and the distance between rows was 70 cm and 30 cm in rows. Trials were planted by hand in 29 May and plants were harvested and threshed by hand in 24 September.

The rainfall and humidity in 2014 is over longer year averages while average temperatures were the same and daily rainfalls in 2014 (Table 1). Drip irrigation was applied and as covering rain shelters, drought stress conditions were set up like below in the experiments (Table 2). Sunflower yield consists three main characters as plant per area, seeds per plant and seed weight the drought stress groups were determined based on these vital periods in the study.

Stress group 1, 2 and 3 were set up in 23.06.2014, in 22.07.2014 and 04.08.2014, respectively. Control: All plant water requests were supplied by drip irrigation (when field capacity reduced until 50%); Stress group 1 (S1): When plants were 50 cm, Stress group 2 (S2): at bud development, Stress group 3 (S3): at the milky stage.

Table 1. Daily rainfalls during the study (mm)

Months and Rainfalls (R) (mm)							
5	R	6	R	7	R	8	R
31st	28,0	4th	38,7	4th	0,9	7th	11,2
		5th	6,6	5th	0,3	18th	5,6
		6th	2,2	16th	39,5		
		26th	42,2	17th	40,1		
				20th	3,0		

Table 2. Irrigation amounts applied in the experiment plots (mm)

Irrigation time	Irrigation (mm)	Irrigation time	Irrigation (mm)
10.06.2014	50	10.08.2014	75
25.06.2014	70	18.08.2014	60
10.07.2014	65	28.08.2014	60
25.07.2014	40		

RESULTS AND DISCUSSION

Based on correlation analysis, there were some positive relationship among yield traits in the stress conditions (Table 3). The negative significant relationships were observed between the total chlorophyll content and some yield traits. Additionally, oil content had some negative significant relationships with thousands seed weight as well as with head diameter and with leaf area. The significant positive relationships were observed that between plant height and other yield traits in almost all of them except total chlorophyll content 1. Similarly, both oil and seed yield exhibited significant and positive relationships also except with oil content and with total chlorophyll content 2 (Table 3).

The highest positive significant correlations were observed in between leaf area and other yield traits in the stress conditions other than connected characters such oil and seed yield as well as flowering and physiological maturity except total chlorophyll content 2. It means that leaf area is the most affected trait from drought stress and also affected much seed and oil yield and other yield trait during the drought stress vial reducing total Chlorophyll content too (Table 3).

Based on regression analysis results between seed yield and other yield traits, there were positive quadratic relationships as well as linear generally in drought stress conditions. The quadratic relationships observed between seed yield and plant height and the decreases were observed until 110 cm in plant height then it started to increase. Similarly, the quadratic relationships also observed between seed yield and head diameter. In smaller heads until 10 cm, there were no effects on seed yield but after 10 cm, larger heads affected seed yield positively. It means that the drought stress mostly affected both plant height and head diameter then at lower amounts reduced also seed yields in the sunflower (Figure 1).

Table 3: Correlation values of sunflower male lines among yield traits under drought stress conditions

	Plant Height (cm)	Head Diameter (cm)	Flowering (day)	Physiological Maturity (day)	Leaf Number	Leaf Area	Total Chlorophyll Contents 1	Total Chlorophyll Contents 2	1000 Seed Weight (g)	Seed Yield (Kg ha^{-1})	Oil Content (%)	Oil Yield (Kg ha^{-1})
PH	1,0000											
HD	0,0928ns	1,0000										
FP	0,2664**	0,4323**	1,0000									
PM	0,2846**	0,4927**	0,8269**	1,0000								
LN	0,2130**	0,5167**	0,5702**	0,6207**	1,0000							
LA	0,4215**	0,5893**	0,5141**	0,5864**	0,6711**	1,0000						
TC 1	-0,0015ns	-0,1496**	-0,1221**	-0,1832**	-0,2985**	-0,2636**	1,0000					
TC 2	0,1432**	0,1890**	0,0395ns	-0,0051ns	-0,0387ns	-0,0494ns	0,4584**	1,0000				
TSW	0,2390**	0,4254**	0,1561**	0,3484**	0,2332**	0,4991**	-0,0877ns	0,0141**	1,0000			
SY	**0,3546****	**0,5812****	**0,3499****	**0,4350****	**0,3931****	**0,7668****	**-0,1449****	**0,0103ns**	**0,5439****	**1,0000**		
OC	0,1630**	-0,3002**	-0,0111ns	-0,1540**	-0,0918ns	-0,1183**	0,1495**	0,2679**	-0,5192**	**-0,1104ns**	1,0000	
OY	0,3680**	0,4744**	0,3399**	0,3803**	0,3609**	0,7007**	-0,1219**	0,0587**	0,3209**	**0,9376****	0,1893**	1,0000

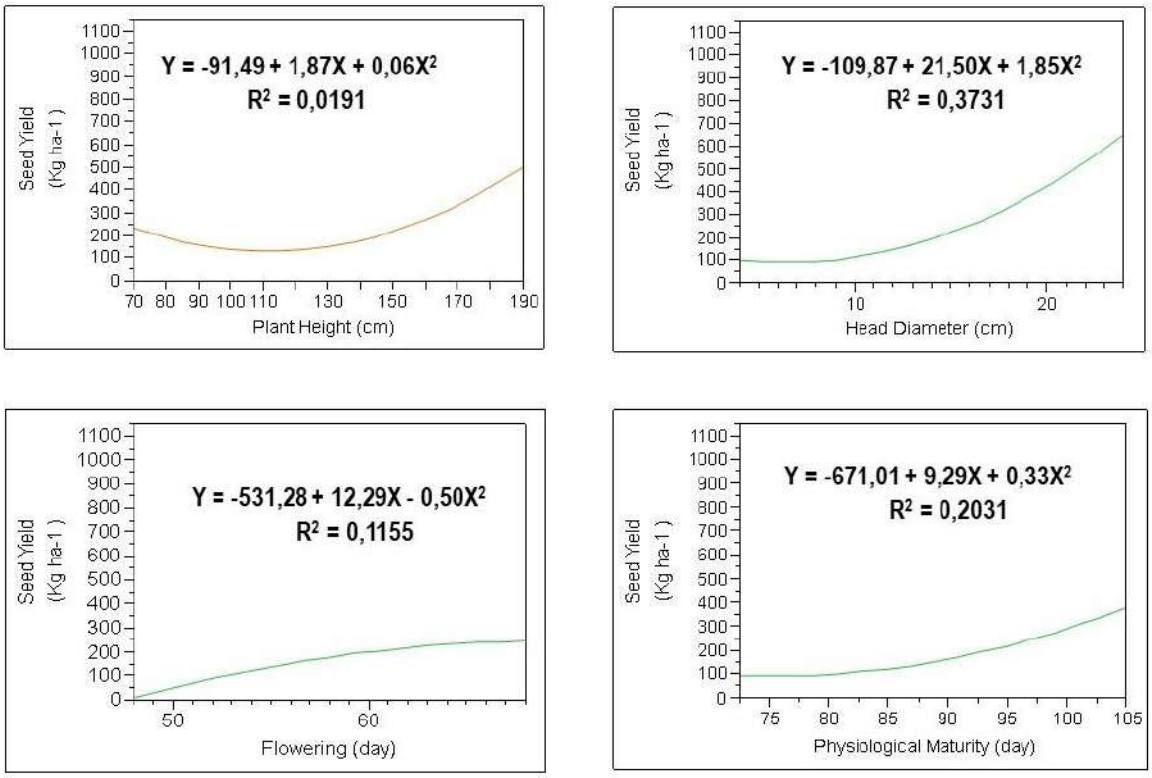

Figure 1: Regression analysis of sunflower male lines among yield traits at all stress

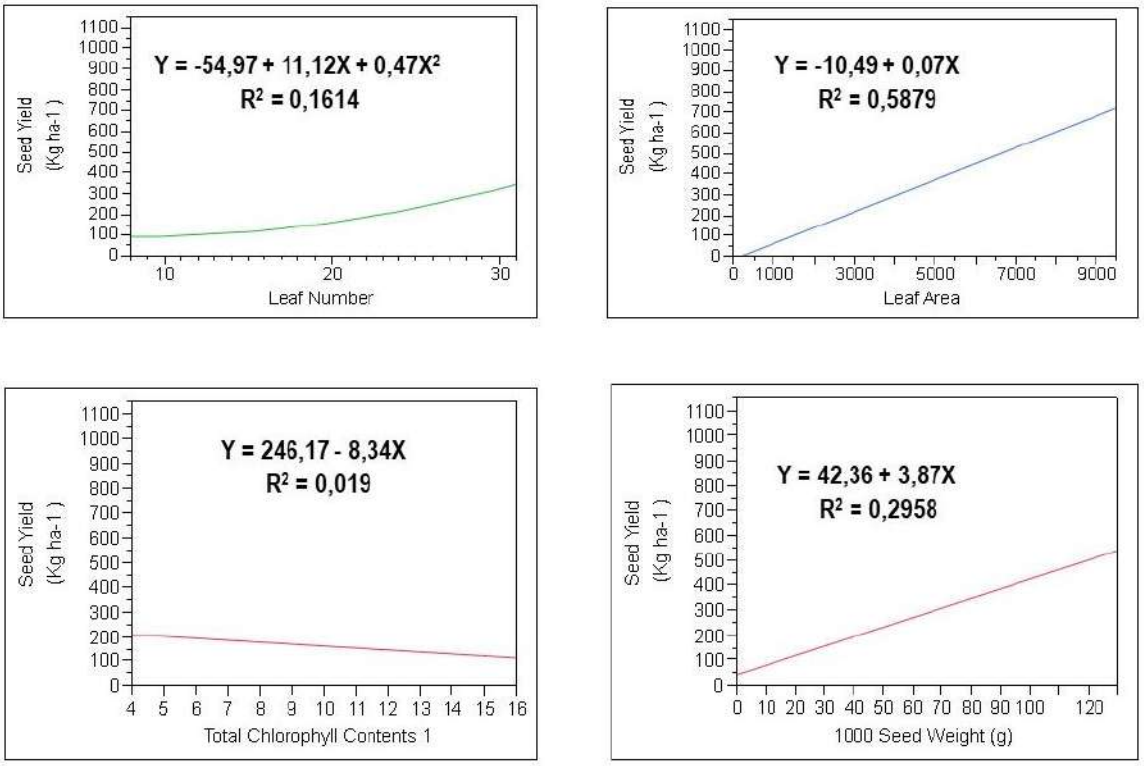

Figure 2: Regression analysis of sunflower male lines among yield traits at all stress

The quadratic relationships were determined in flowering day as well as in physiological maturity. Similar to previous traits mentioned above, there were reduction until earlier 78 days then increases on seed yield were started in physiological maturity. However, in flowering days while increase was lower at the beginning, the later female lines had more seed yield in drought stress conditions. Most probably, later male inbred lines escaped from drought stress coincided at early growth periods (Figure 1). While there was quadratic relationship observed between seed yield and leaf number, linear positive relationships determined between 1000 seed weight and leaf area. However, linear negative relationships were detected between seed yield and total chlorophyll content 1 (Figure 2).

There were linear negative relationships observed between seed yield and oil content but the positive way was between seed yield and oil yield as expected. However, in the relationships with oil content, the regression coefficient was so lower and the inclination detected so lower as well (Figure 3).

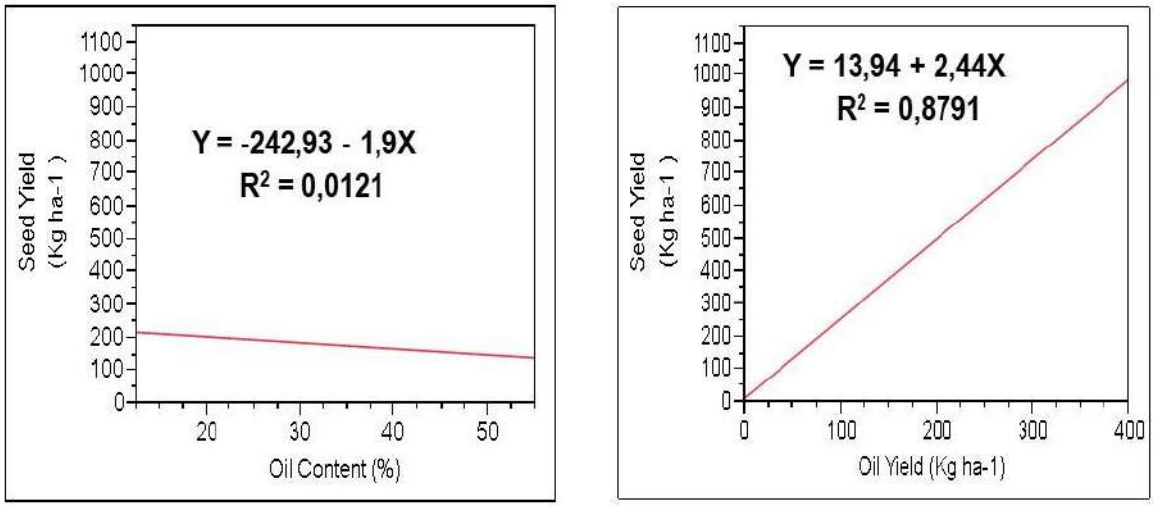

Figure 3: Regression analysis of sunflower male lines among yield traits at all stress

CONCLUSION

As a conclusion, the positive and significant relationships were detected generally among yield traits. Plant height and head diameter exhibited higher and significant correlation values and the most affected yield traits among examined traits in drought stress. There were linear and quadratic relationships between seed yield and other yield traits in sunflower male inbred lines in the study. In the drought stress, sunflower male lines sacrificed from seed yield to get higher oil contents as well as other yield traits such as thousand seed weight and head diameter because in larger heads had bigger seeds but they have lower oil contents in their seeds due to having higher husk contents.

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PERFORMANCE OF *PI40*, A RICE BLAST DURABLE RESISTANT GENE, AND PI40 GENE INTROGRESSED ADVANCED LINES AT FIELD CONDITIONS

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ABSTRACT

This study aimed to determine the reaction of the durable resistant *Pi40* gene against rice blast fungus (*Magnaporthe grisea*) races at different stages of plant development in field conditions in Turkey, and to transfer the *Pi40* gene to high-yielding Kiziltan rice cultivar progenies between 2011 and 2015. *Pi40*-harboring donor lines were introduced from the International Rice Research Institute (IRRI). *Pi40*-harboring material, local varieties, and Kiziltan progenies obtained by crossing Kiziltan and *Pi40*-harboring lines were used as materials in this study. The results of the experiment showed that the *Pi40* gene was field resistant to blast races in all experimental locations. Crosses were created between Kiziltan and *Pi40* gene donor lines, and by using a CAPs marker specifically designed for the *Pi40* gene, the advanced lines of the transferred *Pi40* gene were selected using marker-assisted selection (MAS) for selfing. In this study, high yielding blast-resistant *Pi40* genes were transferred, and promising lines were obtained; these lines were tested in a blast nursery and yield trial in 2015.

Key words: Marker assisted selection, *Pi40* gene, rice blast, *Magnaporthe grisea*

INTRODUCTION

Rice blast disease caused by the fungus *Magnaporthe grisea* (Hebert) is considered the most destructive rice disease across many tropical and subtropical regions, with an estimated crop loss that can be used to feed 60 million people each year (Zeigler et al. 1994). It has been reported that 30.5% of the total yield losses from diseases are caused by blast in China (Shen and Lin 1996). Yield losses in Punshi and KD-2-6 varieties from blast, grown in large areas in India, varied between 60–90% in 1986 (Sing 1987).

Rice blast is the most important rice disease in Turkey; yield losses caused by this disease accounted for 25.7% in the Black Sea region (Gobelez 1953), 90% in the coastal region of the Mediterranean (Tekinel et al. 1980), and 8.3% in the southeastern Anatolia region in 1974 (Oran 1975). The Trakya region of Turkey was affected by a blast epidemic in 1995, causing 20% yield loss in 25,000 hectares; this dramatically reduced the head rice yield (Surek and Beser 1997).

Breeding of blast-resistant rice varieties is the most economical and environmentally sustainable way to control this disease. However, selecting the most suitable resistance genes to be used in the breeding process is fundamental. Although the transfer of resistant genes to improve resistant cultivars for different races of blast (gene for gene) is used as a resistant variety breeding method, most of the genes show resistance to only certain races of diseases, and resistant genes can be broken down in 2–3 years (Surek and Beser 1997). Many japonica-type blast resistant varieties have been developed in South Korea, and similar rice types are

grown in Turkey. However, after a few years, these resistant varieties became susceptible to blast after spreading new races of diseases (Han et al. 2001).

More than 50 disease-resistant genes have been identified and 8 of these have been cloned (Dai et al. 2007; Liu et al. 2007). Except for the *Pi21* gene, most of the resistant genes are dominant (Fukuoka and Okuno 2001). Among the resistant genes, *Pi1, Pi-z5* (*Pi2*), *Pi5*, *Pi9*, and *Pi40* have shown broad-spectrum resistance to many blast races (Jeung et al. 2007). In Mediterranean countries, such as Italy, Spain, Portugal, and Hungary, no virulence was detected for the resistance genes *Pi-b*, *Pi-kp*, *Pi-ta2*, and *Pi-z*, among 41 isolates (Roumen et al. 1997). In Iran, the *Pi5 (t)* gene had the lowest panicle blast severity of 3% (Aram et al. 2013). In Russia, the resistance genes *Pi-ks*, *Pi-a*, and *Pi-i* were easily broken (Aram et al. 2013).

Screening for blast resistance using seedlings in greenhouse tests does not always correctly predict the resistance behavior in the field. For example, although susceptible to most U.S. blast races in greenhouse tests, 'Starbonnet' (1968 to 1984) and 'Cypress' varieties (1994 to 2002) had a good field resistance in the southern USA and were usually grown without requiring special cultural management to control blast. The *Pi-ta* blast-resistance gene present in 'Katy' provided complete blast control from 1989 until 2004. Whereas a race virulent to *Pi-ta* (race IE-1k) was detected in growers' fields since 1994, this new race did not cause significant damage in the *Pi-ta* based variety 'Banks' until 2004. These findings indicate that screening for adequate levels of field resistance is critical for rice blast control (Lee et al. 2005).

Since resistant genes are easily broken down over a few years, and most of the genes show resistance against only 1–2 blast races, studies on broad-spectrum resistant genes have become more important recently. The *Pi40* gene confers durable resistance against most of the blast races, and this gene was transferred to two japonica rice cultivars using marker-assisted selection (MAS) (Suh et al., 2009). The *Pi40* gene was identified in one indica rice line, and the resistant gene was transferred to this indica line from the EE genome of the wild species, *Oryza australiensis* (Jeung et al. 2007). The *Pi40* gene could be the most appropriate gene to improve Turkish rice varieties for durable resistance to blast.

Chemical control of blast is expensive, challenging, and sometimes ineffective. The Kiziltan rice cultivar was developed from a Veneria/Tahinato cross and registered by the Trakya Agricultural Research Institute in 2007. Kiziltan is a short, lodging-resistant, very high-yielding variety with high milling recovery. However, despite all these features, blast has occasionally infected the Kiziltan variety, and chemicals have been used to control blast during production. The objective of this study was to investigate the reaction of the *Pi40* gene to blast races under field conditions at different growing stages of rice in Turkey, to transfer the *Pi40* gene to Kiziltan-cross rice progenies with MAS and to determine rice blast reaction and yield performance of these *Pi40* gene introgressed advanced lines at field conditions.

MATERIALS AND METHODS

The *Pi40* genes harboring IR 83260–1–1-1–7–1–2–B and IR 83260-1-1-1-2-1-22-B rice lines, used in this study were obtained from the International Rice Research Institute (IRRI). Other materials (susceptible check Sariceltik, Kiziltan, Osmancik-97, Halilbey) were obtained from the Trakya Agricultural Research Institute, Edirne, Turkey. Rice cultivar Kiziltan, F_1, and segregating materials of IR 83260-1-1-1-2-1-22-B/Kiziltan and Kiziltan/IR 83260-1-1-1-7-1-2-B crosses, were used as materials in this study. Kiziltan is very high-yielding, lodging-resistant rice cultivar with high head rice milling yield, but it is very susceptible to blast.

In the first study, experiments were conducted to investigate the reaction of the *Pi40* gene against blast races at the different growth stages of rice plants in field conditions in Turkey. The *Pi40* gene harboring materials, together with Trakya Agricultural Institute's registered cultivars and two susceptible checks, Diyarbakir yerli and sariceltik, were planted at six locations at the

main rice growing areas in Turkey in 2011 as follows: in Edirne Province, at 1) Ipsala and Pasali, 2) Ipsala, Sarıcaali; in Balikesir Province, at 3) Gonen and 4) Manyas; and in Tekirdag Province, at 5) Malkara and 6) Hayrabolu. In 2015, a blast nursery was conducted at three locations as follows: in Edirne Province, at 1) Ipsala; in Canakkale, at 2) Biga; and in Balikesir Province, at 3) Gonen, with advanced lines and checks. In 2015, Sariceltik was used to initiate and spread a blast epidemic.

Seeds were sown in plastic trays in the third week of May, and 20-d-old seedlings were transplanted to the field. Each line or variety was transplanted into rows of 1 m length, with a row distance of 25 cm. The test rows were surrounded with the local susceptible checks 'Diyarbakir yerli' and 'Sariceltik' to aid in initiating and spreading a blast epidemic; therefore, excess nitrogen (250 kg/ha) was applied in 2011.

The trials were monitored and scored for the presence of blast disease symptoms at the growth stage 4 (stem elongation), 5 (booting), and 9 (mature grain) in 2011, and at growth stage 9 (mature grain) in 2015. Leaf blast and panicle blast severities were scored according to the Standard Evaluation System For Rice (IRRI, 1996).

The modified CTAB method was used as the DNA extraction method. The CAPs marker 9871.T7E2b primer linked with the *Pi40* gene was used for the molecular analysis of the *Pi40* gene. DNA isolation, PCR, and gel conditions are shown in Table 1.

For the MAS study, Kiziltan/IR83260-1-1-1-7-1-2B and IR83260-1-1-1-2-1-22-B/Kiziltan were crossed in 2011. F_1 seeds were planted in the field to obtain F_2 seeds in May, 2012. Kiziltan/IR83260-1-1-1-7-1-2B and IR83260-1-1-1-2-1-22- B/Kiziltan were crossed again to obtain F_1 seeds as a check material during the MAS selection to confirm marker position in the heterozygote material. F_1 and F_2 materials, together with the Kiziltan cultivar and two donor lines were grown in the plant growth chamber during the 2012–2013 winter. Molecular analysis was performed in all these materials to assess the presence of the *Pi40* gene, and homozygote and heterozygote materials for the *Pi40* gene in F_2-segregating materials were identified using MAS selection. The identified material for the *Pi40* gene was selfed to select other agronomic traits. *Pi40* heterozygote material was also selfed; however, in the following years, it was used in MAS to select the *Pi40* gene.

Table.1 PCR and gel conditions

PCR Preparation		***PCR Profile***
DNA 50 (ng/ul)	2 ul	***Event 1***
F primer (10 pmol)	0.3 ul	Tempetature ^{o}C 95, 3 min.
R primer (10 pmol)	0.3 ul	***Event 2*** (Cycling 3 steps – 35 repeats)
10X PCR buffer with $MgCl_2$	2 ul	Step 1. Temperature ^{o}C 95, 30 s.
1 mM dNTPs	2 ul	Step 2. Temperature ^{o}C 65, 30 s.
ddH_2O	7.4 ul	Step 3. Tempetarure ^{o}C 72, 1 min.
Taq polymeraz	1 ul	***Event 3***
		Temperature ^{o}C 72, 10 min.
		Event 4
		Temperature ^{o}C 25, 20 s.
Mastermix for Enzyme restriction		***Gel Preparation and loading***
PCR products		Gel concentration: 2 % gel
Restriction enzyme (mlucl)	5 ul	10ul Sybr safe gel stain
Restriction buffer	0.5 ul	45 minutes at 130 V.
ddH_2O	1 ul	
Temperature ^{o}C 37, 1 hour	8.5 ul	

Selection was performed for agronomic traits in *Pi40*-transferred homozygote Kiziltan cultivar progenies each year during selfing. Eight *Pi40* gene transferred advanced lines, and one *Pi40* gene not transferred line (IR3260-1-1-2-1-22-B/Kiziltan) were tested with checks at the yield trail in Edirne, and the same advanced lines were also tested in three major rice growing areas (Ipsala-Edirne, Canakkale-Biga, Balikesir-Gonen) in the blast nursery with the susceptible check rice cultivar, Sariceltik, in 2015.

RESULTS AND DISCUSSION

As shown in Table 2, the susceptible checks, Diyarbakir yerli and Sariçeltik, were very susceptible to blast, whereas the *Pi40* gene-harboring materials IR 83260-1-1-1-2-1-22-B and IR83260-1-1-1-7-1-2-B, were resistant to leaf and panicle blast at all eight locations in field conditions in Turkey. However, Kiziltan and other registered varieties from the Trakya Agricultural Research Institute were not resistant to blast. The Sumnu cultivar was the most tolerant to blast, followed by Halilbey and Osmancık-97. Our results provided in Table 2, showed that the Kiziltan variety is susceptible to Turkey blast races and should to be improved against blast. However, *Pi40* gene-harboring lines are resistant to Turkey blast races under field conditions and could be suitable as a resistant parent to transfer the *Pi40* gene to Kiziltan progenies in the Turkish rice-breeding program.

After identification of the resistance of the *Pi40* gene to blast races under field conditions at all locations in Turkey, the *Pi40* gene was transferred to Kiziltan rice cultivar progenies using MAS. The *Pi40* gene primer 9871.T7E2b was used to generate different materials, and it was found that the *Pi40* gene is heterozygous in the F_1 material, homozygous in *Pi40* gene-harboring donor parents, and unavailable in the Kiziltan cultivar. However, it is heterozygous in 6, 8, 9, 10, 14, 15, 17, 18, homozygote in 7, 13, 19 and unavailable in 12, 16, 20 F_2 Kiziltan/ IR83260-1-1-1-7-1-2-B progenies (Figure 1). Thus, it was concluded that the 9871.T7E2b primer could be used in the MAS studies to transfer the *Pi40* resistant gene to the Turkish rice-breeding program. In the three Kiziltan progenies (7, 13, and 19) of the F_2 plants, the *Pi40* gene was fixed as a homozygote. In the following years, selection was performed only for agronomic characteristics of these three *Pi40* gene fixed lines. However, for heterozygote lines, MAS selection was required in the following years. Selection and selfing were performed until 2014 to obtain advanced F_6 plants. Yield trial and blast score results of these advanced lines are shown in Tables 2 and 3.

As can be seen in Table 3; the *Pi40* gene-transferred advanced lines 20111020-TR2959-1-5-2, 2011020-TR2959-3-2-2, 2011020-TR2959-6-4-3, and 2011TR2959-1-6-2 had high yields similarly to their parents Kiziltan. All advanced Kiziltan/ IR83260-1-1-1-7-1-2-B progenies were resistant to blast. *Pi40* gene shows very good resistance to both leaf and panicle blast in all Turkish rice-growing regions in field conditions, and this durable resistant *Pi40* gene should be used to improve rice blast resistant cultivar programs in Turkey. The *9871.T7E2b* primer, together with the methods provided in this study, could be used for molecular analysis when segregating materials for MAS selection in blast-resistant breeding programs to transfer the *Pi40* gene. We improved the blast-resistant high-yielding promising lines obtained from Kiziltan and *Pi40* gene-harboring donor parents cross.

The advanced line, 2011020-TR2959-1-5-2, showed high resistance against both leaf and panicle blast with a 0 score in field conditions. This line also provided the highest yield (9,075 t/ha) among the advanced lines of Kiziltan-crossed progenies. Other advanced Kiziltan progenies, such as 2011020-TR2959-3-2-2 and 2011020-TR2959-5-4-3, showed high resistance to leaf and panicle blast. However, some advanced Kiziltan-derived lines showed high resistance to leaf blast, but not to panicle blast in field conditions. Thus, we improved

high-yielding, blast-resistant advanced lines for both leaf and panicle blast in field conditions with the transfer of the *Pi40* gene using molecular marker-assisted breeding.

Table 2. Blast disease score of rice genotypes for six locations at 4^{th},5^{th} and 9^{th} growth stage

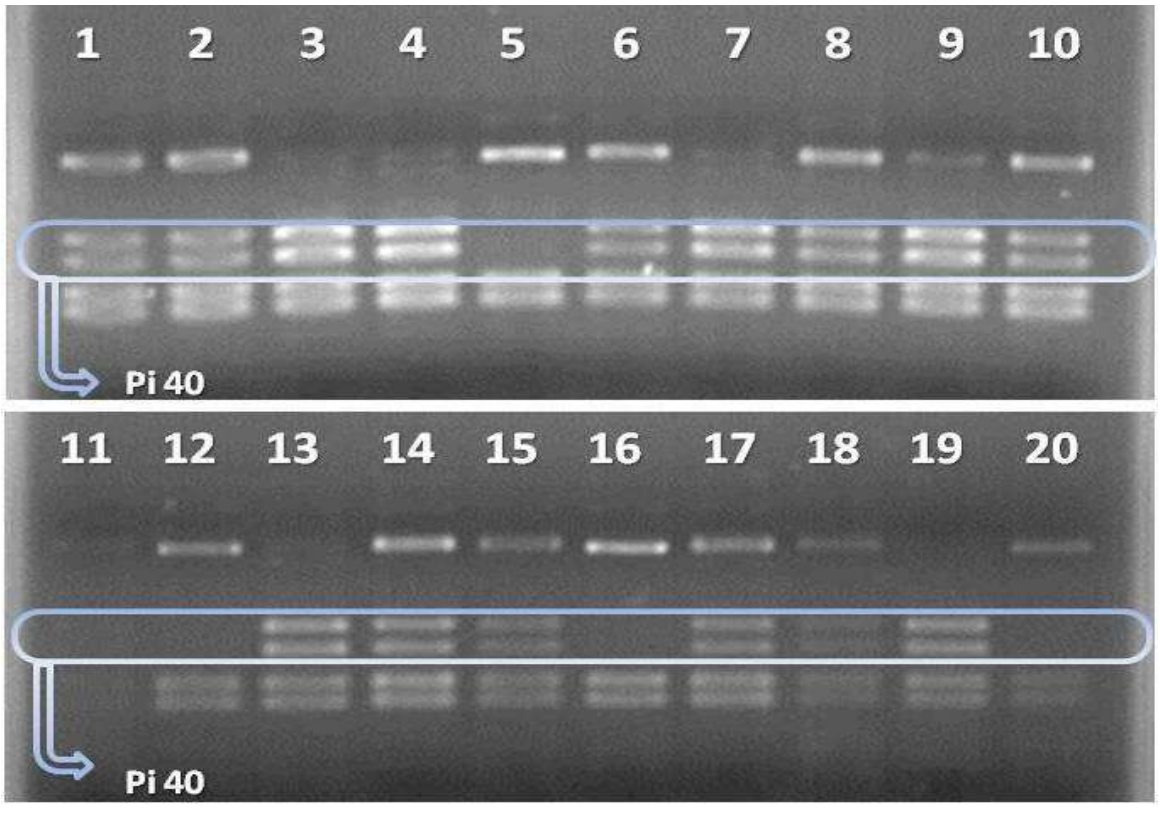
Locations	Growth stage	Blast type	Lines / Cultivars										
			Kiziltan	Osmancık-97	Halilbey	Edirne	Ece	Şumnu	Beser	IR 83260-1-1-1-2-1-22-B	IR 83260-1-1-1-7-1-2-B	Diyarbakir yerli	Sariceltik
Saricaali- Ipsala-Edirne	4	L	1	0	0	1	1	0	1	0	0	3	3
	5	L	9	5	5	7	9	1	7	1	1	9	9
	9	L	9	5	5	7	9	1	7	1	1	9	9
		P	9	3	5	7	9	3	7	1	1	9	9
Pasali- Ipsala-Edirne	4	L	1	0	0	1	1	0	1	0	0	3	3
	5	L	9	5	3	7	9	1	7	1	1	9	9
	9	L	9	5	3	7	9	1	7	1	1	9	9
		P	9	3	5	9	3	7	1	1	1	9	9
Gonen - Balikesir	4	L	1	0	0	1	1	0	1	0	0	3	3
	5	L	7	1	1	3	5	1	5	1	1	9	9
	9	L	9	5	5	7	9	3	5	1	1	9	9
		P	9	5	5	7	9	3	5	1	1	9	9
Manyas - Balikesir	4	L	1	0	0	1	1	0	1	0	0	3	3
	5	L	7	3	3	3	5	3	3	1	1	9	9
	9	L	7	3	3	5	7	3	3	1	1	9	9
		P	7	3	3	5	7	3	3	1	1	9	9
Malkara- Tekirdag	4	L	1	0	0	1	1	0	1	0	0	1	1
	5	L	1	0	0	1	0	1	1	0	0	1	3
	9	L	9	5	1	7	9	1	7	1	1	9	9
		P	9	3	5	7	9	5	7	1	1	9	9
Hayrabolu-Tekirdag	4	L	1	0	1	1	1	0	1	0	0	3	3
	5	L	1	0	0	0	1	0	0	0	0	1	1
	9	L	9	5	5	7	9	1	7	1	1	9	9
		P	9	7	5	7	9	5	7	1	1	9	9

(0: resistant, 9: susceptible, L: Leaf blast, P: Panicle blast)

Table 3. Yield trial results of Pi40 introgressed advanced lines

Line/ cultivar	Pedigree	Yield t/ha
Halilbey (st)	-	9,624 a
Kızıltan (st)	-	9,161 ab
2011020-TR2959-1-5-2	Kızıltan / IR83260-1-1-1-7-1-2-B	9,075 abc
2011020-TR2959-3-2-2	Kızıltan /IR83260-1-1-1-7-1-2-B	9,026 abc
Osmancık-97 (st)		8,848 abc
2011020-TR2959-6-4-3	Kızıltan /IR83260-1-1-1-7-1-2-B	8,803 abcd
2011020-TR2959-1-6-2	Kızıltan /IR83260-1-1-1-7-1-2-B	8,612 bcd
2012040-TR3134-1-1	IR83260-1-1-1-2-1-22-B /Kızıltan	8,178 cd
2011020-TR2959-2-3-2	Kızıltan / IR83260-1-1-1-7-1-2-B	7,855 d
2011020-TR2959-5-4-3	Kızıltan/ IR83260-1-1-1-7-1-2-B	6,846 e
2011020-TR2959-1-6-1	Kızıltan/ IR83260-1-1-1-7-1-2-B	6,844 e
2011020-TR2959-4-3-2	Kızıltan / IR83260-1-1-1-7-1-2-B	6,574 e
	CV %: 6.75, LSD : 948.2	

Means not sharing the letter similar differ significantly



1- Kiziltan/IR83260-1-1-1-7-1-2-B (F_1)
2- IR83260-1-1-1-7-1-2-B/Kiziltan (F_1)
3- IR83260-1-1-1-2-1-22-B (*Pi40* donor)
4- IR83260-1-1-1-2-1-22-B (*Pi40* donor)
5- Kiziltan
6 – 20- Kiziltan/IR83260-1-1-1-7-1-2-B (F_2)

Figure 1. Gel image of genotypes, and segregating materials for 9871.T7E2b primer

CONCLUSION

Based on the results of this study, it was concluded that the *Pi40* gene shows very good resistance to both leaf and panicle blast in all Turkish rice-growing regions in field conditions, and this durable resistant *Pi40* gene should be used to improve rice blast resistant cultivar programs in Turkey. In this study, the blast-resistant *Pi40* gene was transferred to high-yielding promising lines obtained from Kiziltan and *Pi40* gene-harboring donor parents cross with MAS. With this study, high-yielding promising lines, resistant to both leaf and panicle blast in field conditions were obtained.

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