AGRIBALKAN 2023

V. BALKAN AGRICULTURAL CONGRESS

20-23 SEPTEMBER 2023,

EDİRNE, TURKEY

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In Trakya University Balkan Congress Center, Edirne, Turkey

Organized by Trakya University

with

Trakya Universities' Union, Balkan Universities' Union, Namik Kemal University, Onsekizmart University - Turkey, Uludag University, Turkey, Agriculture University of Plovdiv, Trakia University-Stara Zagora - Bulgaria, Democritus University of Thrace – Greece and with contribution of other Balkan Institutions…

Dear Colleagues,

You are welcome to our congress which will be organized by Trakya University supporting with Trakya Universities Union, Balkan Universities Association and together with other Balkan Universities and İnstitutions.

The aim of our international congress is to present the newest research results and research goals, analyze current conditions and perspectives in agriculture.

Conference activities;

Plenary sessions with oral and poster presentations are on 20-23 September 2023.

You are welcome to our congress and Edirne, TURKEY,

Yours sincerely,

Prof. Dr. Erhan TABAKOĞLU

Rector of Trakya University
Honorary Chair of Congress

Prof Dr Yalcin KAYA

Head of Organizing Committee

FOREWORD

Agriculture is so important sector feeding all humankind, but it needs new developments and technologies to supply enough food for increasing world population year by year. Turkey is one leading agricultural economy in the world. Balkan region is one the important agricultural areas of the world having rich soils producing different crops vastly and keeping enormous biodiversity for our future.

As there have been many different scientific meetings around the world, we intended to bring three communities together, namely science, research and private investment, in a friendly environment of Edirne / Turkey to share what they have and get benefit from each other. Trakya University intended to aim that agricultural community in Balkan areas should come together in that important event. Our congress goal is the agricultural subjects should be kept broad in order to provide opportunity to the science community to present their work that can be off value for agriculture.

First Balkan Congress was organized by Trakya University in 2014 as the biggest agricultural congress in Turkey and Balkan region. In the first congress, over 700 participants were presented total 830 papers (650 poster and180 oral presentations) and invited speakers presented country reports from all Balkan countries. 2nd Balkan Agriculture Congress was organized by Tekirdağ Namik Kemal University in 2017. The 3rd Balkan Agriculture Congress was hosted again in Trakya University in Edirne, Turkey in 2021 due to 40th anniversary of Trakya University. There was a worldwide participation from 41 countries with 406 papers contributed by 988 authors with 288 oral, 118 e-poster presentations. In 4th Agribalkan Congress, there was a worldwide participation from 41 countries with 388 papers contributed by 888 authors.

As fifth one, Trakya University will host you again in Edirne, Turkey in 2023. We would like to thank all participants for great interest worldwide to our AGRIBALKAN 2023 congress. We got 488 papers from 25 countries contributing by 1144 authors with consisting of 288 oral and 180 poster papers.

We hope that this congress will help to solve our problems with establishing good network collaborations, joint projects and better relationships among countries with sharing our knowledge and experiences together. We wish success for this meeting and hope a great scientific achievement with your contributions.

Edirne is very nice, lovely and historical city at just the edge of Europe, but just right at the heart of Balkan region and history endowed with monuments reminding imperial past. We are much pleased to host you all in Edirne and in Turkey. We would like to thank you to join this congress and we would like to give also special thanks our sponsors and collaborators for giving us big supports to organize this event.

We wish you nice stay in Edirne for truly rewarding days.

Prof. Dr. Erhan TABAKOGLU
Rector of Trakya University
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CONTENTS

RETROTRANSPOSON-BASED MOLECULAR MARKERS: AN EFFICIENT TOOL FOR GENETIC DIVERSITY ASSESSMENT IN CROP PLANTS

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ABSTRACT

Plant genetic diversity is the main resource of agricultural breeding. Genetic diversity enables plants to adapt to environmental changes and increase their resilience. To cope with the effects of factors such as climate change, it is important to obtain plants with high genetic diversity. Genetic diversity also increases the ability of plants to fight new diseases and pests. Retrotransposons are mobile elements in the genome and cause genetic variation. Therefore, retrotransposon-based molecular markers are powerful and widely used tools to better understand plant genetic variation. These markers are used to analyze genetic differences between plant species, subspecies, and populations by detecting variations in specific regions of retrotransposons. The use of retrotransposon-based molecular markers offers important applications in many areas such as plant breeding studies, conservation of species, management of genetic resources, and monitoring of ecosystems. Inter-primer binding sequence (IPBS), sequence-specific amplified polymorphism (SSAP), retrotransposon-based insertion polymorphism (RBIP), inter retrotransposon amplified polymorphism (IRAP), and retrotransposon-microsatellite amplified polymorphism (REMAP) are the commonly used retrotransposon-based molecular markers**.** This study focuses on the use of retrotransposon-based molecular markers that reveals the genetic diversity of crop plants.

Keywords: Breeding, Genetic Diversity, iPBS, Molecular Markers

INTRODUCTION

The concept of biodiversity constitutes the main source of many disciplines like biotechnology, agriculture, medicine, and industry (Yılmaz et al., 2022). Biological diversity is a whole formed by genes, species, ecosystems (living environments), and ecological events (Selim et al., 2015). Ecological diversity can be defined as the different geographical conditions and soil structures, topographic conditions, climatic conditions, and the relationships between all living things in that region (Çetiner, 2010; Doğan et al., 2010). The disappearance of biological factors and the loss of abiotic factors negatively affect the ecological diversity. In addition to these factors, unfavorable conditions in processes such as water circulation, soil formation, and energy flow affect the diversity negatively (Doğan et al., 2010). Genetic diversity is a basic unit of biodiversity. The diversity in an organism's genetic material is the underlying cause of intra- and inter-species variability. Genetic variation assures the endurance and adaptability, fitness, and evolutionary flexibility necessary for organisms to survive. Genetic fitness is a vital survival mechanism for many organisms due to rapid changes in environmental conditions (Ashry, 2013).

Molecular markers are efficient tools for studying the biodiversity and genetic variation studies. DNA molecular markers basically detect variation in the nucleotide sequence at a particular locus in the genome (Kalendar et al., 2011). The DNA markers directly help identifying genetic variations independent of many factors such as developmental stage, habitat conditions, tissue and organ type (Shi et al., 2023). Molecular markers are considered the most effective technique to evaluate and categorize different sources of germplasm (Jiang et al., 2023). They are also indispensable tools of agricultural genetics by discover DNA fingerprints of varieties and determining genetic differences, as well as determining genus and species phylogeny, selection of lines containing genes with desired characteristics for breeding studies, and creation of linkage maps (Sipahi and Yumurtacı, 2020). Important reasons for using molecular markers can be listed as; accelerating the transfer of desired genes between varieties and species, enabling the transfer of new genes from related wild species, enabling genetic analysis of characters controlled by more than one gene, revealing genetic relationships between plants that cannot be crossed with each other in hybridization studies, facilitating and accelerating gene cloning (Aksu and Şahin Çevik, 2015). This study focuses on the use of retrotransposon-based molecular markers that reveals the genetic diversity of crop plants.

RETROTRANSPOSON BASED MOLECULAR MARKERS

Transposons or transposable elements (TEs) are mobile DNA fragments that have the ability to replace in the genome, change the amount of DNA in the genome and cause mutations. TEs perform the transposition process in the genome by a mechanism called transposition (Karaman and Karlık, 2022). Transposons are found in the genome of almost all organisms. For example, they can make up 90% of the plant genome, 54% of the human genome, and 50% of the mammalian genome (Koçak et al., 2020; Yushkova and Moskalev, 2023). They contribute to genetic diversity through both splice site polymorphism and minor structural rearrangement. TEs are divided into two groups based on the transposition mechanism and mode of propagation; Retrotransposons (class I elements) and transposons (class II elements) (Vershinin et al., 2003).

Retrotransposons (REs) can produce high numbers of copies during evolution and are an important component of plant genome structure (Usai et al., 2020). Transposons (TEs) are DNA sequences that have the ability to migrate within the genome. The cut-and-paste mechanism without an RNA intermediate is used by Tes and they have an open reading frame encoding tansposase enzyme and terminal inverted repeats (TIRs). The enzyme named as transposase is responsible for removing the transposon and placing it in a new location (Yushkova and Moskalev, 2023). The dynamism and distribution of various transposon groups has led to their widespread use as molecular markers (Retrotransposon-based molecular markers) (Kalendar et al., 2011). Several molecular markers based on retrotransposon insertion polymorphisms such as IRAP, REMAP, S-SAP, iPBS, and RBIP have been previously developed (Monden et al., 2014) (Figure 1).

RBIP (Retrotransposon-based Insertion Polymorphism)

In this method, first primer binds to the flanking genomic region at the LTR-RT insertion and second primer binds to the LTR-RT at regions of the 3' or 5' ends of the LTRs, and then this provide detection of retrotransposons in the genome. One of the disadvantages of this method is its high cost compared to other methods. Secondly, RBIP requires knowledge of the sequences of the 5' and 3' flanking regions of LTR-RT insertions. Another limitation of RBIP is wide size range (about 3-5 Kbp) compared to standard PCR. RBIP marker can be a codominant marker when three primers used for detection both the presence and absence of TE insertion, and also can be dominant marker when only two side primers are used (Alzohairy et al., 2014; Alzohairy, 2016).

Searching the Web of Science (WOS) by "RBIP" criteria revealed that some articles were published in pea, jatropha, pear, mango, sweet potato, and coffee during 2010-2021 (Table 1). Meng et al., (2021) used the RBIP molecular marker method to determine intraspecific genetic diversity in sweet potato plants. The results of this study show that the RBIP marker can be used for cultivar identification, genetic diversity assessment and linkage mapping, but that sweet potato germplasms do not diversify well evolutionarily. In Hamon et al., (2011), SSAP, REMAP, and RBIP markers were applied to 182 accessions from 31 *Coffea* species and one *Psilanthus* accession. According to the combined results of markers, the usefulness of markers in estimating *Coffea* genetic diversity and timing of the speciation/differentiation events is reported.

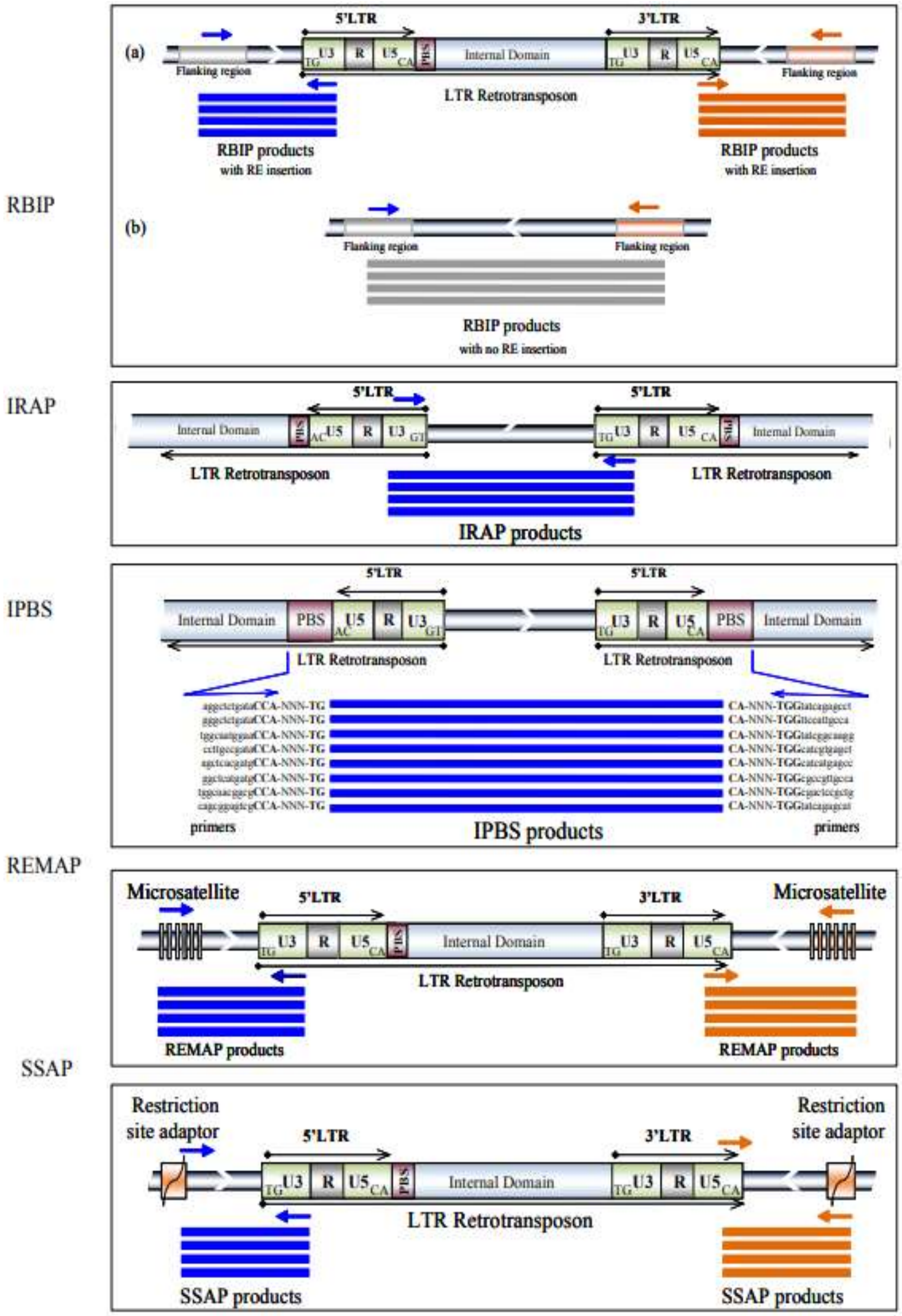

Figure 1. Retrotransposon-based molecular markers (Primers indicated by arrows) (Alzohairy, 2016).

IRAP (Inter Retrotransposon Amplified Polymorphism)

The number and size of the amplified regions of the inter-RT fragments were increased by various retrotransposon insertions. Therefore, we can use them as markers to detect polymorphism of the studied genotype. Primers should be selected as face outward from the LTRs of the LTR-RTs to amplify the region between the two RTs. The same or different RT families can be used for primer design. IRAP can also be performed with a single primer that matches the 5' or 3' end of the LTRs but points outward from the LTR itself. One disadvantages of this marker as follows; IRAP may produce too many fragments and good solubility gels were needed or may not produce products because the target amplification sites are too far to form amplicons. IRAP primers do not require radioactive or fluorescently labeled primers, whereas some markers like SSAP requires. This method has been widely used for genotyping of *Hordeum vulgare* and *Oryza sativa*, as well as *Musa*, *Brassica*, *Spartina*, *Triticum*, and *Solanum* species (Alzohairy, 2016).

Searching the Web of Science (WOS) by "IRAP" criteria revealed that approximately 120 articles in various plant species during 2010-2023, some examples of these studies were given in Table 1. Dilmen et al., (2023) used IRAP and REMAP markers to reveal the effects of salt stress and salicylic acid on retrotransposon polymorphism in wheat. In Ghaffarian and Mohammadi (2023), IRAP and REMAP markers were used for estimate the genetic diversity of *Melissa officinalis* populations from Iran. Both IRAP and REMAP markers had a high level of polymorphism and the average percent polymorphism was 96.35% and 90.78%, respectively. Marzang et al., (2020) used two RT-based marker systems, IRAP and REMAP to assess genetic variability and structure in a collection of 94 durum wheat genotypes from Iranian, Turkish and International cultivars. As a result, it was concluded that the genetic diversity of Iranian durum wheat is low. Shingote et al., (2019) used IRAP and ISSR markers to test the genetic fidelity of micropropagated clones of 47 sugarcane accessions, 20 polymorphic markers representing 98 loci were reported.

iPBS (Inter-primer Binding Sequence)

iPBS is one of the frequently used marker to demonstrate retrotransposon polymorphisms. The iPBS method is based on the amplification of the primary binding site by reverse transcriptase in the LTR retrotransposon (Çayır and Sevindik, 2022). The need for sequence information is a prerequisite for designing LTR-RT-based molecular barcodes. In this marker analysis, PCR amplification occurs between two nested primer binding sites of LTR-RTs. Because plants LTR-RTs are frequently mixed, intertwined, truncated, or inverted in the genome, LTR-RTs can be easily amplified using conservative iPBS primers. Retrotransposon motions and recombinations can also be observed because new inserts or recombinations will be polymorphic and will only appear in plant lines where insertions/recombinations have occurred (Alzohairy, 2016).

Searching the Web of Science (WOS) by "iPBS" criteria revealed that approximately 100 articles in various plant species during 2010-2023, some examples were given in Table 1. Kocak et al., (2023) studied 29 genotypes of the flax from the different sources including agricultural institutes and research centers in Türkiye by 12 iPBS and 33 ISSR markers. According to iPBS and ISSR markers data, there is consistency between agro-morphological results and the genetic diversity and population structure results. Carracedo et al., (2022) conducted study with LTR retrotransposon-based iPBS and IRAP techniques avocado accessions and genetic variations between cultivars have been characterized. In Demirel et al., (2018), the genetic identification and fingerprinting of 151 potato genotypes were determined by 45 iPBS markers. Also, it is reported that iPBS markers are efficient for the fingerprinting of potato germplasm. Baloch et al., (2015) studied 50 accessions from wild and cultivated *Lens* species with iPBS and ISSR markers, and they reported high level of polymorphism, at 82.92% and 51.92% with ISSR and iPBS markers, respectively.

Table 1. Some studies with retrotransposon based molecular markers on agriculture during 2010-2023.

RETROTRANSPOSON MARKER	SPECIES	REFERENCE
RBIP	Pea (*Pisum* spp.)	Jing et al., (2010)
	Pea (*Pisum sativum* L.)	Martin-Sanz et al., (2011)
	Jatropha (*Jatropha curcas* L.)	Alipour et al., (2013)
	Pear (*Pyrus* L.)	Jiang et al., (2015)
	Mango (*Mangifera indica* L.).	Nashima et al., (2017)
	Pea (*Pisum sativum* L.)	Gixhari et al., (2014)
	Sweet potato (*Ipomoea batatas* (L.) Lam.)	Meng et al., (2021)
RBIP, REMAP, SSAP	*Coffea* genus (31 species)	Hamon et al., (2011)
IRAP, REMAP	Durum wheat (*Triticum turgidum* L.)	Marzang et al., (2020)
	Maize (*Zea mays*)	Yigider et al., (2020)
	Artemisia anuua L.	Valizadeh et al., (2021)
	Rice (*Orzya sativa* L.)	Arvas et al., (2022)
	Lemon balm (*Melissa officinalis* L.)	Ghaffarian and Mohammadi (2023)
	Wheat (*Triticum aestivum* L.)	Dilmen et al., (2023)
IRAP	Sugarcane (*Saccharum* spp.)	Shingote et al., (2019)
	Citrus genus	Horibata and Kato (2020)
	Aegilops tauschii Coss.	Sha et al., (2021)
	Pistacia spp.	Chatti et al., (2022)
IRAP, iPBS	Avocado (*Persea americana*)	Carracedo et al., (2022)
iPBS	*Lens* genus (7 species)	Baloch et al., (2015)
	Potato (*Solanum tuberosum*)	Demirel et al., (2018)
	Wheat (*Triticum aestivum* L.)	Pour et al., (2019)
	Bermudagrass (*Cynodon* spp.)	Bülbül et al., (2021)
	Wheat (*Triticum aestivum* L.)	Nadeem (2021)

	Myrtle (*Myrtus communis*)	Tüzün-Kis and İkten (2022)
	Gossypium genus	Çayır and Sevindik (2022)
	Flax (*Linum usitatissimum* L.)	Kocak et al., (2023)
REMAP	Grape (*Vitis vinifera* L.)	Razi et al., (2020)
	Lallemantia genus	Koohdar et al., (2021)
REMAP, SSAP	Pigeon pea (*Cajanus cajan*)	Maneesha and Upadhyaya (2017)
SSAP	Peach (*Prunus persica* (L.) Batsch)	Jiao et al., (2014)
	Malus spp.	Savelyeva et al., (2017)
	Wild barley (*Hordeum brevisubulatum*)	Guo et al., (2018)
	Maize (*Zea mays*)	Roy et al., (2021)

REMAP (Retrotransposon-Microsatellite Amplified Polymorphism)

REMAP combines one primer that binds to the LTRs of LTR-RTs and another primer that binds to adjacent locus-specific simple sequence repeats (SSRs) of the genome. This technique is only applicable when the SSR is located near retrotransposons. Amplification between the retrotransposon and a nearby SSR do not requires restriction enzyme digestion and adapter ligation to generate bands belonging to marker (Alzohairy, 2016).

Searching the Web of Science (WOS) by "REMAP" criteria revealed that approximately 90 articles in various plant species during 2010-2023, some examples of these studies were given in Table 1. Safiyar et al., (2022) used IRAP and REMAP markers to evaluate genetic diversity of *Aegilops tauschii* accessions and its association with *Triticum durum* and *Triticum aestivum*. The study revealed that retrotransposon-based molecular markers are a suitable and reliable method to evaluate genetic diversity and phylogenetic analysis in *Triticum* and *Aegilops* accessions. In Razi et al., (2020), 42 primers belonging to REMAP were used to determine insertional polymorphisms of retrotransposons in 75 cultivated and wild grape genotypes, and polymorphism ratio was calculated as 95.43%. According to their results, there is important level of diversity at molecular and pomological level for future grape breding studies. Koohdar et al., (2021) studied medicinally important plants belonging to *Lallemantia* genus with REMAP markers to reveal the species delimitation and species relationship in genus and investigate discriminating power of the studied markers.

SSAP (Sequence Specific Amplified Polymorphism)

The method is based on technical steps of AFLP (Amplified Fragment Length Polymorphism) technique. SSAP is the first retrotransposon-based barcoding technology. SSAP uses a primer that matches the 3' end of one LTR and the other primer pairs with a restriction site adapter usually for MseI or Pst, like AFLP. Two or three labeled selective nucleotides are necessary for primer pairs. The non-selective primer pair may also be useful when restriction enzymes have a long sequence of recognition sites or when the copy number of LTR-RTs is low. Primers that are highly polymorphic, yielding clear and reproducible SSAP banding patterns are candidate primers for further studies (Alzohairy, 2016).

Searching the Web of Science (WOS) by "SSAP" criteria revealed that approximately 50 articles in various plant species during 2010-2023, some examples of these studies were given in Table 1. In a study by Kour et al., (2014), various regeneration protocol trials were conducted in MS (Murashige and Skoog) media prepared for *Artemisia absinthium* L. plants grown *in vitro*. In order to understand the genetic effects as well as the phenotypic effects, the genetic stability of the plants was examined by using ISSR and SSAP molecular markers. As a result, both markers could detect somaclonal variations in plants regenerated with callus, while no variation was detected in plants regenerated from nodal explants. SSAP was found to be more useful in detecting variability compared to the ISSR molecular marker and concluded that the direct regeneration protocol would be beneficial for the production of plants loyal to the species of this medicinally important plant. Roy et al., (2021) developed SCAR and SSAP markers for maize, and reported that developed markers might be useful especially for the crops with no genetic sequence information.

RESULT AND CONCLUSION

Genetic diversity studies of plant gene resources constitute the guarantee of sustainability in agricultural production. Therefore, very valuable genetic resources should be used as breeding material for the development of new varieties (Erat and Balık, 2022). With the information obtained from genetic marker studies, it has been concluded that which samples should be preserved in the gene banks and the acquisition of genetic information is very effective in the selection of different species and cultivars rather than genetically similar to each other (Demir, 2015).

Transposon elements have extraordinary potential for altering genome structure and gene function, due to their ability to move from one place to another within the genome or to produce new copies of themselves at any genomic location (Vershinin et al., 2003). Retrotransposons are useful for many studies from assessment of biodiversity and genome evolution, gene mapping and estimation of genetic distance/similarity, assessment of basic derivation of varieties, detection of somaclonal variation, traceability and purity of food. These methods are quite extensive, as LTR retrotransposons are ubiquitous (Kalendar et al., 2011).

In plant biotechnology researches, retrotransposons play a significant role in plant genome evolution. Molecular markers based on retrotransposons provide wide applications in genetic diversity determination, genetic mapping, phylogenetic analysis, and variety genetic identification of genotypes. In addition, DNA markers are more effective, accurate and reliable for distinguishing varieties and closely related species when compared with traditional phenotypic markers (Ouyang et al., 2021). It has been determined that retrotransposon-based molecular markers are very effective and easily applicable in determining genetic variation of various crop plants (Aydın, 2016; Coşkun, 2019).

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THE EFFECT OF THE HEATING PROCESS OF HEMP SEED CAKE AND PHYTASE ENZYMES ADDITION IN BROILER DIET ON CARCASS AND INTERNAL ORGAN SIZE

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ABSTRACT

The escalating demand for soybean meal and its inevitable utilization has compelled the feed industry to investigate alternative plant-derived protein feed ingredients actively. This study aimed to evaluate the effect of the utilization of hempseed cake (HC) in the broiler diet on carcass weight and internal organs. A total of 210 unsexed Ross 308-Day-Old-Chick were randomized into 7 treatments with 6 repetitions. Treatments were T1= Soybean meal-based diet, T2= Diet with 15% HC, T3=T2+ phytase enzyme (1000 FTU/kg), T4= Diet with 15% autoclaved HC (120 ^{0}C,10 minutes), T5= T4+phytase enzyme (1000 FTU/kg), T6= Diet with 15% roasted HC (120 ^{0}C,20 minutes), T7= T6+ phytase enzyme (1000 FTU/kg). Dietary HC and phytase enzymes in the broiler diet did not impact the weight of carcass, abdominal fat, liver, duodenum, cecum, and the relative length of the duodenum. Dietary HC and its combination with phytase enzyme increased heart weight percentage ($P<0.05$), while T2 significantly increased ($P<0.05$) the percentage of gizzard and spleen. Ileum weight and relative length of jejunum, ileum, and cecum improved significantly with dietary HC ($P< 0.01$). The Jejunum weight of T2 was significantly higher ($P<0.01$) than that of T1 and T3. It is concluded that dietary HC had no effect on the carcass, abdominal fat, and liver weight. However, the use of HC up to 15% without the heating process and the addition of enzymes has the highest impact on the enlargement of internal organ size.

Keywords: *Cannabis sativa* L, broiler chicken, hemp seed, small intestine, giblets

INTRODUCTION

Currently, the poultry industry and nutrition are constantly evolving, making the exploration of new and sustainable feed sources a complex challenge. One interesting ingredient is hempseed cake (HC) which is derived from the seeds of the versatile hemp plant (*Cannabis sativa*). Although HC has attracted attention for its rich nutritional profile and potential health benefits, recent studies have also shown concerns regarding its antinutritional factors and impact on broiler health.

The emergence of HC as a potential feed ingredient for broilers seemed promising due to its impressive nutritional content. The protein and other nutritional values of HC can vary depending on many factors such as hemp seed variety, climate, and extraction method. HC contains 34.1% crude protein, and 39% neutral detergent fiber (NDF) (Semwogerere et al. 2020). However, this optimism has been tempered by research showing that HC contains antinutritional compounds that may inhibit nutrient absorption, impair organ function, or impact the overall health and performance of chickens. Cannabinoids found in hemp seed, especially Delta-9-tetrahydrocannabinol (THC), are used to a limited extent due to their antinutritional and psychoactive properties. The main antinutritional constituents in hemp seed are phytic acid (22.5 mg/g), tannins (0.23 mg/g), cyanogenic glycosides (3.80 μmol/g), and trypsin inhibitors (2.88 TIU/mg protein) (Pojic et al., 2014). Therefore, pretreatments are needed to reduce the level of these anti-nutrients. Enzymes and heating treatments can be used to reduce the level of antinutrients and crude fiber in feed (Ptak et al. 2015; Konca et al. 2019; Magpantay et al. 2021). This study aimed to evaluate the effects of dietary HC on carcass weight and internal organ size of broiler chickens.

MATERIALS AND METHODS

The study was conducted in the broiler house at the Animal Science Department, Faculty of Agriculture, Ondokuz Mayıs University. A total of 210 unsexed broiler chicks were obtained from a commercial hatchery (Ross Breeders Anadolu, Turkiye) and housed in floor cages. Birds were fed a basic ration based on HC, corn, and soybean meal with nutritional requirements determined according to recommendations for the Ross strain. In this experiment, 42 cages with a size of 1x1.30 m were used for 42 days. By the randomized experimental design, 7 treatments with 6 replicates were designed with 5 animals in each replicate. Treatments were as follows:

T1: Corn-soybean-based basal ration

T2: Ration supplemented with 15% HC

T3: Ration supplemented with 15% HC + phytase enzyme (1000 FTU/kg)

T4: Ration supplemented with 15% autoclaved HC (120 ^{0}C, 10 min)

T5: Ration supplemented with 15% autoclaved HC (120 ^{0}C, 10 min) + phytase enzyme (1000 FTU/kg)

T6: Ration supplemented with 15% roasted HC (120 ^{0}C, 20 min)

T7: Ration supplemented with 15% roasted HC (120 ^{0}C, 20 min) + phytase enzyme (1000 FTU/kg)

The ration was prepared according to the formulation with the ingredients used including corn, soybean meal, HC, vegetable oil, methionine, lysine, threonine, salt, limestone, calcium phosphate, and phytase (Table 1). At the end of the experiment on day 42, the average weight of

5 chickens in each replicate was measured and then one chicken closest to the average was slaughtered. Then, the weight of the carcass, spleen, heart, liver, gizzard, abdominal fat, and intestinal weight and length were determined. The percentage of internal organ weight was calculated by dividing the weight of the organs by the live weight multiplied by 100. The relative length of the intestine was obtained by dividing the length of the intestine by the live weight in cm/kg.

The data were analyzed by ANOVA with IBM SPSS statistics 22 program, and then the significance value at $P<0.05$ was further tested with Duncan multiple range.

Table 1 Ingredients and nutrient composition of broiler chicken diets

Ingredients (%, as fed basis)	Starter (0-10 days)		Grower (11-24 days)		Finisher (25-42 days)	
	Control	HC	Control	HC	Control	HC
Corn	53.20	48.31	59.00	54.00	65.18	60.20
HC (37%)	0	15.00	0	15.00	0	15.00
Soybean meal (44%)	39.50	29.00	34.50	24.00	29.00	18.50
Vegetable oil	3.15	4.00	3.20	4.00	2.80	3.60
Dicalcium phosphate	2.17	2.12	1.76	1.68	1.42	1.39
Dl-Methionine	0.31	0.27	0.27	0.23	0.26	0.22
Limestone	0.80	0.80	0.58	0.60	0.56	0.56
L-Lysine sulfate	0.22	0.02	0.18	0.01	0.27	0.05
L-Threonine	0.18	0.01	0.04	0.01	0.04	0.01
Salt	0.37	0.37	0.37	0.37	0.37	0.37
Vitamin and mineral premix	0.10	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100	100
Nutrient composition (%)						
Metabolizable energy (kcal/kg)	2982	2981	3061	3058	3112	3107
Crude protein	23.5	23.5	21.5	21.6	19.5	19.5
Crude fiber	2.52	5.08	2.51	5.07	2.5	4.46

Ether extract	2.60	4.01	2.80	4.30	2.96	5.06
Lysine	1.32	1.32	1.18	1.19	1.10	1.08
Methionine +cystine	1.00	1.00	0.92	0.92	0.86	0.86
Ca	0.95	0.95	0.78	0.76	0.65	0.66
Avail. P	0.50	0.5	0.43	0.42	0.36	0.36

RESULTS AND DISCUSSION

Dietary HC and phytase enzymes in the broiler diet did not impact the weight of carcass, abdominal fat, liver, duodenum, cecum, and the relative length of the duodenum. Dietary HC and its combination with phytase enzyme increased heart weight percentage (P<0.05), while T2 significantly increased (P<0.05) the percentage of gizzard and spleen. Ileum weight and relative length of jejunum, ileum, and cecum improved significantly with dietary HC (P< 0.01). Jejunum weight of T2 was significantly higher (P<0.01) than that of T1 and T3.

The effect of dietary HC on the weight of the heart, gizzard, spleen, and intestine size of broiler chickens can be attributed to several factors related to the composition of HC and its interaction with the chickens' physiology. It's important to note that while some studies have reported increases in the size or weight of these organs when HC is included in broiler chicken diets, the exact mechanisms behind these effects may not be fully understood and could vary depending on the specific composition and the level of the dietary HC. Besides HC contains various essential nutrients, including vitamins, minerals, and amino acids, HC also has high crude fiber and anti-nutrients. The presumed presence of high crude fiber and anti-nutrients in the feed is supported by the enlargement of the size of the heart, gizzard, spleen, and intestines in this study.

Table 2. Percentage of internal organs weight (%)

Treatments	Carcass	Hearth	Liver	Gizzard	Spleen	Abdominal fat
T1	74.05	0.48^{a}	1.59	1.77^{a}	0.09^{a}	0.86
T2	74.08	0.60^{b}	1.67	2.31^{b}	0.14^{b}	1.34
T3	74.06	0.59^{b}	1.66	2.15^{ab}	0.11^{ab}	1.39
T4	73.97	0.58^{b}	1.61	2.08^{ab}	0.10^{a}	1.28
T5	74.70	0.58^{b}	1.69	1.75^{a}	0.09^{a}	1.45
T6	72.52	0.63^{b}	1.66	1.97^{ab}	0.11^{a}	1.34
T7	74.52	0.55^{ab}	1.60	1.95^{ab}	0.09^{a}	1.30

SEM	0.28	0.01	0.024	0.31	0.03	0.04
P-Value	0.52	0.02	0.07	0.01	0.04	0.87

T1: Corn-soybean based basal ration; T2: Ration supplemented with 15% HC; T3: Ration supplemented with 15% HC + phytase enzyme (1000 FTU/kg); T4: Ration supplemented with 15% autoclaved HC (120 ^{0}C, 10 min); T5: Ration supplemented with 15% autoclaved HC (120 ^{0}C, 10 min) + phytase enzyme (1000 FTU/kg); T6: Ration supplemented with 15% roasted HC (120 ^{0}C, 20 min); T7: Ration supplemented with 15% roasted HC (120 ^{0}C, 20 min) + phytase enzyme (1000 FTU/kg)

Table 3. Weight and relative length of the intestines

Treatments	Weight (%)				Relative length (cm/kg)			
	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
T1	0.57	0.97^a	0.75^a	0.49	13.24	31.74^a	30.66^a	6.12^a
T2	0.69	1.59^b	0.99^{ab}	0.49	14.01	40.58^c	35.05^b	8.97^b
T3	0.87	1.11^a	0.99^{ab}	0.59	14.35	35.07^{bc}	34.76^b	8.14^b
T4	0.71	1.28^{ab}	1.04^{ab}	0.47	12.87	33.18^{ab}	31.84^b	7.61^{ab}
T5	0.51	1.36^{ab}	1.23^b	0.54	13.08	35.67^{bc}	33.86^b	8.33^b
T6	0.68	1.50^b	1.16^b	0.50	14.62	37.16^{bc}	34.86^b	8.76^b
T7	0.69	1.32a^b	1.02^b	0.44	14.93	35.96^b	35.21^b	8.51^b
SEM	0.02	0.05	0.03	0.02	0.31	0.75	0.81	0.19
P-Value	0.42	<0.01	<0.01	0.21	0.45	<0.01	<0.01	<0.01

T1: Corn-soybean based basal ration; T2: Ration supplemented with 15% HC; T3: Ration supplemented with 15% HC + phytase enzyme (1000 FTU/kg); T4: Ration supplemented with 15% autoclaved HC (120 ^{0}C, 10 min); T5: Ration supplemented with 15% autoclaved HC (120 ^{0}C, 10 min) + phytase enzyme (1000 FTU/kg); T6: Ration supplemented with 15% roasted HC (120 ^{0}C, 20 min); T7: Ration supplemented with 15% roasted HC (120 ^{0}C, 20 min) + phytase enzyme (1000 FTU/kg)

HC may influence the size or function of the gizzard due to its fiber content. Hemp seed cake is also a source of dietary fiber, typically comprising around 20% to 30% of its dry weight (Kasula et al. 2021). Dietary fiber can stimulate the development and activity of the gizzard, potentially leading to an increase in its size as it works to grind and digest feed. However, feeding with moderate amounts of insoluble fiber increases chyme retention in the digestive tract, and digestive enzyme production, which in turn increases nutrient digestibility (Mateos et al. 2012). Increasing the fiber component in the diet interferes with nutrient retention and reduces growth performance in broilers because fiber cannot be hydrolyzed by digestive enzymes. Dietary fiber can have a bulking effect on the digestive tract, stimulating the development and enlargement of various parts of the gastrointestinal system, including the jejunum, ileum, and cecum. However, non-starch polysaccharides can be fermented by certain microbes in the cecum to produce H2, CH4, CO2, short-chain fatty acids, and lactic acid (Jha and Mishra, 2021). This may also be responsible for the enlargement of cecum size in this study. Enlargement of broiler heart size is usually caused by the addition or thickening of heart muscle tissue to adjust to excessive contractions where toxins and anti-nutrients in feed affect the condition of the muscle in the heart (Badaruddin et al. 2022). Meanwhile, the enlargement of spleen size may also be due to the negative effect of THC on the immune response of broilers. Indeed, cannabinoids consist of CB1 and CB2 receptors which are commonly found in all birds. CB1 receptors are found mainly in the brain, and in the reproductive system while CB2 receptors are found in the immune system, especially in the spleen responsible for anti-inflammatory effects (Mahmoudi et al.2015). Anti-nutrients have been observed to inhibit nutrient absorption and utilization and may cause damage to some organs such as the liver and spleen (Emiola et al. 2007).

CONCLUSION

It is concluded that dietary HC had no effect on carcass, abdominal fat, and liver weight. However, the use of HC up to 15% without the heating process and the addition of enzymes has the highest impact on the enlargement of internal organs and intestines size.

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THE POTENTIAL OF HEMP SEED CAKE FOR THE BROILER CHICKEN DIET

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ABSTRACT

The increasing demand for plant protein-based feed ingredients has encouraged the feed industry to explore non-conventional feed ingredients. This review is intended to discuss the nutritional content of hemp seed and also emphasizes the application of hemp seed cake as a broiler diet. *Cannabis sativa* L., commonly grown for the fiber industry, has been legalized by many European countries because of its low Tetrahydrocannabinol (THC) content (<0.3%). Hemp seed contains 30-35% oil and 30%-40% crude protein which is abundant in methionine, arginine, and cystine. In addition, hemp seed also contains 75-80% polyunsaturated fatty acids (PUFA), especially Omega-3 and Omega-6. Its application in broiler chicken diets improves productive performances, but it decreases body weight and feed efficiency at high levels. Dietary hempseed cake has been shown to reduce total cholesterol, *low-density* lipoprotein (LDL), and triglycerides, and to increase serum *high-density lipoprotein (HDL).* The current review suggested that hemp seed cake has the potential to be an alternative plant protein source for broiler diets. However, the content of THC and its antinutritional compounds, especially phytic acid, tannins, cyanogenic glycosides, and trypsin inhibitors may limit its use.

Keywords: anti-nutrient, broiler chicken, *Cannabis sativa* L, performances

INTRODUCTION

The increasing global demand for poultry products has driven the poultry industry to constantly explore alternative and sustainable feed ingredients that can provide optimal nutrition to broiler chickens, enhance broiler chicken performance, and optimize production efficiency. Hemp has been cultivated for thousands of years for its versatile applications, ranging from textiles and industrial products to medicinal use. It belongs to the *Cannabaceae* family, which includes both industrial hemp (*Cannabis sativa*) and marijuana (*Cannabis indica* and *Cannabis ruderalis*). While marijuana is known for its psychoactive properties due to its high THC (tetrahydrocannabinol) content, industrial hemp contains very low THC levels and is primarily cultivated for its fibers, seeds, and oil (Nath, 2022). Hemp plant seeds, which constitute approximately 25% of their weight, have recently emerged as a valuable source of nutrition and functional compounds (Pang et al., 2021). After the extraction of hemp oil from the seeds, a residue called hemp seed cake remains, which possesses a well-balanced and nutrient-rich profile. This naturally occurring byproduct contains essential amino acids, beneficial fatty acids, vitamins, minerals, and bioactive compounds (Semwogerere et al., 2020; Singh et al., 2022). This alternative feed ingredient not only helps in reducing dependence on conventional feed source, such as soybean meal and corn, but it may also contribute to the sustainability of the poultry industry by utilizing an underutilized resource from the hemp industry. Given the complexity of broiler nutrition and the multitude of factors that influence growth performance, it is crucial to thoroughly investigate the impact of incorporating hemp seed cake into broiler diets. Various studies have already demonstrated the potential benefits of using hemp seed cake as a feed ingredient, including improved growth rates, feed conversion efficiency, and meat quality attributes. Nevertheless, to fully harness the advantages of hemp seed cake and ensure its safe and effective integration into broiler diets, additional research is warranted.

This review article aims to examine the existing literature on the potential of hemp seed cake in broiler-chicken diets. We analyze the nutritional composition of hemp seed cake and its influence on broiler chicken performance and meat quality.

Hemp Seed Production

Hemp plants can vary in appearance based on their variety and growing conditions. Generally, these plants are tall, and slender and can grow up to 6 meters. The growth cycle of this plant usually lasts about 120 to 150 days, depending on the variety and environmental factors. Hemp plants can grow well in temperate climates with moderate rainfall. It can tolerate a wide range of temperatures but generally thrives in areas with an average temperature of 16°C to 27°C (Visković, et al.2023)

Table 1. Hemp seed production in some countries (tonnes)

Country	Year	
	2018	2019
Chinese	106,200	71,423
Russia	2,117	2,893
Chile	1,522	1,539
Ukraine	647	650
Iranian	202	205
Turkiye	3	20
World	110,691	76,730

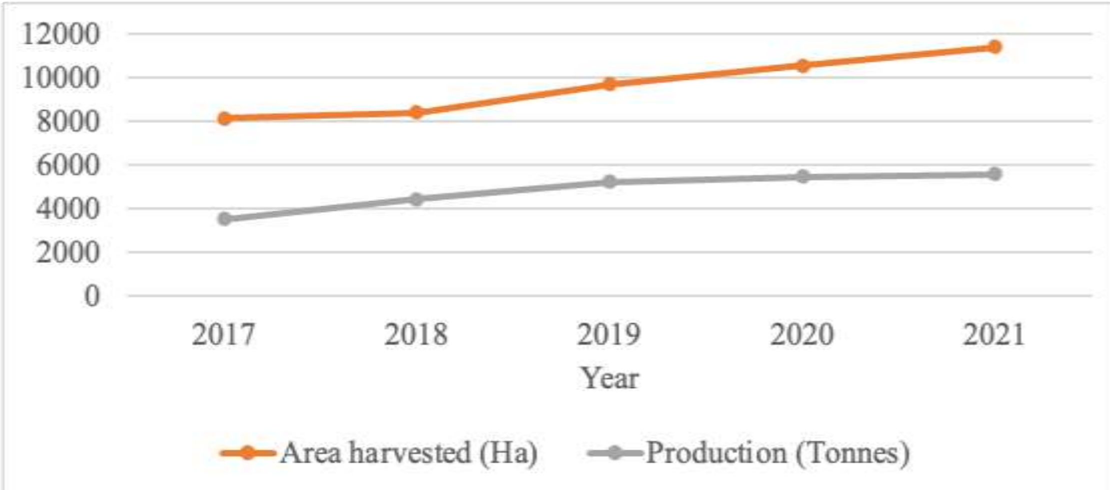


Figure 1. Global production of hemp seed (tonnes)

Hempseed production data from five countries based on the FAO 2023 database is presented in Table 1 and globally in Figure 1. The production of hempseed is significantly growing due to several factors such as regulatory changes, market demand, and farming practices which from 2017 to 2021 started from 3,517.4 tonnes (8,151 ha) to 5,566.02 tonnes (11,422 ha). Additionally, hemp production might not be accurately reported or recorded in some countries due to various reasons, including its association with cannabis and differing legal definitions. Therefore, data from major hemp producers such as Canada is not reflected in the FAO database. In modern times, hemp cultivation has been regulated due to its association with marijuana and its legal status in many countries. However, with the increasing recognition of hemp's economic and ecological benefits, its cultivation has been legalized and regulated in numerous regions. According to FAO data (2023), there are currently 6 countries in the world that produce hemp for seed purposes. Hemp is produced for fiber and seed in many countries around the world. In 2018 and 2019, 11,0691 and 76,730 tonnes of hemp seeds were produced in the world of which China contributes almost 94.50 % of the world's hemp seed production. Furthermore, the largest producers in the world are followed by Russia (2.84%) Chile (1.69%), while Turkiye only contributes 0.01% of the total production from 2018 to 2019.

Nutrient Composition of Hemp Seed Cake

Hemp seed cake is a byproduct obtained after extracting oil from hemp seeds. The nutrient content of hemp seed cake varies slightly based on factors such as seed variety, growing conditions, and processing methods. The nutrient composition of hemp seed cake is presented in Table 2 and Figure 2. Hemp seed contains 30-35% oil, 34% crude protein, 39.5% NDF, and 27.5 ADF (Semwogerere et al., 2020; Singh et al., 2022). Although the crude protein content increases in meal, which is the by-product of seed oil extraction, the energy level decreases compared to whole seed due to a decrease in oil content with an energy of about 385.72 kcal/100g (Singh et al., 2022). Hemp seed cake is distinguished by its impressive protein content, with levels ranging from 25% to 40%. The protein fraction in hemp seed cake is highly digestible, containing all essential amino acids required for optimal broiler growth. The balanced amino acid profile of hemp seed cake, especially its abundant methionine, arginine, and cystine content, can overcome the amino acid deficiencies often found in conventional feedstuffs such as soya meal (Figure 2).

Hemp seed cake is a valuable source of fats, comprising around 10% to 15% of its dry weight. The lipid fraction is notable for its high content of polyunsaturated fatty acids (PUFAs), particularly linoleic acid (omega-6) and alpha-linolenic acid (omega-3). Hemp seed cake contains 13.58 % monounsaturated fatty acids (MUFAs), 75.25 % polyunsaturated fatty acids (PUFAs), 19.08 % omega-3, and 56.18 % omega-6 (Occhiuto et al., 2022). The balanced ratio of omega-6 to omega-3 fatty acids (2.94:1) in hemp seed cake is of significant nutritional interest, as this ratio is known to influence the inflammatory response and overall health of broiler chickens. Incorporating hemp seed cake into broiler diets may help enhance the meat's fatty acid profile and contribute to a healthier product for consumers. Hemp seed cake contains various essential vitamins and minerals, including vitamin E, B vitamins (B1, B2, B3, B6), magnesium (2.3 g/kg), phosphorus (28.0 g/kg), and potassium (5.0 g/kg), and tocopherol (516 mg/100g) (Semwogerere et al., 2020).

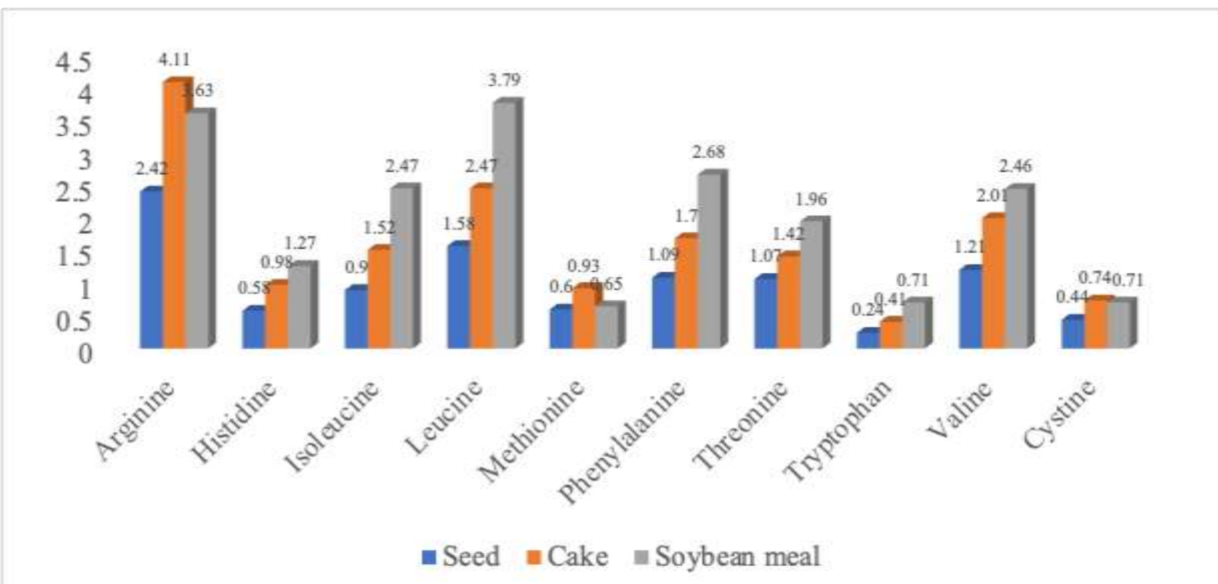
However, the presence of certain anti-nutritional factors, such as trypsin inhibitors and phytates, may limit protein utilization and necessitate careful consideration of inclusion levels. Phytic acid and cannabinoids found in hemp seed such as cannabidiol (CBD), cannabigerol (CBG), and Delta-9-tetrahydrocannabinol (THC) (Figure 3), are used to a limited extent due to their antinutritional and psychoactive properties. Cannabinoids are compounds that are not produced by cannabis seeds but can move from leaves and flowers to seeds and also to cannabis oil when the extraction process. However, it has been reported that if the THC in the hemp plant does not exceed 0.3%, it can be safely used in animal nutrition (Jing et al., 2017). It is reported that no cannabinoid residues are found in eggs, blood, body tissues, and organs (breast meat, abdominal fat, liver, kidneys, spleen) of laying hens fed hemp seed meal (Kasula et al., 2021). The main antinutritional constituents in hemp seed are phytic acid (22.5 mg/g), tannins (0.23 mg/g), cyanogenic glycosides (3.80 μmol/g), and trypsin inhibitors (2.88 TIU/mg protein) (Pojic et al., 2014). Phytic acid can reduce protein digestibility and improve amino acids, endogenous nitrogen, and mineral excretion (Bernardes et al., 2022).

Table 2. Nutrient composition of hemp seed cake

Nutrient composition (%)*	
Dry matter	92.90
Crude protein	34.10
Extract ether	11.60
NDF	39.50

ADF	27.50
Ash	6.80
Fatty acids (%)**	
SFAs	11.17
MUFAs	13.58
PUFAs	75.25
n-3 PUFAs	19.08
n-6 PUFAs	56.18
n-6/n-3 PUFAs	2.94

Source : * Semwogerere et al (2020), ** Occhiuto et al (2022)



Source : Semwogerere et al ,2020

Figure 2. Hemp seed cake amino acids profile compared to soybean meal

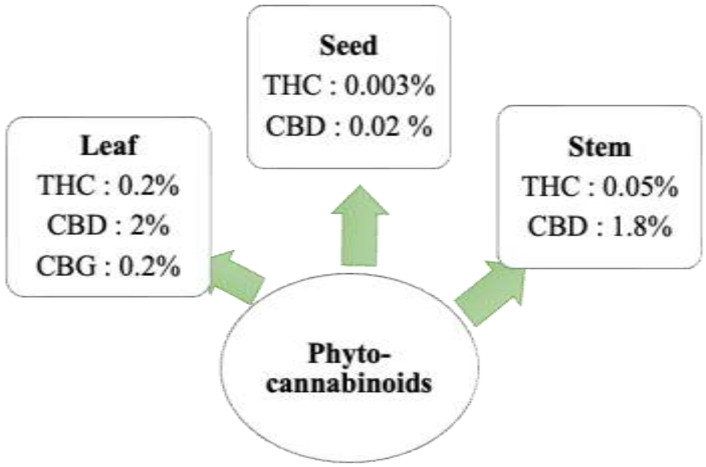


Figure 3. Phytocannabinoid content in the hemp plant (Krüger et al., 2022)

Hemp Seed Cake as Broiler Chicken Diet

Hemp seed cake is a byproduct obtained after extracting oil from hemp seeds. Its nutritional composition plays a pivotal role in determining its suitability as a potential feed ingredient for broiler chickens. Rich in essential nutrients, hemp seed cake has gained attention as a promising source of protein, fats, fiber, vitamins, minerals, and bioactive compounds, making it an intriguing candidate for enhancing broiler chicken nutrition. Studies have highlighted the following effects of hemp seed cake on broiler chickens.

Hemp seed cake's high protein content, balanced amino acid profile, and digestibility contribute to enhanced growth performance in broiler chickens. Hemp seed cake is rich in essential vitamins (e.g., E and B) and minerals (e.g., magnesium, phosphorus, potassium, zinc), which can enhance the overall nutritional profile of broiler diets. The inclusion of hemp seed cake can help meet the birds' nutrient requirements and potentially reduce the need for additional supplementation with synthetic vitamins and minerals. 5% hemp seed meal can be added to broiler diets instead of soybean meal, but 15% hemp seed meal was reported to reduce live weight in broiler chickens (Ondrej et al., 2015). Adding 10 % hemp seed meal to broiler diets was reported to increase live weight and feed conversion efficiency (Khan et al., 2010). 7.5% hemp seed meal containing 0.1% dextran oligosaccharide did not affect broiler performance (Mahmoudi et al., 2015). However, a study by Khan et al (2010) using 20% hemp seed in broiler feed was shown to result in significantly higher body weight gain, lower feed intake, and greater FCR compared to the control. The optimal levels of inclusion may vary depending on factors such as the age of the birds, composition of the basal diet, and processing methods of hemp seed cake.

The composition of whole hemp seed can be used as a source of feed additives while creating functional products for broilers. The high level of linoleic acid and linolenic acid makes full-fat hemp seed an alternative for the production of broiler chickens enriched with n-3 fatty acids. Hemp seed contains 31% oil with more than 80% PUFA and is rich in omega-3 and omega-6 fatty acids in an optimal ratio (1:3) for human health. Omega-3 and omega-6 cannot be produced naturally by the human body. Adding these fatty acids to foods can positively affect rheumatoid arthritis and cardiovascular, psychiatric, and immunological disorders (Callaway, 2004). The unsaturated fatty acid content of hempseed was shown to significantly lower serum total cholesterol, LDL and increase HDL (Mahmoudi et al. 2015). The inclusion of hempseed cake has no impact on the protein and fat content of chicken breast and thigh, but affects the color and odor of broiler meat with a positive response from consumers (Ondrej et al. 2021). The higher level of hempseed cake increases the yellow color of breast meat due to the high carotenoid content in hempseed cake. Changes in meat color are closely related to the content of carotenoids in feed such as beta-carotene, α-carotene, zeaxanthin, and lutein and zeaxanthin (Rodriguez-Amaya, 2016).

CONCLUSION

Hemp seed cake stands out as a nutritionally rich and balanced feed ingredient that has the potential to improve broiler chicken diets. Its high protein content, balanced amino acid profile, valuable fatty acids, dietary fiber, vitamins, minerals, and bioactive compounds make it a compelling alternative to traditional feed ingredients. However, proper evaluation of the inclusion levels, the presence of anti-nutritional factors and crude fiber, and potential health effects are essential to ensure the safe and effective utilization of hemp seed cake in broiler chicken nutrition.

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POTENTIAL OF NEW SUNFLOWER HYBRIDS DEVELOPED AT DAI

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ABSTRACT

Sunflower is a main oil seed crop in Bulgaria. Apart from wheat, it is the second important field crop for the country. Annually, between 6 500 000 and 7 000 000 da of oil seed sunflower are being sown. Unfortunately, due to changes in the market structure during the last 15-20 years, sunflower is grown without observing proper crop rotation. Considering the tolerance of field crops, sunflower is an unstable crop with negative self-tolerance, i.e. it is advisable not to grow sunflower in the same field for two consecutive years. The negative self-tolerance cannot be compensated by applying better agronomy practices since it is brought about primarily by phyto sanitary issues. The aim of this investigation was to demonstrate the level of the Bulgarian sunflower breeding and present the most recent sunflower hybrids and their potential with a view of their future use in practice. The investigation was carried out at DAI – General Toshevo during 2020 – 2022. It included 13 of the most recent sunflower hybrids of the institute. Five female lines and 11 fertility restorers were used to develop them. Some of the traits most important for a hybrid were followed: seed yield, oil content in seed, oil yield per da, plant height and vegetative growth period. Most promising were the hybrid combinations, which involved female lines 3607A and 813A. Their hybrids gave very good results according to the studied traits. Crosses 3607A x 29R and 3607A x 78R combined the best properties of a sunflower hybrid – seed yield, oil content in seed and oil yield. Cross 217A x 102R was submitted for official testing at the national Executive Agency of Variety Testing, Field Inspection and Seed Control and was released as sunflower hybrid Deveda. Mother line 217A and fertility restorer 102R demonstrated very good results according to the parameter oil content in seed and can be used in future high-oleic crosses.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is among the four most important annual crops for edible oil grown worldwide, together with soybean, oilseed rape and peanut (Rauf et al., 2017; Kaya et al., 2019; Mahmood et al., 2019). In Bulgaria, sunflower is a main oil seed crop (Petrov et al., 1994). Due to the specificity of its main organs – root, stem, leaves and head, it is more resistant to abiotic stress in comparison to other field crops and can be successfully grown in many locations. Unfortunately, sunflower is very sensitive to biotic stress (Škorić D, 2016).

Seed yield and oil content in seed are among the most important quantitative traits of each sunflower hybrid. They are determined by genetic factors that can be changed by the growing conditions (Bange et al., 1997; Connor and Hall, 1997; Leon et al., 2003).

Due to the complex interaction of the genotype with the environment, in order to recommend where to grow which variety or cultivar, multiple tests are required under varied soil and climatic conditions and different agronomy practices (Jocković et. Al, 2019; Radić et al.; Stoev, 2020; Drumeva, 2021; Hilli and Immadi, 2021).

The good hybrid should be able to economically and effectively use the factors of the environment. It should be highly adaptable (Nenova et al., 2019; Kalenska, 2020). The level of realization of its biological potential depends both on the growing technology and the climatic conditions of the respective year, and on their interaction (Valkova et al., 2018; McMaster et al., 2012; Ion et al, 2015; Mijic et al., 2020).

Unfortunately, in the past 15-20 years, due to the changed market situation in Bulgaria, sunflower has been grown without observing proper crop rotation. Concerning the tolerance of the agricultural crops, sunflower belongs to the unstable crops with negative self-tolerance. This cannot be improved by applying better agronomy practices due to phyto sanitary considerations.

Therefore, maintaining the same areas where sunflower is grown or their further reduction will put further pressure on the requirement to obtain higher yields as a way of compensation (Tonev, 2006).

The high-yielding sunflower crop is formed under the effect of three groups of factors: environment, genotype, and agronomy factors. Their optimal combination under the conditions of Bulgaria allows the high-yielding sunflower crop to form yields of 450 – 500 kg/da under experimental conditions, which equals 350 – 400 kg/da under production conditions.

The aim of this study was to demonstrate the level of Bulgarian sunflower breeding and to present the most recent sunflower hybrids and their potential with a view of their future use in practice.

MATERIAL AND METHODS

The investigation was carried out in the trial field of Dobrudzha Agricultural Institute – General Toshevo (DAI) during 2020 – 2022. It involved 13 of the most recent sunflower hybrids of DAI. For their development, 5 female lines with cytoplasmic male sterility and very good combining ability were used, as well as 11 fertility restorers with very good specific combining ability. The two types of lines were from the collection of DAI and were developed during the last 10-15 years. The possess resistance to the economically important diseases on sunflower and to some of the new races of the parasite *Orobanche*.

The sunflower hybrids were grown after previous crop wheat according to a well-established technology (Georgiev et al., 1997). Block design was used in this experiment (Zapryanov and Marinkov, 1978), in three replications and plot size of 12 m^2. After harvesting the previous crop, plowing was done to depth 28-30 cm, followed by two spring cultivations. After sowing and prior to emergence of the plants, a herbicide was applied for control of grass and broadleaf weeds, and double hoeing was done during the vegetative growth of the plants, one manual and one mechanized. Fertilization with triple superphosphate and stabilized ammonium nitrate was applied. The experimental plots were harvested by small plot combiner *Wintersteiger*, and the seed yield was equated to 11% seed moisture.

The sowing in all three years of the study was done during the second half of April manually, at crop density 6 200 plants/da. The following parameters were studied: seed yield (kg/da), oil content in seed (%), oil yield per da (kg/da), plant height (cm) and vegetative growth duration (days). Oil content in seed was determined by the method of the nuclear magnetic resonance using Oxford NMR 4000 Analyser, analyzing 10 grams of absolutely dry seeds from each replication.

Based on the phenotype characteristics thus determined, the studied hybrids were compared by using PC analysis (Alberts, 2004; Principal Components Analysis, 2005; Yan et al., 2000). The method allows good differentiation under different environmental conditions (Dimitrova-Doneva et al., 2016). The experimental data were processed with the help of software Microsoft Excel^{xp} and STATISTICA, release 7.0 (StatSoft Inc., 2004).

Characterization of the soil and climate in the region of Dobrudzha

Dobridzha Agricultural Institute (DAI) is located at $43^0 45''$ N and 28^0 E. The soils in the region of DAI are slightly leached chernozems and belong to the most fertile soils in Bulgaria. They are suitable for growing of most of the field crops and have the potential for high yields. They are characterized by a comparatively powerful humus horizon (60-80 cm) and by humus content in the plow layer they belong to the moderately humic soils. The amount of humus in the upper layers is within 3.18 – 3.85 %.

Other typical properties of these soils are neutral reaction, pH = 6.1 – 6.2, and low reserves of available nitrogen, the content of which is 4.1 – 4.3 mg/100g soil. The amount of mobile P_2O_5 is 2.5 – 5.4 mg/100g soil and defines them as having low reserves of this element, while mobile K_2O is 7.3 – 19.7 mg/100g soil, characterizing them as having moderate to good reserves. Their mechanical composition determines a favorable combination of water-physical properties with very good subsoil structure.

Climatically, the region of DAI belongs to the eastern part of the temperate continental area of Bulgaria. In spite of the severe winters, extreme low temperatures are not typical for this area; January is the coldest month. Summer is comparatively cooler than in other regions of the same climatic area. July is the hottest month.

The annual precipitation norm at DAI is 530 mm. The larger amounts of rainfalls (above 55 %) are during April – September.

RESULTS AND DISCUSSION

During the first year of the investigation, the autumn and winter moisture reserves were almost twice lower than the mean long-term value for a 70-year period (Table 1). This was partially compensated by the May and June rainfalls, but there were almost no rainfalls during the second half of the vegetative growth period. All this had an adverse effect on the development of sunflower and it showed lower results from the first year of study.

Table 1. Autumn and winter moisture reserves, rainfalls during the vegetative growth of the crop (mm)

Year	**Autumn and winter moisture reserves (mm)**	**Vegetative growth months Monthly precipitation sum (mm)**						**rainfalls April – September (mm)**
		April	May	June	July	Aug	Sept	
2020	136.4	5.8	48.0	192.2	2.7	3.5	34.1	286.3
2021	298.4	44.6	63.6	162.7	29.7	1.6	2.2	304.4
2022	304.4	76.0	25.6	76.4	40.4	9.2	93.6	321.2
1953-2022	**239.3**	**41.7**	**51.1**	**65.5**	**50.3**	**36.5**	**43.9**	**289.2**

Table 2. Mean monthly air temperature (^{o} C)

Year	**Mean temperature (^{o}C)**					
	April	**May**	**June**	**July**	**August**	**September**
2020	10.0	15.4	19.6	22.3	22.6	19.4
2021	8.8	15.8	18.9	22.8	22.6	16.6
2022	10.8	15.6	20.2	22.7	23.6	17.6
1953-2022	**9.9**	**14.9**	**19.1**	**21.2**	**20.9**	**16.4**

In the other two years of the investigation, the autumn and winter moisture reserves were within the norm, and the vegetative growth rainfalls were sufficient and evenly distributed. The mean monthly temperatures were normal and close to the mean long-term value. There were no periods with extreme high values of this parameter (Table 2). In general, the second and third year were more favourable for the development of the crop in comparison to 2020. This is evident from the obtained results.

The analysis of variances revealed good differentiation of the studied sunflower hybrids. The genotype specificity was with the highest effect on plant height, vegetative growth duration and oil percent in seed (Figure 1).

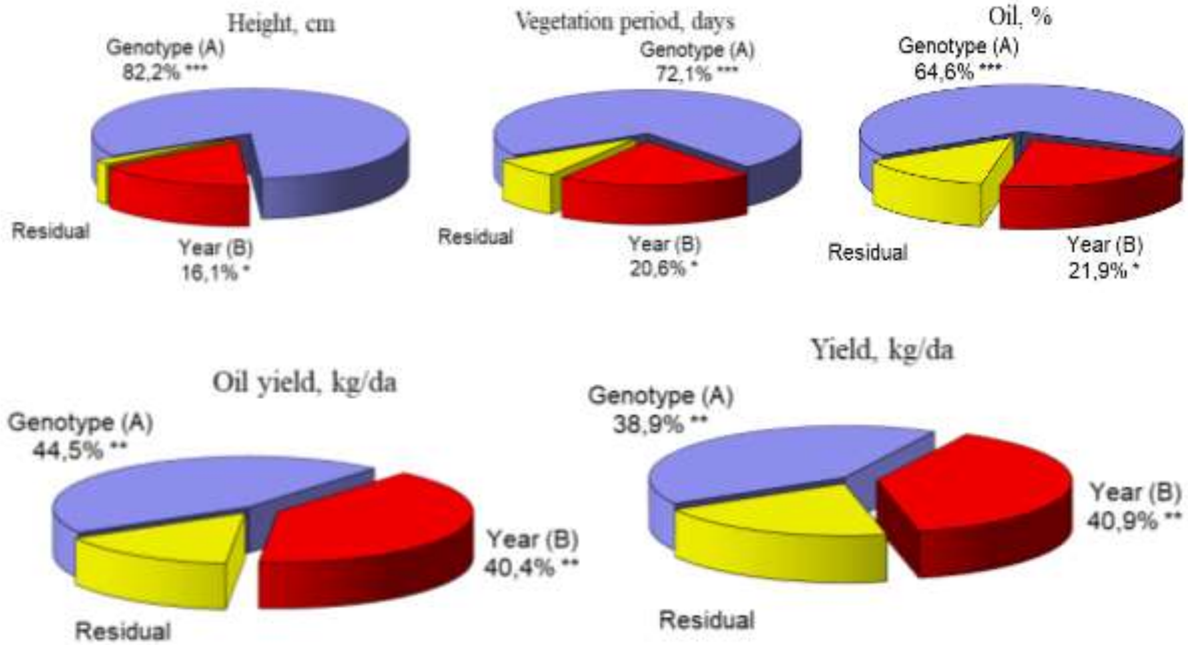

Figure 1. Two-way analysis of variance for the investigated traits according to genotype (factor A) and environmental conditions (factor B).

Lower was the effect of the traits seed yield and oil yield per da; it was equal to the change of the environmental conditions. The correct interpretation of the results requires noting that only the first vegetative growth period (2020) was stressful for the development of sunflower and formation of productivity. The first two principal components constituted 81.53 % of the total variation in the matrix genotype x traits (Figure 2).

The vectors of the traits oil yield per da, seed yield and oil percent were the longest and allowed good differentiation of the studied hybrids. The differentiation was lower with regard to duration of the vegetative growth period and plant height. Such results were expected in relation to the ideotype desirable from a breeding perspective, which would be suitable for distribution under the conditions of Bulgaria, and also from a marketing perspective.

The position of the vectors of the two traits in the bipolar plane indicated that the correlation between them approximated zero, which could be easily broken. The correlation, however, with seed yield was positive. The highest positive correlation was found between oil yield per da and duration of the vegetative growth period. Considerably lower was the correlation between oil yield and plant height. A high correlation of the vegetative growth duration was also found with oil percent. This was probably related to suitable conditions for its accumulation (Onemli, 2012; Andrianasolo et al., 2016). The percent of oil correlated with oil yield per da but not with seed yield. From a breeding perspective, this allows for simultaneous increase of the values of these economic traits. A low negative correlation was observed between oil percent and plant height.

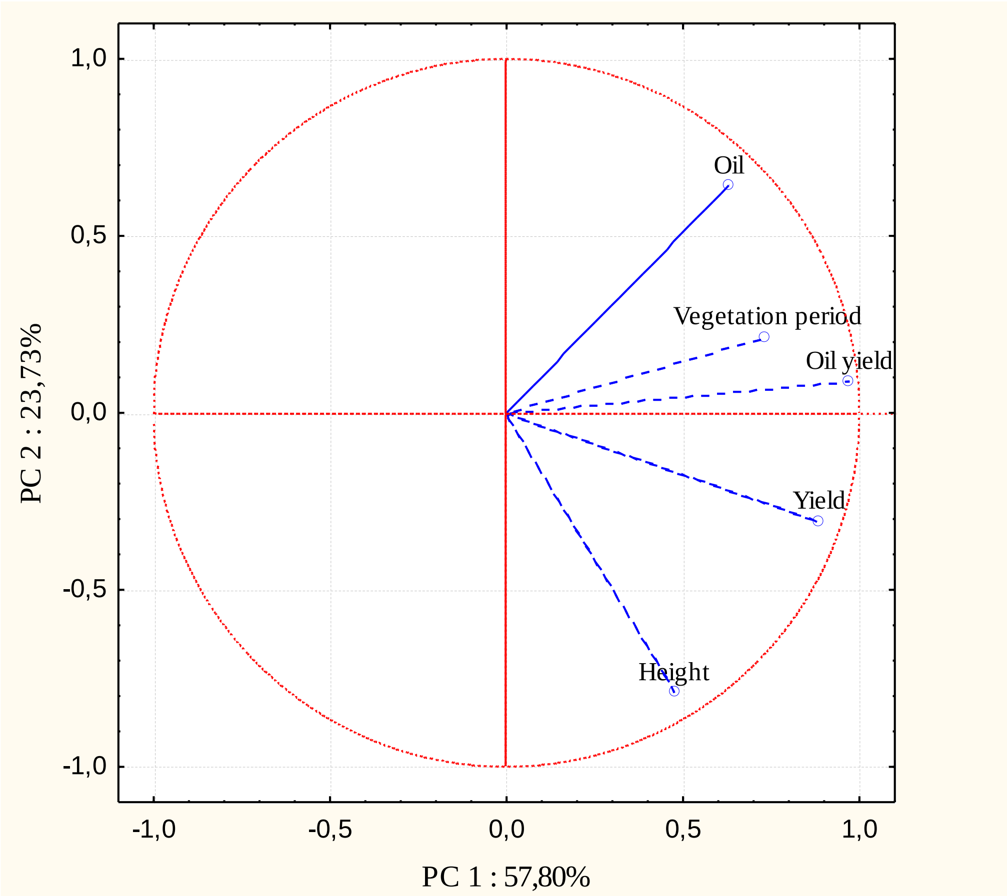

Figure 2. Principal component analysis of sunflower hybrids by their traits.

The comparative characterization between the sunflower genotypes was done on the basis of the mean, minimum and maximum values of the investigated traits (Table 3). Plant height varied slightly within 148.3-180.0 cm, in the hybrid combinations 217A x 102R and 3607A x 28R, respectively. The median of the trait was positioned towards the upper margin indicating that most genotypes had plant height greater than the mean one. For most of them, the differences were significant. It is worth mentioning that it is not possible to estimate the values of the trait by the participation of a specific mother component in the hybrid combinations. In the five tested genotypes involving mother line 3607A, plant height was from 158.3 to 180.0 cm, and with those involving 813A the range was 151.7 – 176.7 cm. The comparison over years revealed that the stressful year 2020 was the reason for the approximately 10 cm lower values (Table 4).

The vegetative growth period varied from 111.0 to 119.6 days. The median and the mean were with similar levels – 115.4 and 116.0 days. With the shortest vegetative growth duration were hybrids 807A x 193R, 813A x 135R and 813A x 175R. The genotype variation over years was low. As expected, the favourable conditions of 2021 and 2022 were related to higher vegetative growth duration, the difference with 2020 being significant.

The mean content of oil was 44.8 %, and this value coincided in practice with the median. The variation range was 43.0 – 49.7 %. Hybrid combination 217A x 102R was with the highest values, and the variation over years, too, was the highest as compared to the other genotypes. It should be noted that the combinations with mother component 3607A have high oil percent. The conditions of year 2021 were the most favorable for accumulation of oil in kernel. Lower were the values in 2022, and comparatively similar to those of 2020. The range of variation was also similar. The difference between the two years was not statistically significant.

Summarizing criteria for assessment of the hybrid combinations were seed yield and oil yield per unit area. The promising hybrids were 3607A x 78R, 3607A x 29R and 3607A x 28R. The comparison of data showed that they combined productivity with high oil percent in kernel. A similar tendency was observed in combinations with another component: 813A x 67R and 813A x 135R. Hybrid 217A x 102R was with oil yield above the average, primarily due to the high oil content in seed, but its mean productivity was below the average. This cross was submitted for official testing in Bulgaria and was officially registered as sunflower hybrid Deveda.

Year 2021 was the most favorable for formation of high values of the two traits, oil yield 194.9 kg/da and seed yield 421.7 kg/da, respectively. In 2022, they were insignificantly lower, while in 2020, they were significantly lower.

Table 3. Mean, minimum, maximum and coefficient of variation values of the investigated traits of sunflower hybrids

Sunflower hybrid		Height, cm	Vegetation period, days	Oil, %	Oil yield, kg/da	Yield, kg/da
807A x 193R	mean	153,3	111,0	44,9	168,4	374,3
	min-max	150-155	108-115	43,1-47,0	146,5-194,6	340-414
	CV, %	1,88	3,25	4,40	14,44	9,96
813A x 53R	mean	151,7	113,0	43,1	170,1	394,7
	min-max	145-155	110-115	42,8-43,4	152,3-194,0	351-450
	CV, %	3,81	2,34	0,70	12,66	12,8
813A x 67R	mean	166,7	114,0	45,3	178,9	394,7
	min-max	160-170	112-115	44,5-46,0	165,5-185,7	371-410

	CV, %	3,46	1,52	1,66	6,47	5,27
813A x 135R	mean	176,7	111,7	44,4	176,6	397,0
	min-max	170-180	110-113	43,3-44	161,9-203,3	374-443
	CV, %	3,27	1,37	3,03	13,12	10,03
813A x 175R	mean	163,3	112,3	43,3	162,1	373,3
	min-max	160-165	108-115	41,4-45,0	142,8-174,2	345-388
	CV, %	1,77	3,37	4,19	10,41	6,57
813A x 193R	mean	166,7	113,0	43,3	163,0	376,3
	min-max	160-170	110-115	41,9-44,3	146,7-174,8	350-401
	CV, %	3,46	2,34	2,85	8,95	6,79
846A x 175R	mean	161,7	116,0	43,0	179,5	415,7
	min-max	155-165	114-118	41,0-45,3	149,7-206,1	365-455
	CV, %	3,57	1,72	5,02	15,79	11,08
3607A x 3R	mean	166,7	117,3	43,2	172,1	397,7
	min-max	160-170	115-119	41,8-44,0	148,0-186,1	354-423
	CV, %	3,46	1,77	2,82	12,18	9,55
3607A x 24R	mean	158,3	119,0	44,1	162,4	367,0
	min-max	155-160	118-120	41,6-46,9	141,9-191,4	341-408
	CV, %	1,82	0,84	6,06	15,91	9,79
3607A x 28R	mean	180,0	117,3	45,1	190,9	423,0
	min-max	170-185	116-118	44,8-45,6	183,8-197,6	403-427
	CV, %	4,81	0,98	0,92	3,62	4,33

3607A x 29R	mean	176,7	118,3	47,6	211,4	441,3
	min-max	170-180	117-119	46,9-48,5	196,4-229,8	405-490
	CV, %	3,27	0,98	1,70	8,02	9,93
3607A x 78R	mean	166,7	119,7	45,4	202,9	446,3
	min-max	160-170	118-121	44,3-46,7	177,2-222,4	400-491
	CV, %	3,46	1,28	2,65	11,45	10,20
217A x 102R	mean	148,3	117,7	49,7	192,0	385,7
	min-max	145-150	116-119	47,2-53,5	167,6-216,7	355-405
	CV, %	1,95	1,30	6,78	12,78	6,96
Mean (2020-2022)		164,4	115,4	44,8	179,3	399,0
Median		166,6	116,0	44,4	176,6	394,6
LSD (0,05)		6,93	2,13	2,85	23,03	37,40

Table 4. Mean, minimum, maximum and coefficient of variation values of the investigated traits over years

Vegetation years		Height, cm	Vegetation period, days	Oil, %	Oil yield, kg/da	Yield, kg/da
2020	mean	158,5	113,2	43,6	160,0	365,7
	min-max	145-170	108-118	41,0-47,5	141,9-196,4	340-405
	CV, %	5,37	3,29	4,94	10,78	6,42
2021	mean	167,3	116,3	46,2	194,9	421,7
	min-max	155-185	110-120	43,1-53,5	167,5-209,2	378-455
	CV, %	6,29	2,56	5,65	7,28	5,89

2022	mean	167,3	116,7	44,6	182,8	409,6
	min-max	155-185	112-121	42,8-48,3	153,8-229,8	369-491
	CV, %	6,29	2,30	3,56	12,3	10,3
Mean		164,4	115,4	44,8	179,3	399,0
LSD (0,05)		5,31	1,90	1,40	18,45	30,67

The applied PC analysis allowed comparing the productivity potential of the hybrids, as well as their response over the years of study. The first two principal components determined 71.80% and 22.92% of the total variation, respectively. The percent of the other components reflecting the residual variation was insignificant. High productivity was registered in 2021 and 2022. It was lower in 2020 (Figure 3).

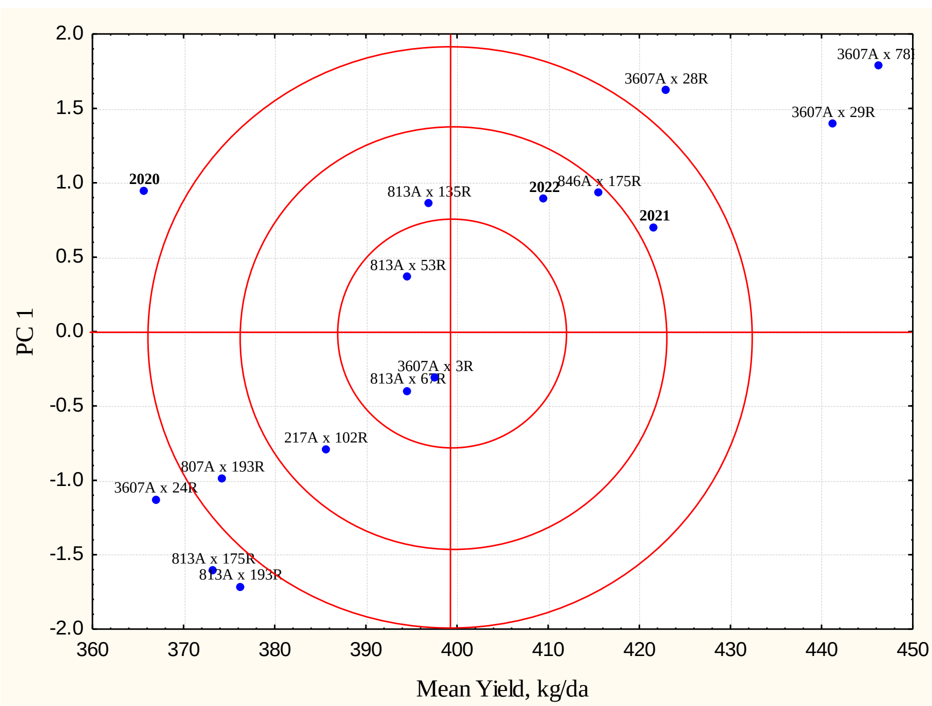

Figure 3. PC 1 and mean yield of sunflower hybrids.

The position of the vegetative growth periods on the bipolar plane showed that the conditions in 2020 and 2022 gave equal opportunity for differentiation of the genotypes, while 2021 was less favourable (Figure 4). Hybrids 3607A x 78R, 3607A x 29R and 3607A x 28R, which have the same mother component – line 3607A, were with the highest mean productivity. Similar response was found in 846A x 175R, 813A x 135R and 813A x 53R.

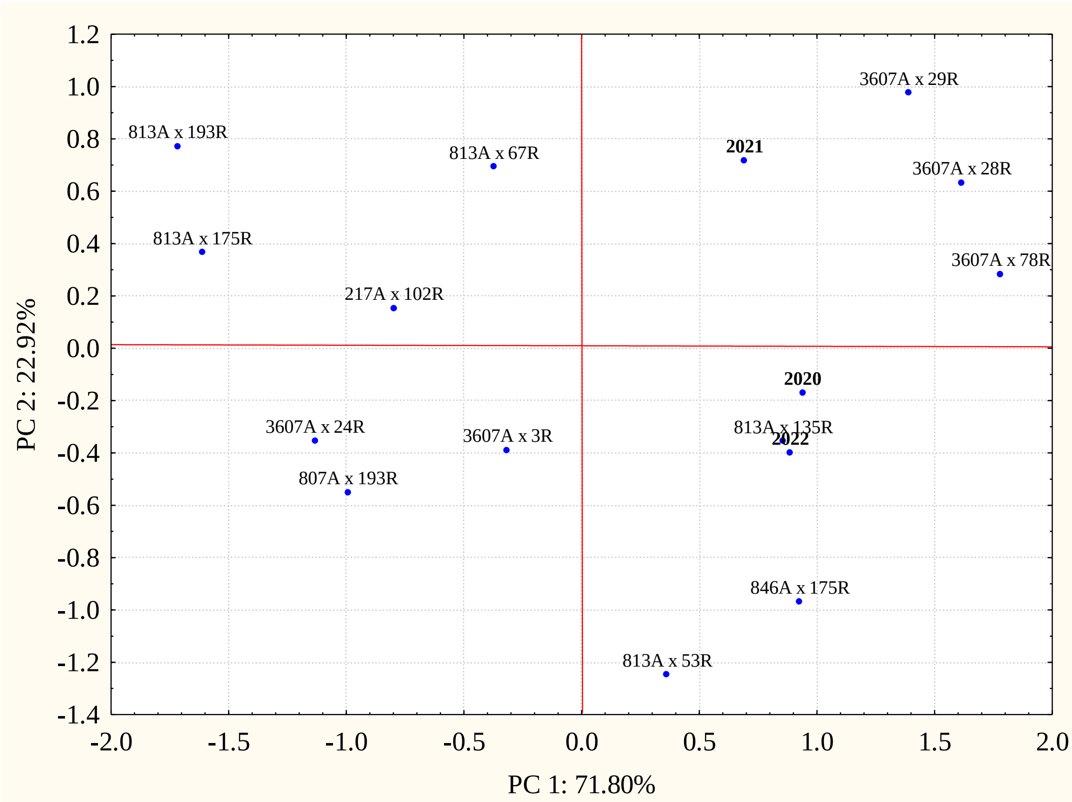

Figure 4. Principal component analysis of sunflower hybrids by yield

The seed yield obtained from them was close to the yield averaged for a three-year period, but the low absolute values of PC2 indicated lower stability.

Hybrid combination 813A x 67R also realized productivity about the average, the variation over years being lower. Similar was the response of genotypes 217A x 102R, 813A x 193R and 813A x 175R. To a separate group belong 3607A x 24R, 807A x 193R and 3607A x 3R; their yield was below the average, with a mean variation. They were characterized by comparatively high productivity during the favourable 2021, and low productivity during the stressful 2020.

CONCLUSIONS

Most promising were the hybrid combinations with the participation of mother lines 3607A and 813A. The hybrids, in which they were involved, gave very good results according to the studied traits.

Crosses 3607A x 29R and 3607A x 78R combined the best properties of a sunflower hybrid – seed yield, oil content in seed and oil yield.

The cross 217A x 102R was submitted for official testing at the national Executive Agency of Varietal Testing, Field Inspection and Seed Control and was registered as sunflower hybrid Deveda.

Mother line 217A and the fertility restorer 102R demonstrated very good results for parameter oil percent in seed and can be used in future high-oleic crosses.

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CATCH STRUCTURE AND CPUE OF THE MAIN FISH SPECIES CAUGHT IN OHRID LAKE

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ABSTRACT

The study aimed the evaluation of catch structure and catch effort of the most abundant fish species of Ohrid Lake. Evaluation of catch, gear used and effort were based on data provided from Fishery Management Organization of Ohrid Lake. The main fishing gear used by fishermen are hook lines and fishing nets. Fishing activity is mainly performed with 4.5-5.5 m length motor boats with engine power ranging 3.5 – 15 Hp equipped with fishing nets (length 500 – 1000 m, with mesh size 26-32 mm and 45-90 mm) and long lines with 150 hooks. The main fish species caught in the Lake are Ohrid trout (*Salmo letnica*), bleak (*Alburnus scoranza*), belushka (*Salmo ohridanus*), chub (*Squalius cephalus*), common carp (*Cyprinus carpio*) and eel (*Anguilla anguilla*). The catches of Ohrid trout made 57% of total catches followed by bleak and belushka with respectively 22% and 13%. The total CPUE for the data collected in the first half of 2023 was calculated 2.48 kg fish/day/boat. The CPUE value for Ohrid trout, bleak, belushka, chub, eel and common carp was respectively 2.2; 2.6; 0.5; 0.85; 0.6 and 0.4 kg fish/day/boat.

Keywords: Ohrid Lake, catch structure, CPUE

INTRODUCTION

Ohrid Lake is of tectonic origin and, having an estimated age of about two to three million years (reviewed in Albrecht & Wilke 2008), is considered the oldest lake in Europe. The lake is located between Macedonia and Albania. Its catchment area (combined with Lake Prespa) comprises about 2,600 km2 (Matzinger et al. 2006 a) and the lake's surface is about 358 km². Its oligotrophic waters conserve over 200 species of plants and animals unique to the lake, including algae, turbellarian flatworms, snails, crustaceans and 17 endemic species of fish including two species of trout, as well as a rich birdlife (UNESCO. 2017 a). Ohrid lake is fed primarily by spring water from the two main surface springs at the southern shores: St. Naum's in Macedonia and Tushemisht in Albania. In addition, there are numerous sub-lacustrine (underwater) springs, which are located at the eastern and, in part, western coasts. The water of the southern (and eastern) surface and underwater springs originates from the nearby Prespa Lake. The four permanent tributaries (rivers Cerava, Velgoska, Koselska and Sateska) influence the lake's water balance to a small degree (mainly in late spring or at times of heavy rainfall). Also there are around 40 temporary creeks and trickles entering from around the lake. At its northern shore, Ohrid Lake drains via River Crn Drim/Drin into the Adriatic See. Significant amounts of water (about 40%) leave the lake by evaporation (Matzinger et al. 2006 b). A remarkable characteristic of Ohrid Lake is its enormous depth of about 289 m; the mean depth is 151 m (Popovska &

Bonacci 2007). In view of nutrient loading, the water is categorized as oligotrophic (Sarafiloska & Patceva 2012, Patceva et al. 2009, Peveling et al. 2015). There are no anoxic layers in the water column and even in the deepest part of the lake, oxygen levels never drop below 6 mg/l (Matzinger et al. 2006 b). Furthermore, the water is exceptionally clear with transparency to a depth of as much as 20 meters (Popovska & Bonacci 2007). According to Stankovic (1960) estimated retention time of the lake water volume is 83 years. Three cities are situated around the lake, two in Macedonia, Ohrid and Struga, and Pogradec in Albania.

The fish fauna of Lake Ohrid is represented by 17 autochthonous species from four families: *Salmonidae* (2), *Cyprinidae* (12), *Cobitidae* (1), *Nemacheilidae* (1) and *Anguillidae* (1) and 6 allochthonous species (belonging to four families). With the exception of eel, none of them is a migratory species. A special characteristic of Lake Ohrid is its high degree of endemism (Albrecht & Wilke 2008). According to Talevski et al. (2009 b) there are seven endemic fish species in the lake, namely *Salmo ohridanus*; *S. aphelios*; *S. balcanicus*; *S. letnica*; *S. lumi*; *Barbatula sturanyi* and *Gobio ohridanus*. Furthermore, occurrence of *Alosa fallax* has been recorded by Tocko 1959, (cited in Talevski et al. 2009 a, Pavlova et al. 2012) and the finding of a single Drin brook lamprey (*Eudontomyzon stankokaramani*) has been reported by Talevski et al. (2009 a).

Figure 1 Locations of fishing boats in Ohrid Lake in Udenisht, Piskupat, Pogradec, Memelisht

MATERIAL AND METHODS

The study aimed the evaluation of catch structure and catch effort of the most abundant fish species of Ohrid Lake. Evaluation of catch, gear used and effort were based on data provided from Fishery Management Organization of Ohrid Lake. The main fish species caught in the Lake are Ohrid trout (*Salmo letnica*), bleak (*Alburnus scoranza*), belushka (*Salmo ohridanus*), chub (*Squalius cephalus*), common carp (*Cyprinus carpio*) and eel (*Anguilla anguilla*). The data collected in the first half of 2023, were the product of a total small-scale fleet consisting of 220 fishing boats. The main fishing gear used by fishermen are hook lines and fishing nets. Fishing activity is mainly performed with 4.5-5.5 m length motor boats with engine power ranging 3.5 – 15 Hp equipped with fishing nets (length 500 – 1000 m, with mesh size 26-32 mm and 45-90 mm) and long lines with 150 hooks.

RESULTS AND DISCUSSION:

In the course of data gathering in the first half 2023 the catches for the main fish species are shown in Figure 2. We have compared this data with the data taken in the same period, the first half of 2022.

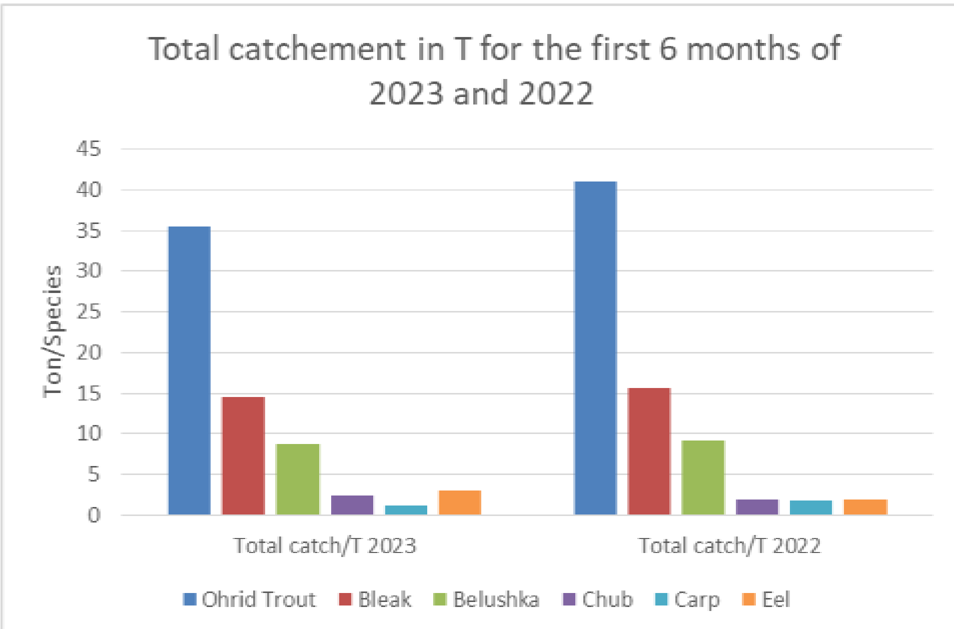

Figure 2. Fish species composition based in the total catches of Ohrid Lake in the first half of 2023 compared with the same period of 2022

The catches for the first 2023 of Ohrid trout made 54% of total catches followed by bleak and belushka with respectively 22% and 13%. And the values for the same period of 2022, of Ohrid trout made 57% of total catches, followed by bleak with 22% and belushka with 13%.

The total CPUE for the data collected in the first half of 2023 was calculated 2.48 kg fish/day/boat. The CPUE value for Ohrid trout, bleak, belushka, chub, eel and common carp was respectively 2.2; 2.6; 0.5; 0.85; 0.6 and 0.4 kg fish/day/boat (Figure 3).

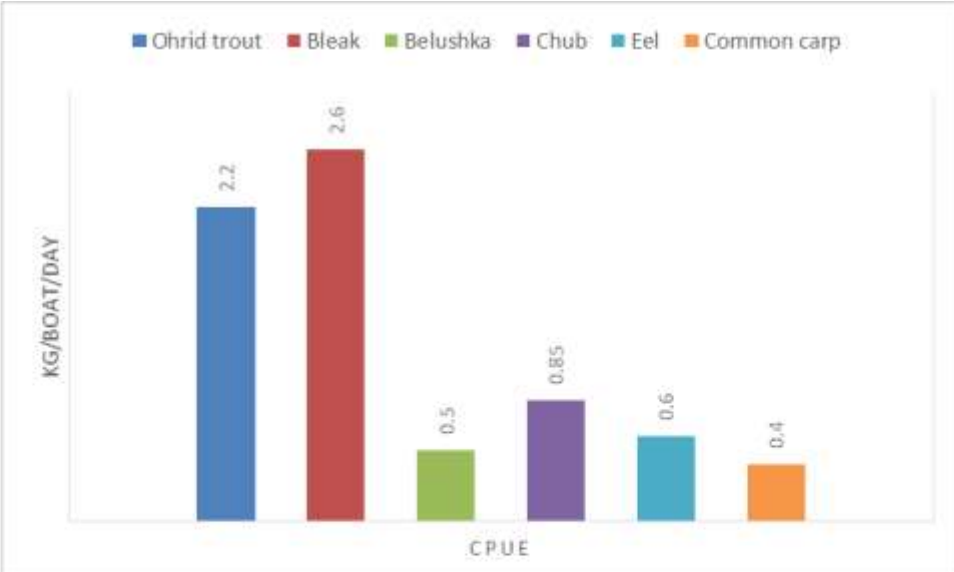

Figure 3 CPUE value for the main fish species in the first half of 2023

CPUE for bleak has increased, from 1.1 kg fish/day/boat in the first half of 2022 to 2.6 kg fish/day/boat in the same period of 2023, which may be an indication that we have had an increase in the reserve for this species. While for the other species there are no strong changes.

CONCLUSIONS

Available fishery statistics for Lake Ohrid shows that, in the past, Ohrid trout (*Salmo letnica*) and belushka (*S. ohridanus*) contributed considerably to the total annual catches of commercial fishers (Spirkovski & Talevski 2002, Anonymous 2004). For this reason, the lake has occasionally been characterised as typical salmonid water or “lake of trout” (Spirkovski et al. 2001, Spirkovski 2004 a). Based in our study, CPUE for some species has increased, which may be an indication that we have had an increase in the reserve for this species. Another reason might be the awareness of fishermen not to fish during closed seasons, for example during spawning periods and the strict and continuous controls of the Fishery Management Organization of Ohrid Lake during this periods.

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POSSIBILITIES OF MONITORING ON THE GROWTH AND DEVELOPMENT OF VEGETABLE PLANTS BY VEGETATION INDICES

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ABSTRACT

Remote sensing technology is based on obtaining information about specific properties of plants without directly coming into contact with them.

The use of remote sensing technology to monitor cultivated crops provides an effective tool for extracting information about their growth and development.

The biophysical properties of vegetable plants can be characterized by spectral images in the form of vegetation indices. Vegetation indices provide information on the biochemical composition and structure of the leaves and the general state of the photosynthetic apparatus. The information obtained is correlated with a database of analytical measurements (in situ) and supports the design of agronomic treatments.

The main aim of this paper is to review vegetation indices from remote sensing and identify those that can be used to monitoring the growth and development of vegetable crops.

Keywords: Vegetable crop production, Remote sensing, Monitoring of plans,

INTRODUCTION

Remote sensing technology is based on acquiring information about certain properties of plants without coming into direct contact with them. Remote sensing of crops provides valuable information on various agronomic parameters. It is possible to make repeated measurements in the same field without destroying the plants. The use of remote sensing technology to monitor cultivated crops offers an effective means of extracting information about their growth and development (Hatfield and Prueger, 2010).

In the field of vegetable production, research on the importance of vegetation indices for predicting the growth and development of vegetable crops has been conducted by Juan et al. (2013), who determined the LAI by imaging an onion plantation with a UAV. Na et al. (2015) investigated the application of UAVs to monitor the growth of onion and garlic. Lee et al. (2016) conducted a comparative study of onion and garlic grading methods using images obtained from UAVs. Na et al. (2016) evaluated cabbage growth in mountainous areas of Korea by using UAVs to determine NDI and accounting for agrometeorological factors. A similar study was conducted by Na et al. (2017) to monitor onion by periodic determination of NDVI. Na et al. (2018) estimated the growth of pepper by vegetation indices generated by UAV. Kim et al. (2018) created a growth model of Chinese cabbage and white turnip by RWB images taken by UAV. Rosio et al. (2018) monitored the biomass accumulation in onion by RGB images from UAV.

According to the critical analysis of Popov (2018), Bulgarian vegetable production demonstrates an insufficient degree of use of new technological solutions related to the production and marketing of production. Prospects for readiness to improve production and marketing technology are assessed as unsatisfactory.

Absence of motivation and opportunities for the application of current and modern technologies in vegetable production and limited scientific information on the use of intelligent decisions in the management of vegetable crop production processes are the main motivation for this publication.

The main aim of this paper is to review vegetation indices from remote sensing and identify those that can be used to monitoring the growth and development of vegetable crops.

MATERIAL AND METHODS

Scientific information was collected by reviewing thematic publications - articles, books, reviews, reference books, catalogues. Mainly scientific on-line databases were used, such as CAB Abstracts, ISI Web of Knowledge, Scopus, Science Direct, Google Scholar.

Based on the study of available scientific literature, the analytical review in this paper was prepared.

The opinion of the authors on study of the possibilities of using vegetation indices for monitoring on the growth and development of vegetable crops are also presented.

RESULTS AND DISCUSSION

According to Pinty et al. (1993) Spectral vegetation indices are mathematical combinations of different spectral bands in the visible and near-infrared regions of the electromagnetic spectrum. These numerical transformations are semi-analytical measures of plant activity and have been shown to vary not only with seasonal variability of green leaves, but also in area. For this reason, they are suitable for detecting temporal and spatial changes within crop fields. The main purpose of spectral vegetation indices is to improve the information available from spectral reflectance data by identifying changes due to vegetation characteristics. Spectral vegetation indices represent a simple and convenient approach to extract information from remotely sensed data due to their ease of use, which facilitates the processing and analysis of large amounts of data obtained from satellite platforms.

As a practical application of remote sensing, scientists have developed vegetation indices for qualitative and quantitative assessment of vegetation cover using spectral measurements. The spectral response of harvested areas is a complex mixture of vegetation, soil brightness, environmental effects, shade, soil color, and moisture. In addition, vegetation indices are influenced by spatio-temporal variations of the atmosphere. More than forty vegetation indices have been developed in the last two decades to improve crop diagnostics, while refining them to avoid biases in their determination and obtain more accurate results (Bannari et al., 1995).

Verstraete et al. (1996) add that most plant indices are usually species-specific and therefore not reliable when applied to different plant species with different leaf architectonics and structure.

Biophysical properties of vegetable plants can be characterized by spectral images expressed as vegetation indices. Vegetation indices provide information on the biochemical composition and structure of the leaves and the general state of the photosynthetic apparatus. The information obtained is correlated with a database of analytical measurements (in situ) and supports the planning of agronomic measures Wojtowicz et al. (2016).

Xue and Su (2017) explain that the solar radiation reflected by plants depends on the chemical and morphological characteristics of the plant. The plant type, its water content and foliage characteristics affect the light reflected in each spectrum differently. Measured reflected light in the ultraviolet, visible (blue, green, red) and near and mid-infrared spectra is commonly used to develop various vegetation indices that provide useful information on the structure and conditions under which plants are developing.

McKinnon and Hoff (2017) define vegetation indices as mathematical expressions that combine measured reflectance across many spectral bands to produce a value that helps estimate crop growth, vigor, and other vegetation properties such as biomass and chlorophyll content. Kamenova et al. (2017) provide a similar definition, adding that their purpose is to reduce additive and multiplicative errors associated with atmospheric effects, solar illumination, soil background effects, and sensor observation geometry.

The same authors have grouped vegetation indices based on their relationship to key plant characteristics (Tables 1, 2, 3).

Table 1. Vegetation indices for chlorophyll and nitrogen estimation (by Kamenova et al. (2017))

Index	**Formulae**
REP	(((R670 + R780)/2 − R700)/(R740 − R700))*40+ R700
MTCI	(R754 - R709) / (R709 − R681)
CI red edge	(R780/R709) − 1
CI green	(R780/R550) − 1
NDRE	(R740 − R705) / (R740 + R705)
NDRE1	(R780 − R705) / (R780 + R705)
CCCI	((R790−R720)/(R790+R720))/((R800−R670)/(R800+R670))
Red edge NDVI	(RNIR – Rred edge) / (RNIR + Rred edge)
WDRVI	(0.3 * RNIR – Rred) / (0.3 * RNIR + Rred)
VARI green	(Rgreen – Rred) / (Rgreen + Rred – Rblue)
VI green	(Rgreen – Rred) / (Rgreen + Rred)

Table 2. Vegetation indices for the evaluation of photosynthesis parameters, (by Kamenova et al. (2017))

Index	**Formulae**
PRI	(R531 – R570)/(R531 + R570)

Table 3. Vegetation indices for biomass estimation (by Kamenova et al. (2017))

Index	**Formulae**
NDVI	(R780 – R670) / (R780 + R670)
NDVI1	(R740 – R705) / (R740 + R705)
NDVI2	(R780 – R705) / (R780 + R705)
SR	R740 / R665
	R783 / R665
	R783 / R740
	R783 / R705
	R945 / R865
REP	700 + 40*((R670 + R780)/2 − R700) / (R740 − R700)
VARI	(R550 − R650) / (R550 + R650 − R470)

Shisodia et al. (2020) evaluated the available vegetation indices and found the relationship with plant diagnostic capabilities and their use in precision agriculture (Table 4). They point out that due to the individual nature of their determination (crop-specific vegetation index values), it is necessary to look for a link with analytical measurements that can be performed in situ to validate the data and to prove the relationship between them.

The most recent classification of vegetation indices is proposed by Omia et al. (2023). They group the main vegetation indices for crop monitoring into three categories according to the basic information that can be obtained from them (Table 5).

Among the typical spectral vegetation indices, NDVI is one of the most suitable for tracking crop development dynamics as it measures the photosynthetically active biomass of plants. It can be used throughout the crop production season, except when plant cover is too sparse and therefore its spectral reflectance is too low. NDVI values are most accurate in mid-season, at the stage of active crop growth. However, this vegetation index is quite sensitive to soil brightness and weathering, which is mitigated by other indices such as EVI, SAVI, ARVI, GCL or SIPI (https://eos.com/make-an-analysis/).

The leaf area index LAI is calculated as the ratio of the one-sided (illuminated) leaf area to the soil surface area they can cover. This vegetation index is important for monitoring crop and forest health, environmental and climatic conditions. The LAI can be scaled for an individual plant, crop(s) in a field, or for an entire region. The LAI leaf vegetation index in remote sensing was introduced for the NASA MODIS sensor to improve the NDVI data. Unlike the latter, it accounts for topographic features, and the spectral bands used for its calculations are subjected to atmospheric correction.

If LAI = 3, then the leaves can cover the surface three times. LAI is considered high at values of 0-3.5. However, its values are affected by clouds and bright objects, which must be accounted for to achieve data accuracy. It is used in plant condition assessment as inputs to a productivity prediction model (https://land.copernicus.eu/global/products/lai).

The fAPAR index quantifies the fraction of solar radiation absorbed by living leaves for photosynthetic activity. The fAPAR depends on the structure of the green vegetative mass of the plants, the optical properties of the plant elements, the atmospheric conditions and the imaging angle. To overcome this dependence, the daily integrated value of fAPAR is estimated. The fAPAR is recognized as an essential climate variable (ECV) by the Global Climate Observing System (GCOS). The fAPAR plays a crucial role in the assessment of the primary productivity of tree canopies, or the leaf mass of herbaceous plants, and the associated fixation of atmospheric CO2 and energy balance in the plant organism. FAPAR monitoring provides information on the quantity and health cycle of vegetation (https://land.copernicus.eu/global/products/).

The fraction of vegetation cover (FCover) corresponds to the fraction of land covered by green vegetation. In practice, it defines the spatial extent of vegetation. As it is independent of the direction of illumination and sensitive to the amount of vegetation, FCover is suitable to replace classical vegetation indices in ecosystem monitoring. It is also a good indicator of the beginning of the growing season, and the time series can be used to compare vegetation condition from year to year for agricultural applications, etc. (https://land.copernicus.eu/global/products/).

Soil Vegetation Index - SAVI was introduced to mitigate the impact of soil brightness. Its creator Huete added a soil correction factor L to the NDVI equation to correct for the effects of soil noise (soil color, soil moisture, soil variability in different regions, etc.) that tend to affect the results." "Key fact: L varies from -1 to +1 depending on the density of green vegetation in the area in question. In areas of high green vegetation density L= 0 and in this case SAVI is the same as NDVI. Conversely, L = 1 for areas with low green vegetation. Most often L is set to 0.5 to accommodate most land covers (https://www.indexdatabase.de).

Table 4. Vegetation indices used for remote sensing in precision agriculture – by Shisodia et al. (2020)

Index	formula	Application for assessment of	Relationship to analytical measurements
Normalized difference vegetation index (**NDVI**)	$R_{NIR}–R_{red}/R_{NIR}+R_{red}$	Biomass, phenotyping, productivity	Fresh weight, dry weight, plant height, chlorophyll content
Green NDVI (**GNDVI**)	$R_{NIR}–R_{green}/R_{NIR}+R_{green}$	biomass	Fresh weight, dry weight, number of fruits
Normalized difference red edge (**NDRE**)	$R_{NIR}–R_{red\ edge}/R_{NIR}+R_{red\ edge}$	Yield, biomass, nitrogen content	Fresh weight, dry weight, number of fruits, chlorophyll content
Red edge normalized difference vegetation index (**RENDVI**)	$R_{NIR}–R_{red\ edge}/R_{NIR}+R_{red\ edge}$	Yield, biomass, nitrogen content	Fresh weight, dry weight, number of fruits, chlorophyll content
Soil adjusted vegetation index (**SAVI**)	$(R_{NIR}–R_{red})(1+L)/R_{NIR}+R_{red}+L$ L-soil conditioning index	Yield, biomass, nitrogen content	Fresh weight, dry weight, number of fruits, chlorophyll content
Modified soil adjusted vegetation index (**MSAVI**)	$\frac{2R_{NIR}+1-\sqrt{(2R_{NIR}+1)^2-8(R_{NIR}-R_{red}}}{2}$	Yield, biomass, nitrogen content, chlorophyll content	Fresh weight, dry weight, number of fruits, chlorophyll content
Renormalized difference vegetation index (**RDVI**)	$(R_{NIR}-R_{red})/\sqrt{R_{NIR}+R_{red}}$	Yield, biomass, nitrogen content	Fresh weight, dry weight, number of fruits, chlorophyll content
Ratio vegetation index (**RVI**)	R_{NIR}/R_{red}	Yield, biomass	Fresh weight, dry weight, number of fruits

Plant senescence reflectance index (**PSRI**)	$R_{680}–R_{550}/R_{750}$	Yield, biomass	Fresh weight, dry weight, number of fruits
Chlorophyll vegetation index (**CVI**)	$(R_{NIR}/R_{Green})*(R_{Red}/R_{Green})$	chlorophyll content	chlorophyll content
Optimized soil adjusted vegetation index (**OSAVI**)	$1.16(R_{NIR}–R_{Red})/R_{NIR}+R_{Red}+0.16$	Yield, biomass, nitrogen content	Fresh weight, dry weight, chlorophyll content
Normalized water index (**NWI**)	$R_{970}–R_{900}/R_{970}+R_{900}$	Biomass, yield, soil moisture	Fresh weight, dry weight, chlorophyll content

Table 5: Main multispectral vegetation indices for crop monitoring by Omia et al.,2023

Category	Type	designation	formula	Measured indicators
Basic vegetation index	Ratio Vegetation index	Ratio vegetation index (RVI)	$RVI = \frac{NIR}{R}$	chlorophyll content
		Green Ratio Vegetation Index (GRVI)	$GRVI = \frac{NIR}{G}$	nitrogen content chlorophyll content
		Chlorophyll index with red edge (CIrededge)	$CIrededge = \frac{NIR}{REG} + 1$	chlorophyll content LAI
	Difference vegetation index	Difference vegetation index (DVI)	DVI = NIR - R	chlorophyll content
		Green difference vegetation index (DVIGRE)	DVIGRE = NIR - G	chlorophyll content

		Red edge difference vegetation index (DVIRED)	DVIGRE = NIR - REG	chlorophyll content
Functional vegetation index	Atmospherically adjusted Vegetation index	Atmospherically resistant vegetation index (ARVI)	$AVRI = \frac{NIR - R_{RB}}{NIR + R_{RB}}$ $R_{RB} = R - \gamma(R - B)$	-
		Green Atmospherically Resistant Index (GARI)	$GARI = \frac{NIR - G + 1.75(B - R)}{NIR + G - 1.75(B - R)}$	chlorophyll content
		Visible Atmospherically Resistant Index (VARI)	$VARI = \frac{G - R}{G + R - B}$	Total biomass
	Soil-adjusted vegetation index	Soil-adjusted vegetation index (SAVI)	$SAVI = \frac{1.5(NIR - R)}{NIR + R + 0.5}$	Съдържание на азот
		Optimized soil-adjusted vegetation index (OSAVI)	$OSAVI = \frac{1.16(NIR - R)}{NIR + R + 0.16}$	chlorophyll content
Modified vegetation index		Modified Soil-Adjusted Vegetation Index (MSAVI)	$MSAVI = \frac{1.5(NIR - R)}{NIR + R} + 0.5$	chlorophyll content
		Normalized difference vegetation index (NDVI)	$NDVI = \frac{NIR - R}{NIR + R}$	chlorophyll content
		Modified simple ratio (MSR)	$MSR = \frac{NIR \div (R - 1)}{(NIR \div (R + 1))^{0.5}}$	chlorophyll content
		Normalized difference red edge (NDRE)	$NDRE = \frac{NIR - REG}{NIR + REG}$	nitrogen content chlorophyll content

Many researchers have worked to clarify the practical application issues of vegindexes, such as Crippen R E. (1990); Friedl et al (1994); Penuelas et al (1997); Blackburn (1998); Gobron et al (2000); Gitelson et al (2001); Pietro Ceccato et al (2002); Gitelson et al (2003); Ferencz et al (2004); Guerric le Maire et al (2008); Ahamed et al (2011); Dang et al (2011).

After reviewing the literature, we can summarize that the practical use of vegetation indices can be expressed by finding the relationship between the main life processes of plants and establishing the values of vegetation indices in the main phases of their development, such as:

Establish the occurrence of the different phenophases in plant development and the simultaneously of crop development. Establish a relationship with NDVI - normalized vegetation index and EVI - enhanced vegetation index. After validation of the data and development of a development model, direct monitoring can be carried out over arable land.

Compare chlorophyll accumulation detected by analysis of plant samples with the CCCI - chlorophyll content index of plant cover.

To determine the relationship of satellite imagery and physiological status of plants- establish basic productivity indices - Leaf area index, Photosynthetic potential, Net photosynthetic productivity and NDVI.

To determinate of the optimum cropping parameters through observations on the plant architectonics and determination of the time to reach the optimum leaf area index and the period during the vegetation for which it is maintained. Determination of the period achieves optimum growth. Establish a relationship with SAVI and NDVI.

The methodological approach for collecting in situ data and establishing relationships with remote sensing observations is presented by Arnaudova et al. (2022).

CONCLUSIONS

New technologies require constant access to detailed information characterizing the environmental conditions under which production takes place. This can be obtained from aerial and satellite imagery at field scale.

The practical use of vegetation indices can be expressed by finding the relationship between the main life processes of plants and establishing the values of vegetation indices at the main phases of their development.

Based on the results of the studies, the practical significance of remote sensing methods and vegetation indices will be determined. The collected scientific information and the establishment of the dependencies of the analyzed data and the spectral analyses of remote sensing will be the basis for expert decision-making for the agronomic management of vegetable production.

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AGRICULTURE OF THE FUTURE: AGRICULTURAL TRANSFORMATION WITH UNMANNED AERIAL VEHICLES

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ABSTRACT
The agriculture and livestock sector is developing more and more every day with technological innovations. One of these innovations is unmanned aerial vehicles (UAV) technology. Unmanned aerial vehicles are used to facilitate agriculture and livestock activities, increase productivity and provide more control to farmers. In the agricultural sector, UAVs are used in many areas such as monitoring agricultural areas, controlling plant health, and application of pesticides. By scanning the vegetation in agricultural lands, UAVs detect plant diseases or harmful insects, thus providing early warning to farmers. Agricultural pesticides can also be applied more effectively with UAVs. UAVs make the spraying process more efficient by providing controlled spraying of the drug towards the target. In addition, UAVs can determine the irrigation needs of agricultural areas. By measuring the humidity level of the agricultural area, it determines the water need and optimizes the irrigation processes. In this way, water resources are used more efficiently and water savings are achieved. In the livestock sector, UAVs provide many benefits. It is used especially in large farms for the control, counting and health monitoring of animals. UAVs can detect animals in large areas, count and check the status of animals. Besides, it can track the location of animals and help them find them back in case of loss. The use of UAVs in the agriculture and livestock sector is especially important for saving manpower. The difficult and dangerous labor that people have to do can be done more safely and quickly with UAVs. In addition, UAVs provide more efficient management of agricultural and livestock activities. Thanks to UAVs, it is possible to access more accurate and timely information. However, there are some difficulties with the use of UAVs. The use of UAVs without the rules and permits governing their flight can lead to various security risks. In addition, the cost of UAVs is a factor limiting their use. The cost of UAVs can be quite high, which may limit the use of drones for small family farms. With regulatory regulations and affordability, UAVs can become an integral part of the agriculture and livestock industry in the future.

Keywords: Unmanned aerial vehicle, agriculture, livestock

INTRODUCTION

People have to produce both animal and plant products to meet their nutritional needs. Agricultural activities are as old as human history. Approaches aiming to increase production in the previous periods have been transformed into production approaches focused on economic profitability, then into quality production perspective and finally into production activities based on sustainable environment and natural resource protection. (Friha et al., 2021). In parallel with technological developments, increases in production amounts have occurred. Agricultural activities have been carried out especially in areas with suitable land conditions and access to water resources. Agriculture is a science that covers a wide range from production to marketing, from quality to protection, from breeding to natural resource utilisation. Agricultural activities are carried out not only to meet nutritional needs but also to create employment and as part of economic activities. Fertilisation, spraying, irrigation, seed selection, mechanisation, labour force are among the important subjects of agriculture. (Zhang and Kovacs, 2012).

The agricultural sector, which seems to be lagging behind in adopting technology, has started to benefit from the advantages and conveniences offered by artificial intelligence technology in the field of rural development. The digital transformation that emerges with the advancement of technology leads to various changes in productivity and employment in agricultural activities on a global scale. According to a report by the Food and Agriculture Organisation of the United Nations, to meet the food needs of the world population in 2050, 70% more food needs to be produced than has been produced since 2006. In order to meet consumer demand, the interest in cereal consumption and sustainable agricultural processes is increasing day by day (Yusuf et al., 2018).

The need to minimise losses by increasing the productivity of the agricultural sector in relation to the growing world population has reached a high point. In response to this demand, drones and other support systems have paved the way for multi-disciplinary work. The raw images imaged by the drone are processed with the help of various programmes or image algorithms (Radoglou-Grammatikis et al., 2020).

Precise imaging techniques provide useful information on plant growth and yield estimation parameters that can be read accurately and estimated with high reliability. By using remote sensing methods, the total number of plants per unit area, the development values of plants subjected to different fertilisations and different issues such as these can be solved quickly. These methods, which save time and money, also help to create data sets that can be used in the coming years (Gnädinger and Schmidhalter, 2017). Drone systems used in agricultural research are shown in Figure 1.

Figure 1. Drone systems used in agricultural research (Teke et al., 2016)

Drone

The growth in the aviation sector with the development of technology has led to many positive developments in the aviation production sector. With the importance given by countries to air defence at the military level, "Unmanned Aerial Vehicles (UAV)" have emerged for various purposes. The use of Unmanned Aerial Vehicles dates back to ancient times and the first unmanned aerial vehicles used were balloons (Kahveci and Can, 2017). However, the first real UAV application suitable for today's usage area started in 1916 when Elmer Speryy demonstrated the gyro-stable flight of the "Curtiss" aircraft of the US Navy (Çetinkaya and Koç, 2023). With the investments made by countries in this field and the use of such aircraft in civil aviation activities, unmanned aerial vehicles have started to have an impact on economic indicators and have created their own sector (Yeşilay and Macit, 2020)

Unmanned aerial vehicles (UAVs) are small aerial vehicles in terms of size and weight, which do not have a human on board for control or travel purposes, and which can fly by remote control or a pre-loaded flight programme. The International Civil Aviation Organisation (ICAO) classifies unmanned aerial vehicles in two categories: military and civilian. According to this classification; UAVs used for armed purposes such as reconnaissance and attack are classified as military; UAVs used for scientific, imaging or hobby purposes are classified as civilian (ICAO (International Civil Aviation Organization), 2011).

The armament of balloons in the early 1900s and the subsequent unmanned flight of small aircraft are considered to be the basis for the use of UAVs and "armed unmanned aerial vehicles (UAVs)". However, after the international political ban on the use of these UAVs for military purposes, the production and development of UAVs and UCAVs stopped. However, technological developments in aeroplanes and aviation have improved the use of UAVs primarily for military purposes, and then, with the confidence in UAVs, UAVs have started to be used in many different fields. With the increase in civilian use, the UAV sector has started to create its own market and economy (Kahveci and Can, 2017).

UAVs are defined as "drone" or "UAV/UAS (Unmanned Aerial Vehicle/Systems)" in the international literature, and they actually mean the same thing except for certain technical features (Kahveci and Can, 2017).

In general, UAVs are classified into two categories: lighter-than-air and heavier-than-air (Figure 2). UAVs used extensively in agricultural areas are fixed-wing and multi-rotor UAVs. In addition, lighter-than-air UAVs are also used by being attached to a rope fixed to the edge of the field (Bozdogan and Yarpuz Bozdogan, 2017). Lighter-than-air and heavier-than-air UAVs are shown in Figure 2.

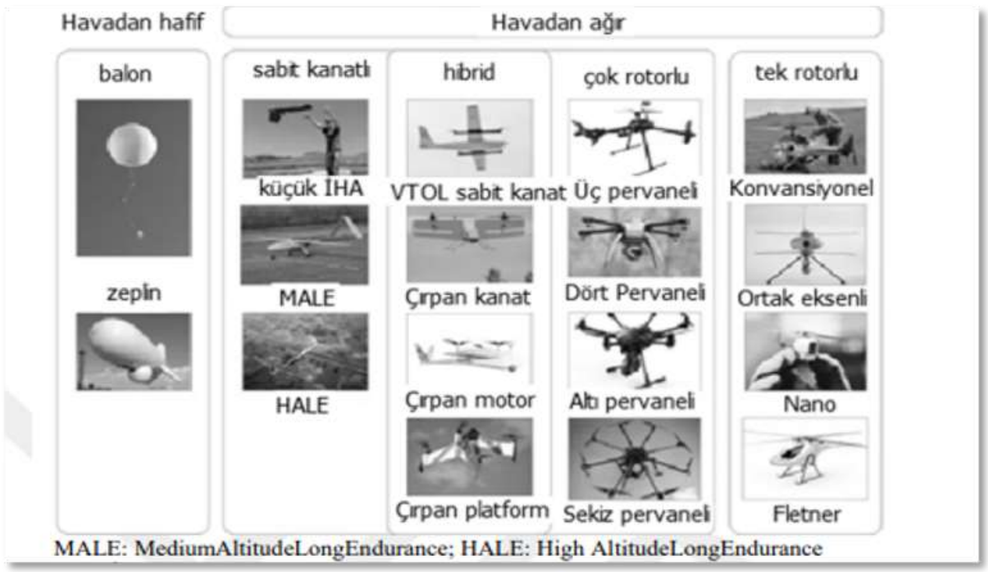

Figure 2. Airborne light and airborne heavy UAVs (Bozdogan and Yarpuz Bozdogan, 2017)

In terms of ease of use and cost, the most widely used UAVs are rotary wing type UAVs. They have different engine numbers, airframe and propeller structures depending on the type of operation, flight duration and flight weight. Although not common in commercial use, they are available in 1-engine (monocopter), 2-engine (bicopter or coaxial copter) or 3-engine (tricopter) designs. The most preferred designs in commercial use are generally 4-motor (quadcopter), 6-motor (hexacopter) and 8-motor (octocopter) structures (Villi and Yakar, 2022).

A drone is a vehicle with four or more propellers in the UAV category, capable of vertical take-off and landing, and capable of remaining stable in the air. Drone has started to be used in agricultural applications in recent years. Studies are carried out for applications such as crop growth monitoring, plant species separation, crop yield determination, automatic harvesting, drought, disease, agricultural pests, etc. damage detection, fruit-vegetable and soil moisture classification, area management, organisation of agricultural activities, agricultural insurance with drone (Tan et al., 2015)

Drone Software Architecture

Drone software exists on three basic backbones. These are system software, user software and a cloud-based control platform. The drone software architecture template is shown in Figure 3.

Figure 3. Drone software architecture template (Inan and Karci, 2021)

The embedded software works as a CPU, manages the hardware, monitors the drone telemetry and partially analyses the data received from the drone sensors.

The operating system allows users to run the firmware partition.

Web and cloud interfaces allow accessing the operating system from remote drone control systems (user applications and cloud control stations) and transferring collected data from embedded software to the cloud or mobile devices.

The cloud-based control platform is used for data processing, storage and analysis. It also enables autonomous reaction actions of a drone. The cloud partition is a must when it comes to complex processes such as, for example, 3D mapping, computer vision, pattern recognition. The cloud-based control platform includes:

- Stream data processor.
- Raw data and big data warehousing.
- Data analysis and machine learning.
- Drone control module.
- Interfaces for communicating with the drone.

A conventional drone consists of standard propellers, pusher propellers, motors, landing gear, electronic speed and stability controls, flight control panel, receiver, transmitter, GPS module, battery and camera components (Figure 4.).

Figure 4. Drone parts (Anonymous, 2022)

1. Standard Propellers, 2. Thruster Propellers, 3. Motors, 4. Landing Gear, 5. Electronic Speed Controls, 6. Flight Control Panel, 7. Receiver, 8. Transmitter, 9. GPS Module, 10. Battery, 11. Camera

Photo Sensors Used in UAVs for Agricultural Purposes

In agriculture, decreasing equipment costs of image processing and computer vision applications, increasing computational capabilities, and increasing interest in quality food evaluation methods draw attention to artificial intelligence technologies (Mahajan et al., 2015). Drones come to the forefront with the advantages of more efficient, precise and reproducible studies in small areas. Drones have recently become an important part of the agricultural sector with the widespread understanding of precision agriculture. With a single flight, hundreds of decares of land can be scanned and data can be obtained. By solving the problem of not being able to take images in cloudy weather, which is one of the disadvantages of satellites, it has made it easier to access data under much more flexible conditions (Türkseven et al., 2016).

The use of unmanned aerial systems (UAS) as sensing and communication platforms is also a breakthrough technology with significant potential in precision agriculture. It is recognised as a low-cost alternative technique for environmental monitoring with high spatial and temporal resolution and low cost of image acquisition. Nowadays, the use of UAVs in agriculture is increasing to assist farmers with monitoring and decision support in agriculture. UAV is used in various agricultural applications such as irrigation, fertilisation, pesticides, weed management, etc. Moreover, the combination of UAV technology with new 3D remodelling techniques has enabled plant-by-plant monitoring of crop growth parameters (Hassler and Baysal-Gurel, 2019; Jang et al., 2020; Manfreda et al., 2018).

By utilising UAVs, remote sensing system based plant monitoring techniques, it is necessary to take images with cameras in order to be used in issues such as disease agent, pest detection, determination of their damage rates, weed flora detection, determination of water stress, determination of harvest time and yield estimation (Koger et al., 2003; de Castro et al.,, 2012). The features of the sensors of the cameras used in these images are extremely important. Visual sensors that provide the closest image to the real image, Lidar sensors that allow us to carry the vagetation to the three-dimensional environment, thermal sensors that distinguish objects on the basis of temperature difference, and multispectral and hyperspectral sensors that can 6 measure infrared wavelength reflections are used. In particular, sensors using infrared wavelength reflections are widely used in remote sensing studies (Türkseven et al., 2016).

Atmospheric sensors such as temperature, pressure, wind, humidity, chemical sensors such as gas, location sensors such as ultrasound, infrared, radio frequency, GPS, microphone etc. sensors can be used in the drone (Metin Özgüven et al., 2022).

Previous generations of UAV platforms were limited to a smaller spectral bandwidth, equipped with only red-green-blue (RGB) sensors or a filter connected to the RGB sensors to obtain manipulated infrared information (Yeom et al., 2019). Recently, UAV platforms with sensors covering the red edge and near infrared (NIR) bands have become available for agricultural applications, allowing plant indices originally developed for conventional remote sensing sensors to be applied to UAV data. Recently, UAV platforms with sensors covering the red edge and near infrared (NIR) bands have been used for agricultural applications (Demir and Başayiğit, 2020).

As remote sensing technologies develop, areas in the field that are diseased or have problems under any stress factor can be quickly identified and specific controls can be carried out for these areas. Thus, unnecessary use of chemical pesticides is prevented, less chemical waste is left to the environment and the maximum product is obtained from the unit area (Mogili and Deepak, 2018).

Developments and innovations in drone technology from unmanned aerial vehicles in agricultural activities attract attention. With drone applications in agriculture, operations such as field mapping, dimensioning, positioning and pesticide spraying are carried out very easily. With the help of drone, farmers can detect a number of problems that are not visible from the ground in their fields from the air and quickly intervene in problematic areas in a timely manner. In addition, farmers can take images of the products in the field from the sky at certain time intervals with the help of drones and analyse whether the products in the field are properly irrigated, the development and yield of the products. A spraying drone is shown in Figure 5.

Figure 5. A drone spraying (Aydogan, 2018)

Use of Unmanned Aerial Vehicles in Agriculture

Product monitoring is very critical in agricultural applications. Today, in many countries, studies on agricultural fields, regulation activities, analysis of yield parameters and land consolidation projects are carried out for sustainability. There is a great need for remote sensing techniques to obtain, calculate and evaluate these studies accurately. Remote sensing methods are developing day by day and are used in wide areas. Drone-based remote sensing is an element of smart agriculture, helping to collect diagnostic information in a timely and efficient manner. UAVs, processed with modern software and controlled by the operator, are recognised as a new remote sensing option in the agricultural field. Emerging sensor technology makes common sensors previously used in satellite systems accessible to UAVs. Precision agriculture practitioners and researchers have started to utilise the available technology for different reasons. According to the results of these studies, data can be obtained in a short period of time and the spatial resolution is better compared to satellite imagery. Drone-based smart agriculture applications equipped with detailed remote sensing sensors are of great importance for sustainability (Milics, 2019).

There is a growing interest in deploying hyperspectral cameras on UAVs to scan agricultural fields at regular intervals in order to increase knowledge of crop and soil conditions and optimise crop productivity. The use of UAVs offers a number of facilities for optimising cropping and checking the condition of arable land. However, hyperspectral cameras bring with them technical challenges, especially related to the lack of spatial information between frames, making it a challenging task to obtain real-time results "off-board" of the captured frames after the end of the flight. The results of the research carried out for this purpose have shown that the developed UAV control has positive results in terms of enabling real-time analysis of the acquired data and the reliability of the results (Guerra et al., 2019).

UAVs capable of vertical and short-range take-off and landing are widely used and have the capacity to cover large areas. These UAVs can be equipped to carry a range of equipment, from NDVI sensors to near full spectrum infrared cameras and different agricultural sensors. Today's modern and advanced technology is crucial for collecting and processing data that is difficult to access in supportable and real-time from the field. Drones are seen as an important technology that will help farmers save time while carrying out agricultural activities in the future. This technology is expected to play a major role in the agricultural areas of the future by enabling agricultural operations to be carried out more effectively (Malveaux et al., 2014).

In recent years, the agricultural sector is becoming a high-tech industry where new companies and investors are involved. Rapid technological progress is not only increasing farmers' production capacities but also developing robotics and automation technologies. Farmers are now using autonomous aerial vehicles and robots for harvest harvesting to increase their production efficiency (Güzey et al., 2020; Zude-Sasse et al., 2016). In a study, it was aimed to carry out the harvest collection process of apples with predetermined targets in agricultural land by means of autonomous unmanned aerial and ground vehicles in communication to minimise time (Güzey et al., 2020). The autonomous harvesting tool is shown in Figure 6.

Figure 6. Autonomous harvesting vehicle (Güzey et al., 2020)

Mogili and Deepak (2018), used drones to prevent serious diseases among farm workers from manually sprayed pesticides. This approach has been recognized as a promising development, especially in rural areas where small areas are the main source of income and any disease can significantly affect household well-being.

Bendig et al., (2014), estimated the fresh and dry biomass of summer barley using plant height from plant surface models in a field experiment with 18 varieties and two nitrogen applications. Super high resolution, very temporal (1 cm/pixel) CSMs are derived from red, green, blue (RGB) images captured from small unmanned aerial vehicle (UAV). At the end of the study, a high correlation was found between pH of CSMs and fresh biomass ($R^2 = 0.81$) and dry biomass ($R^2 = 0.82$).

Roy and De (2022), in their research, developed it to monitor plant pests and diseases based on IoT and UAV, which tried to solve the correlation between pests/diseases occurrence and weather parameters. In the study, rotary devices based on solar perception and UAV were used to obtain solar energy for the Yangtze River Region of China. As a result of the study, the researchers stated that it is feasible to monitor pests and diseases through aerial imaging using low-altitude remote sensing and UAV, and further analysis of climate change will provide some precautionary measures.

UAVs are potential remote sensing platforms for precision agriculture. However, nitrogen status needs to be estimated early enough in the growing season to be useful for in-season management. In a study conducted on potato crops, variations in nitrogen status were analysed. By calculating the NDVI values of the images obtained using UAVs, it was found that all nitrogen treatments could be distinguished in the images. Since there is different chlorophyll content per leaf area, differences between nitrogen treatments were observed (Hunt et al., 2018).

A study has been carried out for vine disease detection using a deep learning segmentation approach in an Unmanned Aerial Vehicle. The methodology of the study is based on the combination of visible and infrared images obtained from two different sensors. The infrared images, which enable the fusion of information from the two sensors, helped to develop a new image registration method to align the visible and the image. The proposed method achieved more than 92% and 87% detection at vine level and leaf level, showing promising perspectives for computing (Kerkech et al., 2019).

Zhu et al. (2010), developed a software for precision spraying of an Unmanned Aerial Vehicle (UAV) used for agricultural spraying. They carried out tests with various aircraft. The results showed that the spray system has the sensitivity to increase the spraying efficiency for grain spraying.

Sahin and Yildirim (2011), a civilian UAV was developed specifically for the afforestation of a cedar forest in Gölbaşı, Ankara, using a fixed wing unmanned aerial vehicle. A model aircraft was modified and an indigenously designed and manufactured seed dispersal system was mounted on the UAV. Two test flights were carried out at altitudes of 9 and 6 metres. According to the results of the test flights, the seed distribution reached a width of 45 and 30 metres respectively, the density of the seed distribution for both tests was consistent with the conditions suitable for seed germination, and according to these results, the UAV proved to be a versatile, economical, safe and highly effective tool in afforestation studies.

Doering et al. (2014), created a special design platform for the design of multiple unmanned aerial vehicles in precision agriculture applications. They tried to determine the positive and negative characteristics of the drones by performing various tests.

In his article, Rokhmana (2015), discussed some practical experiences of using Unmanned Aerial Vehicle-(UAV) based platform for remote sensing. They wrote that some information is needed for land preparation, cadastral boundary, vegetation monitoring, phytosanitary and stock valuation to support precision agriculture mapping. According to the researcher, UAV-based remote sensing system; It should be cost-effective, fast in production, easy to use by local personnel, and good geometry accuracy.

Yallappa et al. (2017), designed and produced a 6-engine drone by using technology on rice and in order not to harm human health in the field conditions where people have difficulty in spraying in India, where agriculture is a very important area. This drone, which has a spraying area of 1300 mm, has a capacity of 5 liters and is powered by 2 battery groups with a capacity of 8000 mAh. They underlined that the drone they produced is very useful in field trials, especially in areas where people cannot reach it, and that it is a fast application and that it can be applied quickly and at low cost without people coming into contact with chemicals that are harmful to human health. They emphasized that a drone with at least 15 liters and 30 minutes of flight time would be more efficient for such applications, for this purpose, where the capacity of 5 liters is insufficient in field applications.

Wang et al. (2019), reported that bird damage is a major problem for grapes worldwide. For the control of bird damage, they proposed a new Unmanned Aerial Vehicle (UAV) drone equipped with a system to disrupt bird psychology with a loud noise. They carried out a study on this in vineyards in the southeast of Australia. As a result, they found that the harmful birds were flying away for a long time in a radius of 50 meters. They stated that while one UAV is sufficient for 25 hectares of vineyard area to protect vineyards, more than one UAV is needed to more effectively protect a large vineyard area.

Mogili and Deepak (2018), conducted a study on the application of drone systems in precision agriculture. By incorporating drone technology into precision agriculture applications, productivity increases, especially in spraying operations; They stated that the spraying processes are carried out very quickly and in a healthy way, and that the spraying process is carried out very easily and effectively in regions where manpower is scarce. Product damage detected by multispectral cameras was determined by GPS coordinates, and spraying was carried out successfully with a drone.

Goswami et al. (2019), on maize genotypes, the genotypes before and after rime were examined. High resolution images of genotypes were obtained by using remote sensing methods. The obtained images were tried to be determined by machine learning methods and different algorithms. Different algorithms were compared with each other and the algorithm that best explained the stress factor was determined. Based on the results of the study on the corn plant, the stress factors could be determined by remote sensing methods and the stress factors were explained numerically.

Gnadinger and Schmidhalter (2017), studied the number of plants using the remote sensing method and the evaluation of plant growth indices after different fertilization applications. According to the results obtained, it was emphasized that remote sensing methods are an effective method in converting plant growth performances into numerical dimensions.

Candiago et al (2015), examined the use of multi-band indices for precision farming applications, as well as the use of UAV imagery to analyze vegetation. They reported that the information collected with multispectral sensors could be useful in grape and tomato cultivation studies. High resolution UAV data and photogrammetric techniques have the potential to collect multi-frame images and evaluate different vegetation index. As a result of the research, they stated that technologies in precision agriculture applications are a fast, reliable and cost-effective resource for the evaluation of products.

Demir et al. (2016), toned digital photographs of vinegar, wild lettuce and lettuce as part of their research at Erciyes University. By simply using digital camera data and image processing techniques, the researchers found that the plants could be sufficient for comparison without the use of a colorimeter based on the collected data.

Altaş (2017), processed the images obtained by UAVs in sugar beet cultivation in Tokat province with the help of MATLAB program and aimed to detect leaf spot and diseases in the plant. The researcher compared the severity of the disease. In addition, he determined that the results of the study using image processing techniques were sensitive that could not be determined by observation and gave the exact value of the diseased areas.

Doğan and Yıldız (2019), thanks to developments in parallel with today's technology, remote sensing methods have gained a new dimension. From these methods, it is possible to perform more than one application at the same time with IHA technology. In these applications, the UAV offers advantages such as being able to fly at the desired height and obtaining clear photos. By using a multispectral camera that can be mounted on the UAV, the plant patterns can be clearly grouped thanks to the featured images. In addition, it was stated that it could give an idea about crop yield and plant health status. Researchers have examined that they can detect the product pattern by using colors such as red, green and infrared obtained from the cameras mounted on the UAV. As a result, they stated that classification can be made in plant pattern using multispectral camera.

Shin and Kadıoğlu (2019), stated that UAVs have developed with technology and have more usage areas in agricultural activities. Image processing techniques such as weed detection and phytosanitary detection can be used with UAVs and control methods are being developed. In addition to the detection processes with UAVs, the researchers mentioned the importance of both in terms of environmental health and in terms of determining the product patterns in a healthier way, thanks to studies such as fertilization and spraying. As a result, it has been reported that studies can be conducted on the importance of weed detection with UAV.

Su et al (2019), focused on the spatio-temporal monitoring of winter wheat inoculated with yellow rust inoculation at various levels throughout the entire growing season in their study for the spatial-temporal monitoring of wheat yellow rust using UAV multispectral images. They designed a custom workflow to acquire time-series five-band (visible infrared) aerial images with a multi-band camera and UAV. The researchers concluded that it would be an important guide for future early spatial temporal yellow rust monitoring at farmland scales.

Castaldi et al. (2017), used UAV multispectral images to classify maize and weeds. Application of classification results in cornfield weed management has resulted in a reduction in herbicide use without harmful crop yield implications.

Use of Unmanned Aerial Vehicles in Animal Husbandry

Unmanned Aerial Vehicles (UAVs), also known as drones, are being extensively researched to improve efficiency in agricultural production. Applications include monitoring fields, pastures and livestock, identifying/controlling potential problems as well as locating these problems in the field (Gómez-Candón et al., 2014). The popularisation of drones is mainly due to the consolidation of technologies such as Global Positioning System (GPS), embedded microelectronics, miniature autopilot systems, mobile communication equipment, compact high-resolution digital cameras and high-power batteries. This makes drones low cost, safe and easy to use (Guo et al., 2018).

One of the most important and challenging tasks in animal monitoring is cattle counting. Soares et al. (2021), The use of Unmanned Aerial Vehicles (UAVs) has been extensively investigated to improve the efficiency of agricultural production and animal monitoring. In this study, they proposed a method to detect and count cattle in aerial images acquired by UAVs based on Convolutional Neural Networks (CNNs) and a graph-based optimisation to remove duplicate animals detected in overlapping images. Their results show that the proposed method is very competitive, outperforms the state-of-the-art in detecting duplicated animals and significantly reduces the computational cost of the overall counting task. A drone used in animal counting is the DJI Mavic Pro. It is shown in Figure 7.

Figure 7. A drone DJI Mavic Pro used in animal census (Soares et al., 2021).

With the advent of deep learning and especially convolutional neural networks (CNNs), it has become more effective to extract relevant information from aerial imagery. Despite technological advances in drone, imaging and machine learning technologies, the application of UAVs for cattle monitoring is far from being comprehensively studied, with many research gaps still persisting. In this context, Barbedo et al. (2019), They set three objectives in their study: (1) to determine the highest achievable accuracy in detecting animals of the Canchim genus that are visually similar to the Nelore genus (Bos taurus indicus); (2) to determine the ideal ground sample distance for animal detection; (3) to determine the most accurate CNN architecture for this particular problem. The results revealed that many CNN architectures are robust enough to reliably detect animals in aerial imagery even at far from ideal conditions, demonstrating the feasibility of using UAVs for cattle tracking. Images from animal detection using UAVs are shown in Figure 8.

Figure 8. Images from animal detection using UAVs (Barbedo et al., 2019)

Monitoring the welfare of cattle and sheep on large pastures can be time-consuming, especially if animals are scattered over large areas of semi-natural pasture. Automated equipment allows continuous monitoring and can provide more information than manual monitoring. Electronic identification ear tags can recognise visits to specific points. Collars with positioning (GPS) units can assess animals' movements and habitat selection, and to some extent their health and welfare. Digitally determined virtual fences, rather than traditional physical fences, have the potential to contain livestock in a predefined area using sound signals in combination with weak electric shocks, although some individuals may have difficulties responding as intended, potentially resulting in reduced animal welfare. Remote technology, such as drones equipped with cameras, can be used to count animals, determine their location and study their behaviour. Drones can also herd and move animals. "Sensors on devices with 'cloud' server connectivity offer great opportunities, but need to be explored further. Possibilities to save battery power by reducing signal sampling and connection intervals without compromising sensitivity and authenticity need to be explored (Herlin et al., 2021).

A feasibility study was conducted on remotely sensed imagery (using datasets from satellites, manned aircraft and UAVs) and deep learning techniques to detect, count, identify and characterise the posture of individual cows in pasture production systems. With these techniques, we focussed on: (1) automatic detection of cattle locations and animal counting; (2) cow postures such as standing, grazing or lying down; and (3) individual cow identification. Detection, identification and posture monitoring of cattle is possible to some extent with remotely sensed imagery. However, high accuracy in cattle detection cannot be achieved on every platform. UAVs are the most suitable and can accurately detect, identify and monitor their posture. Aerial photographs and VHR satellite imagery can only detect and potentially count cattle. Future developments in VHR satellite imagery are promising as they can provide higher spatial resolutions (pixels smaller than 30 cm), which improves the detection of cattle. Satellite data have the advantage that they can be made available almost daily and cover large areas, which is not possible with UAV technology. Current results show that UAV imagery gives the highest accuracy (>95%) in cattle detection due to the high spatial resolution of images with a pixel size of a few centimetres. It shows that drones with RGB cameras are the best use for detecting and identifying cattle in pastures. Based on experiments with Holstein dairy cows it seems possible to detect, identify and monitor the behaviour of individual cows and therefore to gain more information about cattle in terms of flexibility and efficiency in the investigated production systems. These results make camera-mounted drones a promising new technology for monitoring extensive beef production systems (Mücher et al., 2022).

Li et al. (2022), In a study, they proposed a novel robotic herding system based on autonomous barking drones. They developed a collision-free sliding mode-based motion control algorithm that navigates a network of barking drones to efficiently gather a herd of animals when they are highly dispersed and drive them to a designated location. Simulations using a dynamic animal herding model based on Reynolds' rules have shown that the proposed drone herding system can efficiently herd thousands of animals with a few drones. The contribution of this paper is the proposal of the first prototype of herding a large herd of livestock by autonomous drones. A proposed drone herding system is shown in Figure 9.

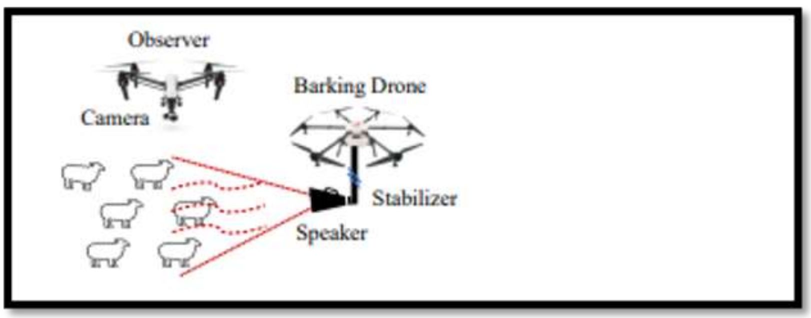

Figure 9. A proposed drone herding system (Li et al., 2022)

Consumer groups are putting pressure on modern farmers to be more efficient, with a focus on better animal welfare. Herding puts farmers' lives at risk, includes stress from farm dogs, and the risks are neglected if not done frequently and intelligently. To adapt mathematical models of shepherding to the new dimension, the behavioural and physiological response of twelve Dorper sheep (Ovies aries) to a drone was studied. The model aims to make it possible for artificial intelligence to enhance the autonomy of farmers and pilots shepherding from the sky. The sheep quickly and positively acclimatised to the drone initiating a ram's flock, regardless of drone speed. The results show that stimulation of sheep auditory awareness during aerial herding leads to altered sheep responses (Yaxley et al., 2021). The expected close response of the sheep to the presence of a Sky Shepherd is shown in Figure 10.

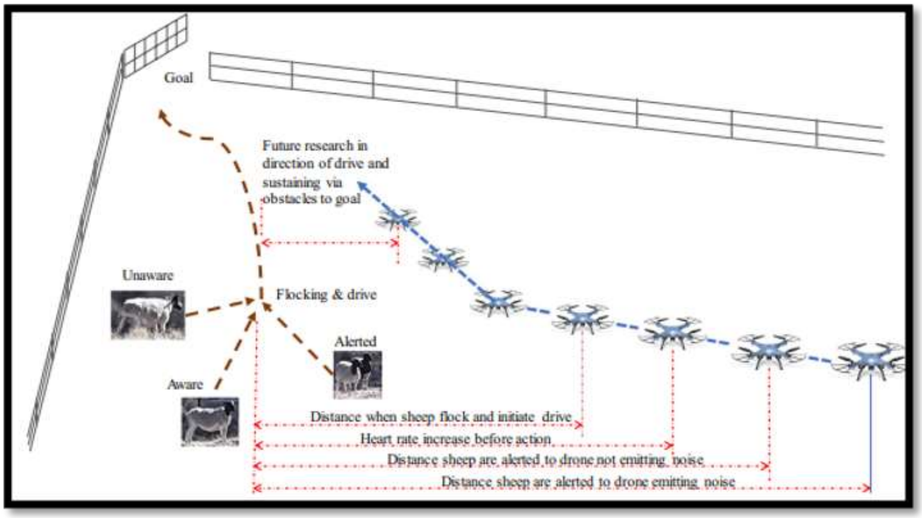

Figure 10. The expected close reaction of the sheep to the presence of a Sky Shepherd (Yaxley et al., 2021)

Conclusion

Although many image processing techniques have recently been developed by researchers for the detection of animal behaviour, further elaboration and enhancement of these techniques with different sensors would greatly contribute to the development of automated management systems that can detect animal behaviour and decide the best solution or instant warning alarm in unusual situations. However, the creation of such a management system is only possible if teams from different research fields such as physiology, zoology and technology are formed.

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LIGHT TRAPS AND PHEROMONES AS FRIENDLY TECHNIQUE FOR CONTROLLING *TUTA ABSOLUTA*

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ABSTRACT

This study focuses on the control of *Tuta absoluta*, a devastating pest that affects tomato crops. The pest has rapidly spread throughout Europe and the Mediterranean basin since it was first documented in Spain in 2006. The study highlights the life cycle and behavior of *Tuta absoluta*, emphasizing its ability to cause significant damage to above-ground parts of tomato plants. To effectively control this pest, the integration of various control measures is necessary, including mass trapping techniques, light traps, insecticides, and biological insecticides. The use of synthetic pheromone lures coupled with Delta traps has been effective in monitoring and managing *Tuta absoluta* populations. The study also introduces Ferolite traps, which combine a water trap, a sex pheromone lure, and a specific light frequency to attract and trap both male and female adults of *Tuta absoluta*. The effectiveness of light traps and pheromones in controlling *Tuta absoluta* is demonstrated through a three-year experiment, where the number of captured moths were monitored. The results show that light traps and pheromones are effective in controlling *Tuta absoluta* and offer an environmentally-friendly alternative to chemical compounds. Overall, the study recommends the use of light traps and pheromones for the control of this devastating tomato pest.

Keywords: *Tuta absoluta*, pest control, light traps, pheromone traps, integrated pest management, tomato crops.

INTRODUCTION

In the current conditions of the market economy where the year-round production of vegetables for fresh consumption is required, the increase of the cultivated surface of greenhouses as well increasing of tomato production.

Vegetable plants as specially tomato is affected by a number of pests and diseases, among which the main ones are tomato moth, whitefly, aphids, leaf miners, and mites.

Cultivation in greenhouses is very suitable for their development, of the harmful pets but most of the products used for spraying, even though they are effective, they are dangerous for the environment and consummators. On the other hand, to keep the pests under the economical threshold, some treatments are carried out with plant protection products, which require not only time and great expenses, but as a result of their use, resistant phenomena are also expressed.

The tomato itself is considered one of the most important plants in the conditions of our country. This plant in Albania is cultivated in all climatic zones and has great economic importance. It is cultivated throughout the year in protected areas and during spring, summer and late autumn in open fields.

Tuta absoluta is an oligophagus pest that attacks all parts of cultivated and wild plants belonging to the family Solanaceae (Desneux et al., 2011). Since this pest was documented in eastern Spain in 2006, it has invaded many other European countries and has spread throughout the Mediterranean basin *(Desneux et al., 2010; Gontijo et al., 2015)*.

The reproduction potential of this insect is also high with a life cycle that is completed within 30 to 35 days based on the environmental conditions (Harizanova et al., 2009). Crop yield losses can be as high as 100 % under high infestation if no control action is taken (Caparros et al., 2013).

The management of *T. absoluta* in tomato is generally based on the use of synthetic insecticides (Abdelmaksoud et al., 2020; Jallow et al., 2020). However, frequent application of pesticides and short developmental time of the pest, favor the development of multiple-insecticide resistance in *T. absoluta* populations, thus making insecticide usage less effect against it (Roditakis et al., 2018; Grant et al., 2019; Mansour et al., 2019).

To compensate the unsatisfactory effectiveness of chemical control, non-insecticide control strategies have been developed, including biological control (Urbaneja et al., 2012), mating disruption (Vacas et al., 2011; Cocco et al., 2012) and mass trapping using light (de Oliveira et al., 2008) and pheromone water traps (Sannino and Espinosa, 2010), (Coco et al., 2012).

The aim of the study is the controlling of *Tuta absoluta* using light traps and pheromones as an alternative and a friendly technique.

MATERIAL AND METHOD

During the years 2021-2023, in a tomato greenhouse with an area of 1 ha located in Gorican, Berat, the experiment was carried out for the mass capture of the tomato moth *Tuta absoluta* by using baited pan traps and light with a density 20 traps per hectare.

To detected the first moths, 2 delta traps which one in 0.5 hectare were placed on this experimental surface. Immediately after the first catches in delta traps, 20 traps per hectare were placed at equal distances on the surface of 1 hectare. At intervals of 10 days, the moths trapped in the baited pan traps and light were counted. All the results are recorded each ten days.

Figure 1. View of experimental field

RESULTS AND DISCUSSION

The area in which Tuta absoluta experiment was carried out has a very strong population dynamic accompanying the tomato in greenhouses during the entire vegetative cycle from planting to harvest.

Tuta absoluta appears immediately after planting in greenhouse and in this area, it is present mainly at the end of February and the beginning of March. When temperatures rise, the number of moths in flight increases rapidly. Mostly Tuta absoluta moths fly flat on the ground.
Based on the experiment's data (Table 1) on the first 10 days of March for the first year result a catch of 63 moths, for the second year 129 moths, for the third year 69 moths in 20 baited pan traps and light. The peak of the catch is reached on the first 10 days of April with 297 moths in the first year, in the second year 198 moths, in the third year 207 moths in 20 baited pan traps and light. Then it is noticed a decrease in catches which corresponds to the end of the first generation.

For the second generation, a maximum of catches is reached on May 19 for the first year with 225 moths, for the second year 180 moth, for the third year 189 moths in 20 baited pan traps and light.

For the third generation, a maximum of catches is reached on May 29, for the first year 186 moths, for the second year 138 moths, for the third year 147 moths in 20 baited pan traps and light.

For the fourth generation, it reaches a maximum on June 28, for the first year 378 moths, for the second year 335 moths and for the third year 414 moths caught in 20 baited pan traps and light.

Table 1. Capture of tomato moth *Tuta absoluta* by baited pan traps and light during three years of the study (2021 - 2023)

Date	**Year I**	**Year II**	**Year III**	**Total**
1 March	0	0	0	0
10 March	63	129	69	261
20 March	186	231	177	594
30 March	243	225	282	750
9 April	297	198	207	702
19 April	405	414	366	1,185
29 April	117	138	135	390
9 May	153	99	144	396
19 May	225	180	189	549
29 May	186	138	147	471
8 June	1,260	1,215	1,287	3,762
18 June	567	549	405	1,521
28 June	378	335	414	1,127
8 July	279	261	189	729
18 July	369	540	252	1,161
28 July	288	342	270	900
Total	**5,016**	**4,994**	**4,533**	**14,498**

In these baited pan traps and light, both male and female sexes are caught. Based on our observations at the end of the production affected leaves and fruits were identified. Although this is an effective and friendly alternative for the environment and the consumer. We can recommend that it is an alternative that shows a good premise, but it cannot alone maintain the dynamics of the population under the economical threshold.

CONCLUSIONS

In Albania situation, where the planted areas are constantly increased and the intensification of production is an increasing trend as well, the phytosanitary issues are becoming very acute. One of these issues is the control of *Tuta absolutes*. In general, farmers are using plant protection products, which in most cases do not have the appropriate effectiveness. In this context, the integration of suitable and compatible techniques and methods is the best alternative of the future. The use of the technique of mass capture through baited pan traps and light is a justifiable alternative not only in terms of technical effectiveness but also in terms of protecting the environment and the consumer as well. For this reason, we recommend to integrate this technique with other control alternatives.

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REVIEW OF INTEGRATED MANAGEMENT TOMATO MOTH (*TUTA ABSOLUTA*) USING MASS CAPTURE TECHNIQUE

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ABSTRACT
The tomato, scientifically known as *Lycopersicum esculentum* and belonging to the Solanaceae family, is a highly productive plant. In Albania, greenhouse tomatoes are commonly available in the entire markets, with the peak production occurring during the summer months, reaching approximately 600-800 quintals per hectare. The tomato moth, *Tuta absoluta* is originated from South America. In July 2009 was observed the first infections of tomato moth in tomatoes field in Levan (Fier) and Novosel (Vlore) regions of Albania. This experiment takes place in a low coastal zone, specifically in greenhouses covering an area of 2 hectares. The main objectives of this study are identifying tomato moth, understand its population dynamics, and determine the appropriate timing for intervention. Due to the significant damage caused by *Tuta absoluta* during this year, it is crucial to implement new techniques to control this pest. So far, the use of chemical compounds has proven inefficiency in controlling it. Instead, pheromone sexual attractants are being employed to determine the right time for intervention. The experiment is divided into four different variants. Another objective of this study is to explore the effectiveness of mass traps as an alternative method for controlling the tomato moth, *Tuta absoluta*

Keywords: *Tuta absoluta*, pest control, greenhouse tomatoes, Intervention time.

INTRODUCTION

T. absoluta is spreading world-wide and has caused damage and losses in Mediterranean basin countries (EPPO, 2008; EPPO, 2009a; EPPO reporting service 2009b; Desneux et al., 2010; Abd El-Ghany et al., 2018; Shahini et al., 2021).

In Albania, greenhouse tomatoes are commonly available in the market, with the peak production occurring during the summer months, reaching approximately 600-800 quintals per hectare (Berxolli et al., 2018b). In July 2009, the first infections of tomato moth were observed in field tomatoes in Levan (Fier) and Novosel (Vlore) regions of Albania (Berxolli et al., 2018a).

The effectiveness of *Tuta absoluta* control is reachable when the appropriate and compatible methods and techniques are used. The implementation of a single method based on many conducted studies has not been successful. In the conditions of Albania, where the climatic parameters are favorable for following the population dynamics of this pest, the implementation and integration shows that it is the most successful alternative.

During the first appearances of the pests, our farmers carried out a large number of chemical treatments for the control of *Tuta absolua*, but the effectiveness was very low. Faced with this situation, a 3-years study was conducted for the low-lying and coastal area using the technique of mass capture combined with biopesticides, which turned out to be a successful alternative.

Tuta absoluta is a pest that in Albania's conditions is evident in open fields and in greenhouses. Affecting a considerable number of plants such as tomatoes, potatoes, pepper, eggplant. Infection of beans and tobacco was also recorded. In the affected leaves, galleries are evident between the two parts of the epidermis, which later turn into spots. On these spots it is easy to distinguish the larvae that develop inside them if the leaf is held in the opposite direction of the light. The pest is also aggressive on tomato stalks and fruits.

Delta traps baited with synthetic pheromone lures are used for male capture and accurately show whether the insect is present or when its seasonal flight period starts, and they are used to arrange the bio-pesticide application period (Witzgall et al., 2010; Caparros Medigo et al., 2013 Shahini et, al., 2021).

Pheromones can also be used in pan traps and are particularly useful in the production of greenhouse tomatoes (Russell IPM, 2009; USDA-APHIS, 2011). Relevant studies about mass trapping effectiveness to control tomato leaf miner have been conducted by Filho et al. (2000), Goftishu et al. (2014), Braham (2014), Refki et al. (2016), and Abd El-Ghany et al. (2016).

The main objectives of this study are to identify the tomato moth, understand its population dynamics, and determine the appropriate timing for intervention.

MATERIAL AND METHOD

This study aims to implement packages for the control of the tomato moth *Tuta absoluta* (Meyrick) in the context of integrated management as an alternative and integral part of integrated production.

In order to monitor the tomato moth *Tuta absoluta* in experimental area, 4 pheromone traps were installed. In our experiment condition are used pheromone lures coupled with Delta traps (0.5 mg E3Z8Z11-14Ac) Product Code PH-937-1RR. The experimental scheme was divided into 4 variants with an area of 0.5 hectare. The used method was the biotechnical one using 10 water traps with lure per 0.5 hectare as well as monitored with pheromone traps. The traps were placed inside the greenhouse, in the center of it with height less than 1 meter. Traps were checked once per week (Berxolli et al., 2017).

Control through mass trapping alone cannot keep the damage level below that of economic injury; thus, it must be combined with other measures, such as double doors or nets (Chermiti et al., 2009; Harbi et al., 2012, Shahini et al., 2021).

The planted cultivar is a round tomato with a size of 220-230 gr, which is characterized by: Regular shape of the fruit, without the presence of branches in the flower; Strong pulp and stable in transport even after harvesting; This cultivar is characterized by an ability to obtain a beautiful red color even in unfavorable light conditions and low temperatures (early March and late

December); Another characteristic of this cultivar is a demand for good taste, as it comes to the market very early and can be used both for export and domestic consumption (Berxolli, 2018).

RESULTS AND DISCUSSION

Based on the research work carried out in the low coastal area of Albania, it turns out that the tomato moth *Tuta a bsoluta* is a major pest, causing massive damage both in protected areas and open fields. Monitoring with sexual pheromones is the most effective way to identify the biological progress of the pest, as well as to determine the time of using the mass capture technique.

From our studies, whether in the area of Durres or Fier, the tomato moth *Tuta absoluta* turns out to have 4 generations for the tomato as a first crop. The first generation begins in the first days of March and ends at the end of the first 10 days of April. The second generation begins on the second 10th day of April and ends in the middle of May. The third generation starts after 10th day of May and ends after the 10th of the second days of June. The fourth generation begins at the end of the 10th day of the third day of July (Berxolli et al., 2018b).

During the three years of the study for the use of the mass capture technique, which is implemented immediately after the capture of the first moths in the traps monitored with sex pheromones, it has been found that the mass capture technique has an effectiveness of 50%-71% in leaves and 52%-72 % in fruit. In these conditions, we come to the conclusion that this technique used alone does not guarantee maximum effectiveness (100%), but it is necessary to combine it with other methods (Berxolli, 2018).

CONCLUSIONS

The use of sexual pheromones is the most effective tool for the evidence of biological progress as well as for determining the time of using the mass capture technique. The use of mass capture should be implemented immediately as soon as the first flights in sexual pheromones are captured. The use of mass capture as a only technique does not guarantee very high effectiveness. The use of the mass capture technique should be combined with other methods and especially with the use of biopesticides. We recomand the use of mass traping in combination with a bio-insecticides, insect-proof net and/or double doors.

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REMOTE SENSING IN HORTICULTURE - SCIENTIFIC INFORMATION AND PRACTICAL IMPLEMENTATION: CASE OF BULGARIA

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ABSTRACT

Vegetable production as a basic element of horticulture is well established in Bulgaria.
The modernization of technologies, the introduction of new varieties and the optimization of growing conditions require the search for new methods to monitoring and assessing the status of vegetable crops. There is a need for permanent observation, almost in real time, to obtain information quickly when environmental conditions change, diseases appear, pests attack or the eco-physiological status of the plant's changes.
In response to these high requirements of modern vegetable production, the use of remote sensing methods is more and more relevant.
The aim of this paper is to review the scientific information on remote sensing in horticulture and to identify opportunities for its use in Bulgaria.

Keywords: vegetable crop production, remote sensing, productivity of plants, vegetation indices

INTRODUCTION

Vegetable farming, as a major sub-sector of crop production, is traditionally present in our country. According to the Department of "Agro-Statistics" of the Ministry of Agriculture and Food, the area planted with vegetables in 2022 amounts to 29.4 thousand ha. The production of vegetables is 629.2 thousand tons (of which 109.7 thousand tons from greenhouses). Tomatoes, cucumbers, peppers, watermelons took a major part of the vegetable production. Cabbages, onions, and pumpkins also occupy a substantial amount of the area. (https://www.mzh.government.bg/bg/statistika-ianalizi/html).

The intensification of technologies, the introduction of new varieties and the optimization of growing conditions require the search for new methods to monitor and assess the condition of vegetable crops to make decisions on the implementation of different agricultural practices in their harvesting.

On the other hand, up-to-date and accurate information on the species composition of crops, their spatial distribution, their biological potentiality, and the limiting factors determining their development and productivity is very important for the rational use of available production resources.

The need for permanent monitoring of the crops, almost in real time is to react immediately and in the most appropriate way to changes in the environmental parameters, the occurrence of diseases or disturbances in the eco-physiological status of the plants.

In response to these high requirements of modern vegetable production, the use of remote sensing methods is an increasingly relevant issue. The efforts of many scientists are directed towards the development of practically applicable methods for crop monitoring and evaluation. The accelerated development of satellite, airborne and ground-based remote sensing is creating the conditions for its increasing use in crop production and in vegetable production.

The aim of this paper is to review the scientific information on remote sensing in horticulture and to identify opportunities for its use in Bulgaria.

MATERIAL AND METHODS

The necessary scientific information was assembled by reviewing thematic issues - articles, books, reviews, reference books, catalogues. Scientific on-line databases such as CAB Abstracts, ISI Web of Knowledge, Scopus, Science Direct, Google Scholar were mostly used.

Based on the study of available scientific literature, the analytical review in this paper was prepared.

The work of the authors' team on investigating the possibilities of using remote sensing information in the context of sustainable vegetable production management is presented. The methodological approach for collecting in situ data and establishing the relationships with remote sensing observations is presented by Arnaudova et al. (2022).

The analyses of Cholakov (2009) and Shaban (2011) were used to identify the main features of vegetable production in Bulgaria. Statistical data are from the “Agro-statistics” department of the Ministry of Agriculture and Food.

RESULTS AND DISCUSSION

According to Kumar et al. (2022), the technical definition of remote sensing is: "A technology for measuring reflected and emitted electromagnetic radiation from the ground, atmosphere, and the aquatic ecosystems across the spectrum from visible to microwaves spectrum". Omia et al. (2023) added that remote sensing technology is the science of acquiring and measuring information about certain properties of objects and phenomena without coming into direct contact with the observed subject. The authors refer to the development of Kundu et al. (2021).

The basic principle described by Kumar et al. (2022) on which remote sensing is founded is that different observed objects emit or reflect electromagnetic radiation in different wavelengths, which specialized sensors detect and present as images. Omia et al. (2023) clarified that the information obtained in this way is carried by electromagnetic radiation, which is propagated in the surrounding space at the speed of light in the form of harmonic wave modes at different wavelengths.

Aggarwal S. (2004) presents a conceptual model of the remote sensing process (Fig. 1). According to the author, some of the images are based on the reflected solar radiation in the visible and near infrared spectrum, while others are based on the measurement of energy radiated from the Earth's surface in the thermal infrared wavelength spectrum. Sensors, or instruments, onboard satellites and aircraft use the sun as a source of illumination or provide their own source of illumination, measuring the energy that is reflected. Systems that make measurements remotely and depend on an external energy source (e.g. sunlight) are passive remote sensing systems.

According to Basso et al. (2004), remotely sensed data can have many variations depending on the electromagnetic energy distribution and can be obtained from different platforms - satellites, aircraft, drones, handheld ground-based measurement instruments. Data can also be collected from various devices - digital cameras, RGB and multispectral sensors.

Depending on the satellite's orbit and sensor design, resolution can vary. There are four types of resolution to consider for any dataset—radiometric, spatial, spectral, and temporal.

- Spatial resolution: refers to the precision of imaging the earth's surface and the objects located on it;

- Spectral resolution: refers to the number of specific waves in the electromagnetic spectrum. This resolution is the basis for the potential use of sensor systems because of the specific differences in the sensors used.

- Temporal resolution: refers to the interval between two consecutive images of the same area.

The determining factors are the type of platform used and the atmospheric conditions (Basso et al., 2004);

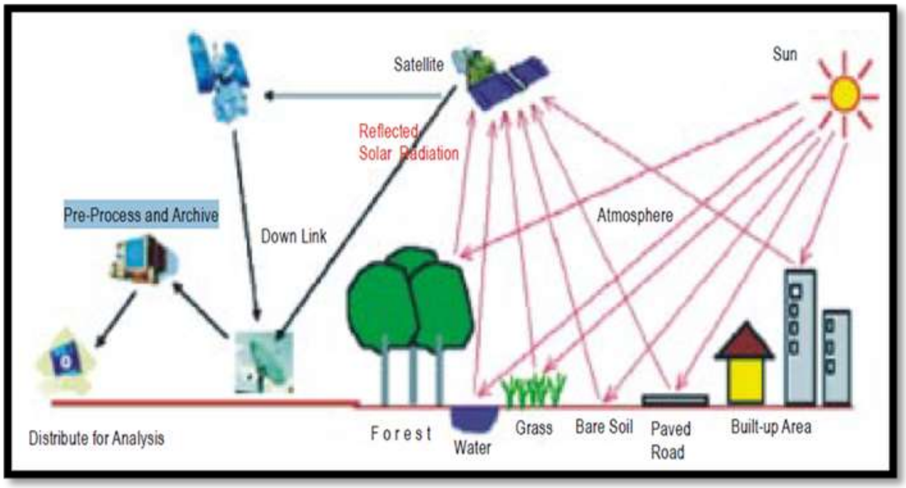

Figure 1. Model of the remote sensing process

Wujtowicz et al. (2016) in their review on the application of remote sensing methods in agriculture describe extensively the different aspects of the ongoing research and the results achieved on the topic. They also give the following examples:

- *For yield prediction*

Remote sensing has been used to predict crop yields mainly based on statistical-empirical relationships between yield and vegetation indices (Thenkabail et al. 2002, Casa and Jones 2005). Information on expected yield is very important for government agencies, market traders and producers in crop planning, storage, transportation and marketing. The more timely and this information is available, the lower the economic risk, leading to greater efficiency and increased return on investment

- *For plant nutrient needs*

The use of remote sensing from the air in agriculture has also been well documented by Goel et al. (2003), who confirm the potential of this technology for determining nitrogen and weed density in maize. The objective of this study was to determine the relationship between reflectance acquired at wavelength bands from 409 to 947 nm and spectral differences resulting from the presence of weeds and different fertilization rates. The results show that the reflectance of maize is significantly affected by the presence of weeds and plant nitrogen deficiency.

Differences in spectral characteristics due to nitrogen stress were most evident at 498 and 671 nm at all growth stages, and the presence of weeds had no interactive effect. Differences in other spectral ranges, whether related to nitrogen, weeds, or a combination of the two, depend on growth stage.

Agüera et al. (2011) compared the performance of nitrogen status estimates derived from multispectral imagery taken by UAVs and data recorded with a ground-based platform. The vegetation NDVI index calculated from both platforms was found to be a good indicator of leaf

N content, but a higher correlation coefficient (R=0.80) was found for the UAV platform than for the ground-based measurements (R=0.71).

There are also numerous examples of the use of satellite imagery to assess crop nitrogen status. For example, Bausch and Khosla (2010) found that multispectral data from the QuickBird satellite can be used to accurately estimate the spatial variability of maize nitrogen status within a field. Similar results were presented by Jia et al. (2011), who demonstrated that single-band reflectance in the NIR (wavelengths 770 - 880 nm, red (640 - 720 nm), and green (520 - 610 nm)) as well as vegetation indices NDVI, GNDVI, RVI, and OSAVI are well correlated with wheat nitrogen status and that high-resolution satellite images are useful tools for determining wheat nitrogen.

- *To detect damage from diseases and pests*

When using imagery to detect infected plants in agricultural crops, it is necessary to select a sensor with appropriate spectral and spatial resolution. Mewes (2010) compared the effectiveness of the identification of wheat plants infected with brown rust (Puccinia recondita f. sp. tritici) with two hyperspectral cameras, one of which (AISA-DUAL, Specim LTD, Oulu, Finland) recorded reflected emission in 498 bands in the spectral range 400 - 2500 nm with a spectral resolution of 2.5 - 5.8 nm and the second (ROSIS, German Space Agency, DLR) in 115 bands in the spectral range 383 - 839 nm with a spectral resolution of 5 nm. The accuracy with which healthy and infested plants were identified in the AISA-DUAL images was higher than in the ROSIS images (84.32 % and 80.33 %, respectively) and was associated with stronger correlations at longer wavelengths in the infrared spectrum. The AISA images were recorded from a lower altitude than the ROSIS images (2300 m and 2880 m, respectively), which resulted in a higher spatial resolution (1.5 m and 2.0 m, respectively) and a stronger signal than AISA due to less atmospheric absorption and scattering of the reflected signal from the field surface.

The spatial resolution of the image data is a key factor in the detection of plant diseases and pests. Better results can be achieved using UAVs, which provide higher resolution images than manned aerial platforms.

The occurrence of diseases and pests on crops can also be detected using satellite imagery. Apan et al. (2004) demonstrated that Hyperion can be used to detect rust *(Puccinia kuehnii*) in sugarcane. Chen et al. (2007) used Landsat multispectral imagery to detect heavy infestations of *Gaeumannomyces graminis* disease in wheat. Franke and Menz (2007) evaluated high-resolution QuickBird imagery to detect powdery mildew (*Blumeria graminis*) and multispectral satellite imagery for leaf rust (Puccinia recondita) in winter wheat. The results indicate that multispectral imaging is suitable for detecting heterogeneous wheat characteristics in the field, especially for later stages of fungal infections, but is only moderately suitable for discriminating early infection levels in wheat.

- *To assess plant water requirements*

Using airborne remote sensing, Champagne et al. (2003) directly estimated tree canopy cover and equivalent water thickness (EWT), which is the weight of water per unit area of leaf area. There is a close relationship between EWT and plant biomass and their LAI, which are important variables in many agricultural applications. The model describing the relationship between EWT and hyperspectral aerial imagery is found to be a good predictor for broadleaf crops such as beans, maize, canola and peas, while it gives poor predictions for wheat.

UAV platforms have proved very useful for irrigation management. The ability to fly at low altitudes allows high spatial resolution thermal images to be obtained, thus eliminating the soil background effect. Gago et al. (2013), using a pixel resolution of the thermal image of 2.5 cm, obtained R2=0.86 for the relationship between the CWSI index and plant water status in vineyards, with an improvement in water stress assessment compared to previous publications.

There are studies that show that accurate estimates of plant water content can also be obtained from the satellite level.

Satellite imagery is particularly useful for estimating vegetation water content and can assist in effective water management by providing information on the total evaporative water requirement of crops. El-Magd and Tanton (2003) estimated ET directly using Landsat ETM satellite data and a modified heat flux sensitive approach. This method is useful for estimating crop water resources and can be used to determine water use efficiency.

- For weed control

Lamb et al. (1999) using hyperspectral emission data from an airborne sensor demonstrated weed detection early in triticale development, and Deguise et al. (1999) successfully mapped weed patches in a field of canola (Brassica napus L.). Peña et al. (2015) explored the possibility of using UAVs to optimize herbicide application based on aerial imagery. Due to very low altitudes (40 m) and high spatial resolution of the images, weeds were detected with an accuracy of up to 91% 50 days after sowing.

Weed detection using high resolution multispectral satellites such as QuickBird and GeoEye with ground resolution of 2.44 and 1.64 m respectively is promising. Detailed maps of Cirsium arvense in sugar beet at the cotyledon stage have been created using QuickBird imagery (Backes and Jacobi 2006). Moderate resolution satellites such as SPOT (20 m) or Landsat TM (30 m) and NOAA-AVHRR low resolution (1100 m) have proven useful for detecting and mapping large groups of weeds due to differences between the spectral properties of weeds and their background (Anderson et al. 1993; Ullah et al. 1989; Peters et al. 1992).

At the end of their review on the current status and potential use of remote sensing, Wujtowicz et al. (2016) indicated that the examples described above are in many cases related to the use of remote sensing in precision agriculture, which has developed rapidly in recent years. The main objectives of this production management method are to optimize the return on inputs while ensuring environmental protection. The highly developed technologies used in precision agriculture require constant access to detailed information characterising the environmental conditions under which crops are grown. Such information can be obtained from aerial and satellite imagery at field scale.

Shanmugapriya et al. (2019) confirms that in the last two decades, remote sensing has been widely applied for crop identification, crop acreage estimation, crop condition/stress assessment, yield estimation in precision agriculture, soil testing, water management, disease and pest presence, drought and flood monitoring, weather forecasting, and agronomic advice.

Kumar et al. (2022) present graphically all the application areas of remote sensing methods in agriculture (Fig. 2).

Usha and Singh (2013) reviewing the potential applications of remote sensing in horticulture identified its main advantages, defining it as a rapid and effective tool for identifying cultivated plants from surrounding vegetation. The use of satellite imagery over a period of time allows the dynamic observation of large areas and the timely recording of land cover changes and quantification of the rate of these changes.

Data collected from satellite, airborne and ground-based sources facilitate monitoring of weed infestation rates and species composition, damage caused by pests and plant pathogens, and can thus be rapidly counteracted. The ability to use remotely sensed data to determine plant fertiliser requirements based on the nutrient content of crops and soils helps to increase yields and improve the quality of harvested seed and fruit, which is important for improving crop profitability. Accurate determination of plant nutrient needs at critical stages during the growing season helps to optimise fertilisation as well as reduce potential adverse impacts associated with the transport of agrochemicals out of the country. Remote sensing is also used to estimate plant water requirements and determine the date of irrigation initiation, which facilitates crop management under water stress conditions (Usha and Singh, 2013).

Figure 2 Remote sensing application in different fields of agriculture (Kumar et al.,2022)

Weiss, et al. (2020) highlighted that remote sensing has the capacity to support the adaptation of agronomic practices by providing replicable information on crop conditions throughout the season and for different stakeholders. They also outline a longer-term perspective, in which the remote sensing and diagnostic methods developed are also linked to climatic changes and the necessary adjustments in crop growing times, as well as the incorporation of new technological practices.According to the authors, remote sensing appears to be an essential response tool, as it offers a non-destructive means of providing information.

Kumar et al. (2022) argue that remote sensing can have wide applications for government authorities and local agencies to make decisions to form new policies to address any national problems and crises related to agriculture. Farmers can also get useful information from the imagery about the health status and possible problems for a particular crop and soil type.

In connection with the increasing interest in scientific research on the application of remote sensing, a national research program on Intelligent Crop Production is being developed in Bulgaria. Its main objective is to conduct basic and applied research to create models for robotic technologies, digital diagnostic and forecasting methods, and digital management of crop farms to ensure a sustainable and efficient food system.

The targeted fundamental research aims at exploring the possibilities and developing models for the use of robotic technologies, satellite imagery and digital methods for diagnosis, prognosis, and production management of quality crop production and without negative effects on the climate and the environment.

Applied scientific investigations are related to the implementation of digital technologies on farms specialized in the production of arable crops, industrial crops, essential oil crops, fruits and vegetables (https://nnp-ir.bg/).

From the review of the development of research on remote sensing and its use in agriculture, the problem is relevant and significant for our time. However, most developments have focused on crops that are strategic for agricultural production, such as wheat, maize, sunflower, cotton, and potatoes. Publications on research conducted with vegetable crops are very limited.

In Bulgaria, at the present stage, vegetable areas occupy about 2.2-2.4% of used agriculture lands (UAL), while the share of fresh vegetables has fallen below 1% of UAL. Despite the minimal share of areas in the production structure, vegetable production accounts for 1/3 of the gross output (GOP) of crop production and about 15% of the GOP in agriculture. This determines the importance of the sector for the country's agriculture. Vegetable production is concentrated in the South-Central region, where 44% of vegetables and 50% of potatoes are concentrated. In the remaining regions, the distribution of vegetables is as follows: in the North-Eastern region - 21 %, South-Eastern region - 13,4 %, North-Central region - 11,6 %, South-Western region - 6,5 % and North-Western region - 4,1 % Shaban (2011).

The specific features of the sector that make the mass application of remote sensing difficult are that the areas under vegetables are fragmented and small. Most vegetable crops have an upgrowth throughout the vegetation period and multiple harvests take place, necessitating periodic monitoring at key phases of their development (Arnaudova et al., 2022).

The prospects for the development of vegetable production in Bulgaria are extremely favorable due to the presence of natural resources, the rich experience and excellent traditions of Bulgarian gardeners, modern varieties with high biological potential, as well as modern, advanced technologies for crop cultivation (Cholakov, 2009).

The efforts of the author's team are aimed at adapting remote sensing to the specific features of vegetable crops. The authors of this publication, together with their colleagues - scientists from the Agrarian University, participate in the scientific team of Component 2 "Diagnosis and Forecasting through Artificial Intelligence" of National Research Program "Smart Crop Production", Work Package 2 " Use of Earth Observation data ".

The following work tasks are performed:

- Investigating the possibilities of synchronizing hyperspectral data (satellite and aerial) with ground-based studies for monitoring the ecobiological status (control of the main soil chemical-physical parameters) of different crops.

- Investigate the decision-making capability of artificial intelligence to use alternative technologies for remote monitoring and diagnostics, depending on the information on climatic conditions from a database of different satellites, aligned in a unified information system to correlate the data with the coordinates of the areas.

- Development of algorithms to jointly use the remotely sensed Earth observation data obtained in combination with the images obtained from the alternative monitoring technologies to train artificial intelligence to recognize/determine the crop type and its extent of development.

- Develop and maintain a software platform (GIS) to integrate data from advanced soil parameter monitoring and yield prediction technologies for major crops and satellite-derived data (such as Soil Water Index (SWI), Surface Soil Moisture (SSM), Normalized Difference Vegetation Index (NDVI), Burnt Area, etc.) as well as imagery from Sentinel-1X, Sentinel-2X, Sentinel-3X, and Sentinel-5P.

- Integrate large volumes of data (from satellite, aerial, and ground measurements) for soil analysis and use artificial intelligence methods to create a National Digital Soil Catalog.

- Analyzing the effectiveness of newly developed technologies by surveying real plots of different crops (arable, vegetable, and perennial crops) to monitor vegetation using precise satellite imagery for vegetation indices. (NNP IR (nnp-ir.bg))

CONCLUSION

The main objective of remote sensing methods in agriculture and vegetable production is to optimize the growing conditions of vegetable crops, including inputs, while ensuring environmental protection.

New technologies require constant access to detailed information characterizing the environmental conditions under which production takes place. Such information can be obtained from aerial and satellite imagery at the field scale.

Data collected from remote sensing facilitate observations on the extent of weed infestation, damage caused by pests and plant pathogens. The ability to use remotely sensed data to determine plant fertilizer requirements based on crop and soil nutrient content helps to increase yields and improve the quality of the resulting crop.

Extensive research work is being developed in Bulgaria in line with the aims and objectives of the National Science Program "Smart Crop Production".

Identification of opportunities for digitalization of management processes in agriculture depending on the specificity and the dynamism of the sector. Development of a model for increasing the competitiveness of crop products using artificial intelligence and digitalization.

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DETERMINATION OF VEGETATION INDICES BY REMOTE SENSING TECHNIQUES OF PEPPER (CAPSICUM ANNUUM) GROWN IN OPEN FIELD

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ABSTRACT

The vegetation indices obtained by various remote sensing techniques are simple and effective tools for quantitative and qualitative assessment of growth and development of vegetable plants. Processing of different sources of data − ground, airborne and satellite are used to generate them. Unmanned aerial vehicles (UAV) are also widely used.

At present, no universal mathematical formulas have been developed for determining all vegetation indices. Specific algorithms are developed depending on the object of the monitoring and the conditions for acquisition of image data.

The main objective of this study is to determine and compare the vegetation indices (NDVI), obtained through processing of image data (HR, VHR, UAV) with different spatial resolutions in similar spectral channels, with the aim of determining the most suitable sensor for the purposes of vegetable production, more specific for pepper grown in open field.

The studies were conducted in Katunitsa village, region Plovdiv, Bulgaria on a cultivated crop field in 2021.

Keywords: Vegetation indices, NDVI, remote sensing, vegetable crop production

INTRODUCTION

Remote sensed information for growth, vigour and dynamics of the agricultural crop can provide extremely useful data for applications in vegetation monitoring, biodiversity conservation, ecological aspects in agriculture, as well as other related fields. This type of information applied to agriculture studies provides not only an objective basis (depending on the resolution) for macro- and micro-management of agricultural production, but also in many cases the necessary information for evaluation of crop growth and yield estimation (Mulla, 2013). These applications of remote sensing data have a relation with developed and well-known category, precision agriculture, and enable to be tracked back the yield and vegetation assessment up to several years (Mulla, 2013; Smith, 2015; Nageswara, 2005).

The multispectral remote sensing images carry essential integrating spectral and spatial features of the objects (Chouhan R., Rao N., 2004). Digital image processing and satellite data and other used techniques provides tools for analysing the image through different algorithms and mathematical indices. Features are based on reflectance characteristics, and indices have been developed to highlight the features of interest on the image (Shikhar et al., 2014). Application of remote sensing and its different VIs, extracted by various techniques, usually relies heavily on instruments and sensors to determine which solution is the best to recognise and solve a particular problem.

There are many indices for highlighting the state of green vegetation on a remote sensing imagery. These vegetation indices provide a measure of the photosynthetic material in vegetation, which is essential for understanding the state of vegetation. Normalised difference vegetation index (NDVI) is the most commonly used index to evaluate and monitor vegetation status (Bhandari et al., 2012; Miura et al., 2006; Muira, 2013; Smith, 2015). This index is widely applied in research, related to global environmental and climate change. NDVI is calculated as a ratio difference between measured canopy reflectance in the red (chlorophyll absorbance) and near-infrared (reflectance peak) bands respectively (Bhandari et al., 2012; Nageswara et al., 2005). NDVI is one of the earliest developed remote sensing analytical products used to simplify the complexity of multispectral imagery, and it is now the most popular index used for vegetation assessment. The widespread use of this index is related to its easy calculation with any multispectral sensor with a visible and a near-infrared band (Meera, 2015; Zhang X. et al., 2009).

Since it was first introduced in 1973 (Rouse et al., 1973), NDVI has been investigated for various applications in a wide range of agro-climatic conditions, crop types and ecological systems, as well as for different scales – local, regional (Hatfield et al., 2010; Rossini, M. et al., 2010; Marti J. et al., 2007) and at a global scale (Shikhar, 2014; Bhandari et al., 2012; Nageswara et al., 2005; Chouhan R., 2004). The launch of many new sensors and satellite systems has increased the generation and analysis of NDVI data. The increasing number of sensors has created a need to evaluate and standardise the NDVI data from different available sensors (Ahmadi H., 2012; Meera, 2015; Karaburun A., 2010; Yang Y. et al. 2010). Inter-sensor comparison of NDVI has been done by a many researchers for different purposes. The main purpose, however, has been to achieve data continuity by temporal infill for the monitoring and modelling of natural resources (Karaburun A., 2010; Yang Y. et al., 2010; Lan Y. et al., 2009; Lan Y. et al., 1997).

In Bulgaria, the vegetable crops are grown on a relatively small cultivated area. The use of remote sensing techniques to estimate the growth stage for many vegetable crops are not well studied.

It is difficult to generalize because there are wide variations in varieties, planting densities, cultural practices, and small size hence the need to use higher spatial resolution. Growth stage and crop size is especially important for horticulture crops because canopy and height are a primary determinant of crop development. Vegetable crops develop from sowing to harvest as a function of meteorological driving variables (e.g., temperature, sunlight, and precipitation). As horticulture regions differ in farm size, crop types and vegetation conditions, compared to the other agricultural land uses, high resolution images are more suitable for analyses in this area. Acquisition of high spatial resolution imageries are freely available and this makes them preferred to be used in agriculture studies.

In 2021, the National Research Programme “Smart Crop Production” funded by the Bulgarian Ministry of Education and Science was initiated. The aim of the programme is to conduct fundamental and applied research, to create models for robotic technologies, digital methods for diagnostics and forecasting, as well as digital management of crop farms to ensure a sustainable and efficient food system. (https://nnp-ir.bg/)

The main objectivities of the programme are:

• Smart agriculture to be involved in the decision-making process to improve the management of different stages of production while dealing with an environmental assessment of profitability and sustainability;

• Higher accuracy in agriculture correlates with the protection of the environment, achieved by fuel efficiency, water, fertilizers, and plant protection products;

• Another aspect of smart agriculture can generate a higher profit margin, by applying less damage to the yield and crop loss;

• Further improving the performance of business operations, thus reducing the production cost;

• Farmers can achieve profitable and ecological production with optimal use of resources. On a global scale, smart agriculture can solve the challenges of food security in the future. This idea will benefit the increase of production and at the same time reduce the pollution applied to the environment.

As a part of NRP “Smart Crop Production” is Work Package 2.2 “Use of Earth Observation data”. The main partners in the WP 2.2 are Risk Space Transfer − Technology Transfer Office (RST-TTO), at the Bulgarian Academy of Scineces and Agricultural University − Plovdiv. The goal of WP is to study and apply the possible solutions and develop models for the usage of satellite images and digital methods for diagnostics, forecasting, and management of production crop quality. Carrying out the pilot projects for the purpose of space transfer technologies in agriculture for strengthen the connection between science, education, business, public authorities, and society. (https://nnp-ir.bg)

During the first year of the NRP, the purpose of WP 2.2 tasks were to research and conduct test studies to determine and specify spatial and radiometric parameters of phenological observations of vegetable crops in real production plots, in order to develop a methodology for conducting synchronised ground and satellite experiments for remote sensing of vegetable crops. Preliminary data on the crops in real conditions were collected to subsequently verify the parameters and characteristics obtained after processing the test images. The aim is to extract typical characteristics to be classified into typical feature classes and to establish the correlation between the observed crop development parameters according to a defined methodology and their identification by remote monitoring.

The purpose of this study is to determine and compare vegetation index NDVI, obtained through remote sensing techniques. To achieve this goal, Earth observation data with different spatial resolutions and with similar spectral bands ((high resolution (HR), very high resolution (VHR), UAV)) were investigated on pepper grown in open field. An analysis and recommendations for the purpose of vegetable production are made.

MATERIAL AND METHOD

Study area

The focus of this study is a vegetable crop – pepper (Capsicum annuum) grown in open field in a typical irrigation environment with total area of 2273 ha. The field is located in the south-central part of Bulgaria in Katunitsa village, municipality of Sadovo, Plovdiv region (Figure 1, Figure 2). The region is dominated by irrigation agricultural land, due to the proximity of the Maritsa River and the presence of high groundwater.

The study area is a part of National Research Programme (NRP) "Smart Crop Production" supported by the Bulgarian Ministry of Education and Science. Research and test studies were conducted to determine and specify spatial and radiometric parameters of phenological observations of vegetable crops in real production plots to develop a methodology for conducting synchronous ground and satellite experiments for remote sensing of vegetable crops.

Preliminary data has been collected for the pepper crop field in real conditions with the aim of subsequent verification of parameters and characteristics obtained after processing the test images. The aim is to extract features to be classified into typical feature classes and to establish the correlation between the observed crop development parameters according to a defined methodology and their identification by remote monitoring. Very High Resolution (VHR) WordView-3 (https://www.maxar.com/), High Resolution (HR) satellite data were provided Sentinel-2 (https://dataspace.copernicus.eu/browser/) and Unmanned aerial vehicle (UAV) − DJI Phanthom 4 Multispectral.

Study period was conducted during two growth stages of the pepper BBCH 59 510 and BBCH 73 703, in 2021 which represent the peak growth time for pepper crops. Plant and soil samples were taken using a randomized method for five target fields. These elementary sites are 50 m^2 having 400 plants each. The collected analytical data represent indicators of biological condition of plants in the production field (stem height -cm; stem weight -g; number of leaves; leaf weight -g; leaf area -cm2; weight of vegetative organs per whole plant and growth indices (total chlorophyll content; leaf area index; photosynthetic intensity; net photosynthetic productivity) (Arnaudova et al, 2022).

The held research was mainly focused on the assessment of the spatial resolution of the NDVI and the phenological observations of pepper carried out during the two vegetation phases. Precision radiometric calibration procedures and additional on-site measurements were not applied.

Figure1. Location of study region and extent of satellite and UAV images in the study

Figure 2. Location of study area L 42° 8'39.59”, B 24°52'53.53" and target fields

All images were co-registered with WGS 84 Zone 35N.

UAV − DJI Phanthom 4 Multispectral - height of the drone flight was 60 m.

The images and NDVI were processing by QGIS 3.10 (http://www.qgis.org).

NDVI values were calculated by formula:

$$NDVI = \frac{NIR - Red}{NIR + Red},$$

where *NIR* is the reflectance of the near-infrared wavelength band and where *Red* is the reflectance of the red wavelength band.

Table 1. Satellite and UAV sensors used in this research

Image	***Satellite/UAV***	***Date of acquisition***	***BBCH growth stages***
1	Sentinel-2	24.6.2021	59 510
2	WorldView-3	26.6.2021	59 510
3	Sentinel-2	16.8.2021	73 703
4	DJI Phanthom 4 Multispectral	18.8.2021	73 703

Table 2. Comparison of sensor attributes

(https://www.dji.com/bg/p4-multispectral/specs,

https://docs.charter.uat.esaportal.eu/missions/opt/worldview-3/,

https://dataspace.copernicus.eu/)

Satellite/UAV	**Spatial resolution (m)**	**Radiometric resolution (Bits)**	**Red band**	**NIR band**
			Range (nm)	**Range (nm)**
DJI Phanthom 4 Multispectral	0,0356	12	634-666	814-866
WorldView-3	0,31	11	630-690	770-895
Sentinel-2	10	12	650-680	785-899

RESULTS AND DISCUSSIONS

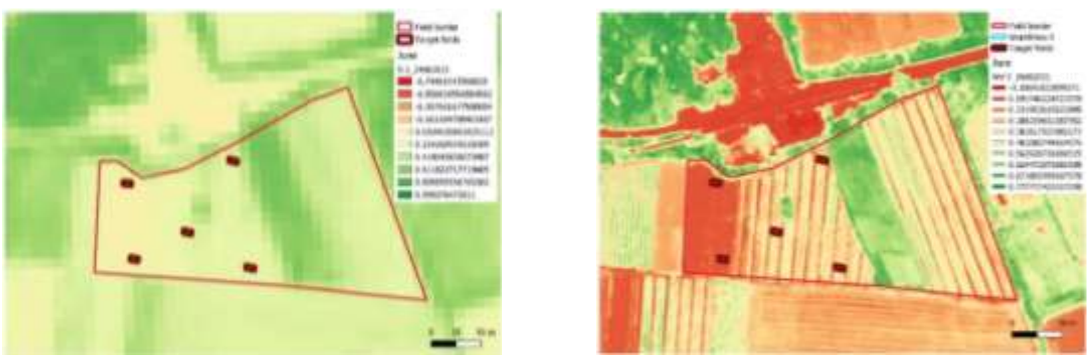
The workflow for the processing of HR and VHR multispectral satellite imagery and multispectral UAV imagery were defined in the frame of the National Research Programme in the first year of the programme. As a final product, vegetation indices were generated that were correlated with in situ observations and measurements during pepper development at different BBCH growth stages. The images used in this study with different spatial resolutions were selected for the peak stages of pepper growth and irrigation.

1. *BBCH growth stage − 59 510*

In the period of BBCH growth stage: 59 510 satellite images were acquired from Sentinel-2 and WorldView-3.

Analysis of Sentinel-2 and WorldView-3 image data shows good convergence of results (Figure 3, Figure 4). The scatter of point values is linearly correlated with very good results R^2=0,78, regardless of the differences in spatial resolution (Figure 5).

In the field observations, it was found no uniformity in the pepper crop due to different planting period. This is also very well represented in the resulting NDVI images (Figure 3).



(*a*) (*b*)

Figure 3. NDVI values: WorldView-3 *(a)* and Sentinel-2 *(b)*

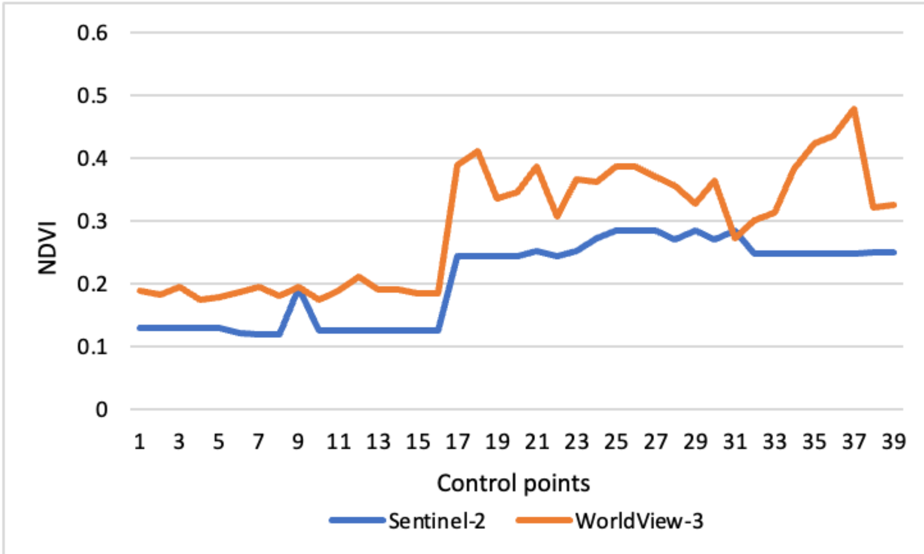


Figure 4. NDVI values for Sentinel-2 and WorldView-3

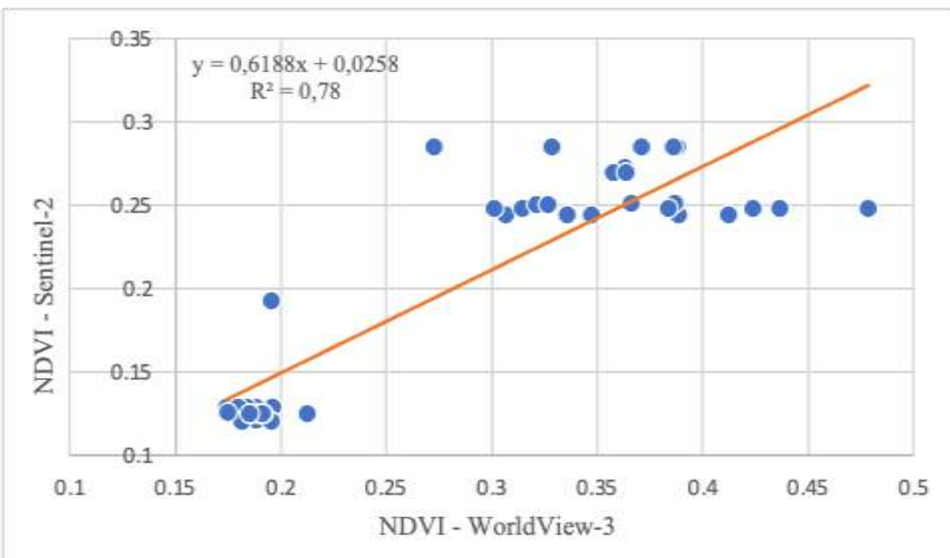
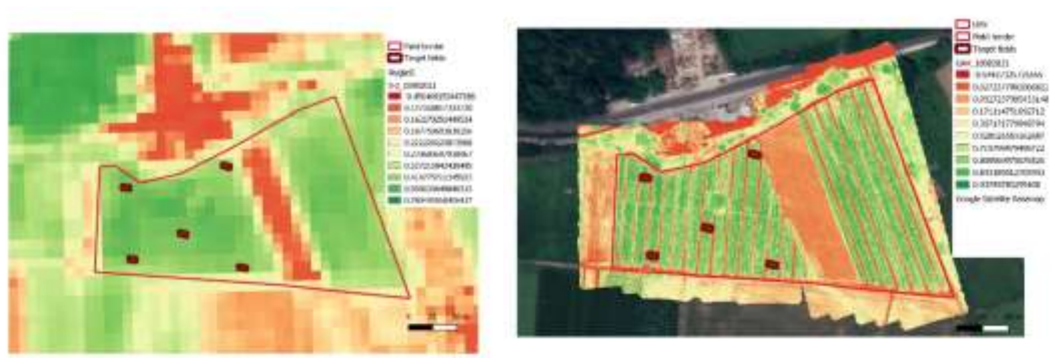


Figure 5. Scatter plot showing the NDVI values from the control points in the target fields from Sentinel-2 and WorldView-3 images

2. BBCH growth stage − 73 703

For the second observational period an UAV and a Sentinel-2 imagery were acquired. In the field verification, very good plant development and uniformity of the pepper growth stages were found. The Sentinel-2 and WorldView-3 images for the NDVI displayed homogeneous pepper plant development. The variations in the plot (Figure 7) have the same high and low values, much more clearly pronounced in the values obtained from NDVI with UAV.



(*a*) (*b*)

Figure 6. NDVI values: Sentinel-2 *(a)* and DJI Phanthom 4 Multispectral *(b)*

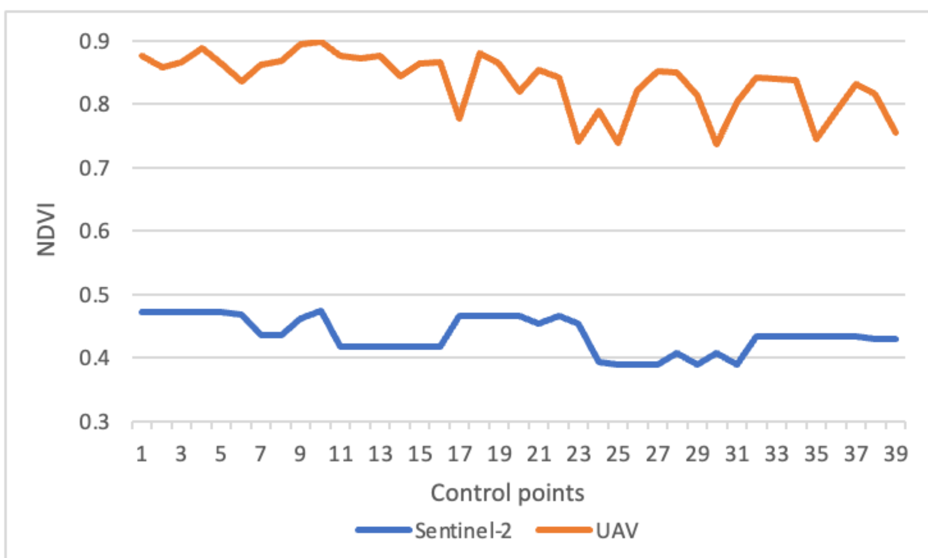


Figure 7. Values of NDVI

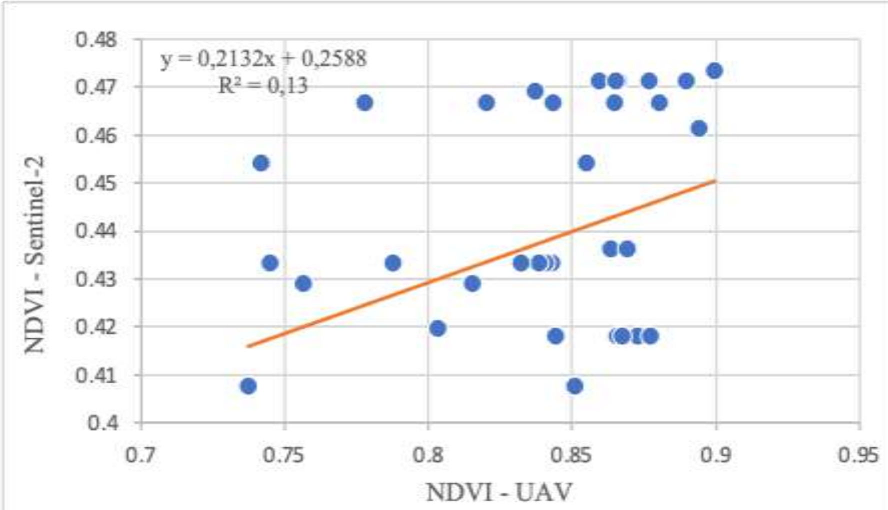


Figure 8. Scatter plot showing the NDVI values for the control points in the target fields from Sentinel-2 and UAV images

Comparison between Sentinel-2, WorldView-3 and UAV's sensors showed that NDVI varied in value from sensor to sensor. On average, WorldView-3 and UAV's NDVI values are higher than Sentinel-2. The average value of WorldView-3 NDVI was 0,291, while the corresponding Sentinel-2 NDVI was 0.206, yielding an absolute difference of 0.075, which was insignificant (Figure 5).

The average NDVI value from the UAV imagery is 0.835, while the corresponding value from Sentinel-2 is 0.436. The difference is 0.399 and is significant. The linear relationship in this case was insignificant R^2=0.13 (Figure 8).

This study compared the field-scale NDVI of three sensors with spatial resolution HR and VHR (Sentinel-2, WorldView-3 and UAV − DJI Phanthom 4 Multispectral). The imagery used in this study were near nadir acquired in clear sky conditions with the assumption that atmospheric corrections are not precisely applied.

A representative pixel approach was used in the studies, where comparisons were made on a pixel-by-pixel basis for representative target fields and control points in the study area.

The remote monitoring parameters were determined to provide essential information on crop condition. Their characteristics (description, ranges of values, their applicability, etc.) and their relationship to ground-based measurements are described.

CONCLUSIONS

The obtained results indicate good convergence and consistency between data with different spatial resolutions and this gives a perspective to prepare synchronized ground spectral measurements using satellite and UAV imaging in the respective growth stages of vegetable crops.

Sentinel-2 imaging gives good results and is a good basis for tracking the phenology of vegetable crops. However, in order to improve accuracy, it is crucial to analyse the possibilities of scaling the parameters in Copernicus - Global Land Monitoring Service (CGLMS) with higher spatial resolution data. To achieve this, the development of a machine-learning algorithm using a globally representative set of simulations from a radiative transfer model of vegetation cover is required.

A full radiometric correction (for the satellite data) and additional calibration for the UAV as well as precise orthorectification can be applied to enhance the obtained results.

Further research needs to investigate, test, and adapt the CGLMS algorithms to be verified by using algorithms developed for cereal to vegetable crops.

Additional ground-based measurements – analytical and agro-meteorological data should be collected and processed together with the data achieved from algorithms, mentioned above, used for developing a methodology for synchronized field, multispectral satellite and UAV campaigns.

ACKNOWLEDGMENTS

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EVALUATION AND STABILITY OF ECONOMIC TRAITS OF HUNGARIAN COMMON WINTER WHEAT VARIETIES IN THE REGION OF CENTRAL SOUTHERN BULGARIA

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ABSTRACT
The research was carried out during the period 2020-2021 in the experimental field and technological laboratory at IRGR "K. Malkov", town of Sadovo. Hungarian common winter wheat varieties were evaluated according to economic traits and their stability was determined. The Bulgarian variety Sadovo 1 was used as a standard. The traits grain yield (kg/da), absolute (g) and testweight (kg/hl) were studied. The results show that the highest average yield for the study period was reported for the Hungarian variety MV-Nemere. The Bulgarian varieties Sadovo 1 and Enola have the highest values for 1000 grain weight and test weight traits. A variance analysis was carried out, proving the influence of the genotype, growing conditions and their interaction on the observed traits. The stability of the studied traits was determined by the variances of stability (σi^2 and Si^2), equivalency (Wi), the criterion of phenotypic stability (Ysi), regression coefficient b_i and general adaptability. Wheat varieties MV-Kaplar, MV-Nador and Sadovo 1 can be singled out as the most valuable from a breeding point of view.

Keywords: Common winter wheat, Evaluation, Economic traits, Stability, Adaptability, Genotype x Environment interaction

INTRODUCTION

In the conditions of constantly changing climatic conditions and variety of varieties, it is necessary to test and establish varieties suitable for a specific region (Delchev and Tashkov, 2000; Georgieva et al., 2004; Yanchev and Yordanova, 2005; Samodova 2013). The construction of a correct varietal structure, depending on the specific agro-ecological conditions of the area, can significantly increase yields and the production quality (Ilieva, 2010). It is of particular importance that the newly created varieties have increased plasticity and stability, which would be a good certificate for their relative constancy in the manifestation of their genetic potential for yield and quality (Dimova et al., 2006).

In this regard, a more in-depth study of the dependence between the variety and the specific weather conditions of a given area is imperative. The yield and its stability are one of the main criteria when choosing a variety in different regions. The term "stability" is commonly used to refer to change in the phenotypic expression of a trait, while the "genotype" itself remains relatively stable (Becker and Leon, 1988). A variety (phenotype) whose yield is not significantly affected by environmental conditions is considered stable (Becker, 1981). One of the most commonly used methods for evaluating variety stability is joint regression analysis (JRA), introduced by Yates and Cochran (1938) and later modified by Finlay and Wilkinson (1963). The analysis includes the mean value of the trait, the slope of the regression line (bi) and the sum of the squared deviation (S_2di). The stability index (Stability Index=SI) according to Kang (1988) is also widely used, representing a sum of the values of grain yield and variance of stability (σ^2_i) according to Shukla (1972). Other methods are the use of the coefficient of variation (CVi), (Francis and Kannenberg, 1978) and general adaptability (GA) also proposed by Eberhart and Russell (1966). A number of authors (Flores et al., 1998; Mohammadi et al., 2016) recommend the application of a complex of stability evaluation methods because each parameter reflects a different degree of combination between yield and stability.

The purpose of the study is to evaluate important economic traits and determine their stability in Hungarian wheat varieties in the region of central southern Bulgaria. In this way, the breeding process will be supported in choosing suitable parent pairs and their further inclusion in the hybridization process.

MATERIAL AND METHOD

The research was carried out during the period 2020-2021 in the experimental field and technological laboratory at IRGR "K. Malkov", town of Sadovo. Seven common winter wheat genotypes originating from Hungary and two Bulgarian varieties of wheat were included in the study. The Sadovo 1 variety was used as a standard. The following economic traits were reported:

- grain yield, kg/da. Varietal experiments were carried out according to a block scheme in three repetitions, with the size of the experimental plot of 10 m^2, and the studied genotypes were compared with the complex standard for the country, the variety Sadovo 1.
- 1000 grains weight, g by weighing two samples of 500 grains each (BDS ISO 520:2003).
- test weight, kg/hl (BDS ISO 7971:2000), equal to the ratio between the weight (kg) and the volume (hl) of the grain, measured with a feed meter (cylinder with a capacity of 1 liter) when applying the method in accordance with the requirements.

The degree of variation of the traits was determined by a coefficient of variation based on average values for the study period. It is accepted that the variation is considered weak if the coefficient of variation is up to 10%, medium - when it is greater than 10% and less than 20%, strong - when it is over 20% (Dimova and Marinkov, 1999).

A variance analysis was performed (Lidanski, 1988), in which the power of influence of the sources of variation - genotype, environment and their interaction - was evaluated. Stability of economic traits and adaptability of common winter wheat varieties was evaluated by stability variances σi^2 and Si^2 according to Shukla (1972), equivalence Wi according to Wricke (1962), criterion of phenotypic stability (Ysi) according to Kang (1993), regression coefficient b_i after Finlay and Wilkinson (1963), general adaptability GA after Eberhart and Russell (1966). The statistical-mathematical processing of the data was carried out with the software products Microsoft Excel and Stabilitysoft.

RESULTS AND DISCUSSION

The agroclimatic conditions during the study are represented by the main meteorological factors for the growth and development of wheat: average monthly air temperature (Figure 1) and monthly sum of the amount of precipitation (Figure 2) during the reporting period.

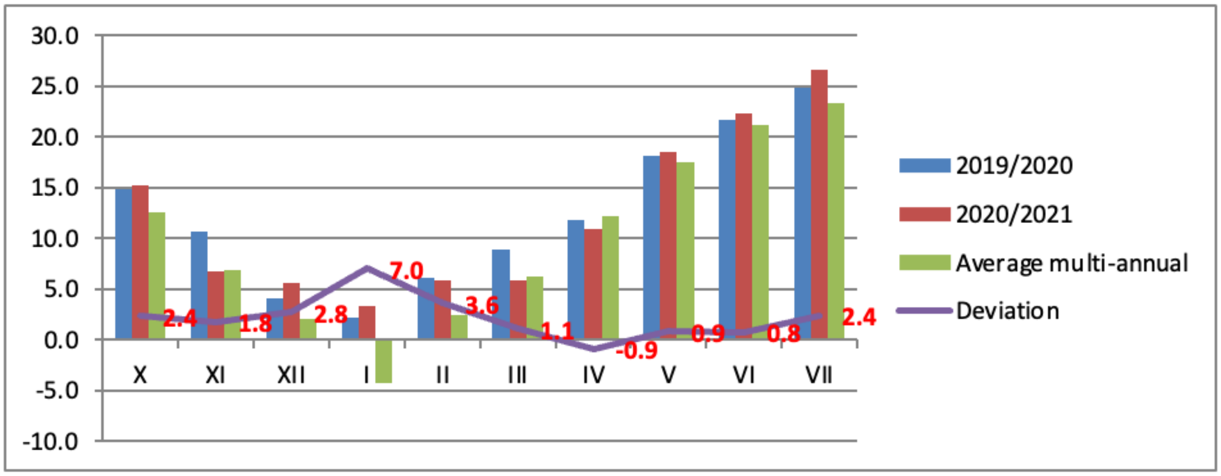

Figure 1. Average temperature (°C) of months during two vegetation years (2019/2020-2020/2021)

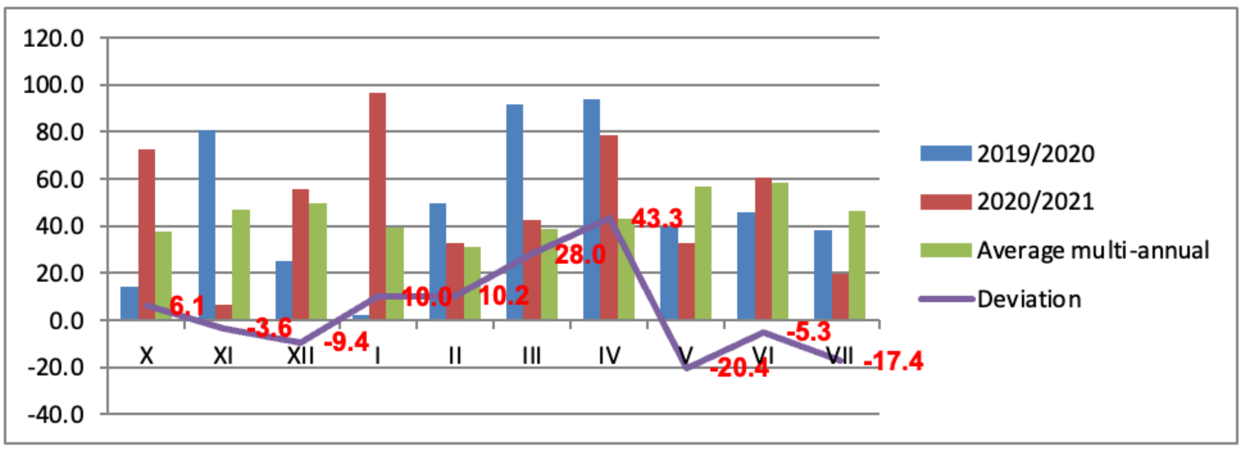

Figure 2. Sum of rainfall (mm) of months during two vegetation years (2019/2020-2020/2021)

During the 2019/2020 growing season, sowing was carried out at the optimal time - the middle of October. The lack of sufficient soil moisture before and after sowing led to a delay in emergence, with mass emergence recorded in mid-November. A snowless winter with higher than normal temperatures is also observed. Rainfall during the months of March (91.5 mm) and April (93.8 mm) helped the rapid development of the plants. The grain filling and ripening phases took place under favorable conditions and sufficient supply of moisture to the plants.

In general, the 2020 harvest year was characterized by higher temperatures and higher rainfall compared to the multi-year period. In the second growing year (2020-2021), the weather conditions differ from the previous crop year. The rains that fell in mid-October delayed sowing and it was carried out at the beginning of November. Mass emergence was reported at the end of November. During heading, flowering, milk and wax maturity, rainfall was about 24 l less than normal. As a result, a smaller and lighter grain is formed. Confirmation of this is the obtained average yields, as in 2020 they are relatively higher compared to the yields of 2021.

Table 1 presents the results of the studied varieties of common winter wheat for trait the grain yield. The obtained data show us that the highest average yield for the studied period was achieved by the MV - Nemere variety (1028.0 kg/da). In all Hungarian varieties, there is a-significant excess of the sign compared to the Sadovo 1 standard. In three varieties, the reported yield is over 900 kg/da.

Table 1. Variation analysis of the traits grain yield

№	Variety	Grain yield,kg/da			± D	Sig.	% to st.
		2020	2021	$\bar{x}$			
1	Sadovo 1-st	899.5	677.5	788.5			100
2	Enola	826.4	923.3	874.8	86.3	+++	111.0
3	MV - Nador	958.6	856.6	907.6	119.1	++	115.1
4	MV - Nemere	1127.0	928.9	1028.0	239.5	+++	130.4
5	MV - Menrot	854.7	911.9	883.3	94.8	++	112.0
6	MV - Mente	858.5	909.9	884.2	95.7	++	112.1
7	MV - Kaplar	962.6	882.5	922.5	134.1	++	117.0
Minimum		826.4	677.5	788.5			
Maximum		1127.0	928.9	1028.0			
Mean		926.7	870.1	898.4			
Std. Error		27.0					
Std. Deviation		71.3					
CV, %		7.9					
GD 5.0 %		97.00					
GD 1.0 %		130.85					
GD 0.1 %		173.98					

+ -,+ + - -,+ + + - - -, significant at GD 5.0 %, GD 1.0 % and GD 0.1 %; n.s. – unsignificant

For the 1000 grains weight, the values of the characteristic (Table 2) are in the range from 36.0 (MV – Kaplar) to 50.1 g (Sadovo 1). Six genotypes are characterized with a - significant difference compared to the standard, and in five of them the reported values are below 40 g.

Table 2. Variation analysis of the trait 1000 grains weight

№	Variety	1000 grains weight, g			± D	Sig.	% to st.
		2020	2021	$\bar{x}$			
1	Sadovo 1-st	49.6	50.6	50.1	0.0		100
2	Enola	44.8	39.6	42.2	-7.9	---	84.2
3	MV - Nador	37.1	39.2	38.2	-11.9	---	76.2
4	MV-Nemere	37.1	36.1	36.6	-13.5	---	73.0
5	MV - Menrot	36.7	39.0	37.8	-12.3	---	75.5
6	MV - Mente	38.7	42.6	40.6	-9.5	---	81.1
7	MV - Kaplar	34.9	37.2	36.0	-14.1	---	71.9

Minimum	34.9	36.1	36.0			
Maximum	49.6	50.6	50.1			
Mean	39.8	40.6	40.2			
Std. Error	0.8					
Std. Deviation	2.1					
CV, %	2.7					
GD 5.0 %	0.48					
GD 1.0 %	0.64					
GD 0.1 %	0.85					

+ -,+ + - -,+ + + - - -, significant at GD 5.0 %, GD 1.0 % and GD 0.1 %; n.s. – unsignificant

The highest test weight (Table 3) was realized by the Bulgarian varieties Enola (79.8 kg/hl) and Sadovo 1 (79.4 kg/hl). In the case of genotypes originating from Hungary, the values of the investigated trait range from 73.9 to 76.9 kg/hl, and their difference with the standard is mathematically ensured at a significance level of GD=0.1%.

Table 3. Variation analysis of the trait test weight

№	Variety	Test weight, kg/hl			± D	Sig.	% to st.
		2020	2021	$\bar{x}$			
1	Sadovo 1-st	81.8	77.0	79.4			100
2	Enola	83.0	76.5	79.8	0.35	n.s.	100.4
3	MV - Nador	78.0	74.1	76.1	-3.35	---	95.8
4	MV - Nemere	76.7	71.2	73.9	-5.49	---	93.1
5	MV - Menrot	78.2	75.2	76.7	-2.69	---	96.6
6	MV - Mente	78.1	75.8	76.9	-2.45	---	96.9
7	MV - Kaplar	78.3	73.0	75.7	-3.74	---	95.3
Minimum		76.7	71.2	73.9			
Maximum		83.0	77.0	79.8			
Mean		79.2	74.7	76.9			
Std. Error		1.8					
Std. Deviation		4.9					
CV. %		12.1					
GD 5.0 %		0.60					
GD 1.0 %		0.81					
GD 0.1 %		1.08					

+ -,+ + - -,+ + + - - -, significant at GD 5.0 %, GD 1.0 % and GD 0.1 %; n.s. – unsignificant

Regarding the coefficient of variation for the studied traits, the results show a slight variation of the 1000 grains weight (CV=2.7 %) and grain yield (CV=7.9 %). The variation in the test weight is estimated on average (CV=12.1 %).

Breeding evaluation of varieties is impossible without the quantitative evaluation of the genotype*environment interaction under a wide range of conditions. (Yan and Hunt, 2001; Gubatov et al., 2016, Golkari et al., 2016). The reason for this statement is based on the term "stability", by which the variety is evaluated, not only as a level of yield, but also as a degree of variation in environmental conditions (Yan and Hunt, 2001; Annicciarico, 2002, Ayciccek and

Yilderim, 2006). A number of authors (Tsenov et al., 2004; Atanasova et al., 2010) point out that quite often high yield stability is associated with low levels of its manifestation, and vice versa.

In our study, the results of the analysis of variance (Table 4) show that the strongest and-significant influence of the genotype factor on yield (40.0 %) and 1000 grains weight (90.3 %). The growing conditions had a primary importance on the test weight (54.5 %). The interaction of the factors genotype x environment are less represented, and their influence on the studied traits is with values of ŋ 32.1 % (yield), 8.8 % (1000 grains weight) and 5.0 % (test weight), respectively.

Table 4. Analysis of variance (ANOVA)

Traits	Source of Variation	SS	df	MS	F exp.	F tabl.	η,%	Sign.
Grain yield	year (A)	33699.6	1	33699.7	10.0	7.6	7.4	**
	genotype (B)	183104.7	6	30517.4	9.1	5.2	40.0	***
	interaction (AxB)	147235.6	6	24539.3	7.3	5.2	32.1	***
	within	94183.1	28	3363.7			20.6	
	total	458223.0	41				100	
1000 grains weight	year (A)	6.2	1	6.2	76.4	13.5	0.7	***
	genotype (B)	855.6	6	142.6	1764.8	5.2	90.3	***
	interaction (AxB)	83.4	6	13.9	172.1	5.2	8.8	***
	within	2.3	28	0.1			0.2	
	total	947.4	41				100	
Test weight	year (A)	211.9	1	211.9	1631.8	13.5	54.5	***
	genotype (B)	153.8	6	25.6	197.5	5.2	39.6	***
	interaction (AxB)	19.5	6	3.3	25.1	5.2	5.0	***
	within	3.6	28	0.1			0.9	
	total	388.9	41				100	

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular; ŋ - force of influence of the factor (%); *,**,*** - significant at α=0.05, α=0.01, α=0.001, n.s. – unsignificant

An important condition for determining the stability and adaptability of genotypes in terms of various economic traits is the presence of a - significant interaction between the studied materials and the conditions of the environment in which they are grown (Uhr, 2015; Ivanov et al., 2018). The results obtained from the analysis of variance show us that there are - significant differences, both between the studied wheat varieties and between the different climatic conditions during the years of the study. This gives us the reason to evaluate the varieties not only by economic traits, but also by their stability depending on the characteristic conditions in individual years.

To evaluate the stability of the studied wheat genotypes, the indices including the stability variances σi^2 and Si^2 according to Shukla, the equivalence Wi according to Wricke and the stability criterion YS_i according to Kang were calculated (Tables 5, 6 and 7). Varieties showing lower values of the traits σ_i^2, S_i^2 and Wi are evaluated as more stable because they interact less with the environmental conditions. The higher the values of σ_i^2, S_i^2 and W_i, the more unstable the respective genotype. The obtained results for the yield stability parameters (Table 5) show us that the Bulgarian varieties Sadovo 1 and Enola are the most unstable and with the highest values according to the stability criteria σi^2, S_i^2 and W_i. The reason for their instability is due to the large differences in grain yields in different climatic years. MV-Kaplar and MV-Nador can be distinguished among varieties with high stability. For these varieties, no great differences in yield values were observed in the individual years of the study. According to Fan et al., 2007, highly stable varieties are very productive and for this purpose it is imperative to use appropriate methods and approaches to combine high productivity with high stability.

Very good information about the value of genotypes is also provided by Kang's YS_i trait for simultaneous evaluation of economic traits and stability. In this trait, the genotypes are arranged in descending order according to their economic value. In our research, MV-Kaplar, MV-Nador and MV-Nemere are the most valuable genotypes according to this criterion. They are characterized by a high and stable yield throughout the study period.

The regression coefficient b_i provides data on the adaptability of varieties to climatic conditions. The higher the value of the coefficient, the more sensitive the variety is to environmental changes (Kosev and Kertikova, 2019). If bi is not significantly different from 1, then the genotype is adaptive to all environments, if $b_i>1$ genotypes are with higher sensitivity to changing environmental conditions and with greater specific adaptability to high-yield environments (Pour-Aboughadareh et et al., 2019). In our study, MV – Kaplar and MV – Nador are the most adaptable varieties to growing conditions.

General adaptability GA (General adaptability) refers to the traits important for the adaptability of the varieties, it represents the difference between the average yield and the regression coefficient b_i. The obtained results for this trait show us that the MV - Nemere and MV - Kaplar varieties are characterized by the highest overall adaptability, and the lowest adaptability was reported at Sadovo 1. Vulchinkov and Vulchinkova (2007) point out that the use of this method is quite limited, due to the presence of more complex parameters for evaluation.

Table 5. Stability parameters for the grain yield

№	Variety	$\bar{x}$	σ^2_i	s^2_i	W_i^2	YS_i	b_i	GA
1	Sadovo 1-st	788.5	18951.3	5836.1	73099.7	14	3.18	785.3
2	Enola	874.8	14473.1	5781.1	57106.1	12	-0.56	875.4
3	MV - Nador	907.6	1227.1	1093.3	9799.1	5	1.56	906.0
4	MV - Nemere	1028.0	7565.3	2167.4	32435.3	6	2.59	1025.4
5	MV - Menrot	883.3	5297.4	410.5	24335.8	9	-0.78	884.1
6	MV - Mente	884.2	4753.0	1436.9	22391.6	7	-0.35	884.5
7	MV - Kaplar	922.5	815.1	1067.4	8327.5	3	1.35	921.2

The calculated parameters of stability of the studied varieties at the 1000 grains weight are presented in Table 6. The results show that the standard Sadovo 1 and the Hungarian variety MV-Nador are characterized by high stability, while Enola and MV-Mente can be indicated as not stable. Among varieties combining good stability and at the same time a high value of the 1000 grains weight according to the YSi parameter, Sadovo 1 and Nador can be distinguished.

Table 6. Stability parameters for the 1000 grains weight

	Variety	$\bar{x}$	σ^2_i	s^2_i	W_i^2	YS_i
1	Sadovo 1-st	50.1	-0.38	0.09	0.70	2
2	Enola	42.2	14.44	0.11	53.60	9
3	MV - Nador	38.2	0.16	0.01	2.61	6
4	MV - Nemere	36.6	0.95	0.04	5.43	11
5	MV - Menrot	37.8	0.55	0.04	4.00	9
6	MV - Mente	40.6	3.72	0.09	15.34	9
7	MV - Kaplar	36.0	0.54	0.05	3.99	10

High stability of the trait testweight (Table 7) was reported for Sadovo 1 and MV – Kaplar. A low level of stability was shown by the MV-Mente variety. The Sadovo 1 standard stands out as a valuable genotype with good stability and high values for the monitored trait.

Table 7. Stability parameters for the test weight

№	Variety	$\bar{x}$	σ^2_i	s^2_i	W_i^2	YS_i
1	Sadovo 1-st	79.4	-0.074	0.010	0.272	3
2	Enola	79.8	1.607	0.050	6.275	7
3	MV - Nador	76.1	0.516	0.276	2.379	9
4	MV - Nemere	73.9	0.395	0.061	1.948	10
5	MV - Menrot	76.7	0.842	0.020	3.545	9
6	MV - Mente	76.9	1.816	0.033	7.023	10
7	MV - Kaplar	75.7	0.169	0.021	1.141	8

CONCLUSIONS

The highest average yield for the study period was realized by the Hungarian variety MV-Nemere. The Bulgarian varieties Sadovo 1 and Enola stand out with the highest absolute and test weight values. The variation of the investigated traits is weak for the 1000 grains weight and grain yield. Average variation was reported for the test weight. The influence of the genotype, growing conditions and their interaction on the observed traits has been significant.

The following wheat varieties can be singled out as the most valuable from a breeding point of view:

MV-Nemere – high yield
MV-Kaplar and MV-Nador – having a combination of stable and high yield
Sadovo 1 and MV-Nador – stability and high value for the1000 grains weight trait
Sadovo 1 – stability and high value for the test weight trait

The released varieties can be used in breeding programs in the process of hybridization when creating new varieties of wheat with a complex of valuable economic qualities.

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FLOUR QUALITY OF HUNGARIAN WINTER WHEAT VARIETIES GROWN IN CENTRAL SOUTHERN BULGARIA

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ABSTRACT

The research was conducted during the period 2020-2021 in the experimental field of the IPGR Sadovo and in the technological laboratories of the IPGR, University of Food Technology (UFT) and Institute of Food Preservation and Quality (IPFQ), Plovdiv. The flours of 7 varieties of common winter wheat were analyzed five Hungarian and two Bulgarian varieties. The Bulgarian variety Sadovo 1 was used as a standard in the study. Basic technological parameters of the flour were determined in order to evaluate their technological quality. The ash content trait of the flour was investigated. The granulometric composition of the flour from the investigated wheat varieties was determined by means of sieve analysis. The purpose of the research is to characterize the flour from Hungarian varieties of common winter wheat compared to the Bulgarian standard ones and their participation in hybridization schemes as donors. The distribution of the size fractions in the flour from harvest year 2020 is uneven, left drawn, monomodal. The fractions in the flour region 0-100μm are presented with the highest weight fraction. The purpose of the research is to characterize the flour from Hungarian varieties of common winter wheat compared to Bulgarian standards and guidelines for their use in various food productions to ensure food security. In the 2021 harvest, a change in the distribution of fractions is reported exclusively in the case of variety MV-Kaplar, where in the area of the finest flour fractions. The color of the obtained flours was analyzed in the color space of the CIE Lab system. In terms of lightness (L), the lightest are flours from variety MV Menrod with 84.88% and variety MV Mente with 84.69%, and the darkest is the flour from variety Enola with 71.62%. As a result of the research, the varieties with the highest technological quality stand out: Mente and Menrot.

Key words: common winter wheat, flour, ash content, grain size composition, color

INTRODUCTION

Bread wheat (*Triticum aestivum L.*) occupies first place as the main cereal crop in world trade. Wheat production and use account for 28% of the world's cereal crops. Therefore, wheat supplies approximately one-fifth of human calories in various forms. It is a key component of human nutrition and therefore increasing its production is an important requirement for food security.

Consumption is constantly increasing due to population expansion and urbanization. One of the main tasks for the improvement of common winter wheat in the new century is the use of local and international sources in breeding programs. (Hongjie et al., 2019; Li et al., 2019; Nazarenko et al., 2020).

The work for grain quality is the second key direction after breeding for productivity in grain production. The high quality of agricultural products, and therefore of food, can help ensure the well-being and health of the population and food security. (Meleshkina, 2018; Kibkalo, 2022).

A major step in the flour production process is the milling of the wheat grain. Its quality composition is of great importance. The content of proteins, gluten, ash content, the size of flour particles, as well as its color. (Aseeva T.A. et al., 2020; Chikpah et al., 2021)

Wheat flour plays an important role in the world grain market due to its high nutritional value. Flour is a fine-grained (with a particle size of about 130 - 165 μm) food product obtained from the milling of grain.

According to Campbell et al. (2007, 2012), the size of the particles obtained as a result of crushing the grain by the first pair of grinding rollers is important in flour production and the milling process and is of great importance during the fractionation and further processing of wheat.

The particle size distribution for bakery products is determined by grain properties (Yuan et al., 2003), and in particular grain hardness (Campbell et al., 2007), their shape (Fuh et al., 2014), moisture content (Fang and Campbell, 2003), as well as from their homogeneity (Satumbaga et al., 1995).

Wheat flour is the most important product of wheat milling. It is used on an industrial scale in baking and production of confectionery, pasta and food concentrates. Ash is one of the main traits of the quality and use of wheat flour (Carson et al., 2009; Cardoso et al., 2019).

Ash content is one of the most significant traits of wheat flour quality, and its content is closely related to the processing accuracy of wheat flour, which is shown as performance and taste. (Li et al., 2023). It characterizes the relations in it of endosperm and bran. The ash obtained from flour consists of mineral compounds of phosphorus, potassium, calcium, magnesium, iron, zinc and copper. Phosphorus (approximately 45%), potassium (approximately 38%), magnesium and calcium (approximately 13% and 3% respectively) are the main elements present in the ash, while the remaining elements amount to only 1% (Kulkarni et al., 2006; Piironen et al., 2009).

Its content in wheat and flour is important for grinding. Millers need to know the total mineral content of the wheat in order to achieve the desired or specified ash levels in the flour. Since ash is mostly concentrated in the bran, its content in the grain is an indication of the yield that can be expected during milling. It also indicates milling efficiency by indirectly revealing the amount of bran contamination. Traditional methods for detecting the ash content of wheat flour mainly rely on physical methods and chemical methods, such as weighing the ash after combustion and configuring reagents to measure sediments. Therefore, these methods are accurate, specific, and sensitive, but most of them are technically challenging, time-consuming, labor-intensive, and unsuitable for rapid screening of a large number of samples (Zhang et al., 2022; Li, et al, 2023). Ash in flour can affect color, giving finished products a darker color. Some types of products require a white color and need a low ash content, while others, such as whole grain flours, have a high ash content (Trajković et al., 1983; Keran et al., 2009).

The color of wheat (*Triticum aestivum L*.) flour is an important characteristic that strongly influences consumer acceptance of the flour itself and also of its final products. (Parker and Langridge, 2000). Flour color behaves as a typical quantitative trait and the efficiency of selection for this trait is relatively low in conventional breeding methods. The direct analysis of flour is based on the CIE colorimetric space (McCaig, 2002; Posner, 2009); color is classified in three dimensions: L *, which measures brightness (0 = black and 100 = bright), a *, where a positive a* indicates redness, and a negative a* indicates greenness, and b*, where a positive b* indicates yellowness, and negative b* indicates blue (CIE Commission Internationale de l'Eclairage, 2004).

CIE flour color is mainly determined by a combination of brightness and yellowness: brightness is influenced by bran content, while yellowness is influenced by endosperm carotenoid content (Hidalgo and Brandolini, 2008a; Oliver et al., 1992).

When creating new varieties of common winter wheat, an important stage is the analysis of the grain and the resulting flour. The optimal combination of protein content, the quantity and quality of wet gluten, ash, color, fineness of grinding, knowledge of the granulometric composition of flour particles of different shapes and sizes give us a complete picture of the quality of the grain and the flour obtained from it.

The purpose of the research is to characterize the flour from Hungarian varieties of common winter wheat compared to the Bulgarian standard ones and their participation in hybridization schemes as donors.

MATERIALS AND METHODS

The research was carried out in the period 2020-2021 in the experimental field of IRGR Sadovo and in the technological laboratories of IPGR, University of Food Technology (UFT) and Institute of Food Preservation and Quality (IPFQ), Plovdiv. The flours of 7 varieties of winter common wheat were analyzed - five Hungarian and two Bulgarian varieties. The Bulgarian variety Sadovo 1 was used as a standard in the study. Flours obtained by grinding grain (on a roller mill) from the studied varieties to a flour yield of 65%. Basic technological parameters of the flour were determined in order to evaluate their technological quality.

- By sieve analysis of flour, the size of the flour particles was determined - according to BDS 754-80/4:2003. It is carried out with planzichters, where the sieves are arranged in a certain way. According to the class of intermediate products, the sieves of the planzichters are grouped as follows:

5.2.2.3.1. Receiving sieves - for separating the mealy overburden

5.2.2.3.2. Semolina sieves – for separating large semolina

5.2.2.3.3. Flour sieves - for separating sifted flour

5.2.2.3.4. Dunst sieves - for separation of dunt sifting and fine semolina.

- ash content of the flour - It is determined according to the standard: BSS EN ISO 2171:2010 "Cereal crops, leguminous crops and their by-products. Determination of ash produced by incineration (ISO 2171:2007)'. This standard specifies the method for determining the ash obtained by incineration of cereals and legumes and their milled products intended for human consumption. The color of the flour is determined by the color space of the CIELab system (Figure 1).

-The color of the flour is determined by means of the color space of the CIELab system.

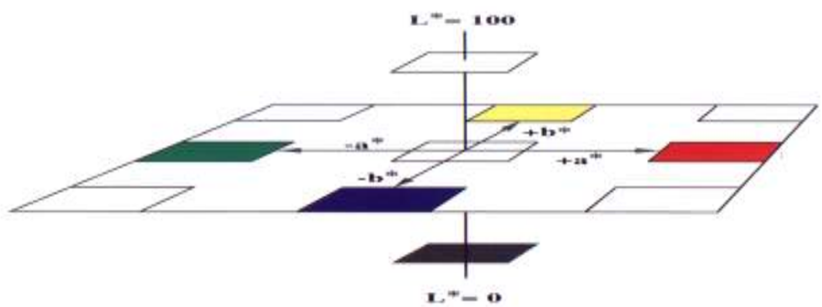

Figure 1. The color space of the CIELab system

The structure of this space is based on the work on the organization of the visual perception system of three oppositions: black - white (achromatic axis); Red green; yellow - blue; The center of this space is the achromatic axis. Calculated for any standard light source. Along the + a* - a* axis, red is in opposition to green. Along the +b* – b* axis, yellow is in opposition to blue, then the LAB system allows the use of cylindrical spatial coordinates TSL with coordinates L*, C*, H*. Where H* is the color tone (hue), C* is the saturation level (chroma) and the lightness value (luma) remains constant. H* = at a ≠ 0. (Read more at: https://minikar.ru/bg/testy/teoriya-cveta---osnovnye-harakteristiki-cveta-chto-takoe-cvet/)

RESULTS AND DISCUSSION

The distribution of the size fractions in the flour from harvest 2020 (Figure 2) is uneven, left drawn, bimodal.

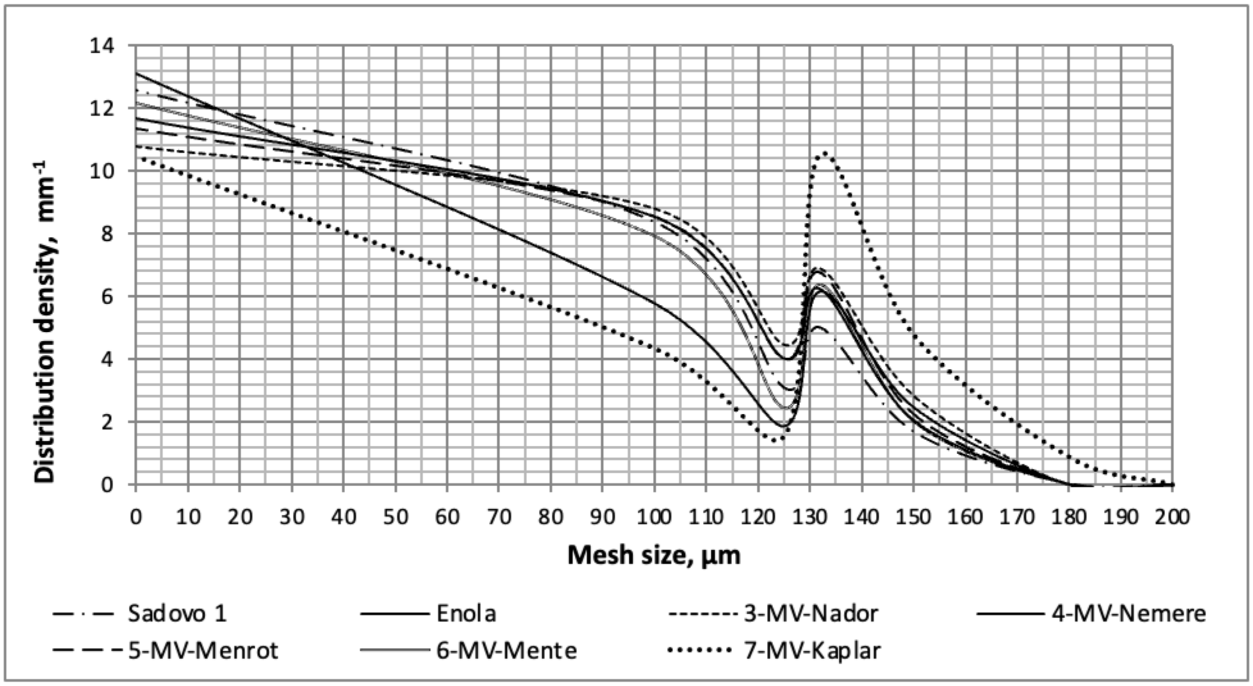

Figure 2. Distribution density of wheat flours from 2020

Two peaks of values stand out in it, which is characteristic of distributions of flours obtained during conventional milling of grain raw materials. In the first peak with the highest mass fraction, the fractions in the area of fine flours with the size of the flour particles 0-100 µm are presented. The second peak is reported in the region 132-150 µm (Figure 3), which confirms that the investigated flours are relatively fine in terms of flour particle size.

The presence of dusts with particle sizes in the range (150-200µm) was reported for all varieties, with their mass fraction ranging between 5.1% (Sadovo 1) and 16.9% (MV-Kaplar). It is characteristic that in this range the distribution curve of variety MV-Kaplar differs in values from the others, with this variety there is a greater share of larger particles, which also affects the values of the average equivalent diameter of the flour particles 95µm, while for the other varieties it varies between 79µm (Sadovo1 and MV-Nemere) and 85µm (MV-Menrot).

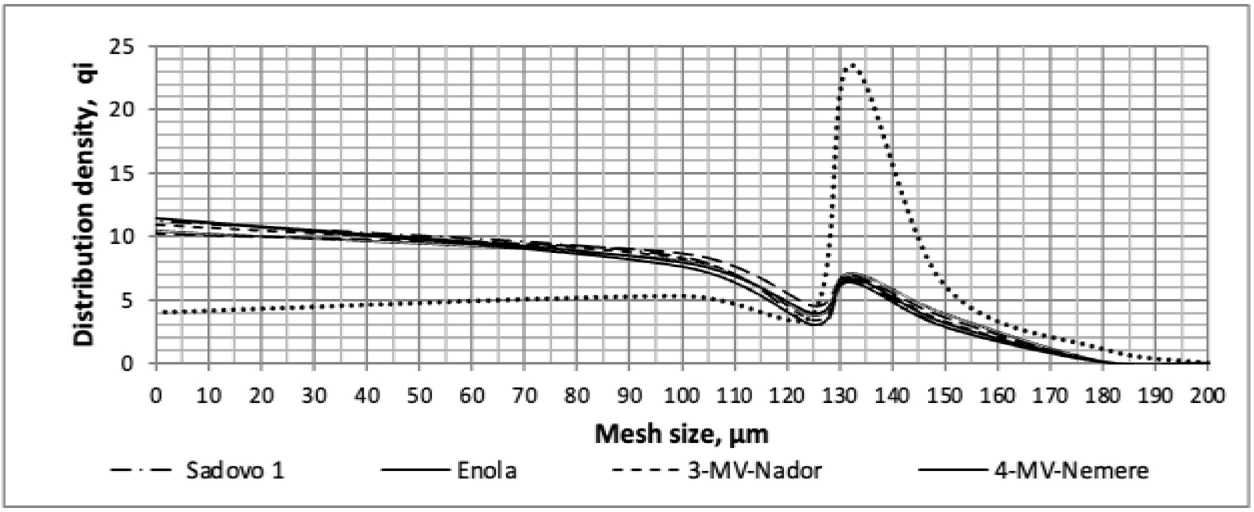

Figure 3. Distribution density of wheat flours from 2021

By comparing the 2020 and 2021 harvests, in general, approximately the same trend and close values are reported in the distribution curves of the size fractions. The most significant is the change in the distribution of fractions in variety MV-Kaplar, where in the area of fine flour fractions, the density of distribution and their quantity is approximately twice lower than that of the other varieties, while in the adjacent area (132-150µm) their amount is about twice as high as the values of the other varieties. Due to these features, the average equivalent diameter of the particles in this variety is the highest 125µm, and in the others it varies between 85µm (Enola) and 91µm (MV-Menrot). These changes indicate a distinct difference in the grain structure of variety MV-Kaplar and its milling behavior, resulting in a flour with larger particles.

The ash content of the flours obtained from the varieties in both harvest years (Figure 4) is in a relatively wide range between 0.52% (MV-Mente) and 0.77% (MV-Nemere). When comparing the obtained average values of ash content for the year 2021, they are lower than those for the year 2020 (with average values of 0.62% and 0.58%, respectively). According to the ash content, the flours obtained from these two varieties can be assigned to type "700" and all others to type "500".

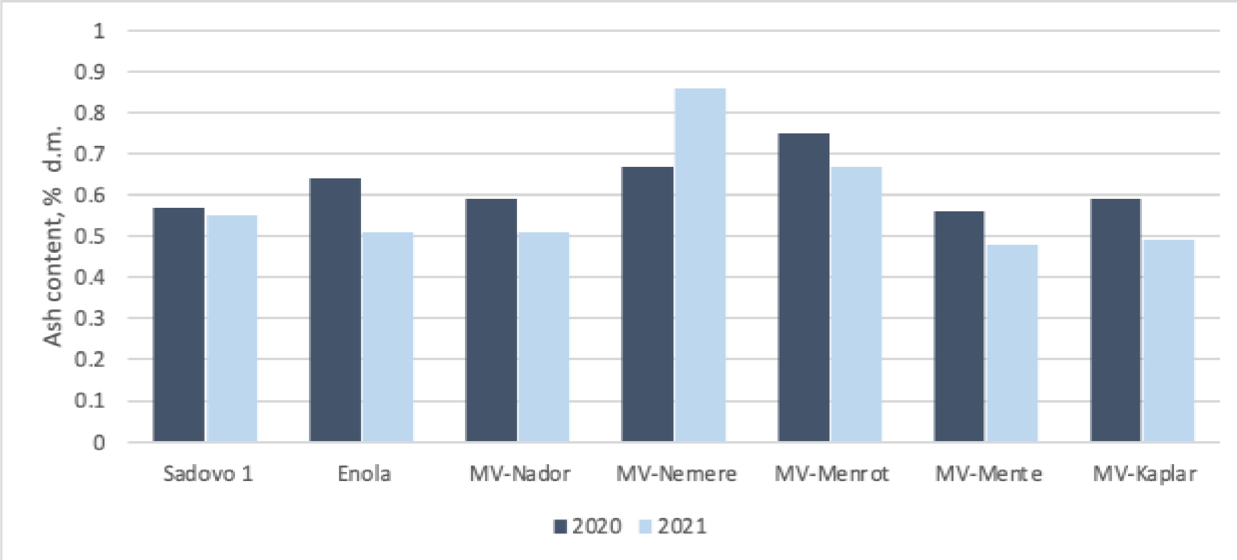

Figure 4. Ash content of wheat flours from harvest years 2020 and 2021

Color is a quality trait that affects the color of the bread of the bread medium (the middle). It is determined by the different reflectivity of the particles and to some extent by the coarseness of grinding. Darker is the flour that has an increased content of bran. In addition, the flour may acquire an uncharacteristic color due to the content of impurities or undesirable changes that occurred during its storage.

Color saturation (C*) of flour is a measure of color purity (deMan, 1990). With the highest color saturation was the flour of MV-Nador and MV-Menrot variety in both years of study. With values close to the control samples of the Sadovo 1 and Enola varieties from the harvest in 2020, there are flours from the MV-Mente and MV-Kaplar varieties, and the flour from the varieties grown in 2021 - MV-Nemere and MV-Kaplar. The color of MV-Menrot variety flour was the most intense compared to the other flours of the different varieties in both years of studies (Figure 5 and Figure 6).

Hue angle (h°) of the flour samples was observed to decrease in the second year of the study ranging from 79.96° to 84.17° (Figure 6) while for this trait in 2020 the values ranged from 84.01° to 86.73°(Figure 5) A hue angle shift from 0° to 90° means a color change from red to yellow, while a change from 90° to 180° means a color change from yellow to green (Francis and Clydesdale, 1975).

In both years of the studies, flours obtained from Hungarian wheat varieties had higher L* values. High values of L* indicate bright color and higher values of b* indicate more yellow. Flour color is affected by the color of the wheat endosperm, particle size, and the ash and protein content of the flour, which often affects the color of the final product (Figure 5 and Figure 6).

The obtained experimental results for flour on the traits a* and b*, reflecting the participation of the red and yellow components in the coloring, show that flour of the MV-Nador variety has the most yellow pigments. The increase in the L* value of the flour may be due to increased protein and ash content, which may reduce their dulling effect on the overall brightness and color saturation of the flour.

An important quality attribute of flour is its color, which affects the appearance and consumer acceptability of products produced from it (Wrolstad and Smith 2010). The increased index of L* corresponds to the increasing values of saturation (C*) for the flours of the new varieties. The hue angle values place the flour in the yellow region of the CIE L* C* H* color space, but as indicated by the lightness and chrominance index, this yellowness is very weak (mild and less intense).

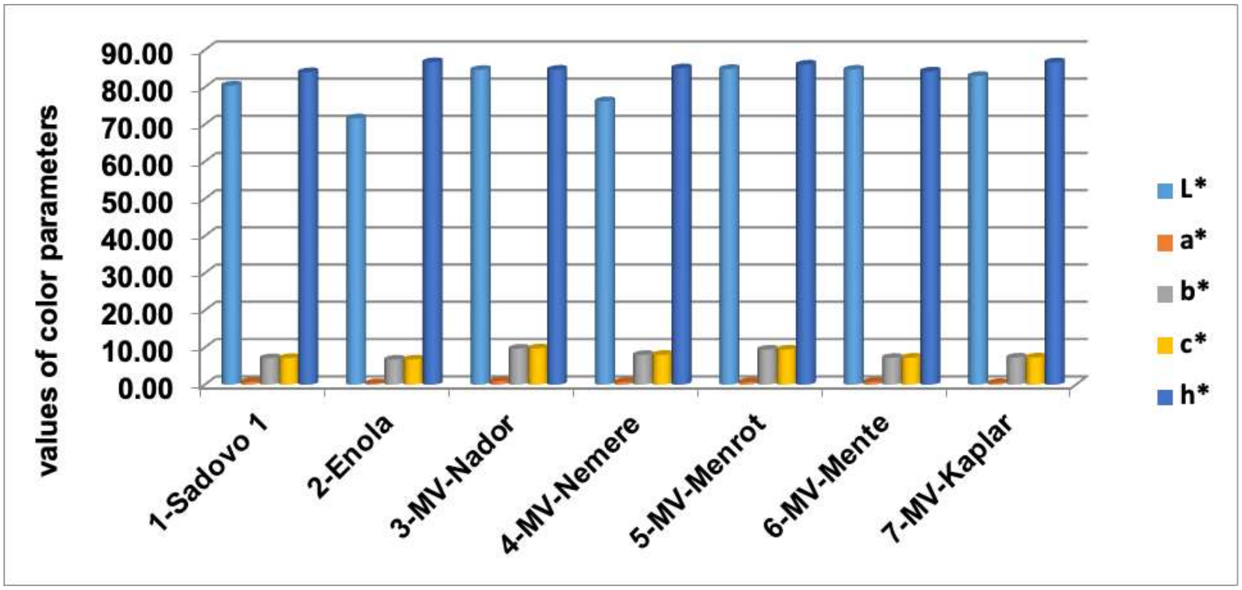

Figure 5. Values of trait color in harvest year 2020

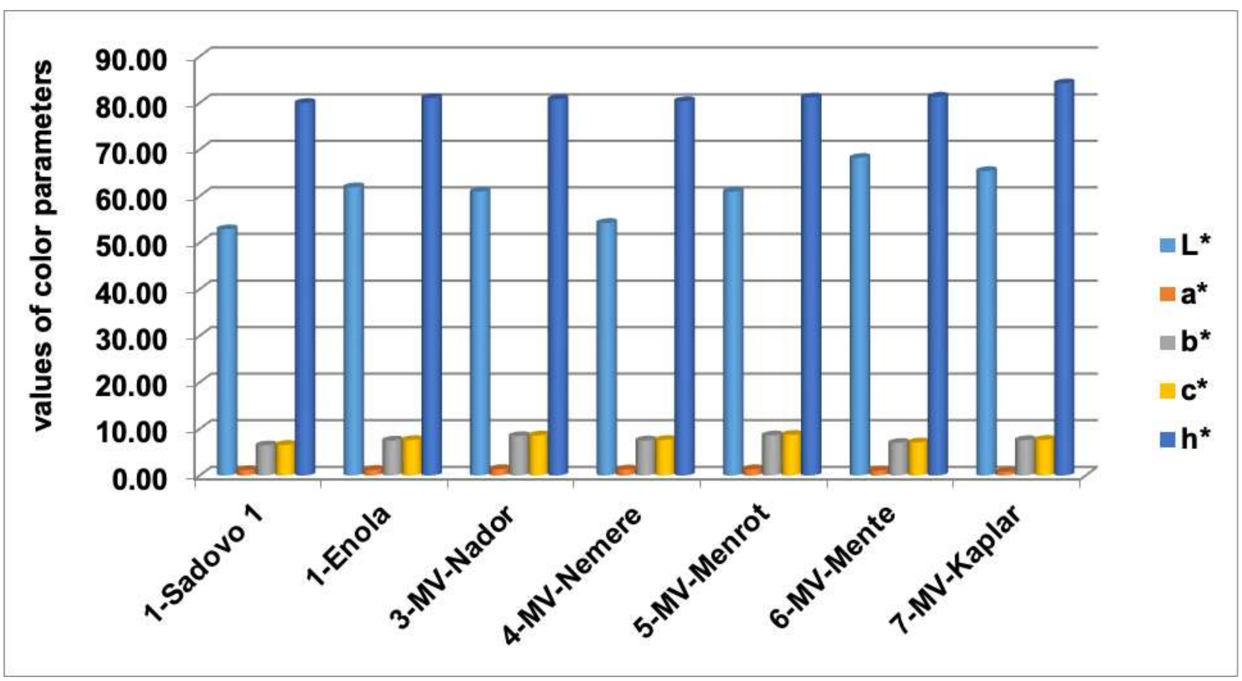

Figure 6. Values of trait color in harvest year 2021

CONCLUSIONS

By comparing the harvests of 2020 and 2021, the most significant change in the distribution of fractions in the MV-Kaplar variety, where in the area of fine flour fractions, the density of distribution and their quantity is approximately twice lower than that of the other varieties. In the neighboring area (132-150μm), their amount is about twice as high as the values of the other varieties.

The ash content in the flours obtained from the varietys in both harvest years was in a relatively wide range between 0.52% (MV-Mente) and 0.77% (MV-Nemere), characterized by a coefficient of variation of 15.9%. According to the ash content, the flours obtained from these two varieties can be assigned to type "700" and all others to type "500".

After the analysis, it was found that the Enola, MV-Nemere and MV-Menrot varieties have proven differences compared to the standard.

The color of MV-Menrot variety flour was the most intense compared to the other flours of the different varieties in both years of studies.

The obtained experimental results for flour on the traits a* and b*, reflecting the participation of the red and yellow components in the coloring, show that flour of the MV-Nador variety has the most yellow pigments. The increase in the L* value of the flour may be due to increased protein and ash content, which may reduce their dulling effect on the overall brightness and color saturation of the flour.

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ASSESSMENT OF WATER STRESS IN STEVIA USING HYPERSPECTRAL DATA BEFORE AND AFTER IRRIGATION

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ABSTRACT

This study aimed to determine the changes in reflectance in stevia at different irrigation levels using hyperspectral measurements. For this purpose, hyperspectral measurements were taken consecutively for 15 days from stevia at six different irrigation levels. The collected data were classified before and after irrigation and statistical analyses were performed. This study was conducted in the research field of Akdeniz University using a randomized block design. Measurements before or after irrigation did not create significant differences in reflectance in the visible wavelength range of the electromagnetic spectrum, but after irrigation, measurements showed higher values in the NIR, R900, and R970 wavelengths. Significant increases in reflectance were observed in the visible wavelength range owing to the increased water stress, whereas no significant differences were observed in the NIR, R900, and R970 wavelengths. The WI/NDVI ratio among the vegetation indices increased depending on water stress, whereas the VI, NDVI, DVI, and WI indices decreased. The results indicated that the DVI and WI indices could be used to determine irrigation scheduling compared to other vegetation indices. Furthermore, the NIR wavelength range, R900, and R970 wavelengths were more effective in determining the water stress in stevia than in the visible wavelength region. Overall, the findings suggest that hyperspectral measurements can be useful in assessing the effects of irrigation levels on stevia and in selecting appropriate vegetation indices to evaluate water stress conditions in these plants.

Keywords: Deficit irrigation, NIR, vegetation indices, visible wavelength

INTRODUCTION

The dynamic nature of relationships on Earth, especially in natural environments, has necessitated the continuous observation of these relationships and the monitoring of changes in short periods of time. To conduct such studies, acquiring new information and data about earth is essential. Today, numerous technologies have been developed to facilitate the timely and accurate access to information about Earth's resources. One of these technologies is Remote Sensing Science and Technology. As defined by many scientists, remote sensing can be described in its most general form as the science of obtaining information about objects by measurements taken from a distance without any physical contact with the objects being observed (Campbell, 1987; Richards and Jia, 1999; Jensen, 2009). One branch of remote sensing technology is ground-based measurement techniques, also called spectroradiometric measurements. Spectroradiometric methods are based on the measurement of reflectance, radiance or irradiance values of energy reflected from any object. The sun or artificial radiation can be used as the radiation source (Başayiğit et al., 2008). Spectroradiometric measurements can be used to determine plant water stress conditions (Ihuoma and Madramootoo, 2019; Sönmez et al., 2015; Zhang and Zhou, 2019). Research aimed at assessing the impact of water stress on plants, particularly through spectroradiometric measurements, holds significant importance in ensuring the efficient utilization of water resources, an absolute necessity for sustainable agricultural production. In this context, hyperspectral measurements are a very effective method for determining plant water stress due to their advantages such as high spatial and spectral resolution, fast turnaround time and low cost (Ihuoma and Madramootoo, 2017).

The aim of this study was to investigate the effect of different irrigation levels on the reflectance rates of stevia plant, one of the important sources of natural sweeteners.

MATERIAL AND METHOD

This study was carried out in the research and application area of the Akdeniz University Faculty of Agriculture. The texture of the experiment soil is clay loam, the field capacity is 26.58%, the wilting point is 17.44%, the bulk density is 1.356 gr/cm^3, EC_e 0.584 dS/m, and pH 8.13.

Stevia plant, which is used as herbal material, is a perennial plant belonging to the Asteraceae family. This plant is one of the most important sources of natural sweeteners without calories. The fact that it has an accumulation of glycosides, which is about 300 times sweeter than sugar cane, makes the plant very important (Cariño-Cortés et al., 2007). The natural habitat of the plant is subtropical areas in the northeast of Paraguay. It shows the best development in areas, with an annual average temperature of 31°C and a rainfall of 1400 mm. The most suitable harvest time is the beginning of flowering, and this period has the highest sweetener level in the leaves.

In the study, six different irrigation levels (I120, I100, I80, I60, I40, and I20) were examined with three replication. I100 represents full irrigation. However, I80, I60, I40, and I20 correspond to 20%, 40%, 60%, and 80% reductions in irrigation compared to full irrigation (I100), respectively. Furthermore, I120 indicates a 20% higher water application compared to full irrigation.Irrigation was applied to all subjects when 45-55% of the available water content was consumed in the 0-30 cm soil profile was consumed in the plots belonging to the control subject (I100) throughout the experiment. The irrigation time and the amount of irrigation water to be applied were decided by measuring the amount of evaporation from the Class A Pan and considering the pan coefficients. Before irrigation, soil samples were taken from the control subjects, and their soil water content was determined by the gravimetric method. These values were used to calculate the required irrigation depth (d, in mm) for the control subjects according to equation (3.1). This depth (mm) was then multiplied by the irrigated area (6.86 m²), and the amount of irrigation water was then converted to liters.

$$d = \frac{(P_{vtk} - P_{vm})}{10} \times D \tag{3.1}$$

Where d is the net irrigation water (mm), P_{vtk} is the field capacity of the soil (%), P_{vm} is the water content in the soil (%), D is the soil depth (mm).

ASD Hand-Held spectroradiometer and plant probe were used to measure the reflectance from the plant. The device measures in the wavelength range of 325-1075 nm. In order to reveal the water stress effect more clearly, blue (450-500 nm), green (501-570 nm), red (610-700 nm) and NIR (701-1075 nm) wavelengths were analyzed separately. In addition, reflections at R900 nm and R970 nm, which are sensitive to the leaf water content were also evaluated. In addition, we calculated vegetation indices using these reflectance values These calculated indices are given in Table 1.

The ANOVA test with SPSS Statistics Base v23 (SPSS Inc., Chicago, IL, USA) was used to determine the interactions between the factors considered (irrigation status and irrigation regimes). The LSD test was then used to evaluate pairwise mean differences at a significance level of $p<0.05$.

Table 1. Formulation of vegetation index

Indices	Formulation		Reference
Bant oranlama indeksi (VI)	$VI = \frac{NIR}{R}$	(3.2)	Aparicio vd. 2004
Normalize edilmiş bitki indeksi (NDVI)	$NDVI = \frac{NIR - R}{NIR + R}$	(3.3)	Penuelas vd. 1997
Bitki ayrım indeksi (DVI)	$DVI = NIR - R$	(3.4)	Teilet, vd. 1997
Su indeksi (WI)	$WI = \frac{R900}{R970}$	(3.5)	Penuelas vd. 1997

RESULTS AND DISCUSSION

The results of the variance analysis regarding the reflectance values of the electromagnetic spectrum at visible, NIR, 900, and 970 nm wavelengths are given in Table 2. According to these findings, the differences in reflectance in the blue, green, and red wavelength regions of the electromagnetic spectrum before and after irrigation (I) were not statistically significant. However, after irrigation, there was an increase of 2.2%, 3.37%, and 2.92% in reflectance at the NIR, R900, and R970 wavelengths, respectively. Penuelas et al. (1997) stated that the reflectance trough at 900-970 nm in the NIR region corresponds to the water absorption band, but 970 nm trough disappears when the plants are exposed to water stress, that is, the reflectance increases in these wavelength regions. Although no difference was determined in the study at 900 and 970 nm wavelengths according to the irrigation levels, the changes in reflectance before and after irrigation support this statement.

Different irrigation levels (IL) caused an increase in reflectance in the visible wavelength region of the electromagnetic spectrum. In the blue and red wavelengths, the highest reflectance (0.082, 0.113) was determined at the I20 irrigation level. The reflectance at blue wavelengths increased by 10%, 8.6%, and 17.1% in treatments I60, I40, and I20, respectively, compared to the control (I100). This increase was 20.7%, 20.7%, and 29.9% for the I60, I40, and I20 tretments at red wavelengths, respectively. In the green wavelength, the highest reflectance was found in the I60 treatment. However, no statistically significant differences were found between the I40 and I20 applications when compared to the I60 treatment. At this wavelength, 19.8%, 15.9%, and 18.3% more reflectance occurred in the I60, I40, and I20 treatments, respectively, compared to I100.

Due to the strong absorption of energy by chlorophyll and other auxiliary pigments in plant leaves within the 400-700 nm wavelength range of the electromagnetic spectrum, the level of reflectance in this wavelength range is quite low (Allen et al., 1969; Carter, 1991; Grace and Gates, 1982; Knipling, 1970; Tucker and Garratt, 1977; Woolley, 1971). This decrease in reflectance is particularly evident in the blue and red wavelength regions of the electromagnetic spectrum. As a result of the decrease in chlorophyll pigments under stress conditions, the amount of energy absorbed in these wavelength regions decreases, leading to an increase in reflectance in the visible region (Aqeel et al., 2011; Blackburn, 2007; Tilling et al., 2007).

Table 2. The effect of irrigation status and irrigation levels practices on plant reflectance

Treatments		BLUE	GREEN	RED	NIR	R900	R970
Before irrigation		0.074	0.139	0.099	0.815 b	0.861 b	0.823 b
After irrigation		0.075	0.140	0.099	0.833 a	0.890 a	0.847 a
$LSD_{irrigation\ (I)}$		ns	ns	ns	**0.012	***0.011	***0.011
I120		0.071 c	0.131 bc	0.089 c	0.829	0.883	0.840
I100		0.070 c	0.126 c	0.087 c	0.827	0.881	0.843
I80		0.071 c	0.135 b	0.094 c	0.821	0.874	0.833
I60		0.077 b	0.151 a	0.105 b	0.831	0.882	0.840
I40		0.076 b	0.146 a	0.105 b	0.815	0.864	0.826
I20		0.082 a	0.149 a	0.113 a	0.814	0.862	0.829
$LSD_{Irrigation\ level\ (IL)}$		0.005***	0.08***	0.007***	ns	ns	ns
Before irrigation	I120	0.070	0.128	0.089	0.818	0.867	0.824
	I100	0.071	0.126	0.089	0.818	0.867	0.830
	I80	0.071	0.133	0.093	0.811	0.859	0.820
	I60	0.075	0.148	0.102	0.822	0.869	0.829
	I40	0.077	0.146	0.107	0.808	0.851	0.816
	I20	0.082	0.153	0.114	0.811	0.853	0.821
After irrigation	I120	0.071	0.133	0.090	0.844	0.903	0.857
	I100	0.069	0.126	0.086	0.838	0.897	0.856
	I80	0.071	0.137	0.094	0.833	0.891	0.846
	I60	0.079	0.155	0.108	0.842	0.898	0.852
	I40	0.076	0.145	0.104	0.824	0.880	0.837
	I20	0.083	0.145	0.111	0.818	0.874	0.837
I x IL		ns	ns	ns	ns	ns	ns

***, ** and ns: significant at 0.1%, 1% level, and non significant, respectively

Considering the interaction between I and IL in the experiment, there was no statistically significant difference in the reflectance.

The calculated vegetation indices, considering reflectance in the near-infrared and red regions have a statistically significant correlation with many plant data (Huete, 1988). In the study, we also investigated the changes in vegetation indices I, IL, and their interaction, I×IL. The results of the statistical analysis of this information are in Table 3.

The differences in the VI, NDVI, and WI/NDVI ratios calculated for the stevia plants before and after irrigation were not statistically significant, whereas the DVI and WI indices were statistically different at the 1% and 0.1% significance levels, respectively. After irrigation, DWI and WI increased 2.5% and 0.5%, respectively.

Irrigation levels resulted in statistically significant differences of 0.01% in VI, NDVI, WI and WI/NDVI, as well as significant differences of 1% in DVI, unlike the data obtained before and after irrigation. In general, all vegetation indices decreased with the increase in stress level. According to the results, the highest VI, NDVI and DVI values at different irrigation levels was in the control treatment, but the I120 treatment was also not statistically different from the I100. However, as the stress level increased, the VI value decreased by 7%, 16%, 18%, and 24% in I80, I60, I40, and I20, respectively, compared to the I100. Similar to the VI, all deficit irrigation applications in the NDVI showed statistically significant differences from the control. Considering the I100 treatment, NDVI values were decreased by 1.7%, 4.1%, 4.7%, and 6.4% in I80, I60, I40, and I20 irrigation levels, respectively. In the DVI index, this decrease was determined as 4% and 5.3% in the I40 and I20 treatments, respectively, compared to I100. Unlike other indices, the highest value in WI was determined in the I120 treatment (1.053), and I80 (1.051), I60 (1.051) irrigation levels were not statistically different from this treatment.

Table 3. The effect of irrigation status and irrigation levels practices on vegetation index

Treatments		VI	NDVI	DVI	WI	WI/NDVI
Before irrigation		8.364	0.784	0.716 b	1.046 b	1.336
After irrigation		8.531	0.788	0.734 a	1.051 a	1.335
$LSD_{irrigation\ (I)}$		ns	ns	0.013**	0.003***	ns
I120		9.305 ab	0.806 ab	0.741 a	1.053 a	1.307 cd
I100		9.517 a	0.809 a	0.741 a	1.047 b	1.294 d
I80		8.809 b	0.795 b	0.728 ab	1.051 ab	1.321 c
I60		7.983 c	0.776 c	0.727 ab	1.051 ab	1.355 b
I40		7.797 cd	0.771 c	0.711 bc	1.047 b	1.358 ab
I20		7.274 d	0.757 d	0.702 c	1.042 c	1.377 a
$LSD_{Irrigation\ level\ (IL)}$		0.551***	0.013***	0.022**	***0.005	***0.022
Before irrigation	I120	9.256	0.805	0.729	1.051	1.307
	I100	9.277	0.804	0.730	1.045	1.299
	I80	8.756	0.794	0.718	1.048	1.320
	I60	8.134	0.780	0.720	1.048	1.346
	I40	7.628	0.766	0.701	1.043	1.361
	I20	7.136	0.753	0.697	1.039	1.381
After irrigation	I120	9.353	0.807	0.753	1.054	1.307
	I100	9.757	0.814	0.752	1.049	1.288
	I80	8.862	0.797	0.739	1.054	1.322
	I60	7.833	0.773	0.734	1.054	1.364
	I40	7.967	0.776	0.720	1.051	1.354
	I20	7.413	0.761	0.707	1.044	1.373
I x IL		ns	ns	ns	ns	ns

***, ** and ns: significant at 0.1%, 1% level, and non significant, respectively

WI/NDVI values, one of the ratios evaluated in water stress studies, increased with increasing water stress. This increase relative to the control treatment was 2.1%, 4.7%, 4.9%, and 6.4% in I80, I60, I40, and I20, respectively.

Penuelas et al. (1997) and Piñol et al. (1998) also reported that WI is highly effective in determining the water status of plants. Many researchers (Çamoğlu, 2010; Danson et al., 1992; Köksal, 2006; Thomas et al., 1971). Generally reported that spectral indices are effective in determining water stress, but they are not sensitive enough to small changes in water stress and therefore they are insufficient to clearly determine the irrigation time.

CONCLUSION

This study was realized to investigate the use of spectroradiometer to determine the water stress status of stevia plants. Measurements were made on the stevia plant for 15 consecutive days, changes before and after irrigation and the effects of different irrigation levels were evaluated. According to the research results, the differences in NIR, R900 and R970 nm wavelengths were significant, especially in the measurements taken before and after irrigation. However, we determined that before and after irrigation measurements caused an increase in reflections in the visible wavelength region of the electromagnetic spectrum.

Vegetation indices formulated using reflectance values from the NIR and red wavelengths were effective in determining water stress in stevia plants. However, between the vegetation indices calculated based on measurements taken before and after irrigation, DVI and WI showed distinctive characteristics compared to other indices.

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SCREENING of SOME CHEMICAL DISINFECTANTS for EXPLANT STERILIZATION during *IN VITRO* MICROPROPAGATION of UCB- 1 (*P. atlantica* x *P. integerrima*)

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ABSTRACT
UCB-1 rootstock is an important clonally propagated *Pistacia* rootstock. The rootstock is stronger than other *Pistacia* rootstocks and high yield is obtained by using this rootstock. Although generative or various vegetative propagation methods are used in the propagation of *Pistacia* rootstocks, with the use of plant tissue culture methods, which is one of the vegetative propagation methods, area can be saved by producing clean plant material free from diseases and pests, high reproduction coefficient, production without depending on the vegetation period and producing thousands of plants in a small area. With the use of plant tissue culture methods, it is possible to reproduce plant species that are difficult to reproduce with appropriate media and sterilization. Sterilization is one of the important stages that affect the success of plant tissue culture method. With sterilization in plant tissue culture, fungi and bacteria on the plant surface are destroyed. However, if the sterilization period is not sufficient or the appropriate sterilization product is not used, fungi and bacteria develop rapidly in the culture medium. As a result, contamination occurs in the culture medium, preventing the development of the plantlet and causing its death. Various chemicals are used in the sterilization stage. In this study, three different sterilization products, namely hypochlorous acid (Crystalin, HOCI), nano silver (Hygo, nAg) and sodium hypochlorite (NaOCI) were used on the UCB-1 rootstock. During the sterilization stage, fresh shoots of the UCB-1 rootstock were taken and the shoots were prepared as micro cuttings in the laboratory environment. The micro cuttings, which were first kept in 70% ethanol for 2 minutes in a sterile cabinet, were then sterilized at 5% and 10% and 10% levels for 10 minutes. Plantlets transferred to culture medium the parameters of the uncontaminated explants (%), shooting rate (%), the number of leaves (pieces/plantlet), shoot quality and shoot length (cm) were examined. The highest spreading rate was observed in 5% NaOCI application.
Keywords: *In vitro*, Rootstock, Micropropagation, Sterilization, UCB-1.

INTRODUCTION

UCB-1 (*P.atlantica* x *P. integerrima*) is an important hybrid rootstock obtained by interspecific hybridization. The rootstock provides high yield when used in areas with irrigation facilities (Ferguson et al., 2005; Ferguson et al., 2016; Pakyürek et al., 2022). The rootstock is stronger than other *Pistacia* rootstocks and is also tolerant to salinity and cold (Epstein et al., 2004; Ahmad et al., 2005).

Pistacia rootstocks are propagated by seeds. However, with the use of this propagation method, the germination percentage is low and since genetic expansion occurs in the seeds, they do not have the same characteristics as the parent plant (Ayfer and Serr, 1961; Ak and Yilmaz, 1990; Değirmenci, 2017). Although *Pistacia* rootstocks are mostly propagated by grafting among vegetative methods (Açar, 2022), by using plant tissue culture methods under *in vitro* conditions, it is possible to produce high reproduction coefficient, clean plant material, regardless of the vegetation period, protect endangered plant species and genetic resources, and also propagate plant species that are difficult to propagate (Babaoğlu et al., 2002; Şaşkın et al., 2022). One of the most important stages affecting the success of plant tissue culture is sterilization. The concentration and application time of the chemicals to be used for sterilization directly affect the survival of the plantlet in the culture medium. Because when sterilization is not sufficient, fungi and bacteria on the plant surface multiply very quickly in the culture environment, causing contamination and causing the death of the plantlet (Babu et al., 2022).

Various chemicals are used for surface sterilization of the explant to be used in plant tissue culture. The chemicals used for sterilization are ethanol, silver nitrate, sodium hypochlorite, bromine water, mercuric chloride, hydrogen peroxide and various antibiotic substances (Bloomfield and Arthur, 1991; Yıldız and Er, 2002). However, if these chemicals are used, they have toxic effects on plant tissue. During the sterilization process of explants, bacteria and fungi on the surface should be cleaned without damaging the plant tissue (Russel and Hugo, 1994; Herman, 1996; Nacheva and Ivanova, 2018). For this reason, the use of alternative sterilization products to the toxic products used in plant tissue culture in recent years will lead to innovative approaches. In addition to the toxic sodium hypochlorite (NaOCI), non-toxic nano silver (nAg) and hypochlorous acid (HOCl) were used in the study.

Sodium hypochlorite is the most commonly used sterilization product for surface sterilization. Sodium hypochlorite, also known as household bleach, is a chemical that is easily available and can be prepared in appropriate concentrations for sterilization studies (Singh and Kumar, 2009; Tyagi et al., 2011). This chemical is effective against many bacteria and viruses (Sawant and Tawar, 2011). It is used at concentrations of 0.5-15% for explants in surface sterilization (Estrela et al., 2002).

It is used at concentrations of 0.5-15% for explants in surface sterilization (Estrela et al., 2002). It is smaller than 100 nm in size and when used in the sterilization phase, it prevents the growth of bacteria, viruses and fungi and effectively destroys them. If the appropriate concentration for sterilization is determined, it does not have a negative effect on the development of the plant (Abdi et al., 2008; Rostami and Shahsavar, 2009; Sarmast et al., 2011; Shokri et al., 2014; Taraszkiewicz et al., 2013; Krupa- Małkiewicz et al., 2019).

Hypochlorous acid (Crystalin, HOCl) is a stronger and more effective antimicrobial agent than other chemicals when used for surface sterilization. It can destroy bacteria, fungi and viruses. It is a pH neutral substance. It is not toxic and does not cause any harm to the environment or people working with this chemical. It is a cheap and easily available substance (Üstünes, 2011; Overholt et al., 2018; Ateş, 2020; Öztoprak, 2021).

In this study, it was aimed to determine the appropriate sterilization product and concentration for the sterilization process of UCB-1 rootstock under *in vitro* conditions by sterilizing explants taken from young shoots of the UCB-1 *Pistacia* rootstock at 5% and 10% concentrations of the sterilization products NaOCI, nAg and HOCl.

MATERIAL AND METHOD

In the study, UCB-1 rootstock in the belonging to collection garden of Harran University Faculty of Agriculture, Department of Horticulture was used. The shoots of the UCB-1 rootstock taken during the active development period were propagated by nodal culture in the tissue culture laboratory. Three replications were constructed with eight plants in each replication, based on random plots. Shoots were prepared as single nodular micro cuttings. In the study, different products and doses were applied for explant surface sterilization. For surface sterilization, they were kept in a sterile cabinet (vertical air flow) for 2 minutes in 70% ethyl alcohol and then in 5% and 10% nAg, HOCI and NaOCI solution in 10 minute, and at the end of these processes, the micro-cutting were rinsed 3 times with sterile distilled water. After surface sterilization, micro cuttings were transferred to tube with Murashige and Skoog (MS) medium. 2 mg/l meta-topolin, 200 mg/l polyvinylpyrrolidone (PVP), 8 mg/l $AgNO_3$, %3 sucrose and % 0.6 agar were added in the shooting medium. 1.2 mg/l Plant Preservative Mixture (PPM) was added to prevent bacterial contamination of explants transferred to the nutrient medium. The explants were kept in the climatic chamber with a temperature of 18-28 ˚C and white fluorescent lamps as the light source, 16 hours of light and 8 hours of darkness.

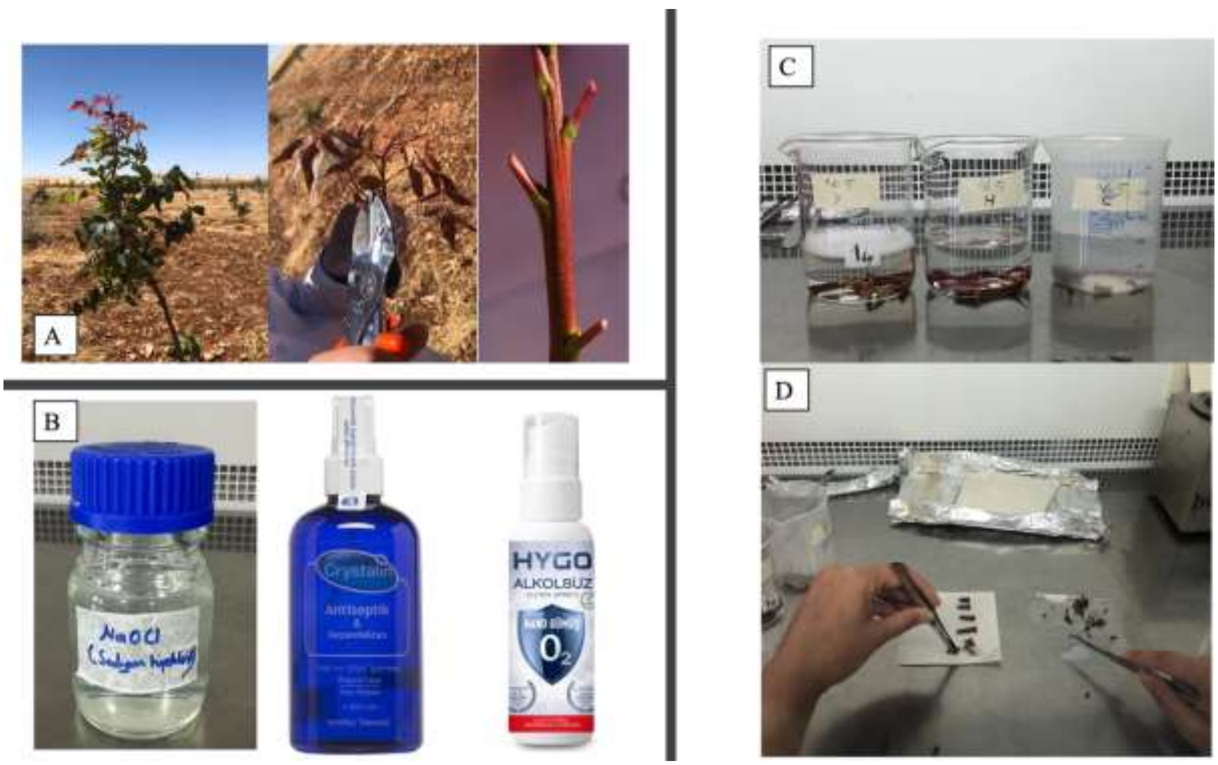

Figure 1. A) Collection and preparation of plant material from the field, B) Products used in sterilization, C) Sterilization of plant materials, D) Preparation of micro cuttings for transfer to the culture medium.

Uncontaminated explants (%), Shooting Rate (%), The Number of Leaves (pieces/plantlet), Shoot Quality and Shoot Length (cm) Measurements

In the uncontaminated explants and shooting rate parameters, the number of uncontaminated nodes and shoot-producing nodes was determined as a percentage by the ratio of the number of nodes cultured. The total number of plant leaves was calculated by counting each leaf collected from each UCB-1 plantlets. Shoot quality was evaluated visually (scored as 1: very poor, 2: poor, 3: fair, 4: good, and 5: very good). Shoot length was measured as the length of newly regenerated shoots per explant in cm.

Data Analysis

In the study, one-way analysis of variance was performed in a randomized parcel design in the JMP Pro 13 statistical program and the statistical difference of the mean values was detected utilizing LSD test ($p \leq 0.05$) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Traditionally, in the sterilization phase, sodium hypochlorite solutions (NaOCl) are generally used, which represent a good option for tissue disinfection. However, this procedure depends on various factors such as explant type, age of the plant, variety and genotype. In the case of plant tissues grown in the presence of a large number of microorganisms, alternative protocols need to be investigated to obtain sterile tissues to initiate an *in vitro* plant tissue culture protocol (Lazo-Javalera et al., 2016; Ak, 2018). The percentage of contamination was not found to be statistically significant depending on the type and concentration of chemical sterilants tested (Table 1). No microbial contamination was observed in T2, T4, T5 and T6 applications and approximately 90% results were obtained in all applications (Table 1). In plant tissue culture, the most important step is to ensure that the tissue to be transferred to the culture medium is free of microorganisms. It has been determined through experiments that NaOCI is very effective against fungi and other microorganisms due to its strong oxidizing properties (Yildiz et al., 2012). Likewise, studies have reported that nAg and HOCl have no toxic effects and have antibacterial and antifungal effects (Nomiya et al., 2004; Sondi and Salopek-Sondi, 2004). In our study, the contamination percentage in line with the practices is parallel to the studies conducted.

The presence of fungi, bacteria and microorganisms not only negatively affects shoot formation but also limits the sustainability of shoot regeneration. Therefore, effective sterilization plays an important role in this regard. Shoot regeneration was found to be statistically significant among the applications made for surface sterilization in node explants of UCB-1 rootstock. The highest application rate was detected in the T1 (33.33%) application, followed by T3 (25%), T2 (16.67%) and T6 (16.67%) applications, respectively. In T4 (0.00%) and T5 (0.00%) applications, no shoots occurred in the node explants in the culture medium (Table 1). Studies have reported that during the propagation of *Pistacia* species under *in vitro* conditions, the explants turn brown and node sprouting is suppressed due to the phenolic substance secreted in the culture medium (Onay, 2000; Ozden-Tokatli et al., 2005). Although there is no contamination in T4 and T5 applications, it is predicted that the shoot rate decreases due to the difference and concentration of sterilant products and the presence of phenolic substances in *Pistacia* species, among the reasons for no shoot formation. Although positive results were obtained with the NaOCI product, it is observed that shoot development is restricted as the concentration increases in T2 (10% NaOCI) application on UCB-1 rootstock (Figure 2). In addition to being an effective sterilant, sodium hypochlorite has a toxic effect and causes tissue death (Figure 2). In addition, some studies have reported that high doses of nAg negatively affect shoot formation and have a cytotoxic effect on cells. Rostami and Shahsavara (2009) reported that immersion of olive explants in very high concentration nAg solution resulted in a toxic effect resulting in severe damage and browning of the explants. Our study also supports that shoot regeneration was negatively affected at a dose of 10% nAg and that high doses have a toxic effect.

A successful in vitro culture protocol begins with effective explant sterilization. Various sterilization products are used to sterilize tissues, and these disinfecting materials are also toxic to explant tissues, and therefore choosing the right concentration is an important step to reduce damage to plants (Rezadost et al., 2013; Al Ghasheem et al., 2018). A statistically significant difference was found in the number of leaves per plantlet between treatments. In the study conducted by Rodrigues et al. (2013), NaOCI concentrations of 5, 10, 15 and 20% were used for 15 minutes and they reported that the best concentration was 5%. In our study, the highest leaf numbers were detected in T1 (5% NaOCI) and T6 (10% HOCl) applications. This was followed by T3 and T2 applications, respectively (Table 1). Although NaOCI solution is an effective disinfectant, studies have also reported that it has a limiting effect on vegetative development as the concentration increases (Mihaljević et al., 2013; Nelson et al., 2015). In our study, it was visually proven (Figure 2) that T2 (10% NaOCI) application resulted in a decrease in the number of leaves as a result of the increasing concentration of sodium hypochlorite.

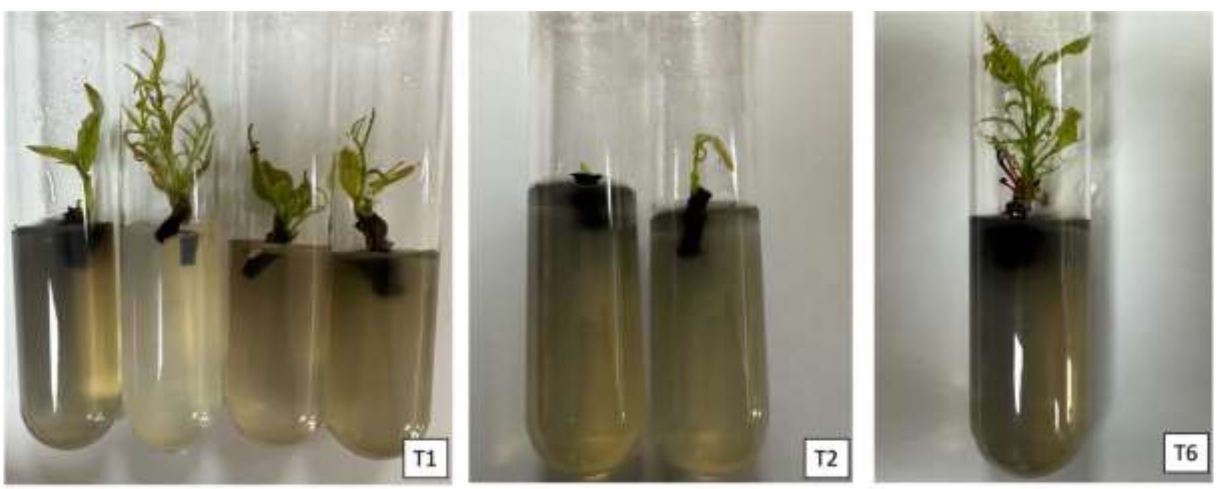

Figure 2. Shoot development in applied explants. T1: 5% NaOCI, T2: 10% NaOCI and T6: HOCl.

While the sterilization process aims to destroy all microorganisms that can easily multiply on the tissue; on the other hand, it should guarantee the vitality and regenerative capacity of the tissue (Yildiz et al., 2012). While trying to prevent contamination with applications performed under *in vitro* conditions, vegetative development should also be ensured to continue sustainably. For this reason, it has been reported by some researchers that the content of the sterilants used and the concentration intensity applied also play an important role in plant viability. Appropriate concentration and sterilant type supported shoot development and positively increased vegetative development in T1 and T6 applications. Shoot quality was found to be statistically significant among treatments.

Shoot quality was found to be statistically significant among treatments. The highest shoot quality was detected in T1, T3 and T6 treatments (Table 1). Since plants do not have a thick protective surface, surface disinfection is difficult, and therefore sodium hypochlorite and similar agents can easily damage sensitive tissues (Oyebanji et al., 2009). Application of these toxic chemicals at high concentrations has harmful consequences for explant development in *in vitro* conditions (Cabrera Jova and Gonzalez, 2014). Therefore, healthy alternative products that protect the plant surface should be tried. It has also been reported by some studies that non-toxic sterilant products play a role in the healthy development of plant material (Safavi et al., 2011; Rudramurthy et al., 2016; Adebomojo and AbdulRahaman, 2020). In our study, it was observed that the nAg and HOCI non-toxic sterilant products we preferred had a positive effect on plant growth, and this supports the studies carried out. It is thought that due to the increasing concentration of NaOCI, which is a toxic substance, the shoot bud tissue on the transferred micro cuttings is damaged and the shoot quality decreases for this reason.

Table 1. Effect of chemical sterilants, percentage of contamination, shooting rate, the number of leaves, shoot quality and shoot length to *in vitro* multiplication (Mean ± SD)

Treatment							
MS medium+chemical sterilants	Treatment no.	Conc. Used (%)	Uncontaminated explants (%)	Shooting rate (%)	Number of leaves (pieces/plantlet)	Shoot length (cm)	Shoot quality
NaOCI (Sodyum hipoklorit) (v/v) (un-autocloved)	T1	5	91.67±14.43	33.33±14.43 a	10.83±4.07 a	3.42±0.88 a	5.00±0.00 a
	T2	10	100.00±0.00	16.67±14.43 ab	1.67±0.58 b	1.33±0.58 b	1.67±0.58 b
nAg- HYGO (Nano Silver Alcohol-Free Hygiene Spray)	T3	5	91.67±14.43	25.00±0.00 a	4.00±1.00 b	1.33±0.58 b	5.00±0.00 a
	T4	10	100.00±0.00	0.00±0.00 b	-	-	-
HOCI-Crystalin	T5	5	100.00±0.00	0.00±0.00 b	-	-	-
	T6	10	100.00±0.00	16.67±14.43 ab	16.67±5.77 a	2.50±0.50 ab	5.00±0.00 a
LSD ($p \leq 0.05$)			ns	19.77	6.03	1.45	0.58

CONCLUSIONS

The results obtained from this study showed that the concentrations of 5% NaOCl, nAg and 10% HOCl used were effective in decontaminating node explants of UCB-1 rootstock with an exposure time of 10 minutes. Contamination control is a key factor of success during *in vitro* plant tissue culture protocols. Due to the importance of contamination control to achieve micropropagation goals and the side effects of frequently used toxic chemical products (NaOCl, HgCl2, H2O2, etc.), the need to consider new antimicrobial nontoxic agents is obvious and would be beneficial. Our study confirmed that these non-toxic (nAg and HOCl) substances have antimicrobial properties. At the right dose, it can be used as an effective tool for removing contaminants from plant tissues. Although it has not yet become a universal sterilization agent, further research on different plant species and different explants is required to expand its use in in vitro culture of other plants.

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BIOCHEMICAL EFFECTS of LIVE FISH TRANSFERS in TURKISH SALMON FARMING and IMPROVEMENT of TRANSFER CONDITIONS

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ABSTRACT

In order to meet the food needs of the increasing world population, aquaculture is one of the most suitable and environmentally friendly methods preferred to obtain low emission and quality protein. Live fish transfers are made in order to increase efficiency by using water resources in the most effective way. However, transfer processes are one of the stress sources that cause death in fish. It is possible to reduce the harmful effects of stress by providing optimum living conditions during the transfer. Correct practices during this process are essential to ensure the health, welfare and success of the transfer operation of the fish. There are some factors to be considered for successful live fish transfers. These factors, which are vital for fish, are; fish health, dissolved oxygen and carbon dioxide levels in the water, pH, water temperature, fish density and metabolic wastes such as ammonia cause changes in fish biochemistry during transportation. In this review, the effects of transfer on salmon candidate trout transferred from dam water to the sea in Turkish salmon farming are discussed.

Keywords: Transportation of live fish, Biochemistry, Water quality, Aquaculture

1. INTRODUCTION

In 2018, the General Directorate of Fisheries and Aquaculture of the Turkish Ministry of Agriculture and Forestry decided to include Black Sea trout in the world fish market under the name Turkish Salmon. The export of Turkish salmon, whose production increases every year, was 16,774 tons in the first half of 2022, and it increased by 35 percent to 22,623 tons in the same period of 2023 (Karakurum, 2023). Turkish salmon undergoes growth in dam lakes until it reaches a weight range of 180–220 grams. Subsequently, it is transferred to farms located in the cold-water regions of the Black Sea, where it continues to mature until it reaches a harvestable weight of 3–4 kilograms (Levent vd., 2021). One of the most important stages in Turkish salmon farming is to manage transport operations correctly. In this period, which is very stressful for fish, it is aimed to reduce post-transport mortality with the right practices. Stressors faced during transportation encompass factors such as pre-transport handling, deterioration of water quality during transit, heightened vulnerability to metabolic shock, stress, and an increased risk of infection and disease post-transport (Vanderzwalmen vd., 2019).

2. IMPORTANCE OF LIVE FISH TRANSFER

Aquaculture stands as the most rapidly expanding sector within the animal food production industry (FAO, 2010). This growth has resulted in a notable increase in the long-distance transportation of live fish, often exceeding eight hours in duration. Such instances arise due to the geographical separation between grow-out sites, such as ponds, cages, and net pens, and the hatcheries or nurseries responsible for providing juvenile fish (Stieglitz vd., 2012). To meet the demands of clients, aquaculture companies must transport substantial quantities of fish through various modes of transportation, including land, sea, and air freight (Harmon, 2009). Live fish transfers are a fundamental strategy in aquaculture. These transfers enable more effective use of water resources and increase aquaculture production (Barton, 2002).

Nevertheless, the expenses associated with shipping and the survival rates of transported fish pose significant challenges to this practice. Consequently, sellers are actively exploring innovative transportation methods aimed at minimizing costs while maximizing the survival rates of the fish (Harmon, 2009; Lim vd., 2003).

3. KEY FACTORS TO CONSIDER BEFORE AND DURING THE TRANSFER

3.1. Fish Health Before Transfer

The quality of fish being transported is a crucial factor. It is imperative that the fish designated for transport are in a state of robust health and overall good condition. In cases where the environmental temperature during shipment is elevated, it becomes particularly essential to cull weakened individuals from the consignment (Feng vd., 2023). Even with a substantial reduction in fish density within the transport container, the presence of subpar-quality fish still leaves the consignment susceptible to losses (Shabani vd., 2016). Notably, weakened fish experience significantly higher mortality rates than their healthier counterparts, especially when the transportation duration is extended (Berka, 1986).

3.1.1. Suggestions for Fish Health Before Transfer

- **Health Assessment:** Prior to the transfer, it is essential to conduct a thorough health assessment of the fish population. This assessment should involve a visual inspection of individual fish to identify any signs of disease, injury, or stress.
- **Disease Screening:** Implement a disease screening process to detect and isolate any fish carrying pathogens or infections. Quarantine measures may be necessary to prevent the spread of diseases to other fish during transport.
- **Stress Reduction:** Minimize stress factors in the days leading up to the transfer. This can include maintaining optimal water quality parameters, providing proper nutrition, and ensuring a suitable environment in holding tanks or ponds.

3.2. Fish Health During Transfer

3.2.1. Water Quality and Metabolic Wastes

One of the most critical needs of fish during transfer is oxygen (Piper vd., 1982). This requirement is particularly important as fish can be in a highly stressed state during transfer, their respiratory rate can increase rapidly and they can produce large amounts of mucus in the transfer tanks. This requirement is especially emphasized during the first moments when the fish are placed in the tank (Das vd., 2015). Trucks used for live fish transfer usually have multiple tanks mounted on the vehicle. These tanks are completely independent systems, each with its own independent water supply (Zhang vd., 2019). Therefore, separate equipment is required to monitor and control each tank.

Usually, just before the transfer process starts, this tank is filled with water, using the fish's source water. This ensures that the fish are transported in an environment with the same water quality as during the initial loading. However, water is not added to the tank as the loading continues. This means that the first load of fish consume dissolved oxygen and the oxygen level in the tank drops. However, to compensate for this drop, an aeration system is placed in the tank, which increases oxygen levels. If not enough oxygen is supplied to the tank, oxygen levels drop rapidly and the fish begin to experience stress (Harmon, 2009). If oxygen levels drop too low, the fish can die (Berka, 1986; Piper vd., 1982). Therefore, it is critical to continuously monitor oxygen levels in live fish transfers and take precautions when necessary. The lower limits of dissolved oxygen concentrations may vary according to water temperature. Accordingly, dissolved oxygen concentrations should not be less than 5.0 mg/L at 5 ^{o}C water temperature, less than 6.0 mg/L at 10 ^{o}C, less than 7.0 mg/L at 15 ^{o}C and less than 8.0 mg/L at 20 ^{o}C (Soderberg, 2017).

Water quality depends on both the stocking density of the fish and the transportation time. It is very important to test the quality of the water supply before starting bulk fish shipments. Dissolved CO_2 in water plays a crucial role as it directly affects pH values and toxic ammonia levels. With longer transportation time, the respiration of the fish leads to increased CO_2 production, which can lower the pH of the water, making it more acidic. Optimum water pH levels typically range between 7 and 8. Sudden fluctuations in pH can cause stress to fish, but the use of buffers can help keep water pH levels stable during fish transportation (Amend vd., 1982).

3.2.2. Suggestions for Fish Health During Transfer

- **Oxygen Monitoring and Aeration Systems:** It is crucial for aquaculture facilities engaged in live fish transfers to invest in reliable oxygen monitoring systems and aeration equipment. These systems should ensure that oxygen levels are maintained at optimal levels throughout the transportation process, especially during the initial moments when fish are loaded into tanks. Continuous monitoring and rapid adjustments are essential to prevent fish stress and mortality due to oxygen depletion.
- **Tank Water Quality Control:** Since live fish transfer trucks often have multiple independent tanks, each with its own water supply, it's imperative to equip each tank with its monitoring and control system. This ensures that water quality parameters, including oxygen levels, can be managed separately for each tank, optimizing the transport conditions for different fish species or batches.
- **Water Quality Testing:** Prior to initiating bulk fish shipments, comprehensive water quality testing of the source water should be performed. This testing should include parameters such as pH, dissolved

oxygen, CO_2 levels, and ammonia concentration. Regular testing helps ensure that the transported fish are placed in an environment Dwith consistent water quality, reducing stress during the transfer.

- **Dissolved CO_2 Management:** Understanding the role of dissolved CO_2 in affecting pH and ammonia levels is critical. With longer transportation times, fish respiration can lead to increased CO_2 production, potentially lowering pH levels and impacting fish health. Effective management of CO_2 through aeration and buffering agents is essential to maintain stable water pH within the optimal range of 7 to 8.
- **Buffering Systems:** Utilizing buffering systems to stabilize water pH during fish transportation is recommended. These systems can help prevent sudden fluctuations in pH, which can induce stress in fish. Properly buffered water provides a more stable and favorable environment for the transported fish.
- **Emergency Protocols:** Develop and implement emergency protocols for situations where oxygen levels drop dangerously low. These protocols should include actions to quickly address oxygen deficiencies, such as increasing aeration or making emergency stops if needed to protect fish health.

By implementing these suggestions, the aquaculture sector can enhance the welfare of transported fish, reduce stress, and minimize the risk of mortality during the transfer process, ultimately contributing to improved fish quality and the success of the aquaculture industry.

4. LIVE FISH TRANSFER of TURKISH SALMON FARMING

In 2020, global aquaculture production amounted to 177.8 million tons, of which approximately 90.3 million tons was obtained through fishing and the remaining 87.5 million tons through aquaculture. According to projections, aquaculture is expected to be around 109 million tons in 2030 (FAO, 2022). In 2021, the shares of the three most cultivated species in Turkey are as follows: Rainbow trout ranks first, accounting for 35% of total production (165,683 tons). Then, sea bass ranks second with 33% (155,151 tons), and sea bream ranks third with 28% (133,476 tons) (Rainbow trout’s tonnage include Turkish Salmon production value.) (TÜİK, 2023). Approximately 27% of the total European production, equivalent to nearly 130 thousand tons, is sourced from Turkish aquaculture facilities, primarily situated in the Black Sea region with its characteristic brackish water. Fish weighing over 2.5 kg at the time of harvest are marketed as "Turkish Salmon." (FAO, 2022).

Salmon farming in the Black Sea is restricted to the winter season, spanning seven months, primarily due to elevated water temperatures during the summer months (Yigit vd., 2023). Cage farming in the Black Sea had its origins in the early 1990s with the production of rainbow trout. Notably, Yigit (1996) observed improved growth performance of rainbow trout in brackish water compared to those raised in freshwater conditions (Yiğit, 1996). Over the years, increasing demand for larger fish has shifted the farm strategy towards salmon production in the Black Sea. Presently, rainbow trout are raised in land-based freshwater hatcheries until they reach a certain size, after which they are transferred to exposed marine sites for the on-growing phase. Fish weighing over 2.5 kg are then supplied to both local and primarily export markets, marketed as "Turkish salmon". The harvest weight depends on the initial fish size introduced to cages in early November, marking the beginning of the production period, which is limited to seven months in the Black Sea, ending in May when surface water temperatures exceed tolerance limits of 23°C (Yigit vd., 2023). In this production method, the highest fish mortality rates are observed after transfer from freshwater to seawater.

5. EFFECTS OF LIVE FISH TRANSFERS ON BIOCHEMICAL PARAMETERS UNDER HYPOXIC AND HYPEROXIC CONDITIONS

Mistakes made during the oxygenation of transport tanks or running out of oxygen tanks during transport cause hypoxic conditions. This usually results in the death of the entire transported fish. During current transfer operations in aquaculture, the dissolved oxygen level in tanks is usually kept at maximum and transfer operations are carried out under hyperoxic conditions. Although there are many studies on stress in fish, there is no study to determine the effects of stress caused by changing the dissolved oxygen level during transfer (Lushchak ve Bagnyukova, 2006; Lygren vd., 2000; Omlin ve Weber, 2010; Ritola vd., 1999).

Rainbow trout undergo metabolic changes to provide sufficient oxygen to tissues in response to hypoxia. These changes include adaptations such as hyperventilation, bradycardia, increased blood oxygen carrying capacity and regulation of blood flow (Diaz ve Breitburg, 2009). Under hypoxic conditions, lactic acid accumulates in muscle tissues as a by-product of anaerobic metabolism. An in vivo study by Omlin and Weber (2010) showed that lactate accumulation in white muscle tissue is much higher than in heart, red muscle, brain and liver tissues. These findings suggest that white muscle is the main source of lactate for trout under hypoxia conditions (Omlin ve Weber, 2010). In a study by Valotaire et al. (2020), an increase in plasma cortisol levels was observed in 3-month-old juvenile trout reared under hypoxic conditions for 25 days when compared to normoxic conditions. However, despite these changes, no significant changes were detected in the behavioral phenotypes and hypothalamic-pituitary-adrenal (HPI) axis reactivity of the fish (Valotaire vd., 2020).

High oxygen concentrations and especially hyperoxic conditions cause the formation of reactive oxygen species (ROS) not only in mammals but also in fish. However, fish have developed biological responses to adapt to hyperoxic and hypoxic conditions, varying between species (Kagan vd., 2004). In a study conducted in aquarium fish, it was reported that fish exposed to hyperoxia showed increased protein carboxylation and oxidative damage in brain, liver, kidney and muscle tissues (Lushchak, 2011; Lushchak ve Bagnyukova, 2006). In another study, juvenile rainbow trout were exposed to hyperoxic conditions (> 40 mg/L O_2) for 1, 4, 12 and 24 hours. A high catalase activity was observed in the gills in the 24 h exposure group and in the liver in the 4 h exposure group, but no significant change was observed in the 1 and 12 h exposure groups. However, SOD, total GSH-Px and GR activities were not affected. In another study by Ritola et al., rainbow trout exposed to hyperoxia for only 5 seconds showed an increase in SOD and catalase activity (Ritola vd., 2002).

Stress factors in fish can cause an increase in ROS in serum and tissue and thus lipid peroxidation (Christie ve Costa, 1984; Lushchak ve Bagnyukova, 2006). MDA is produced as the final product of lipid peroxidation. Increased MDA levels in tissue and serum are indicative of the toxic activity of free radicals (Doyotte vd., 1997). In a study investigating the effect of transfer on serum MDA levels in rainbow trout, samples were taken from juvenile trout before, during and after a 30-minute transfer process (at 6 and 12 hours) and it was reported that serum MDA values increased statistically at 6 hours and decreased to the pre-transfer level at 12 hours.

6. CONCLUSIONS

In this review, we have focused on important factors to consider to increase the success and animal welfare of live fish transfers. Live fish transfers are critical in aquaculture and can be made more efficient with careful planning and correct practices.

We have discussed some key steps to increase the success of transfers and minimize fish stress. First, the transfer process must be carefully planned. All necessary precautions should be taken for the health and welfare of the fish. This is important not only to reduce post-transfer mortality, but also to protect animal welfare, which is an ethical responsibility.

Water quality is a critical factor in live fish transfers and therefore water quality monitoring and management is of paramount importance. Ensuring water conditions as close as possible to the fish's natural habitat plays a vital role during and after the transfer. Regular monitoring of water quality can help to quickly detect anomalies and identify situations that need to be corrected.

In conclusion, live fish transfers are an important practice for sustainable aquaculture. However, minimizing fish stress and managing water quality are critical for successful implementation. Scientific research and good practice methods can guide the achievement of these goals and help sectors such as salmon farming to grow in a more sustainable and ethical way. In the future, it is clear that more research and improved practices are needed on this important topic. This will contribute to aquaculture becoming more sustainable both economically and environmentally.

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THE USE OF SULPHUR IN PLANT DISEASES

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ABSTRACT

One of the mineral originated inputs in agricultural production facilities is sulphur. The chemical is one of the oldest pesticides that is used in the plant both by increasing the resistance against diseases and directly in the combat against biotic disease factors. At the same time, it is a main element as a plant nutrient of producing crops. This valuable element has become even more important in recent years due to its natural origin, as organic agriculture, where there are not many options in the fight against diseases and pest, has gained importance. The sulphur usage alone or in combination with another active substances, is common, especially in powdery mildew besides rust, anthracnose, and mildew in horticultural and field crops. The agent is produced and used in WG, DP, and SC formulations, depending on the other active substances to which it is combined. In the review article, it is aimed to evaluate the studies that have achieved success in plant diseases with the use of sulphur and its compounds, in the last decades.

Keywords: Sulphur, Plant Protection, Pesticide, Plant Diseases

INTRODUCTION

Elemental sulphur is a well-known material in agricultural plant growth and protection steps as fertilizer and pesticide for a long time, and mainly derived from sulphur rich deposits, natural gas, and crude oil (Feely and Kulp, 1957; Lim et al., 2015; Saleh, 2020; MTA, 2023). In pre-modern agriculture organic farming was done naturally and sulphur was an important input of this period, along with copper and arsenic (Zadoks, 2013). The element S is an essential in nearly all biological systems (human, animal, plant, and microorganism) via using in synthesis of sulphur-containing amino acids, vitamins, and other biomolecules in variably (Colovic et al., 2018; Ma et al., 2020).

At the present time, sulphur is known both causing agent as resistance inducer against diseases and direct effector as combating against biotic disease factors. Elemental sulphur is the only inorganic phytoalexin produced by some plant species (Cooper and Williams, 2004). The phytoalexins produced by plants give response to biotic and abiotic stresses (Bizuneh, 2021). Besides the naturally occurring as active defence compounds, elemental sulphur has become even more important in recent years due to its natural origin, as organic agriculture, where there are not many options in the fight against diseases and pests, has gained importance. The sulphur usage alone or in combination with another active substances, is common, especially in powdery mildew besides rust, anthracnose, and downy mildew in horticultural and field crops. The chemical is mostly produced and used in WG, DP, and SC formulations, depending on the other active substances to which it is combined.

Aside from elemental sulphur there are many active substances that sulphur-containing and synthetically produced pesticides against weeds, pests, and diseases in different agrochemical groups such as sulfonylureas, sulfonamides, sulfur-containing heterocyclics, thioureas, sulfides, sulfones, sulfoxides, and sulfoximines, in use on the international or some national markets (Davendar and Yang, 2017).

According to the Fungicide Resistance Action Committee that was founded as an organization to discuss resistance problems (Hermann and Stenzel, 2019), Sulphur's Mode of Action is in M (Chemicals with multi-site activity), target site is multi-site contact, group name is inorganic, and FRAC Code is M 02 (FRAC, 2022).

In the review article, it is aimed to evaluate the studies that have achieved success in only plant diseases with the use of inorganic sulphur and its combination with another active substances, in the last decades.

Sulphur Applications in Powdery Mildew

Powdery mildews are common ascomycetes fungal diseases of many monocotyledonous and dicotyledonous plant species as biotrophs that they can grow and reproduce in nature only in living hosts, and they are called obligate parasites that attack approximately 10,000 species of plants belonging to more than 1600 genera (Saenz and Taylor, 1999; Agrios, 2005). The fungi cause considerable losses in yield (Panstruga & Schulze-Lefert, 2002). The powdery mildew representative order is Erysiphales and has *Blumeria*, *Erysiphe*, *Leveillula*, *Microsphaera*, *Oidium*, *Podosphaera*, *Sphaerotheca*, and *Uncinula* genus that causing powdery mildew (Agrios, 2005). Sulphur is the most common input in combat against the diseases.

Tomato powdery mildew caused by *Leveillula taurica* is the primary mildew of field-grown tomatoes, and a study was conducted during the 2009-2012 tomato seasons in commercial fields or in field of research station in California in the U.S.A. In the study, sulphur dust (80%, 56 kg/ha) was applied weekly beginning at six weeks after transplanting and ended two weeks before harvest. According to the general result, disease severity and foliar necrosis at harvest were always significantly reduced by the sulphur applications. Soluble solid levels in tomato fruits were significantly lowered in non-treated plots in half of the trials although there was no significant reduction in yield of marketable fruit in most trials. In addition, a decrease in the sunburned fruit at the sulphur applied plots, has also been reported (Aegerter et al., 2015).

Pepper powdery mildew caused by *Leveillula taurica* is also an ongoing problem in California coastal regions because of favorable temperatures and humidity since 1992. A study was conducted in 1992-1996 seasons in commercial pepper fields in California. The sulphur applications at different rates and application number, besides Triadimefon, Myclobutanil, Propiconazole, Trilogy, and Azoxystrobin were evaluated. The reached data showed if sulphur applications were done after the onset of pepper powdery mildew, there were not satisfactory control of the disease. Sulphur is a successful in preservation of the disease when it is used prior to the onset of the disease and at frequent intervals with good coverage. Although the other used fungicides showed intermediate or excellent control, they emphasized that sulphur is the principal method for organic growers to control pepper powdery mildew (Smith et al., 1999).

Okra powdery mildew caused by *Erysiple cichoracearum* is a serious disease of okra and most of okra cultivars are susceptible to the diseases. A study has been done to find out the effect of nano-sulphur (by name: IARI) that synthesized under the scientists' institute and the other three commercial products (commercial sulphur-Merck; commercial nano-sulphur-M K Impex, Canada; Sulphur 80 WP-Corel insecticide) on the diseases. Both *in vitro* and polyhouse evaluation showed that IARI-nano-sulphur is the best for inhibition of conidial germination percentage in both food poison technique and detached leaf method. They have emphasized that nanoparticles of sulphur are undoubtedly better for fungicidal property as compared to the used conventional micronized formulations (Gogoi et al., 2013).

Japanese plum powdery mildew (*Sphaerotheca pannosa*) is mostly found in stone fruit such as apricot, cherry, and plum. In Israel the pathogen was newly detected on Japanese plum, and a study has been conducted to compare the efficacy of two sulphur compounds (Helio-Sulfur 70 SC Action-Pin, France; Sulphur 825 SC Cerexagri, France) and some registered fungicides. The study emphasized that spraying of sulphur significantly enhanced the inhibitory effect against the disease. Additionally, the scientists recommended that for an alternative disease management programme, which integrates early and timely applications of effective fungicides, and tank-mixtures of DMI (demethylation inhibitor) fungicides with sulphur, may provide an improved level of powdery mildew control and could be valuable to avoid the spread of powdery mildew and to minimize the development of fungicide resistance (Reuveni et al., 2006).

Apple powdery mildew (*Podosphaera leucotricha*) is one of the common diseases of apple. In Romania the pathogen requires routine fungicide applications at one- or two-weeks intervals as being nearly the rest of the world. Because of rising cost and public corcern about pesticides, a study has been conducted in the country with sulphur base on products (Polisulf, Sulfomat 80 PU) in addition to standard product (Kumulus DF) at the proposed rate in three apple orchards. According to the results highest effectiveness was found in Polisulf and Sulfomat at two different growth stages that applied. They emphasized that the results were close to standard product Kumulus DF and even better (Jalobă & Grădilă, 2019).

Grapevine powdery mildew (*Erisiphe necator*) is one of the major diseases in grapes and caused berry and quality losses. Because of facing to high cost and possible resistance of other fungicides, a study that used sulphur, was done in three distinct locations in Romania. The used sulphur materials were polisulf (sulphur thiosulphuric 3%+ sulphur polisulfhydric 12%), Sulfomat 80 PU (sulphur 800 g/kg), and Kumulus DF (sulphur 80%). The study has showed that the sulphur-based on products (Polisulf and Sulfomat) provided efficient effectiveness to control grapevine powdery mildew in vines, almost equal to standard product Kumulus DF (Grădilă et al., 2020).

Strawberry powdery mildew (*Podosphaera aphanis*) is one of the destructive diseases in strawberry production and caused losses of marketable fruit of highly big percent. Because of phytotoxic effect of sulphur above nearly 27^0C, a study has been realized to determine phytotoxicity of sulphur at temperature 30^0C, 35^0C and 40^0C for periods of one, two, four, and eight hours after applications in both open fields and high tunnels for three cultivars in Florida in the U.S.A. In the study flowable sulphur (Suffa, Drexel Chemical, Memphis, TN) and wettable formulation in addition to standard fungicide with a single application was applied at a single dose besides control. Briefly, in both of sulphur application sites of the study, foliar disease severity and fruit disease incidence were found in reducing in powdery mildew and the observed phytotoxicity was ultimately inconsequential with respect to the overall yield (Onofre et al., 2021).

Wheat powdery mildew (*Erysiphe graminis*) is well known disease in the world and a study was conducted in UK to test the effects of soil- and foliar application of sulphur. For soil application $CaSO_4$ was used as a sulphur source and micronized S (Thiovit 80%) with or without an organosilicon adjuvant was used as a sulphur source for foliar applications besides commercial fungicides. The scientists emphasized that a combination of foliar Sulphur and commercial fungicide appeared to be more effective on powdery mildew controlling than using S or fungicide applications alone (Hussain & Leitch, 2005).

Sulphur Applications in Rust

Plant rusts, cause by Basidiomycetes of the order Uredinales or Pucciniales, are the most destructive plant diseases. They mostly attached to grain crops (e.g., wheat, oats, and barley), vegetables (e.g., bean, asparagus), field crops (e.g., cotton, soybean), ornamental plants (e.g., carnation, chrysanthemum) and trees (e.g., apple, pine). There are about 5000 rust fungi species. *Puccinia*, *Gymnosporangium*, *Hemileia*, *Phragmidium*, *Uromyces*, *Cronartium*, *Peridermium*, *Melampsora*, *Coleosporium*, *Gymnoconia*, *Phakopsora*, and Tranzschelia genera that causing rust (Agrios, 2005; Helfer, 2014). Sulphur can be used in combat against some of the diseases.

Wheat stripe rust (*Puccinia striiformis*) has become a serious problem in wheat production areas. Because of the enormous importance of wheat in all over the world, a study has been conducted consecutive two years in Pakistan to determine effect of sulphur and other fungicides in the management of the disease. Beside sulphur, three commercial fungicides were used. In conclusion they emphasized that all used chemicals showed effectiveness for controlling the rust severity and increasing grain yield over control plots (Ali et al., 2022).

Bean rust (*Uromyces appendiculatus*) is an important leguminous plant disease, and a study was done for integrated disease management via using micronized sulphur as a contact fungicide, potassium sulphate (as a salt), ethyphone (as a growth regulator), and Sumi Eight (systemic fungicide) achieving lower disease occurrence and higher yield. They emphasized that all the chemicals helped decreasing the disease severity but its usage in alteration can be the best choice in integrated control of the bean rust (El-Hamady et al., 2010).

In a study antifungal activity of sulphur-containing salts (ammonium sulfate, magnesium sulfate, potassium metabisulfite, potassium sulfate, sodium metabisulfite and sodium sulfate) against phytopathogenic seven fungi species including *Puccinia triticina*, and *Uromyces appendiculatus* which are rust pathogens, had been studied because of residues effects of synthetic chemicals on food and resistance doubts. The study showed that the efficacy of salts was dependent on used concentration, and an increase in concentration was associated with increased efficacy of salts in reducing the formation of pustules for both rust pathogens. In addition, the pot experiment also showed that sodium sulfate at 1.5% and mancozeb treatments equally and effectively reduced the pustules caused by *P. triticina* and *U. appendiculatus*. In general assessment, all used sulphur-containing salts were in successes, but sodium metabisulfite, potassium metabisulfite and ammonium sulfate were more toxic to the tested fungi than the other salts for mycelial growth, spore germination etc. (Arslan, 2015).

Sulphur Applications in Downy Mildew

Plant true downy mildews, belong to the family Peronosporaceae in an Oomycetes group, are obligate parasites of higher plants, cause plant diseases in cultivated grain, field crops, vegetables, ornamental plants, and grapes etc. *Bremia*, *Hyaloperonospora*, *Peronospora*, *Peronosclerospora*, *Plasmopara*, *Pseudoperonospora*, *Sclerophthora*, and *Sclerospora* are some of the important genera (Agrios, 2005).

Grapevine downy mildew (*Plasmopara viticola*) is well-known problem that bothers the *Vitis vinifera* growers. In most cases copper-based formulations are used. But in organic agriculture copper usage must be in limits. So, a study was performed to find out the alternative formulation to copper. For this aim commercial products of potassium bicarbonate, calcium polysulphide, calcium chlorid, calcium hydroxyide, acid clay, and copper hydroxide were applied a variety of *Vitis vinifera*. At the end of the study, potassium bicarbonate and the chemical with wetting agent can be effective to control the disease. In addition, lime sulphur was found a promising agent as a copper alternative. The results are valuable for think about lime sulphur usage (Lukas et al., 2016).

Onion downy mildew (*Peronospora destructor*) is a major disease of onion, that affects the *Allium* genus. A study was performed to tests some fungicide including active and common sulphur powder besides three formulations that were commercial, at onion fields. The results showed active sulphur succeeded immediately after 70% metil-zineb (Özalp, 1967).

Sulphur Applications in Anthracnose

Anthracnoses are caused by fungi that produce conidia within black acervuli, mostly appear as dark colored lesion on the plant aerial part. The fungi are found in Ascomycetes and Deuteromycetes. *Diplocarpon*, *Elsinoe*, *Glomerella,* and *Gnomonia* are the most common genera. *Glomerella* (conidial stage: *Colletotrichum*) is nearly the most common and well-known genus. Rose, legume, strawberry, grape, citrus, walnut, and some of forest plants can suffer from antrachnoses (Agrios, 2005). Sulphur can be used in combat against some of the anthracnose diseases.

Bean anthracnose (*Colletotrichum lindemuthianum*) is one of the destructive fungal diseases of bean. The pathogen is also can be found in other legumes. A study was done about management of the diseases with three fungicides and three bioagents in addition to sulphur in *in vitro* and with two fungicides and two bioagents in addition to sulphur in *in vivo*. According to the results of the *in vitro* studies, sulphur was found second in all used agents in average fungal growth and in percent inhibition over control. In *in vivo* study, sulphur also was found as third in all used agents either in % disease incidence or in yields in mean of both two years. The study was conducted with a single dose, is important in terms of showing that it is possible to be more hopeful when different doses are used (Singh et al., 2023).

Cucumber anthracnose (*Colletotrichum orbiculare*) is known to cause anthracnose pathogen of various cucurbits including cucumber, melons, and watermelons. A study was conducted on *Cucumis sativus* to find out bio-sulphur effect on the disease in addition to a commercial fungicide. Both *in vitro* and leaf tests showed that bio-sulphur is a successful chemical over control plots and in most cases, results were very closed to commercial fungicide. They emphasized that the bio-sulphur can be useful for eco-friendly growing type (Ko et al., 2019).

CONCLUSION

Plant diseases of agricultural fields and natural areas occurred in microorganism forms such as fungi, bacteria, and viruses. Among the pathogens fungal diseases take an important place. Scientists and farmers always try to find an applicable, ecofriendly, and cheap way to protect and combat against the diseases. In most cases it has been achieved. According to the review article, although elemental sulphur is the oldest, the registered by the most of countries, and eco-friendly inorganic chemical, so many studies were not reached that was hoped. In this manner, further studies need to be realized for help environmental protection with true usage of sulphur and to reduce the number of applications of other commercial products.

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CONVENTIONAL AND NOVEL METHODS FOR MILK AUTHENTICATION

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ABSTRACT

Nowadays, the consumption preference of milk and products changes from bovine to non-bovine, due to the high nutritional value and fewer allergic reactions. The non-bovine dairy products are usually costly and seasonal, which could be a reason for adulteration with bovine milk that is cheaper, prevalent, and available through all seasons. That possibility of adulteration causes few issues like the undesirable sensory and physicochemical properties of dairy products, moreover health problems of people with protein allergies. Therefore, detection of adulteration is significant for consumers for the protection from fraud and mislabeling, but also for producers from unfair competition. This review focuses on the advantages and disadvantages of the conventional methods, and the novel identification methods for milk authentication.

Key Words: Milk, bovine, non-bovine, adulteration, authentication

INTRODUCTION

In recent years, the consumption of non-bovine milks and products are increasing in comparison with bovine milk and products, due to their higher nutritional value and fewer allergic reactions (Mayer, 2005). Moreover, high-quality dairy products produced with different types of milk have been registered as Protected Designation of Origin (PDO). These products are high-cost and seasonal; therefore, adulteration can be seen with bovine milk, which is low priced, prevalent, and available throughout the year (Špoljarić et al., 2013; Trimboli et al., 2017). However, bovine milk addition alters the organoleptic properties and total quality of non-bovine milk and products, and also causes health intolerance, resulting in deception of the consumers concerning about the milk origin, quality, safety, and authenticity (Borková and Snášelová, 2005; Kalogianni et al., 2018). Another concern is the common protein allergy that some people experience after consumption of bovine milk, especially α_{s1}-casein (α_{s1}-CN) which differs between species (Kural, 2013). Also, the production of traditional non-bovine dairy products is usually made on the same production line and with the same equipment as bovine milk, causing a possible adulteration unintentionally. Therefore, detection of adulteration is of great importance to protect the consumers from fraud and misleading labeling, as well as from medical problems like allergenicity, and protect the producers from unfair competition. This review mainly focuses on the conventional methods and highlights the novel methods for milk authentication.

CONVENTIONAL METHODS FOR MILK AUTHENTICATON

Species-specific identification methods with quantitative estimation of adulteration are employed in authenticity controls in milk and dairy products by national and local authorities (Kalogianni et al., 2018). These methods are based either on detection of species-specific proteins/peptides, or DNA sequences.

Protein-based methods

Protein-based methods such as isoelectric focusing (IEF), electrophoresis, immunoassays, and chromatographic methods have been succesfully applied for species-specific identification of milk and products.

IEF

According to the European Community, IEF of γ-casein is the reference method for detection of adulteration of non-bovine milks with bovine milk (EC, 2008). This method is based on the isolation of casein from the sample, differentiation of isoelectric points of bovine γ_2- and γ_3-caseins formed by hydrolysis of β-CN by plasmin and comparing the isoelectric points of these proteins with non-bovine milk. The milk and products are considered adulterated when bovine γ_2- and γ_3-caseins, or the corresponding peak area ratios, are equal to or greater than 1% according to the reference standard. This method has been successfully used for detection of raw and heat-treated bovine milk in fresh and ripened caprine and ovine cheeses (Špoljarić et al., 2013), and bovine milk in asinine milk with 5% limit of detection (LOD) values (Pizzano and Salimei, 2014). IEF has been proven to be accurate and sensitive for detection of bovine milk in mixtures, even though being relatively inefficient and time-consuming (Kritikou et al., 2022).

Electrophoresis

Electrophoresis is used for the separation of negatively or positively charged proteins by migration, with a speed based on their electric charge and size (Borková and Snášelová, 2005). Various forms of electrophoresis are successfully used in detection of adulteration in milk and dairy products. Two-dimentional electrophoresis has been used to identify the adulteration of caprine milk with bovine milk, in raw, pasteurized or ultra high temperature (UHT) milk samples by the help of *κ*-casein (*κ*-CN), with a LOD of 2% (Jamnik et al., 2019). Capillary electrophoresis (CE) is a method that is carried out via free capillary (Borková and Snášelová, 2005). Bovine milk detection and quantification in bovine/ovine milk mixtures with a LOD of 5% (Trimboli et al., 2017), and in Mozzarella cheese by the help of α-lactalbumin (α-La) with a LOD of 1% (Trimboli et al., 2019) has been carried out with this method. Polyacrylamide gel electrophoresis (PAGE) techniques, such as sodium dodecyl-sulfate PAGE and native PAGE have been used to detect the proteins of different species in milk, based on their molecular weights (Poonia et al., 2017). By this technique, bovine milk in caprine milk (Lee et al., 2004), and bovine milk in caprine and ovine milks (Pesic et al., 2011) were easily detected.

Electrophoresis is relatively sensitive, economical, providing good linearity and precision, with replicable and accurate results (Pesic et al., 2011; Trimboli et al., 2017). However, this method is time-consuming and laborious. Detection based on whey protein fraction is strongly affected from the heat treatment used in production of the sample, as whey proteins are more sensitive to heat than caseins (Zachar et al., 2011). Also, excessive proteolysis of ripened cheese samples gives false results for quantification.

Immunoassays

Immunoassays are analytical techniques used for quantification of the antigen in an unknown sample, by the reaction between an antigen and its antibody (Sharma et al., 2014). Electrochemical, optical, piezoelectric, and interferometric biosensors are successfully used for on-site analysis as immunoassays. Interferometric biosensors based on surface plasma resonance and Mach-Zehnder Interferometry (MZI) were used for detection of caprine and ovine milk adulteration with bovine milk (Angelopoulou et al., 2015). A label-free optical immunosensor utilizing Broad-Band MZIs and using an in-house anti-bovine κ-CN rabbit antiserum was developed for the rapid and accurate determination of adulteration of Mozzarella di Bufala Campana and Greek Feta cheeses with bovine milk, with LODs of 0.5 and 0.25% respectively (Angelopoulou et al., 2021).

ELISA is the most widely used form of immunoassays in milk analysis. It is used by employing highly specific antibodies against bovine proteins and peptide fragments (López-Calleja et al., 2005). This method has been used to detect bovine milk in non-bovine milks and cheese by bovine immunoglobulin G (Hurley et al., 2004), bovine milk in asinine milk with a LOD of 0.5% (Pizzano and Salimei, 2014), and bovine milk in yak milk with LOD of 1% (Ren et al., 2014). Commercial ELISA tests such as r-Biopharm Bovine Casein Kit, Tepnel Biokits Bovine Casein Kit, Quantispeed Bov Test: QBT® and Quantispeed Goat Test: QGT® were developed successfully (Costa et al., 2008), however bovine and bubaline milks were indistinguishable due to high homology of proteins (Hurley et al., 2004).

The immunosensors are rapid, sensitive, and labor-friendly, with high accuracy, reproducibility, and robustness (Angelopoulou et al., 2015; Liu et al., 2019; Seddaoui et al., 2022). The small size of the immunosensors makes them suitable for a portable and compact device for on-site detection of adulteration (Angelopoulou et al., 2021). However, for the accuracy of these methods, careful selection of target antigen should be made, considering the denaturation of the proteins with heat treatment or proteolysis during cheese ripening (Mayer, 2005; Costa et al., 2008; Zachar et al. 2011). They are relatively costly because of the disposable expensive antibodies with limited shelf-life (Nicolaou et al., 2010).

Chromatographic methods

Chromatographic methods such as high-performance liquid chromatography (HPLC) and reversed-phase HPLC (RP-HPLC) have been extensively used in the isolation of milk proteins (Borková and Snášelová, 2005). HPLC is an analytical technique that is used for the separation of the components in a mixture with column chromatography, followed by identification and quantification by spectroscopy. RP-HPLC is based on separation of analytes on the basis of their hydrophobic properties. This method was successfully used for detection of 1% bovine milk in human milk using bovine β-lactoglobulin (β-Lg), α-La and *κ*-CN as molecular markers, 1 and 3% of bovine milk in mare milk using α_{s1}-CN and β-CN, and β-Lg respectively, and 2% of bovine milk in two years ripened Pecorino cheeses (Urbanke et al., 1992). HPLC with electrospray ionization mass spectrometry was used for determination of bovine milk in caprine milk based on retention time and molecular mass, using β-Lg, with 5% LOD (Chen et al., 2004).

These chromatographic methods offer a simple and easily automated authentication. Also, identification of the marker protein in every single run with extremely high reliability is achieved (Czerwenka et al., 2010). However, determination of adulteration with HPLC fails between caprine and ovine milks, due to the identical retention times of whey proteins of these species (Poonia et al., 2017), and these tecniques are time-consuming (typically 30-60 min) and laborious (Nicolaou et al., 2010).

DNA-based methods

DNA-based methods have been frequently used for milk authentication, due their extreme sensitivity, specificity, simplicity, and reproducibility (Kural, 2013; Kalogianni et al., 2018). These methods are considered as more reliable than protein-based methods because the stability of DNA under high pressure, temperature, and chemical treatments during food processing makes it the most appropriate analyte for authenticity tests. Also, species-specific variable region of genomic DNA and ease of availability even in very small amount of milk and products increase the importance of these methods.

PCR is the most common and useful method for tracing the species origin in food. PCR technique usually includes the lysis of DNA in the sample by a buffer solution and a proteinase, then the purification for the separation of DNA (Yilmaz, 2015). A PCR cycle consists of denaturation of the DNA into single strands at high temperature, annealing of primers (oligonucleotide probes) to each strand for new strand synthesis at appropriate temperature, and extension of the new DNA strands from primers. This technique is combined with PAGE with intercalating dye staining such as ethium bromide, SYBR green, and GelRed. Restriction fragment length polymorphism analysis (RFLP) is also used, which is a process of breaking the DNA molecule into fragments of different sizes by means of restriction enzymes and imaging the obtained DNA fragment (Koloren et al., 2017). The efficiency of the PCR method may decrease due to chemical decrease during reaction, the competition for primers between the amplicons, and the activity loss of the polymerase enzyme as the cycles increase (Yilmaz, 2015). In order to overcome these issues, real time (quantitative) polymerase chain reaction (qPCR) technique has been developed, which brings along the possibility of quantitative detection at low concentration. qPCR method is based on using different fluorescent chemistries that correlate PCR products concentration to fluorescence intensity to obtain data throughout the process, so combines the amplification and detection steps (Higuchi et al., 1993). DNA intercalating dyes, TaqMan and fluorescent species-specific oligonucleotide probes are used as detection systems. Moreover, multiplex PCR is applied as a modification of PCR, to be amplified more than two gene sequences in the same reaction (Kural, 2013).

PCR method has been applied at first for caprine and ovine milk and cheese adulteration with bovine milk, by amplification of β-CN gene using isolated genomic DNA (Plath et al., 1997). After (RFLP) and PAGE, it was found that there was a different restriction site present in caprine and ovine DNA, but not in bovine DNA. The LOD was significant with a 0.5% since this method had a limitation of identification of bovine milk only. Adulteration of ovine milk with caprine milk (López-Calleja et al., 2005), caprine milk/powder with bovine milk/powder with a LOD of 0.1% (Cheng et al., 2006), caprine and ovine cheeses with bovine milk (Kural, 2013; Klancnik et al., 2016), and caprine, cameline, and equine milks with bovine milk with a LOD of

0.1% and 0.2% in pasteurized and UHT sterilized milks (Deng et al., 2020) were successfully determined by PCR techniques.

PCR-based DNA analysis is rapid, sensitive, and powerful (Plath et al., 1997; López-Calleja et al., 2005). Due to the stability of DNA under several conditions like high temperature, high pressure, and chemical treatment, DNA-based methods are accepted to be more reliable than protein-based ones (Klancnik et al., 2016; Deng et al., 2020). However, PCR techniques are not very suitable for routine industrial use, and quantification of adulteration may be affected by production methods like heat treatment or factors such as mastitis which causes somatic cells to increase in milk (López-Calleja et al., 2005; Cheng et al., 2006).

NOVEL METHODS FOR MILK AUTHENTICATON

Recently, methods such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS), spectroscopic and chromatographic methods coupled with chemometrics, and novel immunoassays have been succesfully applied for milk and products authentication.

MALDI-ToF-MS

This method is a soft ionization mass spectrometry technique, that is basically used for macromolecular analysis (Lu et al., 2023). Determination of adulteration by this method is based on evaluation of the protein patterns of whey proteins used as molecular markers, and it was firstly used by Cozzolino et al. (2001) for the detection of bovine milk in ovine and bubaline milks. Lately adulteration of asinine milk with bovine or caprine milk (Cunsolo et al., 2012), asinine and caprine milks with bovine, ovine and bubaline milks (0.5% LOD, Girolamo et al., 2014), PDO Feta cheese made from ovine and caprine milk mixtures with bovine milk (1% LOD, Kritikou et al., 2022), and caprine milk with bovine milk (1% LOD, Lu et al., 2023) was successfully detected.

Since pretreatment of samples is not required, this method is fast (about 100 analyses in less than 1 h), simple, and easy to proceed for the field analysis of possible adulteration, or even contaminations of milk in farms and dairies (Cozzolino et al., 2001; Cunsolo et al., 2012). It is a reliable and robust method with high sensitivity, as the LOD of this method is comparable to more laborious and time-consuming methods (Girolamo et al., 2014; Lu et al., 2023). Some authors claimed that this method’s reliability and robustness is limited by the potential degradation of proteins with heat treatment, the considerable genetic variability of the milk proteins profile between farms, and processing and ripening conditions of the product such as temperature and the source of rennet used (Kritikou et al., 2022; Rysova et al., 2022).

Spectroscopic and Chromatographic Methods Coupled with Chemometrics

Spectroscopy is the study of the absorption and emission of light and other radiation by matter, dependent on the wavelength of the radiation (Sinha et al., 2023). Chemometrics is a tool that uses mathematical principles to perform data processing, experimental design, pattern recognition of chemical data to unravel the complexity, and find the markers addressing the difference between two set of food samples (Kritikou et al., 2022). The application of chemometrics with spectroscopy techniques such as Fourier transform infrared (FT-IR), near-infrared (NIR), FT-NIR, mid-infrared (MIR), nuclear magnetic resonance (NMR), synchronous fluorescence (SF), mass spectrometry (S), Raman, and chromatographic method LC-MS have been recently employed in detection of adulteration.

FT-IR and FT-NIR spectroscopy coupled with chemometric methods is a rapid technique for detection of fraud (Nicolaou et al., 2010; Dvorak et al., 2016). Adulteration of cameline milk with bovine milk with partial least squares regression (PLS) (Souhassou et al., 2018), breast milk with bovine milk with principal component analysis (PCA), PLS, and discriminant analysis (DA) (Luca et al., 2019), and caprine and bubaline milk with bovine milk (Sen et al., 2021) were successfully predicted based on FT-IR data. FT-NIR spectroscopy, PLS and PLS-DA algorithms were used for authenticity of caprine milks and cheeses (Dvorak et al., 2016; Pereira et al., 2020). MIR was employed with PCA, PLS and multiple linear regression to detect and quantify the amount of bovine milk in bubaline milk (Goncalves et al., 2020). NMR coupled with PCA and DA is another method, which was proved to differentiate bovine, caprine and soy milks, with a LOD of 2% soymilk in bovine and caprine milks, and 5% bovine milk in caprine milk (Li et al., 2017), and to detect 5% of adulteration of caprine milk with bovine milk using N-aceytl carbohydrates as biomarkers (Rysova et al., 2021). SF data obtained were evaluated by PCA and PLS to find out the possible adulteration of bubaline and bovine milks (Velioglu et al., 2017; Ullah et al., 2020). Raman spectroscopy has also been successfully used recently with chemometric methods. Bovine milk in caprine milk (Yaman, 2020; Li et al., 2023), and possible adulteration in raw and pasteurized bovine, caprine and ovine milks (Yazgan et al., 2020) were investigated by Raman with PLS, soft independent modelling of class analogy (SIMCA), and DA algorithms.

Recently, metabolomics based on liquid chromatography (LC) has become a powerful tool for detection of adulteration. LC was firstly employed for determination of bovine milk in bubaline milk and Mozzarella cheese, using β-Lg as a marker (Czerwenka et al., 2010). High resolution MS was coupled with LC for detection and differentiation of milks of eight different species, with a LOD of 0.0005% (Zhang et al., 2022). Also, orotic acid, 4-aminonicotinic acid, and some bovine proteins (B2M and SCGB1D) were considered as bovine milk biomarkers for detecting adulteration in equine milk (Ji et al., 2023).

Spectroscopy and chemometric methods are appropriate for the green analytical chemistry, with minimal or no sample preparation, minimizing the use of chemicals, waste, and energy consumption (Luca et al., 2019; Pereira et al., 2020). These techniques have been preferred due to their low operational costs, simplicity, rapidness, and wide application potential (Dvorak et al., 2016; Goncalves et al., 2020; Sen et al., 2021; Li et al., 2023).

Novel Immunoassay Applications

Sakti et al. (2016) developed a quartz crystal microbalance immunosensor with an antibody specific to a protein at 208 KDa (PSS 208) as an indicator, which is found only in bovine milk and does not exist in caprine milk. The immunosensor detected PSS 208 with a LOD of 0.0001%. Liu et al. (2019) developed a lateral flow immunoassay (LFIA) with the help of a specific monoclonal antibody, labeled with colloidal gold nanoparticles binding to bovine milk casein, and LOD was 0.07% in caprine milk. LFIA with carbon nanoparticles and non-immunoglobulin antigen was also developed for fast detection of adulteration of bovine milk with bubaline milk (Sharma et al., 2021). An enzyme-free immunoassay was conducted using Prussian blue nanoparticles as antibody catalytic labels in a competitive colorimetric microplate immunoassay, for the detection of bovine milk in caprine milk, and 0.01% LOD was achieved (Seddaoui et al., 2022). Moreover, a label-free voltammetric immunoassay for detection of bovine IgG was developed with a LOD of 0.1%. A novel analytical approach was developed by Demiati et al. (2023), with the cyclic voltammetry technique using glassy carbon and evaluated by chemometric analysis. PLS algorithm provided clear discrimination between caprine and bovine milk and predicted the percentage of adulteration. Results show that voltammetric fingerprints and chemometrics may be a simple, low-cost, and fast technique to be used in detection of adulteration.

CONCLUSION

Since authenticity of milk and products is an ethical issue for consumers and producers, the methods used for detection of adulteration are significant and should be carefully carried out. The conventional protein and DNA-based methods of authentication present serious and adequate results, with low LOD values. However, novel methods are much more practical about time and labor. Therefore, further research should focus on rapid, low-cost, labor-friendly, and suitable on-site analysis techniques.

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CURRENT STOCK STATUS OF *Merlangius merlangus (LINNAEUS, 1758)* IN THE SEA OF MARMARA

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ABSTRACT

The distribution and abundance of *Merlangius merlangus* in the Sea of Marmara are presented in this study. Samples were collected between March 2017 and December 2018 at 34 stations from three different depth contours (20 - 50, 50 - 100, 100 - 200) of the trawl net in the Sea of Marmara. Sampling was carried out with bottom trawls according to the Mediterranean International Bottom Surveys (MEDITS) standards. The average speed was 3 miles per hour and the duration was 30 metres. The catch per unit effort (CPUE) of whiting was calculated to be 5.29 kg/h and the stock size per unit area was calculated to be 72.87 kg/km2 in the Sea of Marmara. According to the depth contours in the region, the CPUE and stock amounts are 16.03 kg/h, 220.80 kg/km2 at 20-50 m; 1.59 kg/h, 21.90 kg/km2 at 50-100 m; and 0.35 kg/h, 4.82 kg/km2 at 100-200 m, respectively. The species is the most dominant in the southern part of the Sea of Marmara according to the CPUE data of the stations. This is the first detailed study of whiting stock status in the Marmara Sea.

Keywords: CPUE, Marmara Sea, Whiting, *Merlangius merlangus*

INTRODUCTION

Merlangius merlangus (Linnaeus, 1758), known as whiting, is a species of high economic value belonging to the family Gadidae. It is distributed in the northeastern Atlantic, southeastern Barents Sea, Iceland and Portugal, Aegean Sea, Adriatic Sea and nearby waters, rarely in the northwestern Mediterranean Sea (Froese and Pauly, 2007). The species is found in the Black Sea, Marmara Sea and Aegean Sea in our country (Mater et al., 2003). It lives up to 20 years and reaches a maximum length of 70 cm. It usually inhabits sandy and muddy seabeds and occurs from 10 to 200 m deep (Froese and Pauly, 2007).

Bowers (1954) studied the reproduction and growth of the species in British waters, Hislop et al. (1991) studied the stomach contents and food consumption of the species in the North Sea, Labropoulou and Papaconstantinou (2000) studied the species distribution of deep-water demersal fishes in the northern Aegean Sea, Oven et al. (1995) studied the age composition and diet of the species in the Black Sea, Papaconstantinou et al. (1994) studied the abundance and distribution of the species in the Gulf of Thermatikos and the Thracian Sea in Greece, Pedersen (2000) studied its diet in the North Sea, Pope (1996) studied the stock structure in the North Sea, Seyhan and Grove (1998) studied food consumption, Whitehead et al. (1986) studied the biology of the species. İşmen et al. (2010) conducted a study to determine the bio-ecology and population dynamics of the species in Saroz Bay. Yıldız and Karakulak (2017) reported that whiting were found more at depths of 20-50 m in the Black Sea. Uzer et al. (2017) found that *M.merlangus* was one of the species with the highest catch rate in the Bosphorus. Sağlam and Soyer (2022) found the CPUE value of *M.merlangus* 69.36 in their research in the Black Sea. Karadurmuş (2022) determined that the species was under overfishing pressure in the Maramara Sea. There is no detailed study on the catch per unit effort and stock status of the *M.merlangus* in the entire Sea of Marmara. For this reason our study is important in terms of being a source for future study.

MATERIAL AND METHOD

This study is a part of a research project called "Determination of the population status and the stock estimation of economically valuable demersal fish in the Marmara Sea". Samples were collected by 246 bottom trawl hauls at 34 stasions in the Marmara Sea between March 2017 and December 2018. Samplings was conducted by bottom trawl according to the Mediterraean International Bottom Surveys (MEDITS) standards, with an average speed of 3 miles and a duration of 30 m. Trawl tows were conducted with commercial trawl vessel "Yalçınoğlu", which is 23.5 m in length with 450 hp engine power. Sampling stations covered 3 different depth contours (20-50; 50-100 and 100-200) and broad geographical area.

Catch per unit effort (CPUE) was calculated with the following formula: CPUE = [(ΣCi/Nc)/(Σt/Nc)], where CPUE: Catch per unit effort; Ci: sample catch amount per towing; t: towing time; Nc: operation number (Phiri and Shirakihara, 1999).

CPUA (Catch per unit swept area) was calculated as being the catch weight (Cw) divided by the swept area (a) for the species and for each haul (Spare and Veneme 1992): CPUA = Cw/a The swept area (a) for each hauling was estimated thus: a = D.h.X where h is the length of the head-rope and D is the cover of distance. X is the fraction of the headrope length which is equal to the width of the path swept by the trawl. The value of X varies from 0.4 to 0.66. It is suggested that X = 0.5 is the best compromise value for Mediterranean Sea (Spare and Veneme, 1992).

RESULTS AND DISCUSSION

During the two-year sampling period in the Sea of Marmara, the catch per unit affort (CPUE) of whiting in the whole area was calculated as 5.29 kg/h and the stock amount catch per unit area (CPUA) was calculated as 72.87 kg/km^2. It has been determined that the species is mostly found at depths of 20-50 m. According to the depth contours in the region, the CPUE and CPUA was determined that 16.03 kg/h, 220.80 kg/km^2 at 20-50 m; 1.59 kg/h, 21.90 kg/km^2 at 50-100 m; and 0.35 kg/h, 4.82 kg/km^2 at 100-200 m, respectively. Figure 1 shows the catch per unit effort of whiting in the Sea of Marmara by the stations It was determined that the species was most distributed in the southern part of the Sea of Marmara. The seasonal variation of CPUE were indicated in the Table 1.

The amount of production in our country has been in decline from year to year. In the 2000s, the average production was 1000 tonnes. However, after 2010, it showed a rapid decline (1063 tonnes) and decreased to 110 tonnes in 2021 (TUİK, 2022). Yıldız and Karakulak (2017) reported that the whiting was more likely to be found in the depth range of 20-50 m in the Black Sea. In the present study, it was found in a similar way that the species was found more in the depth range of 20-50 m. In the southern part of the Marmara Sea, Karadurmuş (2022) found that the species was under overfishing pressure. In our research, the high unit catch of the species was found in the South Marmara region. If overfishing was found in this region in 2020-2021, it can be said that the current stock situation for the whole Marmara Sea is more serious. The stocks of the species in the Marmara Sea can be better managed and regional and temporal bans may be applied.

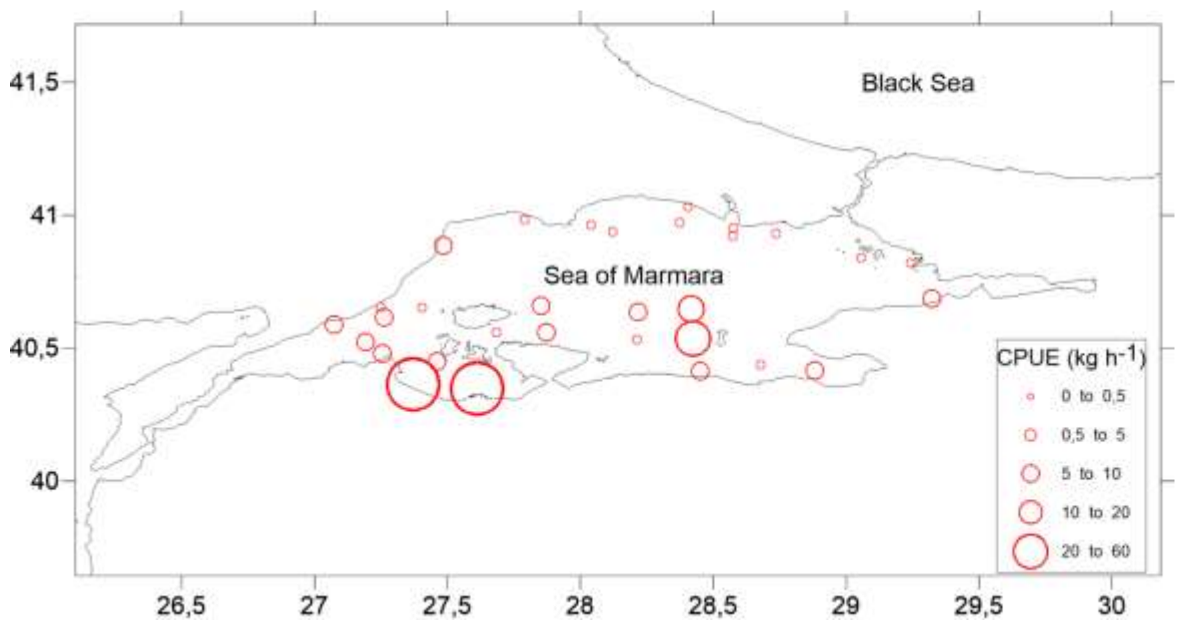

Figure 1. Sampling stations and CPUE values of *M.merlangus* in the Sea of Marmara

Table 1. CPUE values of *M.merlangus* according to depth and seasons

Seasons	CPUE kg h^{-1}			
	20-50 m	**50-100 m**	**>100 m**	**Mean**
Spring 17’	1.97	0.12	0.0	0.67
Summer 17’	74.97	3.12	1.51	22.57
Autumn 17’	18.31	1.80	0.16	6.15
Winter 17’	5.98	3.60	0.80	3.94
Mean 2017	**25.20**	**2.14**	**0.67**	**8.40**
Spring 18’	4.91	0.55	0.0	1.62
Summer 18’	5.83	0.47	0.04	1.81
Autumn 18’	4.41	2.50	0.04	2.69
Winter 18’	2.98	0.16	0.04	0.88
Mean 2018	**4.64**	**0.99**	**0.03**	**1.81**
Mean	**16.03**	**1.59**	**0.35**	**5.29**

CONCLUSIONS

According to this study, when the amount of CPUA and CPUE of *M.merlangus*, one of the species with high economic value in the Marmara Sea, was evaluated; it was determined that the species lived at a maximum depth of 20-50 m and was found much more in the southern part of the Marmara Sea.

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DETERMINING THE APPROACH AND EXPECTATIONS ACCORDING TO THE PROFILE OF ENTERPRISERS IN RURAL DEVELOPMENT SUPPORT: THE EXAMPLE OF THE WEST MEDITERRANEAN

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Abstract

The phenomenon of development is the common goal of both developed and developing countries and can be defined as the advancement of human life in the economic and social field and the increase of welfare by changing the economic, social, and political structures of the countries. The phenomenon of development should be achieved through social cohesion. While the development initiatives, which started with industrialization, manifested themselves in urban areas, rural areas were ignored. However, rural areas should not be excluded from the development initiative. This situation, which is seen as social development, has been prevented by industrialization. However, rural development initiatives that started in the 1960s are steps towards the integration of rural areas with urban areas and their inclusion in social development. Rural development initiatives that started in these years found their reflection in Turkey as well as in other countries of the world. The support given to rural areas has been the main basis for this. These supports to the rural areas were primarily applied on a regional basis, but they did not receive sufficient response due to the differences between the regions and the practices were not suitable for the local area. One of the most important supports to rural development initiatives implemented in Turkey is the Rural Development Investments Support Program (RDISP). Within the scope of this program, it is aimed to increase the income level in rural areas, to improve infrastructure, to ensure the integration of agricultural production and agro-industry, to strengthen food security, to create alternative income sources in rural areas, to increase the effectiveness of rural development activities, to increase the level of basic public services, to increase access to services and to create a certain capacity in rural society, taking into account the protection of natural resources. Although these applications are province-based, they were also applied in the provinces of Antalya, Burdur and Isparta, which are the Western Mediterranean Region. In this study, it is aimed to divide the enterprises benefiting from RDISP in the Western Mediterranean Region into groups, to reveal the profile of each group and to examine the benefits of support elements according to the characteristics of these groups.

Keywords: Rural area, Rural Development, Support, Western Mediterranean

INTRODUCTION

Countries attach importance to development to progress in economic and social fields and to ensure social welfare. Development is recognized as a process that involves increasing the level of social welfare and raising living standards. This process includes economic, social, and cultural dimensions. In these dimensions, economic development includes increasing the production power and raising per capita income, social development includes improving education, health and social services, and cultural development includes protecting and developing the society's own cultural values and heritage. However, development is the growth and development of a country by enabling it to become stronger economically, socially, and culturally. For the country to grow and develop, it is desirable for the society to reach the desired level in economic, social, and political fields. In addition, development is the reduction of human deficiencies emerging in countries by integrating them with ecological balance.

Development is the building of the future of a country. Therefore, in order for development to be balanced, it requires the participation of all dynamics of the country. The human structure, natural resources, economic activities, technological developments, social and cultural structures of the country are these dynamics. Ensuring harmony between these dynamics and developing planning and policies for this is an important factor for development. Countries develop various policies to ensure that there is no discrimination between communities and regions to achieve the desired goals in development. However, the impact of industrialization, the inability to create alternative sources of income in rural areas, the dominance of the agricultural sector in rural areas and the dependence of the sector on nature have caused rural areas to remain in the background. In this framework, rural development policies have started to be seen as a special policy area for individuals living in rural areas to reach humane living conditions, to increase their income levels and to provide them with the opportunities of individuals living in urban areas.

Rural development is defined as "the process of improving the quality of life and economic welfare of people living in rural areas" (Moseley 2003). Rural development is defined differently. According to the Croatian rural development network; "the integral and multi-sectoral and sustainable development of the rural (non-urban) area", according to Atkinson "efforts that are economic and social in nature, aimed at promoting the concepts of retention, growth and expansion in non-urban areas, including improving the quality of life for rural residents" and according to another source; it is explained as "a method of improving the quality of life and financial well-being of individuals living in particularly populated and remote areas" (Anonymous 2023a, Atkinson 2017, Anonymous, 2023b). To define rural development in more detail, it is "the process of increasing people's access to humane living conditions, improving income distribution, increasing income level, ensuring localized developments in social and cultural areas, protecting and utilizing natural resources and reflecting the wealth to the lives of individuals" (SPO 2006).

Rural development policies are policies designed to reveal the efforts made throughout the country to improve the economic, social, social, and cultural opportunities of the communities living in rural areas, to increase the living standards of these communities and to support them to participate in national development (SPO 2000).

Today, both developed and developing countries have increased the importance they attach to rural development. Rural development activities vary from country to country. Even in the USA, there are differences in the rural development program of each state (Gürlük 2001a). In the EU, the basis of rural development activities is to ensure the continuity of production in agriculture, protection of the environment and transparency during the conduct of different economic activities in rural areas (Can 2007). In the Agriculture and Rural Development Report published by the European Union in 2022, it is stated that the European Commission supports rural development through a series of programs and initiatives despite the difficulties encountered, that it is determined to make agriculture and rural development sustainable, and that priorities for the future are set out. The same report calls for continued co-operation between the European Commission, Member States, and stakeholders. In addition, about the rural development activities of the report, it is stated that the Union has helped to improve the quality of life in rural areas and progress has been made in making rural development more sustainable (EC, 2023).

In Turkey, the developments in technology and knowledge level, the increase in the use of machinery in agricultural production have caused the rural labor force to leave agricultural production and employment deficit in rural areas. In addition, the high rate of population growth and limited job opportunities accelerated migration from rural areas to urban areas and Turkey entered a rapid process of distorted urbanization after the 1950s. Migration and rapid urbanization caused by the development differences between rural and urban areas have created problems both in rural and urban areas. With the planned period, national development plans and programs were envisaged within rural development studies.

In this context, rural development projects have started to be implemented throughout Turkey. Rural development projects cover areas such as development of agriculture and animal husbandry, irrigation, improvement of wetlands, construction of village and forest roads, construction of drinking water ponds, provision of drinking water, increasing agricultural and animal production, afforestation activities.

In addition to general and regional activities for rural development in Turkey, various development-oriented programs are also carried out. One of these is the "Program for Supporting Rural Development Investments (RDISP)"(Taşcıoğlu, 2011).

The program aims to determine the procedures and principles for raising the income level in rural areas, improving infrastructure, ensuring integration of agricultural production and agro-industry, strengthening food security, creating alternative income sources in rural areas, increasing the efficiency of the rural development activities being carried out, increasing the level of basic public services, increasing access to services and creating a certain capacity in rural society, taking into account the protection of natural resources (OJ, 2006).

In this study, it was aimed to divide the enterprises benefiting from the program, which aims to create alternative sources of income in rural areas by evaluating the on-site processing of agricultural products to make rural development activities more effective, to reveal the profile and general structure of each group and to determine the opinions of these enterprises on the program.

MATERIAL AND METHOD

The research is supported by secondary data based on the literature but largely based on original data obtained through a survey based on face-to-face interviews with enterprisers of enterprises benefiting from the Rural Development Investments Support Program in the Western Mediterranean Region. A significant number of these enterprisers (e.g. those who benefit from irrigation investments or process their own produce) are also engaged in agricultural production.

The study was conducted in the Western Mediterranean Region. The Western Mediterranean Region is the region called TR61 in the Classification of Statistical Regional Units (IBBS) Level 2, which covers the provinces of Antalya, Burdur and Isparta in the west of the Mediterranean Region. The region has been home to various civilizations since the early ages due to its geographical location, fertile soils, and rich water resources.

In the study, a survey was conducted with the owner/manager (enterpriser) of 47 enterprises in Antalya province, 26 enterprises in Burdur province and 23 enterprises in Isparta province benefiting from the Rural Development Investments Support Program. Face-to-face interviews were conducted with a total of 96 enterprisers benefiting from the support program in the region in question.

In the analysis of the data, simple descriptive statistics and Cluster Analysis were used to divide the enterprisers into groups according to their level of utilization of the program and to reveal the profile of each subgroup and to develop appropriate policy recommendations for the target groups.

Cluster analysis is one of the multivariate statistical analyses that divides units and objects into classes by arranging them in general. Kaufman and Rousseuw (1990) define cluster analysis as a method that enables to classify the units examined in research by gathering them in certain groups according to their similarities, to reveal the common characteristics of the units and to make general definitions about these classes. Hair and Black (2000), after stating that the primary reason for using cluster analysis is to find similar (homogeneous) groups of individuals in any data set, define cluster analysis as a collection of objective methods that quantify the structural characteristics of units in observation clusters.

The aim of the analysis is to reveal the similarities of the units according to certain characteristics and to classify the units on the basis of these similarities and to group the units in such a way that they are like each other.

Although cluster analysis is an analysis based on classification theory, it differs in some respects. The most important of these is that the classification technique is used to divide observations into different subgroups, whereas in clustering, sub-clusters are tried to be formed based on p variables (Kendall 1975).

In this study, clustering analysis was conducted to reveal the preferences of enterprisers in determining the type of support in rural development and to classify enterprisers into groups in terms of their characteristics. The main reason for making this distinction is to reveal which support instruments are preferred by the groups to be formed according to the characteristics of the enterprisers. The aim here is to examine the opinions of the groups to be formed among the enterprisers with the same characteristics about the program and to determine the expectations of the enterprises in such supports to be applied in the future. The results obtained from the clustering analysis will be presented in detail in the findings and discussion section.

RESULTS AND DISCUSSION

In the study, the field research was examined in two stages: general descriptive information about the enterprises benefiting from the program and the results of the analysis. Firstly, information about the enterprises benefiting from the program is given in Table 1.

In the Western Mediterranean Region, the highest number of enterprises benefiting from the program is in Antalya province. Although the region is located in the same geography, it shows differences in terms of climate conditions and soil fertility. While Antalya province has the typical climate conditions of the Mediterranean Region, Burdur and Isparta provinces show the common characteristics of the Mediterranean and continental climate zone. This situation also affects the agricultural sector and agriculture-based industry. In addition, due to the entrepreneurial characteristics of Antalya province and the fact that individuals are in closer relations with agricultural organizations, they have more information about such supports.

Table 1. General characteristics of enterprises benefiting from RDISP in the Western Mediterranean Region

		Rate (%)
Distribution of enterprises by province	Antalya	49.0
	Burdur	27.0
	Isparta	24.0
Legal structure of businesses	Company	56.3
	Cooperative, Union	21.9
	VSPU	18.8
	Sole proprietorship	3.1
Status of interviewees in enterprises benefiting from the program	Business manager	52.0
	Business owner	24.0
	Cooperative, Union President	18.0
	Operating partner	6.0
Education level of the interviewees	Primary School	6.0
	Middle School	12.0
	High School	28.0
	Associate degree	20.0
	University (Undergraduate)	32.0
	Postgraduate	2.0

When the enterprises benefiting from the support in the region are classified according to their legal structures, it is seen that the highest number of investments are made by limited, joint stock and collective companies. It is observed that companies utilize more than half of the total economic investment (56%). After the companies, development and irrigation cooperatives and unions (22%) and Village Service Provision Unions (VSPU) operating under district governorships (19%) made the most investments. Individuals or bilateral partnerships were the least beneficiary enterprises (3%). 56% of the enterprises benefiting from the program are in company status and 80.9% of the total beneficiary enterprises in Antalya province and 52.2% of the enterprises benefiting from the support in Isparta province are in company status in terms of their legal structures. On the other hand, in Burdur province, due to the effective work of the provincial governorship and the fact that they see the program as an opportunity, Village Service

Provision Unions (approximately 60%) were the enterprises that benefited the most from the program.

Within the scope of the survey, most interviews were conducted with people who were in managerial positions such as business managers, accountants, etc. and who were actively working in the application to the program. At the company level, interviews were mostly conducted with company managers or company owners, chairmen or partners in cooperatives and unions, chairmen or managers in VSPU, and partners in sole proprietorships.

It was observed that the education level of the interviewees was generally high. This feature is directly related to the beneficiary status of the program. Because there is a one-to-one relationship between having information about the program and the education level of the people. This situation emerged from the general structure of the interviewees during the observations made during the survey period.

Within the scope of the program, many projects have been supported throughout Turkey. Information on the provinces and fields of activity of the enterprises receiving grant support in the Western Mediterranean Region is given in Table 2.

Table 2. Fields of activity of enterprises benefiting from RDISP by provinces distribution

Activity	**Antalya**		**Burdur**		**Isparta**		**Total**	
	Quantity	Rate (%)	Quantity	Rate (%)	Quantity	Rate (%)	Quantity	Rate (%)
Processing, packaging, storage	31	66.0	12	46.2	16	69.6	59	61.5
Drip irrigation	9	19.1	11	42.3	4	17.4	24	25.0
Capacity expansion	7	14.9	-	-	3	13.0	10	10.4
Sewerage, road	-	-	3	11.5	-	-	3	3.1
Total	**47**	**100.0**	**26**	**100.0**	**23**	**100.0**	**96**	**100.0**

Within the scope of the program, enterprises in the region that determined their own field of activity benefited from grant support for processing, packaging, and storage (61.5%). This situation is valid for all three provinces in the region. Agricultural production is intensive in the region due to the fact that the climate and soil fertility is suitable for fruit and vegetable production compared to other regions of Turkey in general. Intensive agricultural production increases the desire to meet post-production services from within the region. In this context, the program has been an opportunity for the enterprises in the region, and the need for processing and packaging of the products produced has been met to a certain extent. In addition, the need for storage of agricultural products produced in the region has been met through the support program. In this respect, it has been observed that the support program has benefited the rural areas of the Western Mediterranean Region.

The analyses of the enterprises benefiting from RDISP supports were summarized and divided into two clusters according to the non-hierarchical K-means clustering method. Individuals in cluster 1 constitute 54% of the total population and those in cluster 2 constitute 43% of the total population. Information about the clusters obtained is given in Table 3.

Table 3. Group and characteristics of enterprises benefiting from RDISP according to cluster analysis.

Criteria	**1st group**	**2nd group**
Field of activity of enterprises	Activity related to processing, packaging, and storage	Irrigation activity
Project subject of the enterprises	Animal and herbal products	Irrigation activity
Legal structure of businesses	Company	Cooperative and Village Service Union
Interviewees	company owners/managers	president
Education level of interviewees	Elementary and high school	Associate degree and undergraduate
Other sources of income the business	Have additional income	No additional income

Table 3. continued

Criteria	**1st group**	**2nd group**
Membership status of the operator to the agricultural producer organization	no membership	has a membership
How the courses to be organized in the region should be	Practical courses with a subject expert	Meeting, seminar etc.
Reason for businesses to do the project	Being an enterprise that the region needs, Support is an opportunity to establish an economic enterprise, Processing its own product,	Protecting water resources Saving natural resources and energy
Type of investment of enterprises in the project	establishing new businesses and upgrading technology	Increase capacity
Those working on project implementation and reporting	Business managers and private consultant	Personnel working in the company
Businesses' source of information about the program	Meetings organized by the provincial directorates of the Ministry and friends	Provincial Chamber of Commerce and Industry, governorship units
Whether the enterprises have other applications for support	Businesses with more than one application	Businesses with a single applicant
Difficulties encountered by enterprises during project application	Businesses facing difficulties in applying	
Businesses' intention to make other investments in the region in the future	They will make other investments	They will not make any other investments
Investments that enterprises intend to make	Cotton processing, animal husbandry, milking unit,	

in the future	greenhouse cultivation, cold storage, and packaging facility	
Benefits of the program to the region	Collective decision-making and social solidarity	
Problems with the program according to enterprises	Excessive demand for equity capital, long investment period and the necessity of private consultancy	The evaluation period is long, the number of documents required is high, the support rules are strict, and the Ministry staff do not have sufficient knowledge
The idea of businesses to develop environmentally friendly projects and ensure environmental protection	Businesses that do not harm the environment and pay special attention to environmental protection	
Increased job opportunities in rural areas		Businesses arguing that their investments in rural areas will increase job opportunities for people living in rural areas
The situation of using the knowledge and experience of the company owners/managers	Businesses that think that their own knowledge and experience are important in business management	
Experts make business decisions		Businesses that advocate that the final decision on management and other issues in businesses should be taken by specialists
Adequacy of the amount of support		Businesses that agree that the amount of support is sufficient

Table 3. continued

Criteria	1st group	2nd group
Reasons for businesses to choose project topics	Thinking it will bring good income.	Reasons for businesses to choose project topics
Goals that businesses want to achieve with the project	Evaluating products To evaluate the existing resources of the region Increase capacity. The need of the business Contributing to the development of the region Technology innovation Exporting	To utilize the resources of the region The need of the region Protecting water resources Contributing to the development of the region Increasing efficiency Meeting the needs of the producer
Businesses' expectations from the government regarding the overall program	Increasing the monetary amount of support Expansion of area of activity Providing support separately according to sectors Giving to people who will produce. Separate grant for building construction	Expanding the scope of support Benefiting from the same support subject for a second time
Reasons for the continuation of the program according to the company owners/managers	Investment opportunity for businesses Providing financial contributions to businesses Increasing employment	Contribution to the region Making investments that cannot be made in the region. Protection of natural resources Ensuring effective use of

		resources Saving time and labor
Problems with the program according to the company owners/managers	Taxes within the support amount Lack of financial support Too many documents requested. Long evaluation period after application	No problem
Aspects of PSRDI that need improvement according to company owners/managers	The amount of money in support should be increased. Support should be tax-free. The number of required documents should be reduced. Should be given differently according to the sector. Support should be given to projects to encourage production. Facilitate the application process	No need to improve. Support for cooperatives should be diversified and prioritized

CONCLUSIONS

Development is the reflection of the changes to be made in social, economic, and cultural structure on human life, reaching the desired living conditions of people, increasing their income levels economically. In order to realize this, it is necessary to use the natural and human resources and technological structure of the country. For this purpose, the creation of policies should be done in a planned manner.

To realize the development initiative, it should be carried out without regional distinctions. Since the focus of development is seen as urban areas, more importance should be given to rural areas where agriculture and food products are produced, nutrition needs are met, and alternative income opportunities are limited. For this purpose, rural development policies have been implemented.

Rural development activities vary from country to country. Along with the developments in rural development in countries, various policies are developed and implemented for the development of rural areas in the world.

In Turkey, rural development activities and policies have been increasing in parallel with development initiatives in recent years. The "Rural Development Investments Support Program", which was established according to the principles specified in the National Rural Development Strategy (NRDS) issued for this purpose, is one of the most important ones.

The main purpose of this study is to categorize the enterprises benefiting from the RDISP in the Western Mediterranean Region into groups and to reveal the profile of each group. According to the results obtained in line with this main objective, it is examined how the supports for rural development should be on the basis of enterprises, and what kind of priorities and expectations the enterprises prefer in future such programs and/or supports.

According to the clustering analysis applied in the study, enterprises are divided into two groups.

The first group of enterprises benefiting from the support element are enterprises with company status that apply to the program for the processing of agricultural products, which is the next stage after the production of both plant and animal products, or for technology renewal in the existing enterprise. The second group of enterprises are agricultural producer organizations such as cooperatives and unions that support the producers to carry out the irrigation activities necessary for agricultural production.

The first group of enterprises benefited from the program to purchase equipment for processing.

While interviews were conducted with managers and company owners in the first group enterprises, interviews were conducted with the heads of agricultural producer organizations in the second group enterprises.

While the education level of the interviewees in the first group enterprises was primary school and high school, the education level of the interviewees in the second group enterprises was associate degree and bachelor's degree.

In the first group of enterprises, it is seen that the enterprises have different income-generating sources other than the program application, while in the second group, there are no income sources because they are agricultural producer organizations, and they are non-profit organizations in line with the objectives and principles of producer organizations.

It was observed that the first group enterprises were not members of agricultural producer organizations because they were engaged in commercial activities.

While the first group of enterprises wanted the courses for rural areas to be in the form of applications, the second group of enterprises preferred more general applications such as meetings and seminars.

While the first group of enterprises think that the main reasons for doing the project are that there is an enterprise that the region needs, the grant support received is an opportunity to establish an enterprise in the economic sense, and to process their own products, the second group of enterprises are to protect water and natural resources and to save energy.

The first group of enterprises benefited from the support program to establish new enterprises and renew their technologies. The second group of enterprises applied to the program to make use of the water resources of the region, to make the existing irrigation systems work more efficiently and to open more areas for irrigation.

While the first group of enterprises worked with enterprise managers and private consultants in the preparation and reporting of the project in the application to the support program, the personnel of the producer organizations worked in the second group of enterprises. The first group of enterprises encountered various difficulties during the application due to the fact that they made the preparation and reporting of the project through managers, private consultants, and technical staff.

The information sources of the first group enterprises about the support program are the meetings held by the Ministry of Agriculture and Forestry. The second group of enterprises received information about the program from both public institutions and chambers of commerce and industry.

The first group of enterprises applied to benefit from the support program by making more than one application. The second group of enterprises did not have a second application.

The opinions of the two groups of enterprises about the functioning of the support program are as follows.

The first group of enterprises think that they will establish facilities for processing, packaging, and storing agricultural products in the future, thus more people and enterprises will benefit from the support program and alternative income and employment opportunities will be provided to the rural areas. This situation, which is important for rural development studies, is thought that the incomes of individuals living in rural areas will increase and rural development studies and supports will provide the desired effect in rural areas. In addition, this group of enterprises stated that with the increase in the support program, they will transfer other economic investments to the region in the coming years. On the other hand, since the second group of enterprises received support from the program only for irrigation-based projects, they do not have any thoughts about investing in the region in the future.

The first group enterprises add that with such support elements, besides the economic benefit that the enterprises will provide to the region, there will be elements that support social solidarity and collective decision-making.

When the problems encountered within the scope of the support program are examined, it is seen that the most important problems are the high demand for the amount of equity of individuals or companies, the long investment period and the necessity of a special consultancy system, while the second group of enterprises consider the long evaluation period, the excessive amount of required documents and the strictness of the support rules as the most important problems.

The opinions on the development of environmentally friendly projects are considered important for the first group enterprises in terms of developing projects that do not harm the environment and thus creating projects that respect the environment and protect nature in rural development studies.

Within the scope of the opinion on increasing job opportunities in rural areas, especially the enterprises with agricultural producer organizations, which are the second group of enterprises, are in the position of enterprises that advocate that investments to be made in rural areas will provide new job opportunities for the rural community.

When the reasons for the enterprises to choose the project subjects within the scope of the support program are analyzed, the first group of enterprises are of the opinion that such supports will bring alternative and good income to the region and the rural community, the products produced in the region will be evaluated with the establishment of an enterprise that the region needs, the needs of the enterprise will be met with financial support for new technology and modernization, the support will be seen as an opportunity for those who want to establish an enterprise in the region, the enterprises producing in the region will process their own products and increase regional and local production. In the second group of enterprises, the determination of the project subjects came to the forefront due to the fact that an enterprise that is needed in the region will be established by providing services to the rural area, the sustainability of natural resources will be ensured by protecting water resources, water saving will be ensured with irrigation systems and there is a support element within the field of activity of the enterprise.

The objectives that the enterprises want to realize with the project are to evaluate the products of the first group enterprises, to evaluate the existing resources of the region, to improve the business capacity of the region, to reach the elements needed by the enterprise, to contribute to the development of the region, to renew the technology and to export, which will contribute to the development of production and the region in general. In the second group of enterprises, the evaluation of the existing resources of the region, especially the evaluation of water resources, meeting the irrigation needs of the region, protecting water resources and transferring them to future generations, contributing to the development of the region, increasing the yield of agricultural products with irrigation and meeting an important need of the enterpriser has been determined as the target to be achieved in the project.

Enterprises have various expectations in the support program. The first group of enterprises expect that the monetary amount of the support should be increased, the fields of activity should be extended to the whole rural area, the support should be given in different qualities and quantities according to the sectors, the support should be given to the people who will produce, and a separate grant support should be established for the construction of buildings. The second group of enterprises demanded that the scope of the grant capacity should be expanded and that they should be able to benefit from the existing support program for the second time.

When the reasons for the continuation of the program according to the enterprises are examined, the first group of enterprises are the enterprises that argue that the program is an investment opportunity for the enterprises, provides financial contribution to the enterprises and increases the employment opportunities of the region. The second group of enterprises, on the other hand, want the program to continue for reasons such as the protection of natural resources related to the natural structure, efficient use of resources, saving time and labor, contributing to the rural development activities of the region, and making investments that cannot be made in the region.

As for the problems related to the program, the first group enterprises consider the reduction in the amount of support due to the fact that the value added tax rate is included in the grant program, the fact that the amount of support is not sufficient financially for enterprise establishment, capacity increase and modernization, the excessive amount of documents required before the application and at the time of implementation of the project, and the long evaluation process of the application as the most important problems of the program. The second group enterprises argue that there are no problems with the program.

In this study, the expectations of groups with similar characteristics from rural development policies were investigated. Rural development studies have differences from other policy implementations. In rural development policies, the characteristics of the rural area, its potential, the social, economic, and social structure of the rural community, etc. need to be analyzed. It is expected that the analysis of the rural area and the society and the support elements to be made according to the social structure of the social structure, the groups showing similar characteristics in the face of situations and events are classified and the implementation of rural development policies for these groups can increase the effectiveness of existing policies.

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DETERMINATION OF SUPPORT PREFERENCES OF ENTREPRENEURS UTILIZING SUPPORTS POLICIES FOR RURAL DEVELOPMENT BY CONJOINT ANALYSIS

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ABSTRACT

Countries attach importance to development for the continuity and sustainability of societies. Economic, social, political, etc. of development It is expected that it will change in areas, and this will be reflected in society. Development starts with economic indicators and can be achieved with improvement in social indicators. Changes in economic indicators around the world have gained rapid momentum mainly in industry and service sectors. The change in the industrial sector, which started especially in the urban area, caused the rural area to remain in the background. Elimination of the separation of urban and rural areas, which is necessary for social development, has begun to be achieved by supporting rural development studies. Different models have also been used in rural development studies, and they have changed due to the general characteristics of the rural area. Various studies have been made and are being carried out for the development of rural areas in Turkey. In recent years, various programs have been implemented to support rural development studies. The main purpose of these programs is to increase the income level in rural areas, to improve the infrastructure, to ensure the integration of agricultural production and agro-industry, to strengthen food security, to create alternative income sources in rural areas, and to increase the effectiveness of the rural development studies, considering the protection of natural resources. The most important of these programs is the Rural Development Investment Support Program (RDISP). In the program, which includes the support given on a provincial basis, it is aimed to process and evaluate agricultural products, and to ensure the integration of agriculture and industry in rural areas. However, within the scope of the support, the preferences of the business owners/ manager (enterprisers) in rural development supports were not considered. Support preferences of entrepreneurs are important in terms of entrepreneurial activity. Support elements and types are a situation that encourages entrepreneurs to start businesses.

This study, it is aimed to determine the support preferences of the enterprisers benefiting from Rural Development Investment Support Program in the Western Mediterranean Region, which includes the provinces of Antalya, Burdur, and Isparta, by using conjoint analysis. Thus, it is aimed to determine the degree of influence of the policy set that maximizes the utility of the business owners/ manager and the characteristics of the manufacturer in this policy preference.

Keyword: Rural development, Support policies, Conjoint analysis

INTRODUCTION

Development is the common goal of developed and developing countries. Development can be defined as the change in the economic, social, and political structures of countries and the progress and welfare of human life in the economic and social fields. Harris (1992) defines development as the growth of the economy, change in its structure, improvement of income distribution and improvements in the political and cultural spheres. In the Special Specialization Commission (SPC) Report of the State Planning Organization (SPO) for the 9th Development Plan, development is defined as "the process of increasing people's access to decent living conditions, improving income distribution, raising income levels, ensuring locally appropriate developments in social and cultural areas, protecting and using natural resources, and reflecting wealth on the lives of individuals" (SPO, 2006a). Within the framework of the human dimension, development is the mobilization of the existing power to reduce the human deficiencies that arise in countries to a great extent and to increase the welfare of people in material terms. In the light of comprehensive definitions, development is not only the increase in the income of individuals in economic terms, but also social and cultural developments and the phenomenon of living in greater social welfare.

For development to be balanced, it is necessary to ensure harmony between elements such as population dynamics, natural resources, economic activities, technology level, social and cultural structures of the country. As a result of the analysis of these factors, planning and policy formulation and development processes have an important role in the success of development. To achieve the expected goals in development, improving the qualifications of the society in terms of education, health, and manpower, raising the standard of living, eliminating the differences between regions and settlements should be one of the most important goals (Anonymous, 2002).

The economic dimension of development started especially with the industrial revolution. This change in the industrial sector has led to the development of the economic structure and its reflection on human life and the concentration of the population in urban areas where the industrial sector is intense. The inability to create alternative sources of income other than the agricultural sector in rural areas has caused this change to bring urban areas to the forefront and rural areas to be ignored.

The first foundations of rural development were started to be established with the traditional rural development approach, which started in the 1960s, with the increase in ideas about the lack of distinction between urban and rural areas for social development. Today, different models and methods of rural development approaches have been applied, rural development policies have been harmonized with sustainable development policies and changes in rural structure have been tried to be achieved.

The change in the industrial sector in the world has also been experienced in our country, especially with migration and rapid urbanization, which has led to the emergence of various problems in both rural and urban areas and the development at the national scale has not reached the desired dimensions.

With the Planned Period, various strategies were developed and put into practice in order to increase infrastructure and public services for rural areas and to accelerate rural development (SPO, 2006b). In addition to the basic legal regulations on rural areas, issues such as rural development, village problems and village development have been mentioned in all national development plans, and the priority targets for rural areas have been determined in these plans and programs.

The main purpose of rural development policies is to improve the economic, social, and cultural opportunities of the communities living in rural areas, to bring these communities to the national level of living, and to ensure their full participation in national development (SPO, 2000). A significant portion of the world's population lives in rural areas, and these communities provide their development in economic and socio-cultural areas, especially in the agricultural sector, with their own means or through external support (Taşcıoğlu, 2011).

With the planned period in Turkey, approaches to rural areas and rural development were generally different from the previous period. In addition to the basic laws enacted in the previous period, industrialization, modernization in agriculture and urbanization were considered in rural development and it was emphasized that rural development was a part of national development and should be handled together (Çağlar, 1986). In addition, regional development projects were implemented during this period and continue to be implemented.

In addition to general and regional activities for rural development in Turkey, various development-oriented programs are also carried out. One of these is the "Program for Supporting Rural Development Investments (RDISP)", which was established according to the principles set out in the National Rural Development Strategy (NRDS) published in 2003 and put into practice after being published in the Official Gazette dated 06.04.2006 and numbered 26131. The Program aims to determine the procedures and principles for raising the income level in rural areas, improving infrastructure, integrating agricultural production and agro-industry, strengthening food security, creating alternative sources of income in rural areas, increasing the efficiency of ongoing rural development activities, increasing the level of basic public services, increasing access to services and creating a certain capacity in rural society, taking into account the protection of natural resources (OJ, 2006).

The scope of the program is determined as the issues related to what needs to be done in order to encourage and support the economic activity investments of real and legal persons for the processing, evaluation, and marketing of agricultural products and the investments of organizations for the rehabilitation of existing infrastructure facilities in order to ensure economic and social development in rural areas within the provinces determined for the projected investments based on equity capital to be made individually and/or collectively by agricultural enterprisers (business owners/managers) in rural areas within the framework of development plans and programs and the National Agricultural Strategy.

Within the scope of RDISP, projects for village-based Irrigation facilities that ensure participation with a bottom-up approach, develop local capacity and organization, have the potential to create employment, increase and diversify entrepreneur incomes, encourage the increase in the level of education and entrepreneurship of the female population, and are based on the development and expansion of small and medium-sized industries based on agriculture are supported.

This study, it is aimed to determine the support preferences of the enterprisers benefiting from Rural Development Investment Support Program in the Western Mediterranean Region, which includes the provinces of Antalya, Burdur, and Isparta, by using conjoint analysis. Thus, it is aimed to determine the degree of influence of the policy set that maximizes the utility of the enterprisers and the characteristics of the entrepreneurs in this policy preference.

MATERIAL AND METHOD

The research was carried out with original data, supported by secondary data based on the literature, but largely obtained through a survey based on face-to-face interviews with enterprises benefiting from the Rural Development Investments Support Program in the Western Mediterranean Region.

The study was conducted in the Western Mediterranean Region. The Western Mediterranean Region is the region called TR61 in the Classification of Statistical Regional Units (IBBS) Level 2, which covers the provinces of Antalya, Burdur and Isparta in the west of the Mediterranean Region, with Muğla and Denizli in the west, Afyon and Konya in the north, Karaman and Mersin in the east, and the Mediterranean Sea in the south. The region has been home to various civilizations since ancient times due to its geographical location, fertile soils, and rich water resources.

A "field survey" covering the enterprises benefiting from the Rural Development Investments Support Program was conducted in the area called TR61 according to the Statistical Regional Units Classification (IBBS) Level 2, which includes the provinces in the Western Mediterranean Region (Antalya, Budur and Isparta). Information on these enterprises was obtained from the Support Branches of the Provincial Directorates of Agriculture.

In the study, 47 enterprises in Antalya province, 26 enterprises in Burdur province and 23 enterprises in Isparta province benefiting from the Rural Development Investments Support Program were surveyed. Face-to-face interviews were conducted with a total of 96 enterprises benefiting from the support program in the region in question.

Conjoint Analysis methods, one of the multivariate analysis techniques, were used to analyze the data. Conjoint, as a word, means collective participation. The word Conjoint was formed by combining the words consider and joint (Churchill and Lacoubicci, 2002). If a Turkish equivalent is desired, it can be called "Analysis of Relationships", "Association Analysis" or "Composite Analysis" (Yiğit, 2008). With conjoint analysis, it is possible to define the service as combinations of quality levels and to determine the quality levels and the detailed judgments of individuals towards that service (Gill and Sanchez, 1997). Conjoint analysis is a multivariate analysis technique used to analyze individuals' preferences for different combinations of measured and unmeasured attributes. According to another definition, Conjoint analysis is defined as a method of systematically evaluating and estimating a decision maker's choice of a limited number of alternatives (Joel, 2002). This analysis is a method that tries to determine which features a newly developed or already existing product or service should have, to reveal the preference behavior of individuals who benefit from this service and to determine the most desirable features of the service.

In this analysis, it is assumed that the value people place on a service corresponds to the sum of the benefits they derive from all its identified attributes, and that they will then use that service in proportion to the benefits they derive from it. Utility is a highly subjective phenomenon that varies from person to person. It would therefore be difficult to know without the help of Conjoint analysis. The analysis is widely used in a wide range of fields and can be used in new service planning to determine the impact of innovations and in efforts to improve existing achievements.

The starting point of Conjoint analysis is based on "Total Benefit Theory". In the partial benefit contribution model, the partial benefits of each attribute level of the product are independent of each other and the sum of the partial benefits of these attribute levels constitutes the total benefit.

In Conjoint analysis, two different calculation methods are used to determine the importance levels of policy-related features. The first one is to determine the difference between the partial utility values of each attribute. The other way is to calculate the relative importance levels of the combinations. The difference between the partial utility values of the attributes is the difference between the two attribute levels with the highest and the lowest partial utility value. This value shows the relative importance of each level of each combination in the combination. In measuring the relative importance between combinations, the partial benefit change values calculated for each combination are proportioned to the total partial benefit change value.

When applying Conjoint analysis, it is important to determine the variables and measurement methods at the beginning. The stages start with defining the problem and determining the research purpose, and end with determining the variables and levels and collecting and evaluating the data accordingly.

The purpose of Conjoint analysis is to determine the priorities and options that affect the outcome in the decision phase (Schweikl, 1985). The first step in the analysis is the selection of the preference function that will determine the effect of the factor characteristics that have an impact on the preferences of the people participating in the analysis on the decision. This function is the basis for determining the partial values of the factor attributes that affect the preferences of the participants in the analysis (Gutsche, 1995; Green and Srinivasan, 1978). The most used models are the ideal vector model, the ideal point model and the partial benefit model (Gustafsson, 2003).

As in all statistical studies, the first step in conjoint analysis is to determine the decision mechanism and objectives of the research problem. The point to be considered at this stage is that the research problem can be solved by defining preferences between variables and variable levels.

Within the scope of the Conjoint study, the selection of the factors and their levels to be included in the cards to be shown to the interviewee is a critical step. For this reason, the researcher should pay attention to the following points while determining the characteristics and levels of the product or service:

- Factors should be determinative in a way that they could influence individuals' choice. Any factor that is not related to choose should not be included in the study. However, the inclusion of factors that are important but do not create differences between preferences will make it difficult for the respondent to decide (Hair et al. 1995).
- Factors should provide complete and meaningful information about the service and be realistic.
- Factors should be practical and represent a single concept. The use of factors that include more than one dimension such as quality should be avoided.

➢ Factors should be easily communicated by the interviewee to enable a realistic assessment.

➢ The number of factors included in the analysis directly affects the reliability and statistical validity of the results. In addition, when the number of factors and factor levels is increased, the increased number of parameters will either lead to the presentation of more cards or to a decrease in the validity of the parameters.

In addition, many factors may cause respondents to be reluctant to participate in the research, as it would take too much time.

In this study, conjoint analysis was used to determine the support preferences of the enterprisers benefiting from RDISP in agricultural policy and rural development policy. Thus, the policy set that maximizes the benefit of the enterprisers and the degree of influence of the characteristics on this policy preference of the enterprisers were determined. At this stage, first of all, 5 factors required for the policy were determined and these are support type, support amount, support area, investment period and tax exemption. While determining the factors and factor levels, the factors and factor levels previously given in the supports for this field and given within the scope of this program were used. Factor levels according to these factors are given in Table 1.

Table 1. Factors and Factor Levels Used in Conjoint Analysis

Factors	**Factor Levels**			
	1	**2**	**3**	**4**
Support area	Animal husbandry	Greenhouse cultivation	Irrigation	Manufacturing industry
Type of support	Cash payment	Building construction	Machinery purchase	-
Support amount (rate)	25%	50%	75%	-
Investment period (months)	9	12	15	-
Tax exemption	None	2 years	3 years	-

The combinations to be used in the analysis according to factors and factor levels were determined as 16 in the SPSS package program. Accordingly, the combinations were formed as shown in Table 2.

Table 2. Conjoint Analysis Combinations

Card No	**Type of support**	**Support amount (rate)**	**Support area**	**Investment period (months)**	**Tax exemption**
1	Machinery purchase	50%	Greenhouse cultivation	12	None
2	Machinery purchase	50%	Animal husbandry	9	3 years
3	Cash payment	50%	Animal husbandry	9	2 years
4	Building construction	75%	Animal husbandry	15	None
5	Cash payment	25%	Irrigation	9	None
6	Cash payment	25%	Greenhouse cultivation	15	None
7	Building construction	25%	Greenhouse cultivation	9	3 years
8	Cash payment	75%	Manufacturing industry	12	3 years
9	Cash payment	75%	Greenhouse cultivation	9	2 years
10	Machinery purchase	75%	Irrigation	9	None
11	Building construction	25%	Irrigation	15	None
12	Machinery purchase	25%	Manufacturing industry	15	2 years
13	Cash payment	25%	Animal husbandry	12	None
14	Building construction	50%	Manufacturing industry	9	None
15	Cash payment	25%	Manufacturing industry	9	None

16	Cash payment	50%	Irrigation	15	3 years

RESULTS AND DISCUSSION

The study was examined in two stages: general descriptive information about the enterprises benefiting from the program and the results of the analysis. First, information about the enterprises benefiting from the program is given below.

In the Western Mediterranean Region, the highest number of enterprises benefiting from the program was in Antalya province. In Burdur and Isparta provinces, the need for agriculture-based industry differs from Antalya due to the nature of the investments made, and this is also reflected in the program.

When the enterprises benefiting from the support in the region are classified according to their legal structures, it is seen that most investments are made by limited, joint stock and collective companies. More than half of the support program (56%) was used by companies. After companies, development and Irrigation cooperatives and unions (22%) and Village Service Provision Unions (VSPU) operating under district governorships (19%) made the most investments. Individuals or bilateral partnerships benefited the least from the program (3%) (Table 3). In Antalya and Isparta provinces, companies benefited the most from the program, while in Burdur province, Village Service Provision Unions (approximately 60%) benefited the most from the program due to the effective work of the provincial governorship and the fact that they saw the program as an opportunity (Table 3).

Table 3. Distribution of legal structure of program enterprises by province

Legal Structure of Support Beneficiaries	**Antalya**		**Burdur**		**Isparta**		**Total**	
	Quantity (person)	**Rate (%)**	**Quantity (person)**	**Rate (%)**	**Quantity (person)**	**Rate (%)**	**Quantity (person)**	**Rate (%)**
Company	38	80.9	4	15.4	12	52.2	54	56.3
Cooperative, Union	7	14.9	5	19.2	9	39.1	21	21.9
VSPU	1	2.1	15	57.7	2	8.7	18	18.8
Sole proprietorships	1	2.1	2	7.7	-	-	3	3.1
Total	**47**	**100.0**	**26**	**100.0**	**23**	**100.0**	**96**	**100.0**

The survey was conducted with the people who are in the managerial positions such as business manager, accountant, etc. in the enterprises benefiting from the program and who are actively working in the application to the program. This also shows that the results are directly proportional to the legal structure of the enterprises. At the company level, interviews were mostly conducted with company managers or company owners, chairmen or partners in cooperatives and unions, chairmen or managers in VSPU, and partners in sole proprietorships.

The education level of the interviewees was generally high. This feature is directly related to the beneficiary status of the program. Because there is a one-to-one relationship between having information about the program and the education level of the people. This situation emerged from the general structure of the people interviewed during the observations made during the survey period.

Within the scope of the program, enterprises that determined their own field of activity in the region benefited from grant support for processing, packaging, and storage (61.5%). Agricultural production is intensively carried out in the region since the climate and soil fertility is suitable for fruit and vegetable production compared to other regions of Turkey. Intensive agricultural production increases the desire to meet post-production services from within the region. In this context, the program has been an opportunity for the enterprises in the region, and the need for processing and packaging of the products produced has been met to a certain extent. In addition, the need for storage of agricultural products produced in the region has been met through the support program. In this respect, it has been observed that the support program has benefited the rural areas of the Western Mediterranean Region.

Within the scope of the program, other supports other than processing, packaging and storage grants were also benefited from. While 25% of the enterprises benefited from the support for drip Irrigation, 10.4% received grants for capacity increase and 3.1% received grants for infrastructure works such as sewerage and road construction.

Accordingly, the evaluations of the surveyed individuals about each alternative were taken and the evaluation of the individuals on the subject was made on a 10-point scale. In the scoring system, 1 point was accepted as the highest score for the alternative preferred by the individuals.

Individuals who benefited from the support were asked to rank the cards obtained as a result of the orthogonal design according to their preferences. Everyone’s ranking was subjected to the Bretton-Clark Conjoint Designer process and the partial utility coefficient, the degree of importance calculated for each factor and the preference ranking of everyone were calculated.

When the results of the analysis are evaluated, it is revealed that the most important factor in the support preference of individuals is the "support area". The degree of influence of the support area on the decision of individuals to benefit from support was calculated as 38.23%. After the support area, the second most important factor in the decision of individuals to benefit from support is "investment period". The degree of influence of the investment period on the decision of individuals to benefit from support was calculated as 16.25%. The third most important factor in the decision of individuals to benefit from support is "type of support". The degree of influence of the type of support on the decision of individuals to benefit from support was calculated as 15.57%. "Tax exemption" is the fourth most important factor in individuals' decisions to benefit from support. The degree of influence of tax exemption on individuals' decision to benefit from support was calculated as 15.42%. Finally, the fifth and last factor in individuals' decisions to benefit from support is the "amount of support". The degree of influence of the investment period on the decision of individuals to benefit from support was calculated as 14.53% (Table 4).

Table 4. Results of Conjoint Analysis

Factors	**Factors levels**	**Partial utility (Part worth value)**	**Significance levels (%)**
Support area (SA)	Animal husbandry (SA1)	**0.925**	**38.232**
	Greenhouse cultivation (SA2)	0.917	
	Manufacturing industry (SA3)	-0.291	
	Irrigation (SA4)	-1.551	
Investment period (months) (IP)	9 (IP1)	**0.331**	**16.246**
	12 (IP2)	-0.081	
	15 (IP3)	-0.250	
Type of support (TS)	Machinery purchase (TS1)	**0.666**	**15.572**
	Cash payment (TS2)	-0.265	
	Building construction (TS3)	-0.401	
Tax exemption (TE)	3 years (TE1)	**0.102**	**15.418**
	2 years (TE2)	0.086	
	None (TE3)	-0.188	
Support amount (rate)(SAR)	50% (SAR1)	**0.419**	**14.532**
	75% (SAR2)	0.023	
	25% (SAR3)	-0.442	

Total			**100.000**
Pearson's R Value = 0.983	Significance = 0.0000		
Kendall's tau Value = 0.833	Significance = 0.0000		

Within the framework of the findings obtained in the research region, it can be said that the most important feature in the optimum policy choice that gives the highest total benefit in the support decision of the enterprisers benefiting from RDISP in the Western Mediterranean Region is the "area of support" to be provided to the region. It is seen that enterprisers and administrators primarily pay attention to the area of support in the investments to be made in their regions. This situation shows that the bottom-up implementation in the EU in recent years, especially in rural development studies, is also suitable for the region in question. As a matter of fact, in relation to the investments to be made in a region, cooperation with local stakeholders or non-governmental organizations of that region is requested first. This is based on the fact that local stakeholders have knowledge about the shortcomings and potential of the region. It is seen that the individuals who will benefit from the support first pay attention to the area to be supported and prefer to benefit from the support accordingly.

The partial utility values of each factor level show the effect of those levels on individuals' preferences. The factor level with the highest partial utility value is the most preferred option by individuals. Accordingly, the factor level with the highest partial utility score in the support area factor is "animal husbandry" with 0.925. Animal husbandry is followed by "greenhouse farming" with a benefit score of 0.917, "manufacturing industry" with a factor score of -0.291 and finally "Irrigation" with a benefit score of -1.551. These data show that in the selection of the support area, the livestock breeding activity of the enterprisers benefiting from the program in the region is the factor level with the highest partial benefit for the region.

In the investment duration factor, the factor level with the highest partial benefit score is "9 months" with 0.331. This factor level is followed by "12 months" with a benefit score of -0.081 and "15 months" with -0.250. In the selection of the investment period given in the supports, enterprisers who benefit from RDISP in the region prefer a period of 9 months.

Table 5. Total Utility Values of combinations in Conjoint Analysis

No	TS	Partial utility	SAR	Partial utility	SA	Partial utility	IP	Partial utility	TE	Partial utility	Total utility
2	TS1	0.666	SAR1	0.419	SA1	0.925	IP1	0.331	TE1	0.102	2.443
1	TS1	0.666	SAR1	0.419	SA2	0.917	IP2	-0.081	TE3	-0.188	1.733
3	TS2	-0.265	SAR1	0.419	SA1	0.925	IP1	0.331	TE2	0.086	1.496
9	TS2	-0.265	SAR2	0.023	SA2	0.917	IP1	0.331	TE2	0.086	1.092
7	TS3	-0.401	SAR3	-0.442	SA2	0.917	IP1	0.331	TE1	0.102	0.507
4	TS3	-0.401	SAR2	0.023	SA1	0.925	IP3	-0.250	TE3	-0.188	0.109
13	TS2	-0.265	SAR3	-0.442	SA1	0.925	IP2	-0.081	TE3	-0.188	-0.051
14	TS3	-0.401	SAR1	0.419	SA3	-0.291	IP1	0.331	TE3	-0.188	-0.130
6	TS2	-0.265	SAR3	-0.442	SA2	0.917	IP3	-0.250	TE3	-0.188	-0.228
12	TS1	0.666	SAR3	-0.442	SA3	-0.291	IP3	-0.250	TE2	0.086	-0.231
8	TS2	-0.265	SAR2	0.023	SA3	-0.291	IP2	-0.081	TE1	0.102	-0.512
10	TS1	0.666	SAR2	0.023	SA4	-1.551	IP1	0.331	TE3	-0.188	-0.719
15	TS2	-0.265	SAR3	-0.442	SA3	-0.291	IP1	0.331	TE3	-0.188	-0.855
16	TS2	-0.265	SAR1	0.419	SA4	-1.551	IP3	-0.250	TE1	0.102	-1.545
5	TS2	-0.265	SAR3	-0.442	SA4	-1.551	IP1	0.331	TE3	-0.188	-2.115
11	TS3	-0.401	SAR3	-0.442	SA4	-1.551	IP3	-0.250	TE3	-0.188	-2.832

In the form of support factor, the factor level with the highest partial benefit score is "machinery purchase" with 0.666. Machinery purchase is followed by "cash" with a benefit score of -0.265 and "building" with -0.401. In the choice of the type of support, enterprisers who benefit from RDISP in the region prefer to receive machinery directly.

In the tax exemption factor, the factor level with the highest partial benefit score is "3 years" with 0.102. This factor level is followed by "2 years" with a benefit score of 0.086 and "none" with -0.188. In the choice of taxation in the supports provided, enterprisers who benefit from RDISP in the region prefer that the business they will establish be exempt from tax for 3 years.

Finally, the factor level with the highest partial utility score in the support amount factor is "50% grant" with 0.419. This factor level is followed by "75% grant" with a benefit score of

0.023 and "25% grant" with -0.442. According to the enterprisers benefiting from the program in the region, the factor level with the highest partial benefit when choosing the support amount is the support amount with "50% grant" rate (Table 5).

The average and total utility values of the combinations (question cards) presented to the enterprisers within the scope of Conjoint analysis and the priority order of individuals in policy choice are given in Table 5. The total utility value is the sum of the factor level scores and the combination with the highest total utility value is defined as the policy set that provides optimum utility for individuals. The combination with the lowest total utility value provides minimum benefit to the enterprisers.

According to the enterprisers, the optimum policy pattern that provides the maximum utility is card or combination number 2 with a total utility value of 2.443. The second most preferred combination by the enterprisers is card number 1. As can be seen from the above, machinery and cash grants are the most preferred forms of support for the owner, manager, shareholders, or heads of cooperatives/unions. As for support, 50% and 75% grants are preferred by the enterprisers. Animal husbandry and greenhouse cultivation are the most preferred sectors in the region. However, keeping the investment period short is seen as a preferred practice by individuals. The policy support set that provides the minimum (least) benefit to individuals is determined as combination number 11 with a total benefit score of -2.832. This result shows that individuals do not prefer building construction, 25% support rate and irrigation investments (Table 5).

CONCLUSIONS

Development is the process of increasing people's access to humane living conditions, improving income distribution, increasing the level of income, ensuring localized developments in social and cultural areas, protecting, and utilizing natural resources and reflecting the wealth to the lives of individuals. Development is a target that countries want to reach and a series of developing movements.

Developments and development initiatives in countries have been to the detriment of rural areas and in favor of urban areas. For centuries, it has been accepted that urban areas are the focal points of development and progress. However, in recent years, this idea has started to change in many countries, especially with the demonstration that no distinction can be made between urban and rural areas for social development.

Policies have been established to make rural development efforts more efficient. These policies are the policies that reveal the efforts made on a national basis to improve the economic, social, and cultural opportunities of the communities living in rural areas, to increase the living standards of these communities, and to support them to participate in national development. However, these efforts have ceased to be the domestic policy of countries and have become an international issue in the world. Rural development activities vary from country to country. With the developments in rural development in countries, various policies are developed and implemented for the development of rural areas in the world. In Turkey, rural development activities started in the early years of the Republic and rural development policies were implemented with various regulations in the following years. One of these practices is the "Rural Development Investments Support Program". The program aims to create a certain capacity in rural society.

In this study, a "field research" covering the enterprises benefiting from the Rural Development Investments Support Program in the provinces in the Western Mediterranean Region was conducted. A survey based on face-to-face interviews was conducted with a total of 96 enterprises benefiting from the support program in the said region. According to this

When the results of the Conjoint analysis were evaluated, it was revealed that the most important factor in the support preference of individuals was the "support area". The degree of influence of the support area on the decision of individuals to benefit from support was calculated as 38.23%. After the support area, the second most important factor in the decision of individuals to benefit from support is "investment period". The degree of influence of the investment period on the decision of individuals to benefit from support was calculated as 16.25%. The third most important factor in the decision of individuals to benefit from support is "type of support". The degree of influence of the type of support on the decision of individuals to benefit from support was calculated as 15.57%. "Tax exemption" is the fourth most important factor in individuals' decisions to benefit from support. The degree of influence of tax exemption on individuals' decision to benefit from support was calculated as 15.42%. Finally, the fifth and the most important factor in the decision of individuals to benefit from support is the "amount of support". The degree of influence of investment duration on the decision of individuals to benefit from support is calculated as 14.53%. According to these results, the enterprises benefiting from the support in the region preferred the support combination of machinery in the form of support, 50% in the support amount (rate), livestock breeding in the support area, 9 months in the investment period and 3 years in tax exemption. In addition, according to the enterprisers, this combination is accepted as the policy pattern that maximizes their total benefits.

Conjoint analysis reveals the results obtained in the field of agricultural policy and the opinions of the people who benefit from the support. The study has created a entrepreneur-oriented approach that reveals the thoughts of the enterprisers on how the support for rural areas should be and can answer the question of how the support should be. As a result of this approach, the study is an important resource for policy makers.

In line with the results obtained from the study, the objectives of the support program, scope, support area, investment period, form of support, amount of support, what kind of support they benefit from the support program due to what kind of features of the support, according to which features the enterprises benefit from the support to operate in rural areas and which support combination the enterprises prefer were carried out through the example of enterprises benefiting from RDISP in the Western Mediterranean Region. With the findings obtained, the creation of support units according to the wishes, expectations, and potential of local knowledge in support policies for rural development will ensure that rural development efforts will achieve the desired success.

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EFFECT OF DIFFERENT FLIGHT PARAMETERS ON SPRAYING EFFICACY IN PESTICIDE APPLICATIONS WITH UNMANNED AERIAL VEHICLE IN SUNFLOWER

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ABSTRACT

The use of unmanned aerial vehicles in pesticide applications has increased rapidly in Turkey and the world in recent years. The biggest problem experienced in pesticide applications with unmanned aerial vehicles is the low uniformity of distribution and penetration due to the low volume application. In this study, the spraying of unmanned aerial vehicles (UAV) with different flight parameters during the sunflower flowering period, and the parameters with the best uniformity of the droplet distribution were tried to be determined. Flight parameters were determined as 2 different heights (2 m and 1.5 m from the top of the plant), 2 different spraying rates (10 l/ha and 20 l/ha), and 2 different travel speeds (11.2 km/h and 19 km/h), and a total of 8 flights were made combining them with each other. Wind speeds, temperature, and relative humidity values were recorded during flight and throughout the trial. In each experiment, 6 sunflower plants on the flight route of the unmanned aerial vehicle were randomly selected and water-sensitive paper was placed on these plants in 4 different areas: behind the head, inside the head, middle leaf and lower leaves. Tap water was used as a spraying liquid. The papers were scanned and transferred to a computer environment, and droplet analyses were performed with the DepositScan software. In droplet analyses, the average droplet diameter (Dv0.5), the number of droplets per unit area (droplets / cm^2) and the percentage coverage (% area) were calculated. The results show that the average droplet diameter was between 250-300 µm. The least accumulation was observed on the front of the head and on the lower leaves and the highest on the paper behind the head. The accumulation of droplets increased as the spray rate increased, whereas the accumulation of droplets decreased as the flight height increased. According to the results of the experiment, the application with a spray rate of 20 l / ha, a flight speed of 11.2 km / h and a height of 2 meters gave the most successful results in terms of droplet distribution and droplet penetration.

Keywords: unmanned aerial vehicle, sprayer, sunflower, pesticide, drone spraying, droplet

INTRODUCTION

The use of technology in agriculture is becoming more intense each year. Technologies such as precision agriculture technologies, field-based applications, application of GPS technology in agriculture, Internet of Things, Blockchain, Remote Sensing are being adapted to agriculture and making farmers' lives easier.

Unmanned aerial vehicles (UAVs), also known as drones, are widely used in areas such as photography, imaging, remote sensing, battlefield surveillance, and cargo transportation. Drones, which first emerged in World War I to take images, were limited to the military field for a long time and then spread to areas such as the entertainment sector, photography, civil defence, and civil defense, and have become widespread in agriculture in recent years (Stehr, 2015). UAVs are used in agriculture for remote sensing, in-season plant health monitoring, weed and pest monitoring, herd management, irrigation system planning (Veroustraete, 2015), pesticide applications, fertilization applications (Önler et al., 2023a) and seed planting. Especially in pesticide applications, both in Türkiye and throughout the world, unmanned aerial vehicles with a liquid tank and spray nozzles serve as an alternative or an assistant to ground spraying machines.

Sunflower (Helianthus annus L.) is the most important oil seed crop in southern Europe and the Black Sea region. As of 2019, it is produced on a total area of 26 million hectares in countries in the Black Sea region and 51 million tons of sunflower is produced. This production volume represents 75% of the world sunflower production (Kaya, 2020). In Türkiye, in 2022, sunflower was cultivated on 980,000 hectares of land, 80,000 of which were for snacks and 900,000 for oil, and 200,000 tons of sunflowers were produced for snacks and 2,350,000 tons of sunflowers for oil (TUIK, 2023). 73% of sunflower production areas in Türkiye are in the Marmara Region, 13% in Central Anatolia, 19% in the Black Sea Region, 3% in the Aegean Region and 1% in the Eastern and Southeastern Anatolia Region (Meral, 2019).

Unlike conventional applications, various problems arise in unmanned aerial vehicle pesticide applications since the application rate is lower. Factors such as droplet distribution uniformity, spray drift, covered area of the target surface, and penetration can become risky in aerial pesticide applications. However, the downward airflow generated by the propellers of the drone can also provide better drop distribution (Carvalho et al., 2020). According to Matthews (2018), ULV pesticide applications with drones can be effective, but require narrower droplet spectra. The characteristics of the droplet distribution are also the main criterion to measure the success of pesticide application success (Zhang et al., 2020). In pesticide applications with unmanned aerial vehicles, flight speed, flight altitude, application rate, and nozzle type are the main parameters that affect the droplet distribution characteristics.

Researchers have conducted studies with different parameters to determine the distribution and thus the application efficiency in pesticide applications with unmanned aerial vehicles. Zhang et al. (2020), in experiments with different spray rates, flight heights, and flight speeds, obtained the most uniform droplet distribution in the application with 15 L/ha spray volume, 3 m flight height, and 4 m/s flight speed. In a similar study, Zhang et al. (2020) reported a flight height of 6 metres and 2.5 m/s flight speed as the most efficient application in spraying applications with unmanned aerial vehicles in sugarcane fields. Martin et al. (2019a) also obtained the largest average droplet diameter at a flight height of 3 metres and a flight speed of 5 m/s flight speed. Ahmad et al. (2020) obtained the highest droplet deposition at a flight speed and 2 meters height.

For sunflower, which is an important oil seed crop in the Thrace region, pesticide and plant nutrition applications are carried out with conventional sprayers (Ozyurt et al. 2020). There is no study in the literature on the application of these practises with UAVs. In this study, the aim was to determine the droplet distribution uniformity and penetration of different application parameters in spraying applications with unmanned aerial vehicles in sunflower plants.

MATERIAL AND METHOD

Unmanned Aerial Vehicle

The agricultural unmanned aerial vehicle DJI Agras MG-1P RTK model was used in the trials (Figure 1.). The UAV has a fully autonomous flight mode. It can communicate with an external RTK station and fly in vertical and horizontal positions with high precision. After the plot boundaries, work width, height, and spraying rate are determined via the remote control, the route is automatically created, and the UAV can take off from the take-off area, perform the spraying operation with the specified parameters, and land back to the take-off area.

Figure 1. DJI Agras MG-1P UAV Sprayer

There are 4 Teejet XR11001VS (Spraying Systems Co.) flat fan nozzles on the unmanned aerial vehicle. This type of nozzle produces fine droplets according to the ASAE S572.1 standard. (ASAE, 2009). The technical specifications of the unmanned aerial vehicle are shown in Table 1. and the specifications of the nozzles are shown in Figure 2.

Table 1. Technical specifications of the UAV

Dimensions (m)	1460 × 1460 × 578 mm (with arms extended, without propellers) 780 × 780 × 578 mm (arms folded)		
Total weight (kg)	44.751,00	Max power consumption (W)	6400
Flight time (minutes)	20,00	Min. height above plant (cm)	150
Number of rotors	8,00	Load weight (liter)	10
Spray nozzle type and number	XR11001VS 4 units	Max. spray nozzle flow rate (l/s)	0.379
Battery weight (kg)	4,00	Max flight speed (m/s)	12
Max working speed (m/s)	7,00	Battery capacity (mAh)	12000

	bar	DROP SIZE 80°	DROP SIZE 110°	CAPACITY ONE NOZZLE IN l/min	l/ha 50cm 4 km/h	5 km/h	6 km/h	7 km/h	8 km/h	10 km/h	12 km/h	16 km/h	18 km/h	20 km/h	25 km/h	30 km/h	35 km/h
XR8001 XR11001 (100)	1.0	F	F	0.23	69.0	55.2	46.0	39.4	34.5	27.6	23.0	17.3	15.3	13.8	11.0	9.2	7.9
	1.5	F	F	0.28	84.0	67.2	56.0	48.0	42.0	33.6	28.0	21.0	18.7	16.8	13.4	11.2	9.6
	2.0	F	F	0.32	96.0	76.8	64.0	54.9	48.0	38.4	32.0	24.0	21.3	19.2	15.4	12.8	11.0
	2.5	F	F	0.36	108	86.4	72.0	61.7	54.0	43.2	36.0	27.0	24.0	21.6	17.3	14.4	12.3
	3.0	F	F	0.39	117	93.6	78.0	66.9	58.5	46.8	39.0	29.3	26.0	23.4	18.7	15.6	13.4
	4.0	F	VF	0.45	135	108	90.0	77.1	67.5	54.0	45.0	33.8	30.0	27.0	21.6	18.0	15.4

Figure 2. Properties of the spray nozzle used in the drone (Spraying Systems Co.)

Trial Area and Conditions

The trials were carried out on 26.07.2022 in the sunflower fields of Tekirdag Viticulture Research Institute (40 ° 58’18.0’N, 27 ° 28’21.3’E). The total size of the field were 1.5 ha and 0.1 ha was allocated for the experiment. The sunflower cultivar planted in the field was Pioneer P64LP130. At the time of the experiment, the sunflowers were in the R6 stage (grain formation was completed and flower drying started) (Schneiter and Miller, 1981). This stage was chosen because it is the stage where the canopy density of sunflower reaches the highest value and it is the most difficult for pesticide droplets to reach the lower leaves.

The weather was clear and sunny during the trials. Temperature and humidity values were measured with a Testo 605-H1 thermohydrometer (Testo SE & Co. KGaA, 2023) and wind speed with a Lutron AM 4202 anemometer (Lutron Instruments, 2023). The average temperature was 32.5 degrees and the relative humidity was 52.5%. The average wind speed was 1.3 m/s; then the highest was 1.6 m/s and the lowest was 0.9 m/s.

Method

In the sunflower field, a 16 m x 50 m parcel was selected and the parcel boundaries were determined by remote control of the UAV. The working width was determined to be 4 metres and a flight route was generated. The UAV started spraying 5 metres before the plot boundaries and finished spraying 5 meters after the plot boundary in order not to affect the spraying rate changes that occurred at the end of the headland turn, spraying starts and ends. Six individual plants were randomly selected within the plot, and water-sensitive paper was placed in four different areas, namely, inside the flower, behind the head, middle leaves, and lower leaves, to measure the drop distribution in the plant. The placement of water-sensitive papers in the experimental area and in the plant is shown in Figure 3.

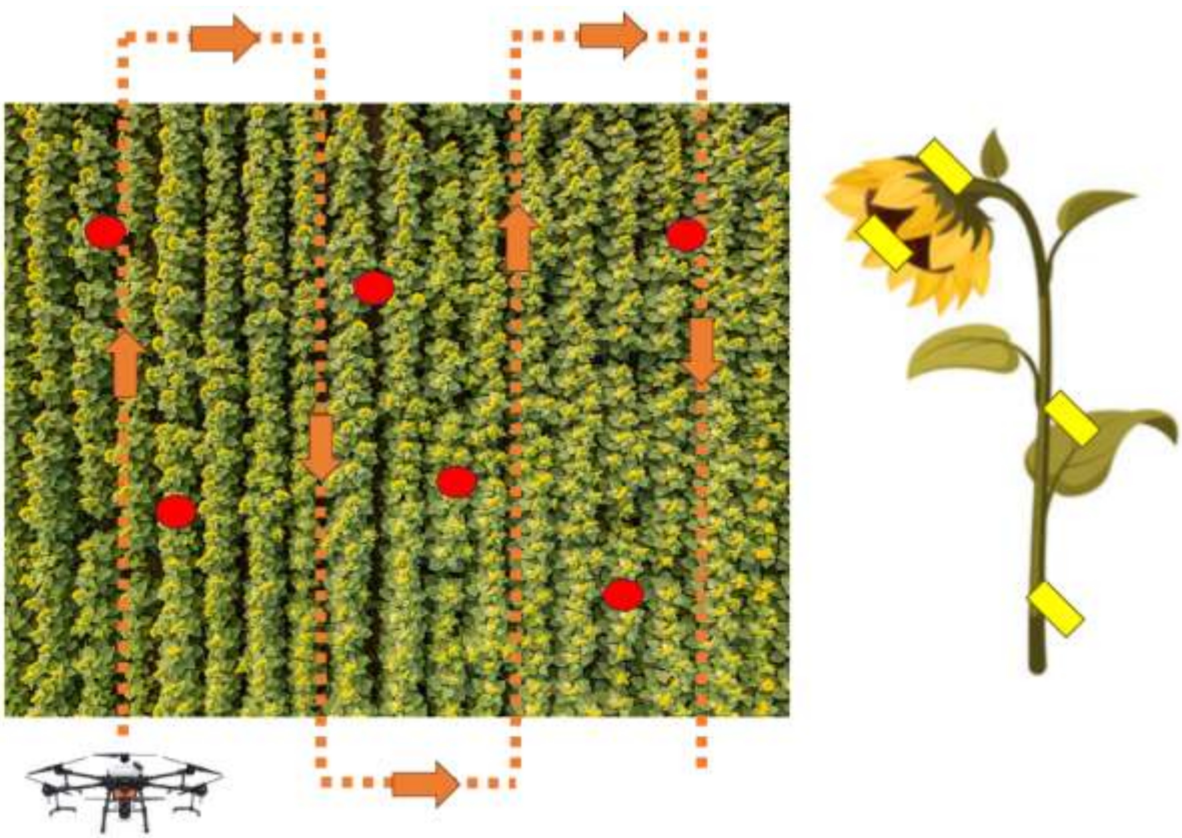

Figure 3. Experimental area and placement of water-sensitive papers on the plant

A total of eight trials with two different heights (1.5 m, 2 m from the top of the plant), 2 different spraying norms (10 L/ha, 20 L/ha), and 2 different flight speeds (11.2 km/h, 19 km/h), 24 flights were made with the UAV, 3 repetitions for each trial. The spray pressure was 3 bar in all flights. Since only a drop distribution was to be observed in the trials, tap water was used as a spray liquid. The trial plan is shown in Table 2.

Table 2. Trial Plan

Number of Trial	Flight Altitude (m)	Spray Rate (l/ha)	Flight Speed (km/h)
1	2	10	11,2
2	2	10	19
3	2	20	11,2
4	2	20	19
5	1,5	10	11,2
6	1,5	10	19
7	1,5	20	11,2
8	1,5	20	19

Data Collection and Analysis

After each flight, the wet water-sensitive papers were collected from the plant after drying and placed in sealed containers. New water-sensitive papers were placed on the plant for the next test flight. The collected water-sensitive papers were scanned on a scanner with a resolution of 600 dpi scanner and transferred to a computer. Droplet analysis was performed on the images scanned with DepositScan software and the average drop diameter Dv0.5, the percentage coverage (%), and the number of droplets per unit area were measured (Zhu et al., 2011a). For each trial, the parameters with the most uniform droplet distribution were determined by calculating the standard deviation and coefficient of variation for drop analyses from a single plant and between plants.

RESULTS AND DISCUSSION

After 24 flights in triplicate for 8 different trials, all water-sensitive papers were subjected to drop analysis with the DepositScan software and the results were shown in graphs and tables.
First, looking at the distribution of average droplet diameters, all trials produced an average drop diameter in the range of 173-260 µm. According to ASABE, 572.1, drop diameters in the range of 106-235 µm are classified as "fine" and the range of 236-340 µm is classified as "medium". Looking at the nozzle manufacturer's table in Figure 2, it can be seen that although the nozzle used was committed to producing droplets in the "fine" droplet class, some trials produced medium class drops with higher diameters (Table 3). Since the nozzle manufacturer conducted its tests under spraying conditions with a conventional sprayer, it is normal for the droplet sizes to vary slightly in the applications with the unmanned aerial vehicle. Because the unmanned

aerial vehicle flies autonomously, even if the spray pressure is set constant, it can make small increases or decreases in pressure to apply the targeted spray rate at the specified speed. This can lead to a change in the diameter of the droplets. There were no significant differences in the average droplet diameters between the trials. In Trial 2 and Trial 7, the low drop diameters in the inner flower (A) (155,100, respectively) and lower leaves (D) (110, 220, respectively) regions are due to the fact that the accumulations are very low and fall below the threshold value in the analysis software (Figure 4.). In addition, the lowest coefficient of variation was calculated in trial 5 and the highest coefficient of variation was calculated in Trial 7. In all trials, except for the last two trials, as the uniformity of the flight speed increased, the droplet distribution decreased. Table 3 shows the mean, standard deviation and coefficient of variation values of mean droplet diameter, number of droplets per unit area, and percentage of coverage.

Table 3. Values of mean, standard deviation, and coefficient of variation obtained in the trials

	Volumetric Median Diameter ($Dv_{0,5}$)			*Number of droplets/cm^2*			*Coverage (% of WSP Area)*		
Trial No.	Average	Standard Deviation	CV (%)	Average	Standard Deviation	CV (%)	Average	Standard Deviation	CV (%)
Trial 1	232,3	26,9	11,6	12,7	9,8	77,4	0,9	0,7	80,2
Trial 2	173,4	40,4	23,3	4,1	2,6	64,8	0,3	0,3	86,0
Trial 3	255,8	23,5	9,2	30,9	18,6	60,2	2,3	1,7	70,8
Trial 4	248,0	72,8	29,3	23,5	22,4	95,6	2,2	2,6	115,0
Trial 5	241,6	20,8	8,6	20,0	15,8	79,1	1,2	0,8	70,5
Trial 6	248,0	34,5	13,9	24,1	12,4	51,5	1,7	1,0	55,9
Trial 7	232,8	69,5	29,9	31,8	27,5	86,6	2,8	3,2	115,6
Trial 8	262,6	36,7	14,0	20,8	7,9	38,0	1,7	0,7	42,2

Second, when the number of drops per unit area and coverage percentages were analysed, it was observed that the highest accumulation of droplets was observed in the area behind the head in the evaluation made with a single plant. Due to the genetic structure of the sunflower variety, during the application phase, the back of the head faces upwards and the inside of the flower faces towards the ground as a result of tilting the head towards the soil after grain filling is completed. Therefore, the area where the droplets accumulate the most is the back of the head at the top (zone B). In the inner flower zone (Zone A), the accumulation was low because the water-sensitive papers were facing toward the ground. In the mid-leaves zone (zone C), the depositions were close to the average. In the lower leaves, the penetration of pesticide droplets into the lower leaves decreased as the leaf area index increased at the R6 stage of sunflower. In all trials, increasing the flight rate decreased the average droplet deposition (Figure 5). With increasing spray rate, droplet deposition increased. In trials treated with 20 L/ha, the mean droplet deposition (30,23,31,20 droplets/cm^2, respectively) was higher than in trials treated with

10 L/ha (12,4,20,24, respectively). Although no specific correlation was observed between flight height and the amount of droplet deposition, the coefficients of variation (CV) (Table 3) were lower (51%, 38%) for treatment at 1.5 metres height.

One for the criteria of spraying efficacy in pesticide applications is the coefficient of variation. A low coefficient of variation means that the application is homogeneous. In a successful pesticide application, the coefficient of variation is expected to be less than 25% (Woldt et al., 2018; Martin et al., 2019b). However, the results found in studies on UAV spray performance of UAVs also do not reach 25% (Zhang et al., 2020; Richardson et al., 2019; Wang et al., 2018; Liao et al., 2019; Önler et al. 2023b). The researchers claim that this is due to factors such as the small diameter of the droplets, production, wind, and drift caused by the downward airflow created by the drone. In this study, the lowest coefficient of variation was obtained in trial 8 (38%) and the highest coefficient of variation was obtained in trial 4 (95%). It was observed that the coefficient of variation decreased as the flight speed increased in trials other than trial 4.

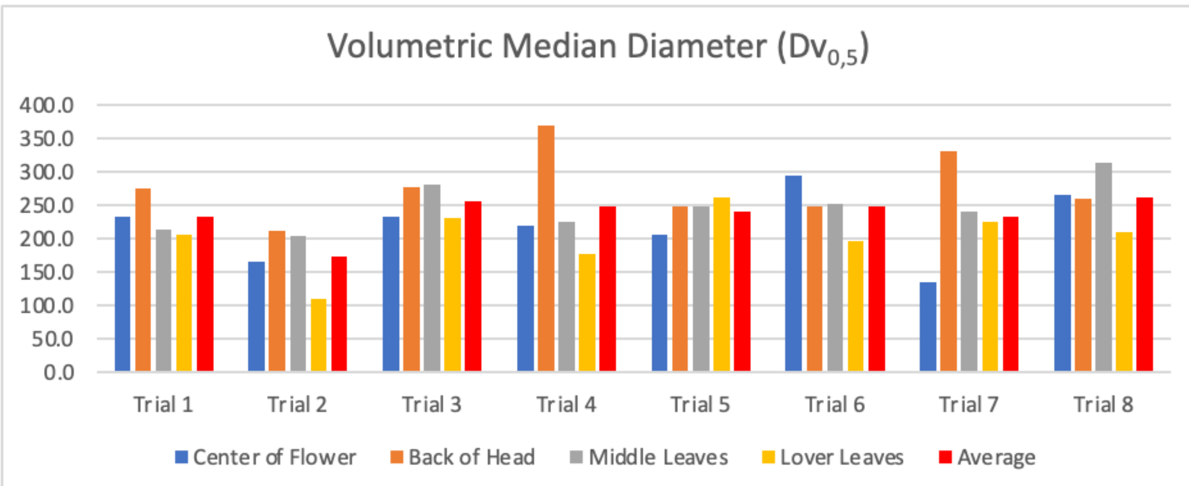

Figure 4. Variation of the Average Droplet Diameter according to Different Parameters

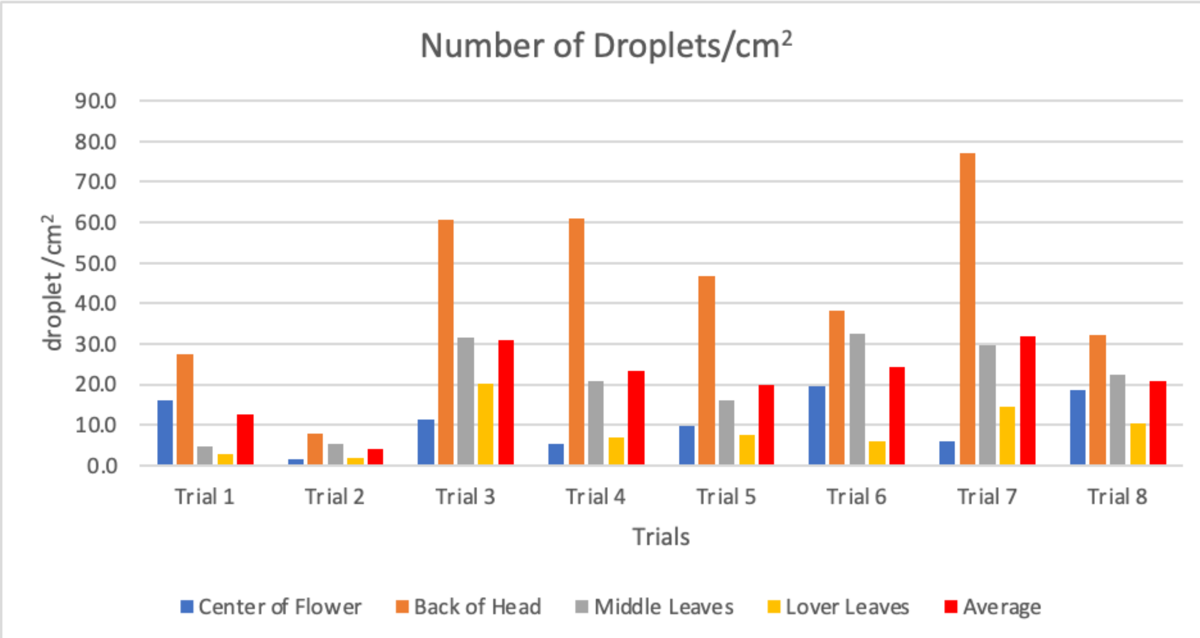

Figure 5. Variation of the number of droplets per cm^2 according to different parameters

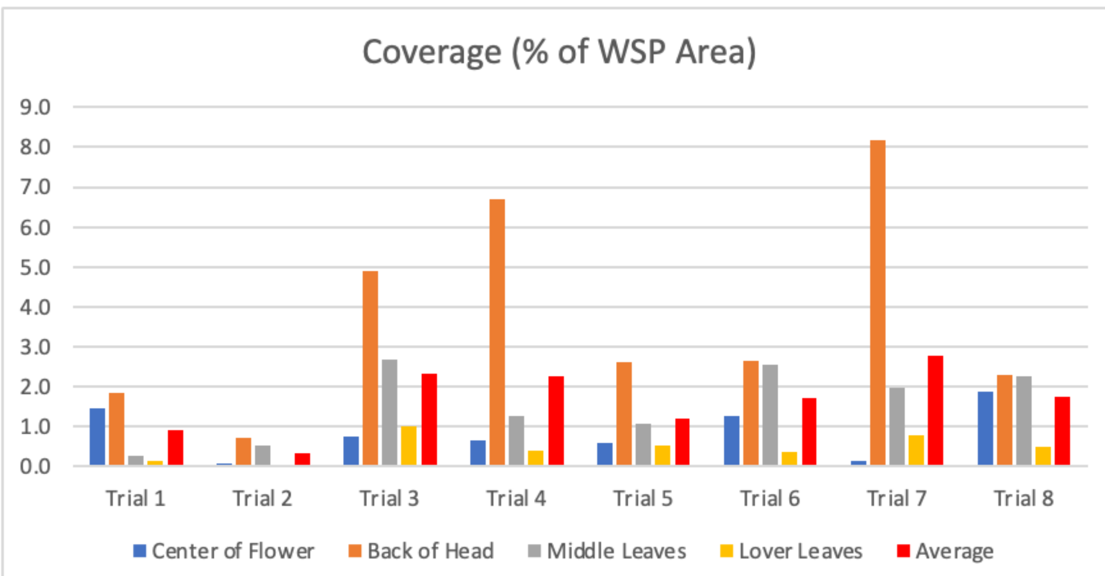

Figure 6. Variation of the droplet coverage percentage according to different parameters

The number of droplets per unit area and the percentage of coverage are important for spraying efficiency. For all types of pesticides, the droplet density required for spraying efficacy is different. Syngenta Crop Protection AG (Basel, Switzerland) recommends a minimum droplet density of 20-30 drops/cm^2 for insecticides and preemergence herbicides, 30-40 drops/cm^2 for herbicides and 50-70 drops/cm^2 for fungicides (Zhu et al., 2011b). Table 3 shows that the highest average deposition was observed in Trial 7 (31.8 droplets/cm^2) and the lowest in Trial 2 (4.1 droplets/cm^2). Pesticide applications with the application parameters used in trials 1 and 2 will not be effective because droplet deposition is very low. With the parameters used in the other trials, an effective pesticide application can be obtained only for insecticides and preemergence herbicides. Figure 5 shows that the deposition of droplets in the inner flower (A) and lower leaf (D) zones of the plants was below the threshold value of 20 drops/cm^2 . Therefore, it can be concluded that penetration was low for the parameters used in the trials.

CONCLUSIONS

Using the Agras MG-1P UAV Sprayer, 8 different trials were conducted with different speeds, spray rate, and the uniformity of the flight altitude, and spray and droplet characteristics were analysed for each trial. In these trials, it was observed that in stage application of pesticides to be made at the R6 in sunflower plants, there was less deposition of drops on the lower leaves and areas facing the ground, resulting in low penetration. The average diameter of the droplets produced by the UAV showed a uniform distribution in all trials. As the spray rate increased, the droplet accumulation increased and decreased with increasing height and speed. Therefore, a higher spray rate, lower altitude, and lower speed result in a higher drop deposition in UAV pesticide applications. However, the uniformity of the distribution was low as a result of factors such as wind, air flow, and low droplet volume. According to the results of the experiment, the flight height of 1.5 metres, the spray rate of 20 L / ha and 19 km/h flight speed give the best results in the application of pesticides for sunflower plant when droplet distribution and droplet deposition amount are considered. However, these results may not be sufficient to increase the effectiveness of herbicide and fungicide applications. Therefore, future studies should investigate the biological efficacy of pesticides with different application parameters.

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EFFECT OF USING BUCKWHEAT IN QUAIL DIETS ON PERFORMANCE AND EGGSHELL STRENGTH

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ABSTRACT

This study was carried out to determine the effect of using buckwheat at the levels of 0, 15 and 30% in the diet on the performance and eggshell breaking strength of laying quails. For this purpose, 48 female quails of 10 weeks old were randomly distributed into 3 groups with 4 replicates. As a result of the study, the use of buckwheat in the diet linearly decreased the egg weight. Eggshell breaking strength was quadratically affected by the dietary buckwheat and improved at 15% of buckwheat, but it was minimum at 30% (Q=0.011). Egg production, feed intake, and feed efficiency were not affected using buckwheat in the diet. According to the results of this research, it was determined that the use of buckwheat at 30% in laying quail dies reduces egg weight and eggshell breaking strength and can be used up to 15% in the diet.

Keywords: Quail, buckwheat, eggshell breaking strength, performance

INTRODUCTION

Buckwheat, which belongs to the *Fagopyrum* species of the Polygonaceae family, is a grain-like (pseudo-cereal) annual and Central Asian plant (Zhang et al., 2012). The most produced buckwheat variety in the world is *Fagopyrum esculentum moench* (Wijngaard and Arendt, 2006). According to 2021 data, buckwheat, which is produced approximately 1.9 million tons/year, is mostly grown in Russia, followed by China and Ukraine (FAO, 2023).

The presence of bioactive compounds including polyunsaturated fatty acids, rutin and quercetin (Dizlek et al., 2009), flavones, flavonoids, phytosterols and myo-inositol makes buckwheat a good source of antioxidants (Holasova et al., 2002; Zhang et al., 2002). It is also rich in essential amino acids (especially lysine) relative to wheatgrass grains, and poor in antinutritional factors such as gluten and water-soluble non-starch polysaccharides. However, buckwheat also contains antinutritional factors such as high cellulose, phytic acid, and enzyme inhibitors (Alvarez-Jubetea et al., 2010; Krkošková and Mrázová, 2004).

Buckwheat, which is an alternative starch source in human nutrition today, was widely used in the feed of layer hens in the past (Steadman et al., 2001). However, interest in the use of buckwheat in poultry nutrition has started to increase again. Chowdhury and Koh (2019) reported that buckwheat can be used to increase the available phosphorus content due to its high phytase content. Hasić and Basić (2014) and Hasić et al. (2022) stated that buckwheat was effective in improving egg production and traits.

The aim of this study is to determine the effect of buckwheat use in the diet on performance and eggshell breaking strength of laying quails.

MATERIAL AND METHOD

In the research, 48 female quails at 10 weeks of age were equally distributed in three treatment groups. Each treatment group was composed of four replicates with four quails each. During the eight-week trial, the quails were fed with treatment diets containing of 0% (control), 15% or 30% buckwheat. The diets were prepared according to the nutritional requirements of the layer quails reported by NRC (1994) (Table 1). During the experiment, 16-hour lighting program applied and feed and water given ad-libitum to the quails. The authors declare that the study was carried out in accordance with the animal welfare rules specified in Article 9 of the Law No. 5996 of the Republic of Turkey.

Table 1. Treatment diets and calculated nutrient contents

Ingredients	**Buckwheat level, %**		
	0	**15**	**30**
Buckwheat	0.00	15.00	30.00
Corn	53.00	39.40	25.80
Soybean meal	35.40	32.9	30.30
Soybean oil	4.04	5.15	6.35
Limestone	5.60	5.58	5.56
Dicalcium phosphate	1.14	1.15	1.17
Salt	0.35	0.35	0.35
Premix	0.25	0.25	0.25
Methionine	0.22	0.22	0.22
Lysine			
Calculated nutrient contents			
Metabolizable energy, kcal/kg	2902	2899	2903
Crude protein, %	19.99	20.02	20.00
Calcium, %	2.50	2.50	2.50
Available phosphorus, %	0.35	0.35	0.35
Lysine, %	1.11	1.07	1.03
Methionine, %	0.45	0.45	0.45

[1]Premix provides the 80 mg manganese (manganese oxide), 60 mg iron (iron carbonate), 5 mg copper (copper sulphate pentahydrate), 1 mg iodine, 0.15 mg selenium, 8800 IU vitamin A (trans-retinol acetate), 2200 IU vitamin D3 (cholecalciferol), 11 mg vitamin E (tocopherol), 44 mg nicotinic acid, 8.8 mg Cal-D-Pan, 4.4 mg Vitamin B2 (riboflavin), 2.5 mg thiamine, 6.6 mg vitamin B12 (cyanocobalamin), 1 mg folic acid, 0.11 mg biotin, 220 mg choline to per kg of diet.

Method

Body weight change was determined as g by group weighing at the initial and final of the trial. During the experiment, egg production was recorded daily and calculated as %. The feed was given to the quails by weighing, and at the final of the experiment, the remaining feeds were weighed and subtracted from the total feed, and the feed intake was determined as g/day/quail.

The weights of the eggs collected in the last two days of the trial were determined as g. Egg mass was calculated as g/day/quail with the formula (egg production x egg weight)/100. Also, feed conversion ratio rate was determined feed intake/egg mass formula as g feed/g egg.

Damaged eggs were recorded during the trial and calculated as percentage of the number of eggs. Eggshell breaking strength was assessed by applying supported-systematic pressure to the blunt of the eggs (N) (Egg Force Reader, Orka Food Technology, Israel).

A one-way ANOVA and orthogonal contrasts were applied to data. If ANOVA showed significant differences among means (main effects), a planned multiple comparison of means was examined by Duncan's multiple range test. All statistical analyses were carried out using the SPSS Package 23.

RESULTS AND DISCUSSION

The effect of using buckwheat at 0, 15 and 30% levels in quail diets on performance parameters and eggshell breaking strength was given in Table 2.

Table 2. Effect of using buckwheat in the diet on performance and eggshell breaking strength in laying quails

Parameters	**Diet buckwheat level, %**			**Standard error**			
	0	**15**	**30**		***P***	***L***	***Q***
Initial body weight, g	244.88	250.25	247.88	7.152	0.962	0.880	0.822
Final body weight, g	257.25	264.13	264.13	6.324	0.897	0.694	0.820
Body weight change, g	12.38	13.88	16.25	1.736	0.698	0.413	0.913
Egg production, %	87.40	89.02	87.53	1.411	0.893	0.973	0.645
Egg weight, g	13.07	12.99	12.08	0.201	0.065	***0.035***	0.269
Egg mass, g/day/quail	11.43	11.55	10.57	11.18	0.191	0.191	0.263
Feed intake, g/day/quail	30.77	31.89	30.67	0.552	0.650	0.945	0.368
Feed conversion ratio, g feed/g egg	2.71	2.76	2.91	0.068	0.520	0.283	0.752
Damaged egg rate, %	0.00	3.93	1.62	0.830	0.151	0.397	0.079
Eggshell breaking strength, N	13.88[a]	14.41[a]	11.34[b]	0.470	***0.002***	***0.004***	***0.011***

[a,b;] Means with in the same rows without common superscripts are significantly different ($P<0.05$).

Body weight, body weight change, egg production, egg mass, feed intake, feed conversion ratio, and damaged egg rate were not affected by the use of buckwheat in the diet (P>0.05). Egg weight decreased linearly from 13.07 g to 12.08 g with the use of buckwheat in the diet (L=0.035). However, previous studies reported that buckwheat or its by-products had no effect on egg weight (Benvenuti et al. 2012; Chowdhury et al. 2017; Chowdhury and Koh 2019). The use of buckwheat bran in these researches may be the reason for the difference among the studies.

Eggshell breaking strength was affected quadratically by using buckwheat in laying quail diets (Q=0.011). Eggshell resistance increased with 15% level of buckwheat (14.41 N) compared to the control group (13.88 N) but decreased with 30% (11.34 N) level. However, Chowdhury and Koh (2019) reported that the use of buckwheat (10, 15, and 20%) in laying hen diets decreased the eggshell resistance. Benvenuti et al. (2012) stated that the eggshell thickness and weight of laying hen eggs were not affected by buckwheat.

According to the results obtained from the study, it can be said that the use of buckwheat at the level of 30% in quail diets negatively affected egg weight and eggshell resistance and can be used at the level of 15%, but further studies are needed.

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EFFECTS OF PROLINE AND HUMIC ACID APPLICATIONS ON STRESS TOLERANCE INDICES OF WHEAT SEEDS UNDER DIFFERENT SOIL SALINITY LEVELS

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ABSTRACT

Salt stress is one of the most important environmental problems limiting plant development and productivity. In particular, exposure to salt stress during seedling developmental stages limits sustainable agricultural production. In this study, the effects of control (K), proline (P, 15 mM), humic acid (HA, 5 lt/ha), and proline + humic acid (P+HA) applications on seedling development characteristics of wheat seeds were investigated at four different soil salinity levels (0.28, 4.17, 9.33, and 15.23 dS m^{-1}). The responses of wheat seedlings to soil salinity were evaluated using the parameters of proportional tolerance index (RTI), root/shoot ratio (RSR), shoot weight ratio (SWR), root weight ratio (RWR), stress tolerance index for plant height (PHSI), Shoot Fresh Weight Stress Tolerance Index (SFSI), Root Fresh Weight Stress Tolerance Index (RFSI), Shoot Dry Weight Stress Tolerance Index (SDSI) and Root Dry Weight Stress Tolerance Index (RDSI) were evaluated. According to the results, PHST ranged from 51.6% to 113.5%, SFSI ranged from 22.3% to 116.4%, RFSI ranged from 13.6% to 168.5%, RDSI ranged from 12.7% to 136.4%, and RTI ranged from 37.3% to 157.0%. The highest seedling development traits in wheat seeds were observed in the order P+HA>P> HA compared to the control. In agricultural production areas with high salinity, it is recommended that wheat seed be pretreated with 15 mM proline and 5 lt/ha humic acid be incorporated into the soil before sowing to ensure salt-tolerant and high-quality wheat seedling characteristics.

Keywords: Salt stress, Germination, Growth Performance, *Triticum aestivum* L.

INTRODUCTION

Adequate and balanced nutrition for the rapidly increasing population, with the production obtained from fragmented and decreasing agricultural areas, is becoming increasingly difficult daily (Kiremit et al., 2022). For this reason, it is important to identify genotypes well adapted to the ecological conditions of the region, morphologically and physiologically suitable, with good yield and quality characteristics to meet the increasing nutritional needs. Among the basic needs, wheat provides the most important nutritional requirement in human nutrition (FAOSTAT 2019) and is the most widely grown crop globally (Giraldo et al., 2019). Compared to the previous year, wheat production in Turkey increased by 11.9% and yielded 19.8 million tons (TUIK, 2022). Salt stress is an important abiotic stress factor that affects plant growth and limits crop productivity, and the plant's response to this stress can vary according to the amount, type, and duration of exposure to salt and the species and variety of the plant (Munns 2002). In a study on wheat, Şenay et al. (2005) reported that increasing salt concentrations decreased germination rate by 21%, seedling length by 42.5%, and root length by 74.4% on average. They reported that increasing salt decreased root/stem length and fresh and dry weights, and wheat was more affected than barley at the same salinity rates.

Plants were more sensitive to salt stress at germination and seedling development than other stages (Ashraf, 1994). All these features that improve product competitiveness directly correlate with seeding vigor, a complex agronomic trait controlled by multiple genetic and environmental factors (Rajjou et al., 2012; Jisha et al., 2013). Seed priming is an environmentally friendly technique that allows plants to respond faster and stronger when exposed to biotic and abiotic stresses (Aranega-Bou et al., 2014; Lutts et al., 2016). Seed priming reflects the natural cycle of irrigation and drying and can be considered the pre-emergence of seeds under harsh environmental conditions (Kranner et al., 2010). Seed priming stimulates metabolism before germination, increasing water uptake and thus preventing full seed germination by controlling metabolic processes normally activated in the early stages of germination; this process positively affects water uptake and pre-germination metabolism (Paparella et al., 2015).

In recent years, there have been numerous papers on the effect of exogenous compounds such as hormones, mineral elements, and amino acids in alleviating salinity stress during seed germination (Atia et al., 2009; Dallali et al., 2012; Rizwan et al., 2015; Coskun et al., 2016). Proline is an organic osmoprotectant that accumulates in relatively high concentrations in plant tissues exposed to stress (Hayat et al., 2012). It has been widely reported that proline is a multifunctional amino acid that acts at different plant growth stages (Szabados and Savouré, 2010). Similarly, 50 mM proline treatment improved seed germination of two *S. bicolor* cultivars under saline conditions (Nawaz et al., 2010). In *Z. mays* under salt stress, proline applied to the leaf increased the number of seeds per plant, total grain weight, and 100-grain weight (Alam et al., 2016). Despite acting as an osmolyte, proline is also considered a potent antioxidant defense molecule, a metal chelator, a protein stabilizer, a ROS scavenger, and an inhibitor of programmed cell death (Dar et al., 2016; Adejumo et al., 2021). Humic acid (HA) is an organically charged biostimulant that significantly affects plant growth and development and increases yield (Rajpar et al., 2011). Previous research has suggested that the beneficial effects of HA can be attributed to activating several metabolic enzymes, improving plant water status, maintaining ion and redox homeostasis, and promoting secondary metabolite assimilation (Hagagg et al., 2013). HA also helps plants absorb nutrients, and it is particularly crucial for the movement and availability of micronutrients (Bohme and Thilua, 2013).Çimrin et al. (2010) reported that humic acid doses applied to pepper seedling shoots positively affected plant growth parameters and nutrient uptake regarding salt tolerance, and N, P, K, Ca, Mg, S, Mn, and Cu contents increased significantly.

The present investigation aimed to alleviate the detrimental effects of different soil salinities on the ability of wheat seeds to grow by priming the seeds with proline or only by adding humic acid to the soil and combining both techniques. Moreover, the growth performance of wheat seeds under different soil salinity conditions was evaluated with stress tolerance indices to understand how different treatments affect morphological features relative to the control condition.

MATERIALS AND METHODS

Experimental area and design

This study was conducted between December 11, 2022, and February 02, 2023, in a greenhouse area of Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation. Seeds of the Falado variety were used as plant material in the study.

The soils used for the experiment were obtained from Ondokuz Mayıs University, Faculty of Agriculture, Research and Experiment Area. The physical-chemical properties of the collected soil are shown in Table 1.

Table 1. The physical-chemical properties of the collected soil

Physical Properties								
	Clay (%)	Silt (%)	Sand (%)	Texture	Field Capacity (%)	Permanent Wilting Point (%)		
	18.2	29.8	52.0	Loam	23.2	11.4		
Chemical Properties								
Organic matter (%)	N (%)	P (ppm)	Ca (meq/100g)	Mg (meq/100g)	K (meq/100g)	EC (dS m^{-1})	pH	$CaCO_3$ (%)
1.80	0.08	20.5	32.4	14.8	0.41	0.28	7.7	2.05

N: Nitrogen, P: Phosphor, Ca: Calcium, Mg: Magnesium, K: Potassium, EC: Saturated soil salinity, $CaCO_3$: Calcium carbonate

The research was carried out in 48 pots with four treatments (control, proline, humic acid, and humic acid x proline combination), four different soil salinity (0.28, 4.17, 9.33, and 15.23 dS m^{-1}), and three replications according to the randomized blocks design. The collected soils were crushed to pass through a 4 mm sieve, and (S1: 0.28 dS m^{-1}) were set aside as the control treatment. The 50 kg of dried and sieved soils were prepared with three irrigation water salinity levels (6, 12 and 18 dS m^{-1}) by adding equal amounts (1:1:1) of NaCl, $CaCl_2$ and $MgSO_4$ to tap water and three different types of salts (8, 12 and 20 dS m^{-1}) were determined to suit the subjects (S2: 4.17 dS m^{-1}), (S3: 9.33dS m^{-1}) and (S4: 15.23 dS m^{-1}). Three pots of 50 kg soil content were irrigated with full and equal saline water for one month. At the end of one month, soil samples were taken to determine the average soil salinity in the three pots, and the soil samples' electrical conductivity (EC) was determined (Rhoades, 1992). Saturated soil salinity values were 4.17, 9.33 and 15.23 dS m^{-1}. The prepared saline soils were filled in 48 pots made of polyethylene material with 15 cm and 18 cm dimensions. Before the soil was filled, mulch was cut and placed in the landfill to allow drainage at the bottom of each pot.

Seed sterilization and treatments

Before germination, seeds were surface sterilized using 5% hypochlorite. Sterilized seeds were primed in proline solution with a concentration of 15 mM for 24 hours and then dried at room temperature. After pretreatment, 288 seeds primed with proline were sown in 24 pots under different saline soil ratios. Detailed information about treatments is given in Table 2.

Table 2. The detailed information for experimental treatments

Saturated soil salinity levels	**Treatments**	**Description**
S_1 : 0.28 dS m^{-1} S_2 : 4.17 dS m^{-1} S_3 : 9.33 dS m^{-1} S_4 : 15.23 dS m^{-1}	Control	Wheat seeds were sown without priming with proline solution or adding humic acid to the soil.
S_1 : 0.28 dS m^{-1} S_2 : 4.17 dS m^{-1} S_3 : 9.33 dS m^{-1} S_4 : 15.23 dS m^{-1}	Proline	Wheat seeds were primed with 15 mM proline solution and then sown to the soil.
S_1 : 0.28 dS m^{-1} S_2 : 4.17 dS m^{-1} S_3 : 9.33 dS m^{-1} S_4 : 15.23 dS m^{-1}	Humic Acid	Just 5 lt/ha HA solution was added to the pots before seed sowing and after thinning.
S_1 : 0.28 dS m^{-1} S_2 : 4.17 dS m^{-1} S_3 : 9.33 dS m^{-1} S_4 : 15.23 dS m^{-1}	Proline + Humic Acid	Wheat seeds were primed with 15 mM proline solution, and 5 lt/ha humic acid was added to the soil prior to seed sowing.

Agronomic practices and measurement

Before sowing the seeds in the pots, three pots were filled with experimental soil (S_1: 0.28 dS m^{-1}). These pots were then saturated with tap water, and the pot surfaces were covered with plastic. When the drainage water flow had stopped, each pot was weighed on a precision balance, and the mean value was taken as the pots' field capacity. All pots were irrigated with 0.18 dS m^{-1} water during the experimental period. Irrigation was performed when 50% of the available soil moisture was depleted in the S_1 treatments. The twelve seeds were sown in each pot. When the plants had two leaves, the thinning was carried out, and the study was continued by leaving seven plants in each pot. Accordingly, 0.623 g of triple super phosphate fertilizer was applied as base fertilizer, and 0.182 g of urea fertilizer was applied immediately after seedling emergence. All plants in each pot were harvested 111 days after sowing. All plants in each pot were harvested, and plant height, stem diameter, above-ground fresh and dry biomass, and root fresh and dry weights parameters were determined. Plant height was measured in centimeters (cm) using a ruler, considering the distance from the soil level to the top of the plant. To determine the fresh root weight of each pot, each pot was rinsed with tap water to remove soil, and the roots were collected and weighed. The plant and root fresh weights were weighed separately on a precision balance, then dried in an oven at 70 °C (Nüve, EN 400) for 24 hours, and the dry weight of the plant and root were weighed. Before harvesting, stomatal conductance was assessed using a porometer (AP4 Porometer Delta-T, Cambridge, UK) from three points on fully developed upper leaves of three different plants within each pot and then averaged (Sezer et al., 2021).

Different ratios and stress tolerance indexes

Root/shoot ratio, shoot weight ratio, and root weight ratio were calculated as follows using the equations below (Hunt 1982);

$$\text{Root shoot ratio (RSR)} = \frac{\text{RDW}}{\text{SDW}}$$

$$\text{Shoot weight ratio (SWR)} = \frac{\text{SDW}}{\text{TDW}}$$

$$\text{Root weight ratio (RWR)} = \frac{\text{RDW}}{\text{TDW}}$$

RDW is the root dry weight, SDW is the shoot dry weight, and TDW is the total dry weight.

To observe the stress tolerance index for plant height, shoot fresh and dry weight root fresh and dry weight was calculated by the following equations, according to Ashraf and Harris (2004). Also, the relative tolerance index was calculated according to Pantoja-Benavides et al. (2021) to understand how stomatal conductance differed according to treatments.

Plant Height Stress Tolerance Index

$$\text{PHSTI (\%)} = \left[\frac{\text{Plant height of } S_x \text{ for each of C, P, HA, P+HA treatment}}{\text{The average plant height of } S_0}\right] \text{x}100$$

S_x shows the plant height at S_2, S_3, and S_4 soil salinities for C, P, HA, and P+HA treatments.

S_0 indicates the average plant height under control treatment at the S_1 soil salinity level.

Shoot Fresh Weight Stress Tolerance Index

$$\text{SFWSTI (\%)} = \left[\frac{\text{Shoot fresh weight of } S_x \text{ for each of C, P, HA, P+HA treatment}}{\text{The average shoot fresh weight of } S_0}\right] \text{x100}$$

S_x shows the shoot fresh weight at S_2, S_3, and S_4 soil salinities for C, P, HA, and P+HA treatments.

S_0 indicates the average shoot fresh weight under control treatment at the S_1 soil salinity level.

Shoot Dry Weight Stress Tolerance Index

$$\text{SDWSTI (\%)} = \left[\frac{\text{Shoot dry weight of } S_x \text{ for each of C, P, HA, P+HA treatment}}{\text{The average shoot dry weight of } S_0}\right] \text{x100}$$

S_x shows the shoot dry weight at S_2, S_3, and S_4 soil salinities for C, P, HA, and P+HA treatments.

S_0 indicates the average shoot dry weight under control treatment at the S_1 soil salinity level.

Root Fresh Weight Stress Tolerance Index

$$\text{RFWSTI (\%)} = \left[\frac{\text{Root fresh weight of } S_x \text{ for each of C, P, HA, P+HA treatment}}{\text{The average root fresh weight of } S_0}\right] \text{x100}$$

S_x shows the root fresh weight at S_2, S_3, and S_4 soil salinities for C, P, HA, and P+HA treatments.

S_0 indicates the average root fresh weight under control treatment at the S_1 soil salinity level.

Root Dry Weight Stress Tolerance Index

$$\text{RDWSTI (\%)} = \left[\frac{\text{Root dry weight of } S_x \text{ for each of C, P, HA, P+HA treatment}}{\text{The average root dry weight of } S_0}\right] \text{x100}$$

S_x shows the root dry weight at S_2, S_3, and S_4 soil salinities for C, P, HA, and P+HA treatments.

S_0 indicates the average root dry weight under control treatment at the S_1 soil salinity level.

Relative Tolerance Index

$$\text{RTI (\%)} = \left[\frac{\text{Stomatal conductance of } S_x \text{ for each of C, P, HA, P+HA treatment}}{\text{The average stomatal conductance of } S_0}\right] \text{x100}$$

S_x shows the stomatal conductance at S_2, S_3, and S_4 soil salinities for C, P, HA, and P+HA treatments.

S_0 indicates the average stomatal conductance under control treatment at the S_1 soil salinity level.

Statistical analysis

The present study was conducted using a completely randomized design with two factors (soil salinity levels and treatments) and three replicates. Statistical analyses were performed using JMP software version 13.2 (SAS Institute, Inc., Cary, NC, USA). Root/shoot ratio, shoot weight ratio, and root weight ratio parameters were analyzed by two-way analysis of variance (ANOVA), and significance differences between means were evaluated by LSD test at the %5 probability level. Similarly, the significant differences between the means of the relative tolerance index and stress tolerance indexes were tested by one-way ANOVA with an LSD test at a %5 probability level. Microsoft Office 365 software was used to draw bar graphs for the stress tolerance index parameters, and the graphs were presented as means and standard errors of the three replicates.

RESULTS

As depicted in Table 3, wheat's root/shoot ratio was observed with increased soil salinity.

The highest root/shoot ratio (0.65) was obtained from the CxS2 treatment, and the lowest value (0.22) was obtained from the (P+HA)xS4 treatment. However, no significant difference was found between CxS_2, CxS_1, PxS_1, $HAxS_2$, and $(P+HA)xS_1$ treatments.

Table 3. Effects of different treatments on root/shoot ratio of wheat seedlings under different soil salinity conditions

Soil salinity	**Treatments (T)**				
(SS)	**Control**	**Proline (P)**	**Humic Acid (HA)**	**P + HA**	**Mean**
S_1	0.62 ab	0.59 abc	0.52 d	0.63 ab	**0.59 A**
S_2	0.65 a	0.58 bc	0.61 ab	0.55 cd	**0.60 A**
S_3	0.43 e	0.39 e	0.27 gh	0.32 fg	**0.35 B**
S_4	0.38 ef	0.38 ef	0.31 g	0.22 h	**0.32 C**
Mean	**0.52 A**	**0.49 B**	**0.43 C**	**0.43 C**	
	*$LSD_{0.05}$ SS = 0.03**; $LSD_{0.05}$ T = 0.03**; $LSD_{0.05}$ SS×T = 0.06***				

**: P < 0.01. Means in each column with lowercase letters are statistically significantly different according to the soil salinity and treatment interaction (P <0.05). Means marked with different capital letters in vertical and horizontal columns are statistically significantly different according to soil salinity and treatments, respectively. (P <0.05). Means with the same letter were not statistically significant at P ≤ 0.05. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

In Table 4, when examining the effect of various treatments on the shoot weight ratio of wheat seedlings under different soil salinity conditions, it was observed that as soil salinity increased, the shoot weight ratio of wheat increased. The highest value was obtained in S4 treatment. No significant differences was observed between S1 and S2 treatments The highest shoot weight ratio value was obtained for (P+HA)xS4 with 0.82, and the lowest shoot weight ratio value was obtained for (P+HA)xS1 with 0.61.

Table 4. Effects of different treatments on shoot weight ratio of wheat seedlings under different soil salinity conditions

Soil salinity (SS)	Treatments (T) Control	Proline (P)	Humic Acid (HA)	P + HA	Mean
S_1	0.62 fg	0.63 fg	0.66 e	0.61 g	**0.63 C**
S_2	0.61 g	0.63 efg	0.62 fg	0.64 ef	**0.63 C**
S_3	0.7 d	0.72 d	0.79 b	0.76 c	**0.74 B**
S_4	0.72 d	0.73 d	0.77 bc	0.82 a	**0.76 A**
Mean	**0.66 C**	**0.68 B**	**0.71 A**	**0.71 A**	
	*$LSD_{0.05}$ SS = 0.014**; $LSD_{0.05}$ T = 0.014**; $LSD_{0.05}$ SS×T = 0.028***				

***: $P < 0.01$.* Means in each column with lowercase letters are statistically significantly different according to the soil salinity and treatment interaction ($P < 0.05$). Means marked with different capital letters in vertical and horizontal columns are statistically significantly different according to soil salinity and treatments, respectively. ($P < 0.05$). Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

As shown in Table 5, the effect of different treatments applied to wheat seedlings at various soil salinity levels on the root weight ratio was evaluated. The findings indicated that an increase in soil salinity led to a decrease in the root weight ratio of wheat. However, no statistically significant difference was found between the S1 and S2 conditions and the humic acid and P+HA applications. The highest value was observed under the CxS2 condition, while the lowest was under the (P+HA)xS4 condition.

Table 5. Effects of different treatments on the root weight ratio of wheat seedlings under different soil salinity conditions

Soil salinity (SS)	Treatments (T) Control	Proline (P)	Humic Acid (HA)	P + HA	Mean
S_1	0.38 ab	0.37 ab	0.34 c	0.39 a	**0.37 A**
S_2	0.39 a	0.37 abc	0.38 ab	0.36 bc	**0.37 A**
S_3	0.3 d	0.28 d	0.21 f	0.24 e	**0.26 B**
S_4	0.28 d	0.27 d	0.23 ef	0.18 g	**0.24 C**
Mean	**0.34 A**	**0.32 B**	**0.29 C**	**0.29 C**	
	*$LSD_{0.05}$ SS = 0.014**; $LSD_{0.05}$ T = 0.014**; $LSD_{0.05}$ SS×T = 0.028***				

***: $P < 0.01$.* Means in each column with lowercase letters are statistically significantly different according to the soil salinity and treatment interaction ($P < 0.05$). Means marked with different capital letters in vertical and horizontal columns are statistically significantly different according to soil salinity and treatments, respectively. ($P <0.05$). Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

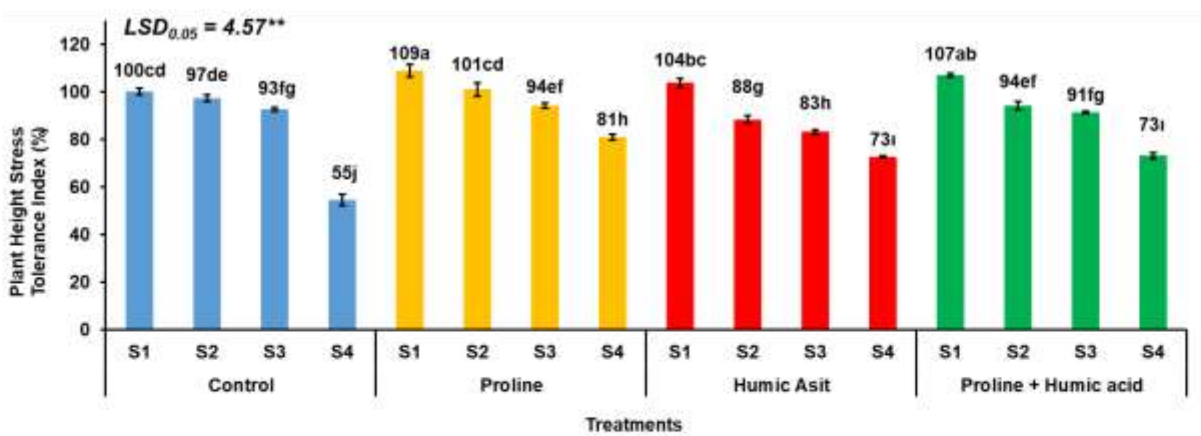

Figure 1. Response of plant height stress tolerance index of wheat seedling to different treatments and soil salinity levels. Results on each vertical bar graph are shown as the mean of three replicates ± standard errors. The least significant difference test was used to compare means based on analysis of variance (ANOVA) at the %5 probability level. Means with the same letter were not statistically significant at *$P \leq 0.05$*. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23, and 15.23 dS $m^{-1,}$ respectively.

As soil salinity increases, plant height stress tolerance index values have decreased across all treatment conditions (Fig. 1). The conditions where plants are most exposed to stress are the s4 conditions. As depicted in Figure 1, compared to the CxS1 condition, the CxS4 condition exhibited a decrease of 45%. However, the PxS_1, $HAxS_1$, $(P+HA)xS_1$ treatments showed a tolerance of 9%, 4%, and 7%, respectively, compared to the CxS1 condition.

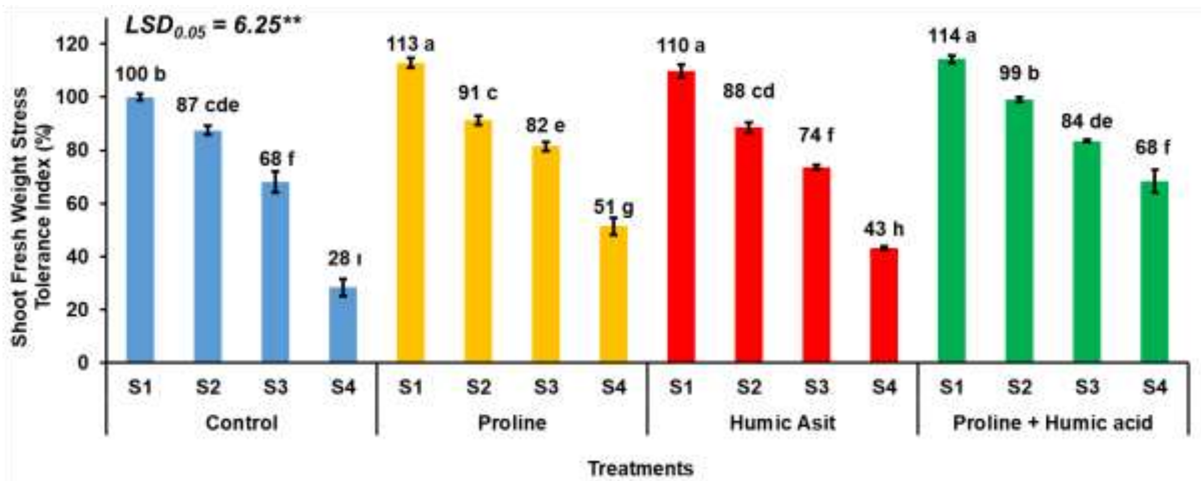

Figure 2. Response of shoot fresh weight stress tolerance index of wheat seedling to different treatments and soil salinity levels. Results on each vertical bar graph are shown as the mean of three replicates ± standard errors. The least significant difference test was used to compare means based on analysis of variance (ANOVA) at the %5 probability level. Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

With an increase in soil salinity, there has been a decrease in shoot fresh weight stress tolerance index values across all treatment conditions (Fig. 2). As shown in Figure 2; it is observed that in comparison to the CxS_1 condition, the values have increased by 14% in the case of the $(P+HA)xS_1$ interaction, by 13% in the PxS_1 condition, and by 10% in the $HAxS_1$ condition. The lowest shoot dry weight stress tolerance index values were found under the S_4 conditions.

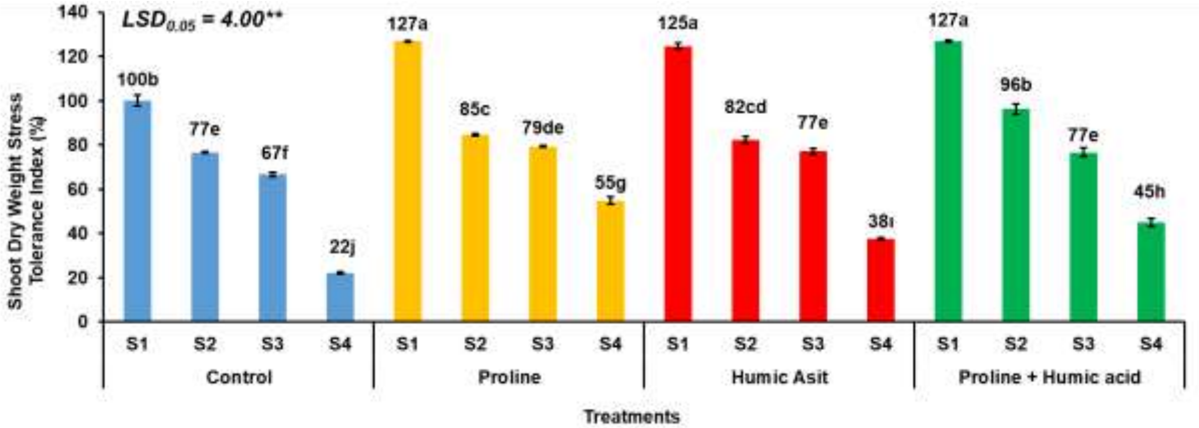

Figure 3. Response of shoot dry weight stress tolerance index of wheat seedling to different treatments and soil salinity levels. Results on each vertical bar graph are shown as the mean of three replicates ± standard errors. The least significant difference test was used to compare means based on analysis of variance (ANOVA) at the %5 probability level. Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

With an increase in soil salinity, there has been a decrease in Shoot dry weight stress tolerance index values across all treatment conditions. Figure 3 shows that the highest root fresh weight stress tolerance index value was found at the (P+HA)xS1 treatment, while the wheat seedling was more depressed at the CxS4 condition. Additionally, it has been observed that the (P+HA)xS_1 condition shows a 27% increase in comparison to the CxS_1 condition,

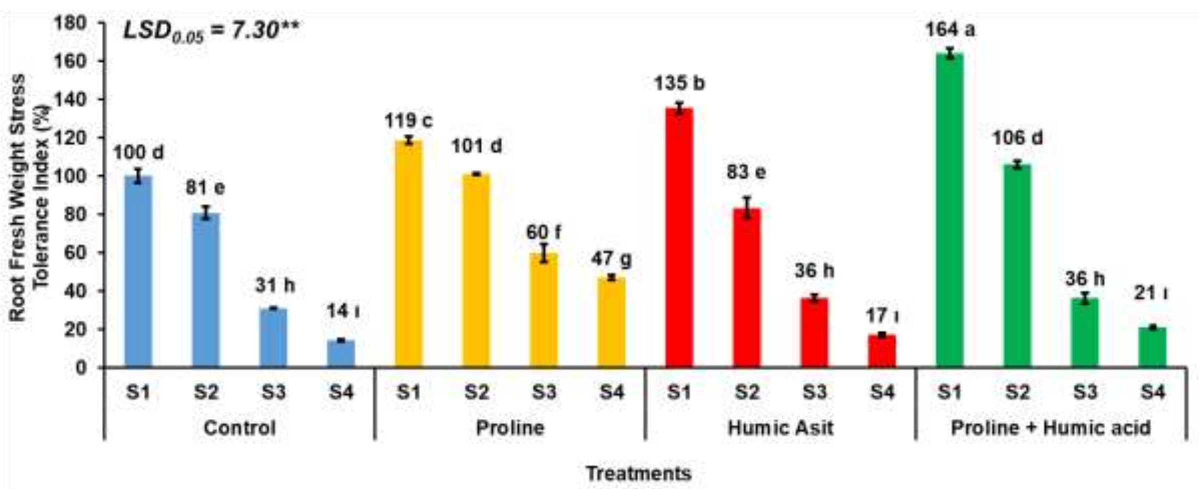

Figure 4. Response of wheat seedling's root fresh weight stress tolerance index to different treatments and soil salinity levels. Results on each vertical bar graph are shown as the mean of three replicates ± standard errors. The least significant difference test was used to compare means based on analysis of variance (ANOVA) at the %5 probability level. Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

Compared to the CxS_1, considering root fresh weight stress tolerance index values, the lowest values were obtained under the S_4 conditions, while the highest values were observed under the S_1 conditions (Fig. 4). Regarding all treatments, wheat seed priming with proline and application of humic acid to soil increased root fresh weight stress tolerance of wheat seedlings, meaning that P+HA treatment was more effective in improving root fresh weight than C, P, and HA treatments. Moreover, Considering the S2 treatments, the P+HA application had a higher effect on root fresh improvement than the rest treatment, and it also increased the 6% root fresh weight than the CxS1 treatment. (Fig. 4).

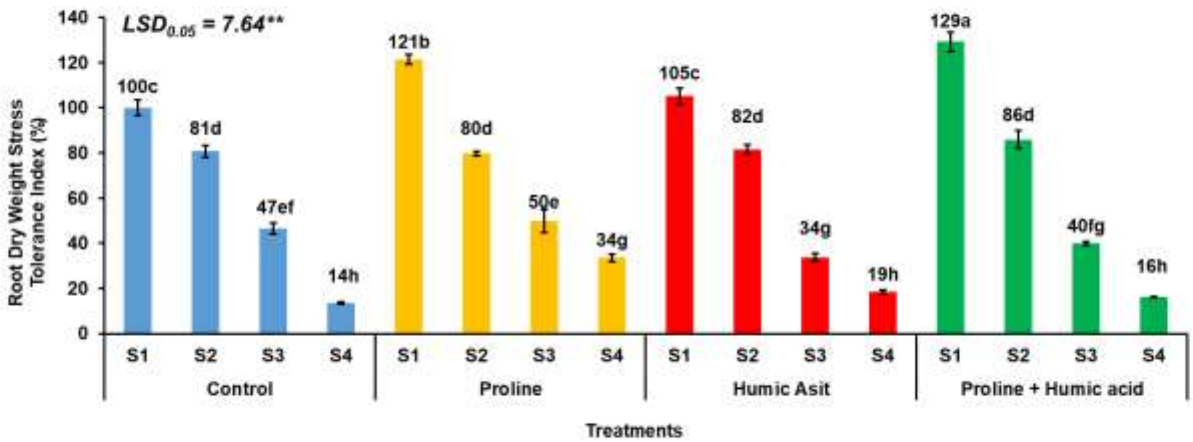

Figure 5. Response of wheat seedling's root dry weight stress tolerance index to different treatments and soil salinity levels. Results on each vertical bar graph are shown as the mean of three replicates ± standard errors. The least significant difference test was used to compare means based on analysis of variance (ANOVA) at the %5 probability level. Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

As depicted in Fig. 5 plant root dry weight stress tolerance index values decreased with increasing soil salinity. The highest decrease was observed under the (CxS4) condition, while the highest increase was seen under the $(P+HA)xS_1$ condition. It was found that the seedlings grown under the (P+HA)xS1 condition had a 29% increase in root dry weight stress tolerance index value compared to the CxS1 condition (Fig. 5).

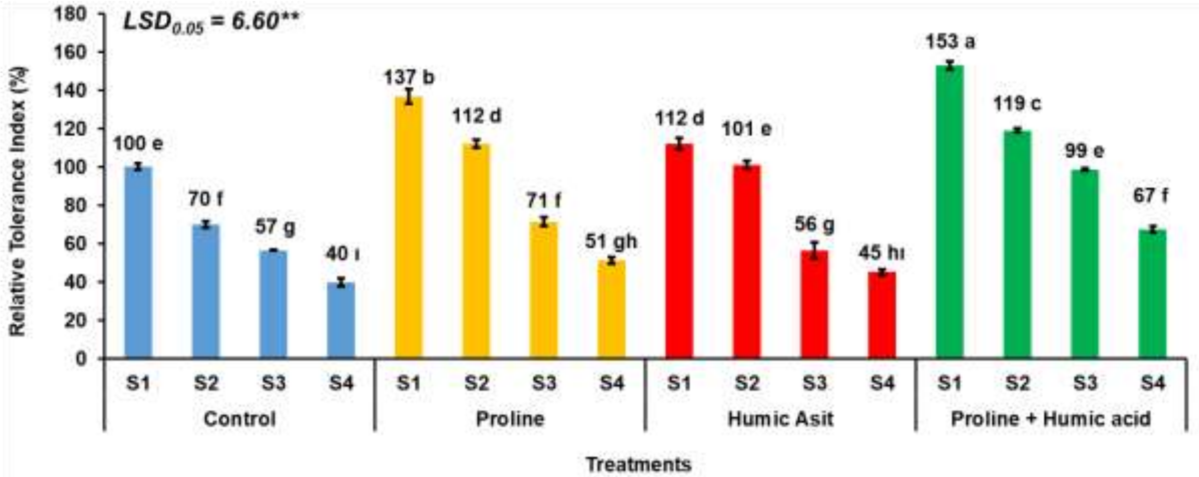

Figure 6. Response of relative tolerance index of wheat seedling to different treatments and soil salinity levels. Results on each vertical bar graph are shown as the mean of three replicates ± standard errors. The least significant difference test was used to compare means based on analysis of variance (ANOVA) at the %5 probability level. Means with the same letter were not statistically significant at $P \leq 0.05$. S1, S2, S3, and S4 represent saturated soil salinities of 0.28, 4.17, 9.23 and 15.23 dS m^{-1} respectively.

An increase in salinity stress leads to a decrease in the plant's relative tolerance index values (Fig. 6). The highest tolerance, with a 53% increase, was found in the (P+HA)xS1 treatment compared to the CxS1 condition (Fig. 6). However, the wheat seedling was more depressed at the CxS4 condition, with a 60% decrease compared to the CxS1 treatment. Furthermore, it was observed that proline application alone is effective in increasing plant tolerance, but its interaction with humic acid significantly increases the tolerance of wheat seedlings up to the S2 level. The (P+HA)xS2 treatment showed a 19% increase in tolerance compared to the CxS1 condition (Fig. 6).

DISCUSSION

The results of this study provide important insights into how priming, as a pretreatment method for seeds, and adding humic acid to the soil can alter the effects of varying soil salinity levels on the growth and developmental abilities of wheat seeds. Firstly, by stimulating seed metabolism before germination and initiating metabolic processes, the priming method can contribute to more effective growth and development of seeds. While these processes are typically activated in the early stages of germination, they might hinder full germination. Priming, especially with proline, can notably enhance wheat seeds' tolerance capacity to salt stress. Proline, an organic osmo protectant, accumulates in plant tissues under stress.

Additionally, proline serves as an antioxidant defense molecule, metal chelator, protein stabilizer, ROS scavenger, and inhibitor of programmed cell death. These attributes suggest that proline can enhance salt stress resilience and positively impact growth. On the other hand, humic acid (HA) is recognized as a biostimulant with significant effects on plant growth and development. Its positive effects can extend to protecting plants from toxic impacts and aiding in drought and salt stress mitigation. By influencing root development, humic acid assists plants in adapting to soil conditions. This study also investigates how different soil salinity levels can modify these positive effects. The findings depicted that plant growth is negatively affected as soil salinity increases. Exposure of wheat seeds to salt stress may contribute to increased root/shoot ratios, particularly evident at higher salinity levels. Based on the findings, an increase in soil salinity leads to an increase in the root/shoot ratio of wheat plants. The highest root/shoot ratio was observed in the CxS2 condition, while the lowest was in the (P+HA)xS4 condition. However, no significant differences were observed among conditions such as CxS2, CxS1, PxS1, HAxS2, and (P+HA)xS1. When examining the shoot weight ratio of seedlings, an increase in soil salinity was associated with an increase in this weight ratio. The highest shoot weight ratio was obtained under the S1, S2, S3, and S4 conditions, with no significant difference between S1 and S2.

Evaluation of the root weight ratio revealed that an increase in soil salinity led to a decrease in this ratio. However, no statistically significant differences existed between the S1 and S2 conditions and humic acid and P+HA applications. The highest root weight ratio was observed in the CxS2 condition, while the lowest was in the (P+HA)xS4 condition. As soil salinity increases, a decrease in the plant height stress tolerance index values is observed. The highest decrease is noted under the S4 conditions, but comparisons across treatment conditions indicate that the (P+HA)xS1 condition shows a higher tolerance. Similarly, in the shoot fresh and dry weight stress tolerance index values, an increase in soil salinity leads to a decrease. The highest values are associated with the (P+HA)xS1 condition for the shoot dry weight stress tolerance index, while the lowest is associated with the CxS4 condition. When examining the root fresh weight stress tolerance index values, an increase in soil salinity results in lower values, with the highest values observed under the s1 conditions. The application of P+HA has been found to increase root fresh weight values under the S2 conditions compared to the CxS1 condition. Lastly, increasing soil salinity decreases the plant's relative tolerance index values. The highest increase in tolerance is observed in the (P+HA)xS1 condition, while the lowest value is associated with the CxS4 condition.

CONCLUSIONS

The present investigation reported the changes in salinity tolerance indices of 'Falado' wheat seedlings according to proline, humic acid and their interaction under different soil salinity conditions. The results confirmed that only wheat seed priming with 15 mM proline was more effective than 5 lt/ha humic acid applied to soil in improving the salt tolerance of wheat seedlings. In contrast, both wheat seed priming with 15 mM proline and 5 lt/ha humic supplying to the soil was more effective than priming seed with proline or humic acid application to the soil in boosting seedling fresh weight, root growth ability, and increasing relative tolerance index under soil salinity conditions. Therefore, before sowing wheat seeds, priming with 15 mM proline and adding 5 lt/ha humic acid to the soil is recommended to increase salt tolerance and improve wheat seedling growth performance in high salt-affected soil areas.

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EFFECTS OF SUNFLOWER MEAL FERMENTED WITH RUMEN LIQUID ON NUTRIENT COMPOSITION

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ABSTRACT

In this study, it was aimed to improve the nutritional composition of sunflower meal by fermenting it at different fermentation times with the solid state fermentation method. The study was divided into five groups (Control, 1, 3, 5 and 7 days of fermentation) and a total of 15 samples, three replicates in each group, were used. Sunflower meal was ground to a size of 1 mm, and then nutritional salt was added and sterilized after adjusting 80% ambient humidity. Rumen liquid was collected from 2-year-old cattle and prepared for inoculation in a sterile laboratory environment. 1 *N* HCl was used to maintain a pH of 6.0 in the fermentation medium. Fermentation was carried out at 38 °C. After the fermentation period was completed, the pH of the samples was measured and then dried at 60 °C and prepared for analysis. Samples were analyzed for dry matter, ash, crude protein, ether extract, and crude fiber content. Results indicated that fermentation had a positive impact on the nutrient composition of sunflower meal. The highest crude protein and ash were found on the first day ($P<0.001$), the highest ether extract was found on the fifth day ($P<0.001$), and the lowest crude fiber was found on the first day ($P<0.001$) among the fermented groups, and the difference was significant. Based on these findings, it can be concluded that a one-day solid-state fermentation of sunflower meal using rumen liquid is the most effective time.

Keywords: Solid state fermentation, rumen liquid, nutrient composition, sunflower meal

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a plant that exhibits high adaptability to diverse climatic and soil conditions and is cultivated for the production of oil, meal, and biodiesel fuel (Kallivroussis et al., 2002; Yegerov et al., 2019). Approximately 90% of the sunflower seed produced in the world is processed for oil, and the average oil yield is 43%. The remaining part, sunflower meal (SM), is used as animal feed. The chemical composition of SM can vary depending on plant characteristics (seed composition, hull-to-kernel ratio, dehulling potential, growth stage, and storage conditions) and processing techniques (dehulling, mechanical, and solvent extraction) (Golob et al., 2008). In hulled expeller meals, crude protein can be as low as 24%, while fiber content can reach up to 32% (Clef & Kemper, 2015). Fat content in this type of SM can range between 7% and 10%. However, in solvent-extracted or press + solvent-extracted

SM varieties, crude protein ranges from 24% to 44%, with corresponding fiber contents of 12% to 25% (Clef & Kemper, 2015). SM, with 44% crude protein, is comparable to soybean meal (SBM) in terms of protein.

Despite its potential as a valuable feed ingredient for poultry in countries unsuitable for SBM cultivation, SM contains certain undesirable factors that limit its inclusion level in diets or its use as a substitute for SBM in animals. Compared to other oilseeds, SM has a higher content of non-starch polysaccharides (NSPs), which are indigestible by poultry (Dusterhoft & Voragen, 1991). Cell wall components such as beta-glucans, xylans, arabans, pectins, and oligosaccharides lead to increased viscosity in the digestive tract, reduced feed intake, and depressed growth in chicks (Moghaddam et al., 2012). The use of only SM in diets negatively affected the morphological parameters of the small intestinal mucosa of roosters, especially in the ileum, resulting in decreased nutrient absorption (Hamedi et al., 2011). Therefore, even if the effects of the factors mentioned above are eliminated, SM cannot become competitive with SBM. To reduce the high fiber content and enrich the amino acid profile of SM, alternative strategies must be implemented. One such approach could be the use of solid-state fermentation (SSF).

SSF refers to microorganism growth and metabolic activity on moistened solid substrates without free water (Mitchell et al., 2000). SSF enables the conversion of nutrient components through enzymatic interactions of microorganisms on the feedstuff (Steudler et al., 2019). This process improves the nutrient composition of the feedstuff, reduces anti-nutritional components, and increases bioavailability. SSF also increases the digestibility of the feedstuff (Özlü & Altop, 2023) and the bioavailability of nutrients (Güngör & Erener, 2023). In fermentation studies, bacteria, fungi, yeast, or their combinations are generally preferred as inoculants (Adeyemi et al., 2008; Akinfemi, 2010; Ari & Ayanwale, 2012; Ari et al., 2012). In addition, there are studies in which rumen liquid was also used as an inoculant. In these studies, it was reported that the nutrient composition of the feedstuffs improved (Özlü et al., 2022a; Özlü et al., 2022b; Altop et al., 2022; Koç et al., 2021).

The objective of this study is to reduce the crude fiber content, an anti-nutritional factor for poultry, and to enrich the protein content of SM through solid-state fermentation using rumen liquid.

MATERIAL AND METHOD

The SM used in fermentation was obtained from a local feed mill, brought to the laboratory, ground to 1 mm, and stored at +4 °C until the research was carried out. Rumen liquid was obtained from 2 years old cattle fed mainly on roughage. The rumen liquid was then brought to the laboratory and prepared for analysis in a sterile environment.

The study was carried out in 15 samples, including a non-fermenting group and four different fermentation times (1, 3, 5, and 7 days) and three replicates per group.

After mixing SM with nutrient salt, a fermentation medium with 80% humidity was prepared. The initial pH of the fermentation medium was adjusted to 6.0 using 1 N HCl after sterilization. Inoculation was then performed in a sterile environment at a rate of 1% per 100 g of SM.

The samples were kept at 38 °C until the fermentation period was completed, and then the pH of the samples was measured. After the samples were dried at 60 °C and then stored at +4 °C until the analysis period. Dry matter, ash, crude protein, crude fiber, and ether extract analyses were performed according to the method described by Akyıldız (1984).

The data obtained at the end of the research were analyzed using SPSS 21.0 (SPSS Inc., NY, and the USA) statistical package program. Duncan test compared the differences between groups after the ANOVA test for the data variance. Results were considered significantly different at $P<0.05$.

RESULTS AND DISCUSSION

At the end of the study, a significant decrease in pH values was observed in all groups. The lowest pH was obtained from samples fermented for one and three days, and the difference was significant ($P<0.001$). This decrease in pH values is consistent with previous fermentation studies using rumen liquid (Özlü et al. 2023; Güngör et al. 2023) and indicates the effectiveness of microorganisms in fermentation.

Fermentation positively affected the nutrient composition of SM. The highest crude protein and ash were determined on the first day ($P<0.001$), the highest ether extract was determined on the fifth day ($P<0.001$), and the lowest crude fiber among the fermented groups was determined on the first day ($P<0.001$). In fermentation studies using rumen liquid, an increase in crude protein and a decrease in crude fiber have been reported, resulting in an increase in feedstuff nutritional value and a reduction in anti-nutritional factors (Güngör et al., 2023).

CONCLUSION

In conclusion, the nutrient composition of SM was improved through fermentation with rumen liquid. Based on the results, a one-day solid-state fermentation of SM using rumen liquid was determined to be the most effective fermentation time.

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ANALYSIS OF THE STRUCTURES AND MANAGER'S PROFILES OF AGRICULTURAL DEVELOPMENT COOPERATIVES IN ÇANAKKALE

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ABSTRACT

Cooperatives are essential tools in providing rural development. The role of cooperatives is also essential in strengthening the agricultural sector. The characteristics of the cooperative manager are effective in the success of a cooperative. On the other hand, the structure of the cooperatives is essential. This study was carried out specifically for agricultural development cooperatives in the province of Çanakkale. The institutional structures of the cooperatives in the area were examined. In addition, the socio-economic characteristics of cooperative managers and the problems of cooperatives were investigated. According to the results, cooperatives have been operating for an average of 28 years. The average number of members of the cooperatives was determined as 91. 15.54% of the cooperatives have businesses, and 43.6% have financial difficulties. Cooperative managers are, on average, 49 years old, and their management experience is an average of 10 years. 78.1% of the managers attend meetings related to agriculture, and only 54.5% of them have received training on cooperatives.

Keywords: Agricultural development cooperatives, Manager, Cooperative assets

INTRODUCTION

The development of rural areas, stopping rural-urban migration, maintaining sustainable agricultural production and ensuring justice in income distribution are common goals of all countries. The most appropriate organization to achieve this goal is agricultural cooperatives. The most important task of agricultural cooperatives is to protect the economic rights of farmers. In addition to the economic benefits they provide to farmers, agricultural cooperatives play an important role in local and regional development. Considering that the agricultural structure in Turkey is generally dispersed and consists of many small enterprises, horizontal and vertical organization of farmers through cooperatives is necessary for development (İnan and Serikli, 2007). In developed countries, cooperatives are actively involved in the agricultural sector, but in Turkey, it is clear that agricultural cooperatives are mostly developed numerically and are not sufficient in terms of management (Acar and Yıldırım, 2000). The origin of the agricultural cooperative movement in Turkey is based on the peasants' demand for inputs, cash and product marketing needs. Meeting the input demands of agricultural producers and marketing their products are still among the most serious problems of the agricultural sector. The most effective and easy solution to these problems is for producers to act together under the roof of cooperatives (Başaran and Irmak, 2018).

Agricultural development cooperatives are one of the most common types of agricultural cooperatives in Turkey. They have important tasks for the development of villages. In addition, agricultural development cooperatives are important organizations that can provide producer organization to meet the increasing demand in the production of milk and meat products, which have an important place among animal foods for a balanced and healthy diet of the society (Bayramoğlu and Direk, 2006). These cooperatives are independent organizations that provide multi-dimensional development of producers (Dedeoğlu and Yıldırım, 2006). However, these cooperatives cannot fully fulfill their important tasks in rural development, especially due to the financial difficulties they face and the lack of ownership of the cooperatives by the members.

The aim of the research is to analyze the general situation of agricultural development cooperatives in Çanakkale province and to examine the socio-economic status of their managers. The research was conducted in Çanakkale province. Çanakkale is a province where agricultural production and cooperatives in agricultural production are developed. Agricultural development cooperatives are the most established cooperative type in Çanakkale.

MATERIAL AND METHOD

Material

The main material of the study was primary data obtained from the managers of Agricultural Development Cooperatives in Çanakkale province through a questionnaire. Primary and secondary data were utilized in the research, and the data obtained as a result of the survey constituted the primary data. On the other hand, the secondary data of the research consisted of the data of the relevant Ministries, the results of the research conducted by other researchers on the subject, official statistics, data obtained from compilations, reviews and theses.

Methodology

Methodology Used in Data Collection

According to data from Çanakkale Provincial Directorate of Agriculture and Forestry, there are 307 Agricultural Development Cooperatives in Çanakkale province as of the date of the survey (Çanakkale Provincial Directorate of Agriculture and Forestry, 2019). The managers of these cooperatives constituted the population of the study. The number of farmers to be surveyed was determined by proportional sampling method (Newbold, 1995). While determining the sample volume, a 90% confidence interval with a 10% margin of error was used and the sample volume was found to be 55 according to the formula below.

$$n = \frac{N * p * q}{(N-1) * \sigma^2 p + p * q} = \frac{307 * 0.5 * 0.5}{(307-1) * 0.00369 + (0.5) * (0.5)} = 55$$

$\sigma 2p = (r / Z_{\alpha/2}) = (0.10/1.645)^2 = 0.00369$

n = Number of cooperative managers in the sample

N = Main population size

p = Main population proportion

var ² px = Variance of the main mass ratio

The determined sample volume was distributed proportionally among the districts. Accordingly, the distribution of the number of questionnaires by districts is as shown in Table 1.

Table 1 Distribution of interviewed cooperatives by districts

Name of District	Number of Agricultural Development Cooperatives	Number of Cooperative Managers interviewed
Ayvacık	14	3
Bayramiç	30	5
Biga	81	15
Çan	40	7
Eceabat	6	1
Ezine	12	2
Gelibolu	21	4
Lâpseki	22	4
Merkez	18	3
Yenice	63	11
Toplam	307	55

Methods Used in Data Analysis

In the study, basic descriptive statistics such as number, percentage, minimum, maximum and mean values were used to evaluate the data.

RESULTS AND DISCUSSION

General Information about the Cooperatives Participating in the Survey

According to the data, cooperatives have been in operation for an average of 28 years. Therefore, there is a long-established cooperative tradition in the study area. The minimum number of members in cooperatives was 18, the maximum number of members was 231 and the average number of members was 91. 89.1% of cooperatives are engaged in livestock (milk collection), 14.5% in crop production, 10.9% in irrigation, and 1.8% in forest products. The majority of cooperatives, 94.6%, operate only in their own villages. It was reported that 56.3% of cooperatives rent their buildings, and the majority of the rented buildings belong to the village legal entity. Everest et al. (2019) also found that 52% of the cooperative buildings of agricultural development cooperatives were rented, 42.67% had their own buildings, and 5.33% had no buildings. It is observed that cooperatives generally do not have much office equipment. Everest et al. (2019) also found that cooperatives have weak institutional structures. Except for milk tanks and generators, the cooperatives have very poor equipment and machinery, and therefore the use of common agricultural machinery is not developed. It was found that 85.5% of the cooperatives did not have any economic enterprises. General information about the cooperatives participating in the survey is shown in Table 2.

Table 2 General information about the cooperatives participating in the survey

Criteria	***Number***	***Rate (%)***
How many years they have been in business		
1-10 years	5	9
11-20 years	18	33
21-30 years	9	16
31-40 years	9	16
41-50 years	13	24
50-60 years	1	2
Minimum: 6, Maximum: 53, Mean: 28 Standard Deviation: 14,20		
Number of members		
10-49	16	29,09
50-99	17	30,91
100-249	22	40,00
Minimum: 18, Maximum: 231, Mean: 91.49, Standard Deviation: 54,46		
Gender of members		

Female	477	9,4
Male	4555	91,6
Working areas		
Livestock (milk collection)	37	67,3
Livestock (milk collection) and crop production	4	7,3
Livestock (milk collection) and irrigation	4	7,3
Crop production	3	5,5
Crop production and cold storage management	1	1,8
Livestock (milk collection)and weighing scales	2	3,6
Livestock (milk collection), weighing scales and forest products	1	1,8
Livestock (milk collection), irrigation and milling	1	1,8
Forest products	1	1,8
Irrigation	1	1,8
Number of villages in the operational area		
1 village	52	94,6
2 villages	2	3,6
4 villages	1	1,8
Ownership status of administrative buildings		
Rent	31	56,3
Own	21	38,2
Absent	3	5,5

From the data obtained, it can be observed that the majority of cooperatives sell their products without processing them, in other words, they do not engage in value-added production. When asked about the problems of the cooperatives, 67.3% of the cooperatives stated that they had problems, while 32.7% stated that they did not have any problems. The cooperatives that reported problems mostly reported financial problems and high input costs. The problems of the surveyed cooperatives are shown in Table 3.

Table 3 The problems of the surveyed cooperatives

Criteria	***Number***	***Rate (%)***
Problems		
Financial trouble	12	21,8
High input costs	12	21,8
Members' milk production decreased	8	14,5
Members do not take care of the cooperative	7	12,7
Milk cooling costs must now be covered by the cooperative	5	9,1
Low milk prices	3	5,5
No suitable buildings for milk pouring	1	1,8
Milk storage tanks are insufficient	1	1,8
Cooperatives are not adequately audited	1	1,8

Socio-Economic Status of Cooperative Managers

The average age of cooperative managers is 49. The youngest manager is 29 and the oldest manager is 70 years old. Şahin et al. (2015) found that the average age of agricultural development cooperative members was 48.0 years, with the youngest member being 24 years old and the oldest member being 70 years old. The average length of time that cooperative managers have been a member of the cooperative is 16.8 years. Everest (2015) also found that the average number of years of farmers' membership in agricultural credit cooperatives was 17 years. The minimum number of years of membership was 1 and the maximum number was 46 years. Cooperative managers have been in cooperative management for an average of 10 years. Cooperative management experience was determined as minimum 1 and maximum 30 years. The majority of cooperative managers, 89.1%, were married. It is observed that 56.4% of cooperative managers are primary school graduates. The majority of cooperative managers, 78.1%, have attended a meeting on agriculture in the last year. The majority of cooperative managers (81.8%) do not have a membership to an agricultural journal or publication. The majority of cooperative managers (78.2%) use the internet to access agricultural information. More than half of the cooperative managers have received cooperative education. In Everest's (2015) study, farmers were asked whether agricultural credit cooperatives organized training on cooperatives, and 29.97% of farmers stated that no training was organized. It is observed that 72% of those who did not receive cooperative training would like to receive this training. In Everest's (2015) study, 73.93% of the farmers were asked whether they would like to receive cooperative training if cooperative training was not organized by the cooperative, and 73.93% stated that they would like to receive training on cooperatives. The socio-economic status of cooperative managers is shown in tables 4 and 5

Table 4 The social statuss of cooperative managers

Criteria	***Number***	***Rate (%)***
Age Level (Years)		
≤49	27	49,1

>49	28	50,9
Minimum:29, Maximum:70, Mean:49.34, Standard Deviation:9.39		
Duration of cooperative membership (years)		
<16	35	63,6
≥16	20	36,4
Minimum:1, Maximum:46, Mean:16.86, Standard Deviation:9.97		
Management Experience (Years)		
<10	23	41,8
≥10	32	58,2
Minimum:1, Maximum:30, Mean:9.74, Standard Deviation:6.71		
Marital Status		
Married	49	89,1
Single	6	10,9
Education level of managers		
Primary school	31	56,4
Secondary School	8	14,5
High school	13	23,6
Higher Education	3	5,5
Participation in a meeting on agriculture		
Participated	43	78,1
Not participated	12	21,9
Membership in an agricultural journal or publication		
Yes	10	18,2
No	45	81,8
Managers' use of the internet to access agricultural information		
Yes	43	78,2
No	12	21,8

Have cooperative managers received cooperative training?		
Yes	30	54,5
No	25	45,5
Whether those who have not received cooperative education would like to receive cooperative education		
Yes	18	72,0
No	7	28,0

It was observed that 81.8% of cooperative managers own their own land, 54.5% rent land for cultivation and 7.3% cultivate land with partners. The average size of the land owned by the cooperative managers is 86.4 decares, the average size of the land rented is 67.1 decares, and the average size of the land cultivated with partners is 41.3 decares. It is observed that 72.7% of cooperative managers own cattle and 27.2% of them own ovines. The average number of cattle owned by cooperative managers is 26.9 and the average number of ovines is 36.1. It is observed that 61.8% of cooperative managers have non-agricultural income. Everest (2015) also found that 63.76% of the members of agricultural credit cooperatives had non-agricultural income. Everest and her colleagues (2019) also found that 65.89% of managers of agricultural development, irrigation and aquaculture cooperatives had non-agricultural income.

Table 5 The economic status of cooperative managers

Criteria	***Number***	***Rate (%)***
Land sizes (decares)		
≤100	45	81,8
>100	10	18,2
Animal assets	***Average number of animals (head)***	***Ownership rate (%)***
Cattle	26,9	72,7
Ovine	36,1	27,2
Annual Agricultural Income (TL)		
<10.000	6	10,9
10.001-20.000	12	21,8
20.001-50.000	19	34,5
50.001-100.000	14	25,5

>100.000	4	7,3
Does he/she have non-agricultural income?		
Yes	34	61,8
No	21	38,2

CONCLUSION and RECOMMENDATIONS

The majority of cooperatives are engaged in milk collection, have poor machinery and equipment, do not sell value-added products, are not financially strong, and the majority of members are men. The majority of cooperatives are active in only one village. It is known that cooperatives generally do not have a great deal of activity on their own and most of their policies are determined by their upper unions. Each cooperative is also a type of company according to the Turkish Commercial Code No. 6102. Having a separate cooperative in each village means that each cooperative hires a separate accountant and is taxed separately. In this case, although the number of agricultural cooperatives in the province is high, their effectiveness is very weak. Regulations should be made to prevent the establishment of separate agricultural cooperatives in each village and encourage the establishment of cooperatives that cover many villages with high efficiency.

Cooperative managers in Çanakkale province are middle-aged farmers, generally primary school graduates, with long experience as cooperative managers, and engaged in medium-scale agricultural production. Arrangements should be made to ensure more professional management of agricultural development cooperatives by imposing restrictions such as upper age limits and minimum education requirements on their managers.

As a result of the study, it is seen that the inventory of tools and machinery of cooperatives is quite low. The fact that each farmer buys his own tractor and buys and uses his own tractor materials causes him to incur a lot of costs. Instead of each farmer buying their own agricultural machinery separately, cooperatives buying these machines and using them jointly will provide a significant cost advantage to our farmers and will allow them to use more modern machinery. Since maintenance and repair costs will also be shared in the joint use of machinery, the level of income will increase. The use of common machinery should be explained especially to agricultural development cooperatives and tax advantages should be provided to agricultural cooperatives that use common machinery.

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CHARACTERIZATION AND PATHOGENICITY OF *RHIZOCTONIA* AG P CAUSING DAMPING-OFF ON TURFGRASS

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ABSTRACT

The genus *Rhizoctonia* consists of species that have one, two (binucleate) and multinucleate hyphae that are pathogenically, morphologically and genetically different from each other. These species are named and divided into subgroups depending on the anastomosis reactions between their hyphae and they show different pathogenic characteristics according to their hosts. Binucleate *Rhizoctonia* spp. generally live in soil and plant debris as saprophytes, but some form parasitic relationships with plants. In this study, surveys were conduct in parks in Kocaeli province in 2022. As a result of isolations from 52 turfgrass plants, two binucleate *Rhizoctonia* AG P isolates were identified in accordance with the rDNA-ITS sequences analysis. The rDNA internal transcribed spacer (ITS) regions of the isolates were amplified by polymerase chain reaction (PCR) using universal fungal rDNA primer pair ITS1/ITS4. The ITS1-5.8S-ITS2 region of isolates were sequenced and performed BLAST analyzes. Pathogenicity tests were performed two times in pots using turfgrass seeds in greenhouse conditions. As a result of the pathogenicity tests, it was determined that both isolates cause damping-off on turfgrass.

Keywords: *Rhizoctonia* AG P, Turfgrass, ITS region

INTRODUCTION

Rhizoctonia genus that has wide host range is a soilborne pathogen and it includes subgroups composed of a lot of pathogen and non-pathogen called as anastomosis groups. The anamorph stage of the genus called as "*Rhizoctonia"*, which includes three major groups: multinucleate (MN) *Rhizoctonia* (teleomorphs *Thanatephorus* and *Waitea*), binucleate (BN) *Rhizoctonia* (teleomorphs *Ceratobasidium* and *Tulasnella*), and uninucleate *Rhizoctonia* (teleomorph *Ceratobasidium*). These groups divided into different subgroups called as anastomosis groups (AGs) based on hyphal fusion (Sharon et al., 2007). *Rhizoctonia solani*, which is the most popular among the multinucleated *Rhizoctonia* species, is divided into 13 (AG

1-13) anastomosis groups. Binucleate *Rhizoctonia* isolates are grouped into 19 AGs (AG A, B, C, D, E, F, G, H, I, K, L, O, P, Q, R, S, U, V, W) (Hyakumachi et al., 2005; Misawa and Kurose, 2018; Sharon et al., 2008; Yang et al., 2015; Dong et al., 2017; Zhao et al., 2019). Binucleate *Rhizoctonia* spp. represent a diverse group of organisms that have been isolated from soil and plants. They generally live saprophytically in soil and plant debris, some binucleate isolates are in mycorrhizal relationship with orchids. Each of these groups has host specificity, and some of them cause disease in some plants and are not pathogenic in others (Sneh et al., 1996). Among the binucleate *Rhizoctonia* groups AG A (on strawberry, sugarbeet, bean, pea, sunflower, tomatoe, melon, cucumber, spinach, lettuce, peanut, potato, tobacco), AG B (on rice, dandelion) AG D (on barley, wheat, sugar beet, cotton, potato, soybean, bean and turfgrass), AG E (on bean, pea, lettuce, onion, tomato, horseradish, lima, soybean, peanut, sugar beet, cowpea, azalea, pine), AG F (on pistachio, corn), AG G (on strawberry, sugar beet, bean, pea, sunflower, peanut, tobacco, melon), AG I (on strawberry), AG K (on chickpea) , AG P (on tea, kiwifruit), AG Q (on turfgrass), AG R (on bean, lima bean, soybean, peanut, black-eyed pea, tomato, lettuce, radish, onion, pea), AG-V (on taro, ginger), AG-W (on potato) were isolated from plants (Oniki et al., 1986; Sneh et al., 1996; Yang et al., 2015; Alaei et al., 2017; Dong et al., 2017; Türkkan et al., 2018; Türkölmez et al., 2019; Basbagci and Dolar, 2020).

Some BN *Rhizoctonia* species have been considered as pathogens causing significant damage in different plant species, but there is no detailed study describing the binucleate AGs and subgroups of *Rhizoctonia* spp. causing on turfgrass areas. The aim of this study is identification and cultural, morphological and pathogenic characterisation of a pathogen BN *Rhizoctonia* AG obtained from turfgrass plants showing patch, root and stem rot symptoms in Kocaeli province of Türkiye, by the rDNA-ITS sequences analysis.

MATERIAL AND METHOD

Collection of Samples and Isolation of Fungi

Surveys were carried out to determine fungal diseases in turfgrass areas including parks, recreation areas and refuges in Kocaeli province and diseased turfgrass samples were collected from parks, recreation areas and refuges. Isolations were made by samples which were taken from diseased roots with yellowing, browning, undersized plants, samples from bare, yellow and brown patches. Brown and undersized roots surfaces were sterilized for 1 minute in 1% NaOCI rinsed for 30 s in sterile water, dried on filter paper, and placed on PDA (Difco, USA) amended with streptomycin sulfate (100 µg/ml).

Pathogenicity Assays

For inoculum production, 125 ml of sterile distilled water was added to bottles containing 500 gr of wheat seeds and autoclaved twice at 90°C for 1.5 hours, one day apart. Then, 10 agar discs of 7-8 mm in diameter with mycelia of each isolate were added to these bottles and incubated at 25°C for one month. After incubation, the inoculum was air-dried and smashed using blender. Then, infested wheat seeds (5%) were applied to the sterilized (two consecutive days at 121 ºC for 45 minutes) sand, soil and fertilizer mixture (1:2:1). There were three replicate pots (12x12 cm) for each treatment. Control pots were not inoculated with fungi. The pots were covered with polyethylene bags and incubated for 72 hours. After 72 hours, thirty *F. arundinaceae* seeds were planted in pots and covered with about 1 cm of sterile soil. After planting, 15 ml of distilled water was added to each pot. After one month, the turfgrass plants were examined. Disease assessments were evaluated on modified the scale of 0 to 5 (Ichielevich-Auster et al., 1985). These scale values were converted to disease severity values using the Townsend and Heuberger formula, 0-5 Scale: 0= healthy plant;1= 1-10%, hypocotyl infection and/or shortening of plant height; 2 = 11-30%, hypocotyl infection and/or shortening of plant height; 3= 31-50%, hypocotyl infection and/or shortening of plant height; 4= 51-80%, hypocotyl infection and/or shortening of plant height; 5= dead plant and/or ungerminated seed.

Determination of Nuclear Numbers of Isolates

Hyphae tips from *Rhizoctonia* isolates were transferred to lamella water agar medium. Lamella water agar medium was prepared by immersing the coverslips sterilized by burning in 95% ethanol and immersed in soft PDA medium containing 0.5% agar and placing them in water agar medium containing 1.5% agar. Petri dishes were incubated for 24-48 hours in the dark at 25°C and samples were examined at the end of the incubation period. A drop of 0.5% Safranin O solution was dropped on a slide for examination. Then, the coverslip taken from the water agar medium with the coverslip was placed on the solution in the slide (Bandoni, 1979). In the preparations prepared in this way, the number of nuclei was determined under the light microscope (×100 and ×400) considering the number of nuclei in at least 15 cells in the hyphae. Three petri dishes were used for each sample (Ogoshi et al., 1990, Carling et al., 1994, Karaca et al., 2002).

DNA isolations and PCR analysis of isolates: DNA isolations were made by using QIAGEN Blood and Tissue Kit, according to the company's instructions for use. In the polymerase chain reactions (PCR) study, general primer pairs ITS1 and ITS 4 were used. (White et al., 1990). For PCR analysis, the reaction mixture was prepared as 50 µl; 2 µl of primers (10 mM), 4 µl of BSA, 13 µl of double-distilled water, and 25 µl of GoTaq® Hot Start Green Master mix (2x) (Promega, USA). After this mixture was distributed to the PCR tubes of each isolate, 4 µl of the DNA template of each isolate was added. The PCR cycling protocol consisted of an initial denaturation step at 94 ºC for 4 min, 30 cycles of 45 s at 94 ºC, 45 s at 55 ºC and 2 min at 72 ºC, and finally 10 min extension at 72 ºC. PCR products were subjected to direct sanger sequencing in a private R&D Laboratory. Sequence results were compared with isolate sequences in GenBank after BLAST analysis at NCBI.

RESULTS AND DISCUSSION

In consequences of the survey studies in Kocaeli province in 2022, 52 plant samples were collected from the study sites with lesions in the root and root collar and dwarfing symptoms. As a result of the isolations made from the plants, a total of 2 binucleate yellowish cream *Rhizoctonia* isolates were obtained. On Potato Dextrose Agar (PDA), isolates were initially yellowish cream and turned buff-coloured in 3 weeks (Fig. 1a). Sclerotia were 0.3–1 mm in diameter, almost globose, produced singly or in clumps, and light brown when young, turning brown with age (Fig. 1a). The nucleus numbers that were determined in each hypha cells were two, and width of the main runner, hyphae were less than 4-5µm (Fig 1b). According to the cellular nucleus number, width of the main runner hyphae, colony morphology and the morphological features of two isolates on PDA were suitable with descriptions of *Rhizoctonia* AG P in Sneh et al. (1996).

Molecular studies were performed with the isolates according to ITS 1 and 4 regions. As a result of the molecular identification studies, identified two species showed 100% similarity with the isolate (Accession number: KC782937.1) belonging to *Rhizoctonia* AG P group in NCBI.

As a result of the pathogenicity tests performed in the pot experiments carried out twice under greenhouse conditions, it was determined that both factors caused the turfgrass to settle and it was 100% pathogenic (Fig. 2)**.**

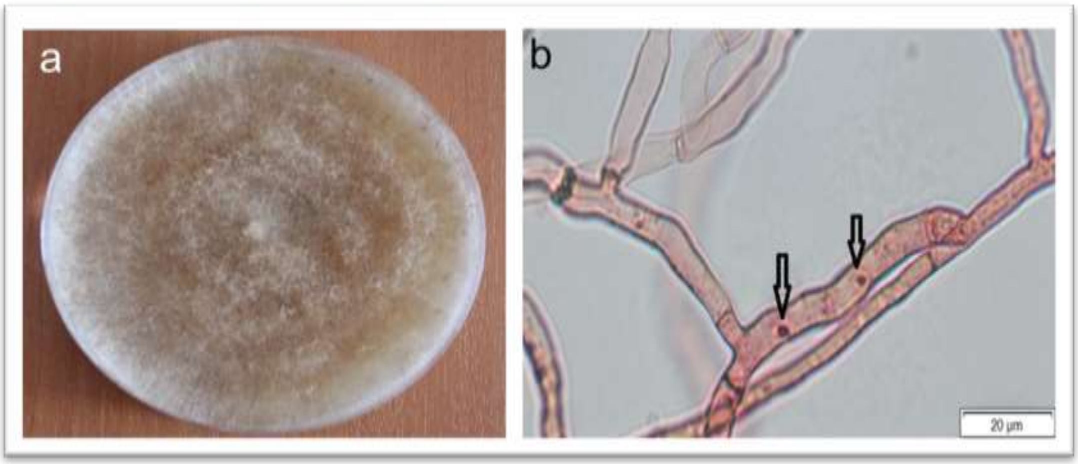

Figure 1. (a) Colony appearance of binucleate *Rhizoctonia* AG P isolate on potato dextrose agar, (b) Binucleate *Rhizoctonia* AG P cell

Figure 2. In pathogenicity studies *Rhizoctoni* AG P induced damping-off on turfgrass

Binucleate *Rhizoctonia* AGs are generally weakly pathogen or nonpathogen. Some of the binucleate isolates have mycorrhizal life. At the same time, non pathogen BN isolates use in biocontrol of pathogen agents (Sneh et al., 1996). However, several studies reported that some BN *Rhizoctonia* AGs were pathogenic on some economically important agricultural and ornamental plants.

BN *Rhizoctonia* isolates of AG P and AG R caused wirestem symptoms on red birch (*Betula nigra* L.) seedlings in China (Yang et al. 2006). Polizzi et al. (2009) reported that AG A caused crown and root rot on *Dodonaea viscosa* in Italy. In 2017, Alaei et al. demostrated that BN AG F isolates have been caused root and stem rot symptoms on pistachio (*Pistacia vera* L.) seedlings in Iran. Aiello et al (2017) determined that AG R, AG A and AG G and AG V were pathogen to different ornamental plants, while *Rhizoctonia* AG F were nonpathogen. AG G caused root rot of japanese bay tree (*Machilus thunbergii*) in Korea (Lee et al., 2023), AG U caused black scurf on potato tubers in Japan (Misawa and Kurose, 2018), AG P significantly reduced plant height, shoot and root dry weights and root length on kiwifruit (Türkkan et al., 2018), AG F caused stalk and root rot of corn (*Zea mays*) in Türkiye (Türkölmez et al., 2019), AG K caused root rot on chickpea (Basbagci and Dolar, 2020) in Türkiye, AG G and AG K caused root rot on strawberry in Kyrgyzstan (Erper et al., 2022), AG G and AG R caused damping-off on tobacco seedlings in Cuba (García et al., 2009).

Up to the present, BN *Rhizoctonia* AG DI, DII, DIII and Q have been reported as pathogens in turfgrass areas in studies carried out so far (Oniki et al., 1986; Sneh et al. 1996).

CONCLUSIONS

In this study, it was determined that BN AG P caused damping-off in turfgrass in Türkiye. Much study needs to be done on the virulence of BN *Rhizoctonia* anastomosis groups on plants.

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DETERMINATION OF THE EFFECT OF HYPERACCUMULATOR PLANTS GROWN ON SOIL CONTAMINATED WITH ZINC ON THE BIOLOGICAL PROPERTIES OF SOILS

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ABSTRACT
Soil pollution caused by heavy metals has emerged as one of the most significant environmental problems in the world. In soils, specific plant species are able to grow, adapt and absorb heavy metals. Phytoremediation is an emerging technology in which higher plants are used to reclaim the contaminated environment. It is important to strongly emphasize that the ultimate goal of heavy metal remediation process must be not only to remove the heavy metals from the soil (or instead of this to reduce their bioavailability and mobility) but also to improve soil quality. In this study, it was aimed to determine the effect of some hyperaccumulator plants such as *Brassica juncea, Raphanus sativus* and *Silene vulgaris* grown in zinc contaminated soils on the biological properties of soils. Changes in microbial properties such as microbial biomass carbon (C_{mic}), soil respiration (SR), dehydrogenase activities (DHA), urease (UA) and β-glycosidase (β-GA) activities in soils taken at harvest were evaluated.
It has been determined that there are significant improvements in the biological properties of the soils in the removal of Zn pollution using phytoremediation technology, but the biological properties are far from reaching to the initial level in the case of 1-year hyperaccumulator plant cultivation. While it was determined that the negative effect of Zn contamination was partially eliminated in the case of growing hyperaccumulator plants from some of their biological properties (SR, DHA, UA, β-GA and C_{mic} /C_{org} ratio), the negative effect on C_{mic} continued.
Keywords: phytoremediation, Brassica juncea, Raphanus sativus, Silene vulgaris, biological properties

INTRODUCTION
Heavy metals are natural constituents of the Earth's crust but human activities have drastically altered their geochemical cycles and biochemical balance in the biosphere (Giachetti and Sebastiani, 2006). In recent decades the development of industry and agriculture and activities such as mining and smelting of metal ores, industrial emissions and applications of agrochemicals and fertilizers have all contributed to elevated levels of heavy metals in soil metal pollution has become an environmental issue of great public concern (Hu et al., 2007). Heavy metals are non-biodegradable and therefore display long-term persistence in aquatic and terrestrial ecosystems. They are potentially harmful to all biota and tend to accumulate in the food chain so that heavy metal contamination represents one of the most pressing threats towater and soil resources and to human health (Yoon et al., 2007). Heavymetals exhibit toxic effects

towards soil biota: they can affect key microbial processes and decrease the number and activity of soil microorganisms (de Mora et al., 2005).

Many physical, chemical and biological techniques are used to improve heavy metal contaminated soil. However, among these various methods, phytoremediation is considered to be the most economical and environmentally friendly method (Prasad, 2003; Padmavathiamma and Li, 2007). Phytoremediation is a term applied to a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aim of restoring area sites to a condition useable for private or public applications. To date, phytoremediation efforts have focused on the use of plants to accelerate degradation of organic contaminants, usually in concert with root rhizosphere microorganisms, or remove hazardous heavy metals from soils or water. This procedure is relatively inexpensive compared to other remediation techniques (Wan et al., 2016) and also leads to less environmental degradation since it produces less secondary sewage (Cunningham and Berti, 2000). The rhizosphere, defined as the root-soil interface, is a dynamic system where plant roots, soil, and microorganisms interact and can significantly alter the soil properties (Wenzel, 2009; Wenzel et al., 2003).

Plants have evolved a multitude of life forms and, though somewhat less visible, of physiological realizations. Overthepast 20 years, our fundamental molecular and functional understanding of plants has been revolutionized, taking advantage of a focus ona few modelplants. In a few plant taxa, the concentrations accumulated in aboveground biomass of the transition metals or metalloids Ni, Zn, Cd, Se, As, Mn, Co, Cu, Pb, Sb, or Tl are more than one, and up to four, orders of magnitude higher than in other adjacent plants (Krämer, 2010). Hyperaccumulators take up particularly high amounts of a toxic substance, usually a metal or metalloid, in their shoots during normal growth and reproduction. Metal hyperaccumulator plants accumulate and detoxify extraordinarily high concentrations of metal ions in their shoots. Hyperaccumulators constitute an exceptional biological material for understanding mechanisms regulating plant metal homeostasis as well as plant adaptation to extreme metallic environments (Verbruggen et al., 2009). Whether phytoremediation technology can be used in a region depends on a variety of factors, such as the suitability of the plants for the ecology of the region or the concentration of the metal in the soil that has the ability to accumulate. *Silene vulgaris* is a plant commonly found in many metal-rich soils in Europe. This plant is tolerant to high heavy metal concentrations and is capable of accumulating heavy metals. It can also produce a vast amount of biomass fast, and the root system is quite large (Nadgórska-Socha and Ciepal, 2009). *Raphanus sativus* is used as a model plant in laboratory toxicology studies for various pollutants and is preferred in phytoremediation due to its rapid growth, large biomass, and sensitivity to heavy metals (Hamadouche, 2012). *Brassica juncea* is considered one of the most promising species for plant breeding. It is an oilseed plant with a root system known to excessively accumulate certain heavy metals (Goswami and Das, 2015). *Silene vulgaris* and *Raphanus sativus* are commonly found in Black Sea region (Mumcu and Korkmaz 2018; Ozbucak et al., 2006). *Brassica juncea*, on the other hand, can spread in the ecological conditions of Turkey (Güner et al., 2012). These three plants may be preferrable for field applications due to their easy availability.

To date, emphasis has mostly been placed on physical and chemical soil properties as indicators of soil health, but biological parameters are becoming increasingly used due to their being more sensitive to changes in the soil, as well as to their capacity to provide information that integrates many environmental factors (Singh et al., 2009). Many biological parameters have been proposed as bioindicators of soil health, such as microbial biomass, basal and substrate-inducedm respiration, mineralizable nitrogen, soil enzyme activities, abundance of soil microflora and fauna, root pathogens, structural and functional biodiversity, food-web structure, plant growth and diversity, and so on (Alkorta et al., 2003). Cleaning soils contaminated with heavy metals involves not only reducing the concentration of heavy metals in the soil but also replacing the disrupted microbial balance of the soil. Microbial indicators of soil health are therefore important tools used to evaluate the success of phytoremediation processes (Singh et al., 2009; Jiang et al., 2010; de Mora et al., 2005; Gómez-Sagasti et al., 2012; Hernández- Allica et al., 2006).

In this study, it was aimed to determine the effect of some hyperaccumulator plants such as Brassica juncea, Raphanus sativus and Silene vulgaris grown in zinc contaminated soils on the biological properties of soils.

1. MATERIAL AND METHODS

1.1. Materials

The research has been carried out on land belonging to the Bafra Agriculture District Directorate in Bafra District of Samsun Province, Turkey (41°34'34"N 35°53'53"E). Since the trial area soils show little pedogenetic horizon development and are located on the flood plains on the alluvials brought by Kızılırmak, they are defined as "Typic udifluvent" (Yüksel and Dengiz, 1996). In the Bafra Plain, summers are generally hot, and winters are warm and rainy. The plain has a warm and temperate climate. In winter, the Bafra Plain receives more precipitation than summer. According to the Köppen-Geiger climate classification, it can be referred to as a Csa climate (Mediterranean climate). The annual average temperature is 13.6 °C, and the annual average rainfall is 730 mm (https://tr.climate-data.org/asya/tuerkiye/samsun/bafra-8522/).

The sewage sludge used in the study was obtained from Bafra Municipality Sewage Water Treatment Facility. The solid matter ratio of the cake coming out of the facility is 41.39%. The zinc required to ensure Zn pollution in the experiment was obtained from Ekmekçioğulları Incorporated Company in the form of $ZnSO_4.7H_2O$ (22% Zn). *Brassica juncea, Silene vulgaris*, and *Raphanus sativus* were used as phytoremediation plants. These plants are winter plants and are part of the natural ecology of the region. However, the seeds of *Brassica juncea* and *Silene vulgaris* were obtained from abroad (www.herbiseed.com) as certified, and for Rapanus sativus was obtained domestically.

1.2. Methods

2.2.1. Establishment of trials

The trials were set up with 3 replications based on the randomized block experimental design in the form of 2 trials side by side in the field on 18.10.2004. One of the experiments was created with a constant level of sewage sludge and increasing levels of Zn application while the other by only increasing the levels of Zn application. The organic matter contents of the trial area (1.53% organic matter) and the organic matter contentssewage sludge (52.86% organic matter) used as the material in the experiment were determined, and the amount of sewage sludge required to increase the organic matter content of the soil up to 3% was calculated on dry weight and applied equally to each plot and mixed with soil. In order to determine the Zn levels in the application, the Zinc-buffering capacity of the soil was determined by adding Zn at increasing levels under laboratory conditions to the soil samples taken from the experimental area. This level was determined to be 650 μg g^{-1} in the soil sample. Based on this level, Zn pollution levels in the experiment were determined as 0-75-150-300-600-1200 μg g^{-1}, respectively. During the trials, the application doses determined in Zn applications were performed to cover 0-75-150-300-600-1200 μg g^{-1} Zn by preparing 15 Lt aqueous solutions of zinc sulfate. After these applications, the area was fallowed for one year. In the first year of the experiment, no cultural practices were undertaken (irrigation, fertilization, spraying, etc.) after the addition of the trial materials (sewage sludge and Zn) to the plots, and the weeds grown in the plots were cleaned by hand at the beginning of their development.

At the end of the first year of the experiment (18.10.2005), the plots belonging to each application were divided into 3 sub-plots of 1 m^2 and the second year trials were established. The second year trials consisted of 108 plots. Certified seeds of *Brassica juncea, Silene vulgaris,* and *Raphanus sativus* determined as hyperaccumulator plants were planted in these sub-plots. Plants were thinned immediately after emergence so that an equal number of plants were found in each plot (25 plants/plot). In the second year of the experiment, no other cultural practices (irrigation, fertilization, spraying, etc.) but weeds grown in the plots were cleaned by hand at the beginning of their development.

Soil sampling was carried out in the last month of the first year of the trial, before planting (18.10.2005) and at the harvest (26.06.2006). In these samples, microbial biomass carbon (C_{mic}), soil respiration (SR), dehydrogenase activities (DHA), urease (UA) and β-glycosidase (β-GA) activities in soils were determined.

2.2.1. Analysis Methods of Soils

In order to determine some physical and chemical properties of the soil where the experiment was established, the soil samples; clay, silt and sand fractions by hydrometer method, soil reaction (pH) 1:1(w/v) soil: in distilled water by pH-meter, Electrical Conductivity (EC) 1:1 (w/v) soil: distilled water In the mixture, the organic matter content was determined by the Walkey-Black method, the lime content ($CaCO_3$) was determined volumetrically by the Scheibler calcimeter (Rowell, 1996). The total Zn content of the soil was determined in atomic adsorption spectrophotometer (AAS) using the extract obtained with 1:10(w/v) soil: aqua regia (Kick et al. 1980).

The waste sludge used in the experiment, organic matter content by dry-ashing method (Ryan et al., 2001), pH 1:5 (w/v) waste sludge: distilled water suspension (Peech, 1965), EC 1:5 (w/v) waste sludge: in distilled water suspension (Bower and Wilsox, 1965), total N by Kjeldal method (Bremner, 1965), total Zn, Pb, Cu, Ni, Cd, Cr content HNO_3:HCl (1:4, v/v) with the extract obtained as a result of method of wet digestion was determined by atomic adsorption spectrometry (Kacar, 1990).

At the end of the harvest, the soil samples taken from the plots were brought to the laboratory in a +4 C portable cooler and analyzed, and the samples that came to the analysis were stored in the +4 C refrigerator until processing. In the soil samples taken from the plots, the % moisture was determined and the results of the biological analyzes were expressed in terms of dry soil. In the soils taken after harvest; soil respiration was determined as reported by Anderson (1982), microbial biomass carban was determined as reported by Anderson and Domsch (1978), dehydrogenase activity was determined as reported by Pepper et al. (1995), urease (EC 3.5.1.5) activity was determined as reported by Hoffmann and Teicher (1961), β-glycosidase (EC 3.2.1.21) activity was determined as reported by Eivazi and Tabatabai (1988). C_{org}/C_{mic} ratios of soil samples were calculated as the ratio of total organic carbon to microbial biomass carbon.

2.2.2. Statistical analysis

The statistical evaluations were made using the TARIST package program based on the predictions by Yurtsever (1984).

2. RESULTS AND DISCUSSION

2.1. Properties of soils and sewage sludge

The soil used in the experiment was loamy in texture (17.40 % clay, 34.29% silt, 48.31% sand), medium calcareous (11.08%), unsalty (0.32 dS m^{-1} EC), had low organic matter content (1.53%) and a slightly alkaline reaction (8.25 pH). Its N content was 0.10% and total Zn was 88.49 µg g^{-1}.

The Total N, organic carbon, C/N ratio and pH of the sewage sludge were found as 2.20%, 28.7%, 13.1, and 6.65, respectively. It contained 647 µg g^{-1} Zn, 45 µg g^{-1} Pb, 121 µg g^{-1} Cu, 53 µg g^{-1} Cr, 2.1 µg g^{-1} Cd and 58 µg g^{-1} Ni.

2.2. Changes in Biological Characteristics of Soil Samples Taken at the End of Harvest of Plants

2.2.1. Microbial biomass C

The changes in microbial biomass C (C_{mic}) of soil samples taken at the harvest of hyperaccumulator plants grown in parcels containing increasing levels of Zn added to soils with and without sewage sludge are given in Figure 1. At the end of the statistical evaluations; sewage sludge application ($P<0.001$), increasing levels of zinc application ($P<0.05$), changes in C_{mic} content ($P<0.001$) in soil samples taken at the harvest periods of plants were found significant. It was determined that the parcels with sewage sludge addition were at a higher level in C_{mic} than those without sewage sludge addition ($P<0.001$) in harvest periods of plants and Zn application doses. This is due to the fact that the high amount of organic matter in the sewage sludge is a source of nutrients and energy for microorganisms.

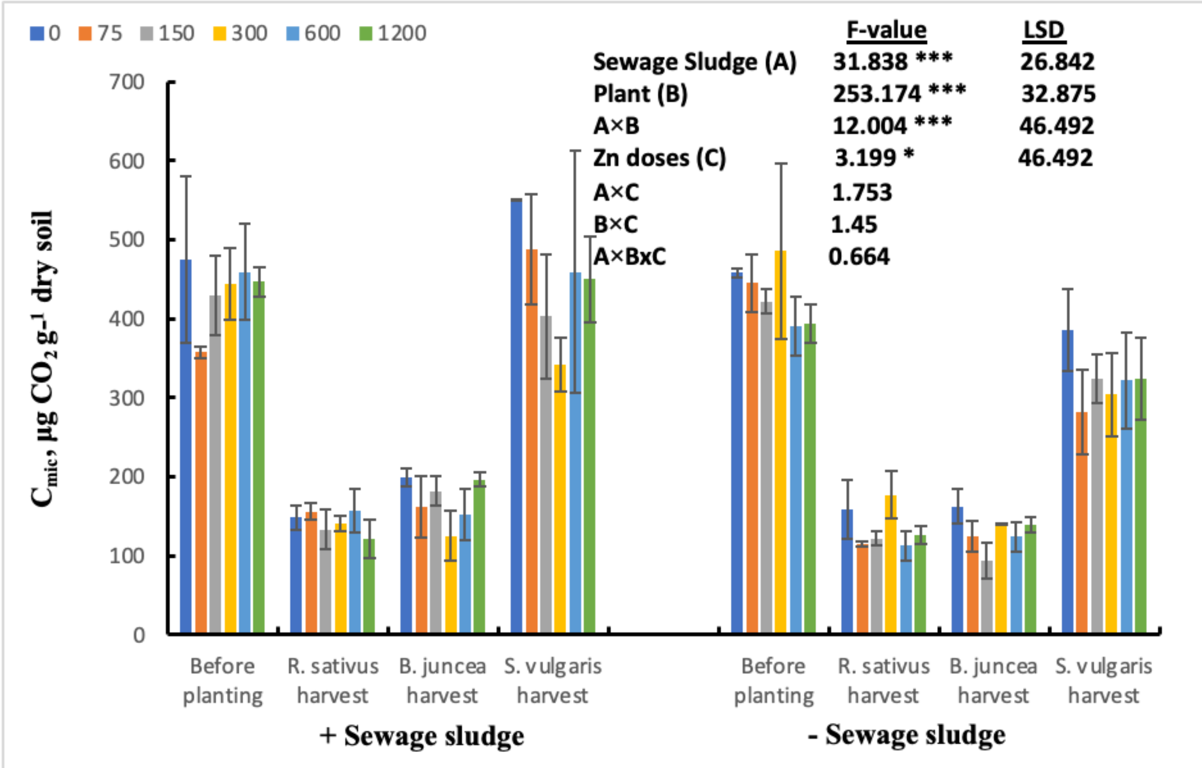

Figure1. Changes in microbial biomass C of soil samples taken at the harvest of hyperaccumulator plants by increasing levels of Zn application with and without sewage sludge

Significant differences were determined between plants for C_{mic} value in all Zn application doses and with and whitout sewage sludge parcels ($P<0.001$). The C_{mic} amount determined in the soil samples taken at the harvest of *Raphanus sativus* and *Brassica juncea* at all Zn doses in the sewage sludge applied parcels was lower than the soil samples taken before planting. At 150 and 300 mg kg^{-1} doses, C_{mic} amounts in soil samples taken at *Silene vulgaris* harvest were also determined at low levels in soil samples taken before planting, but high at all other application doses. On the other hand, in the parcels where sewage sludge was not applied, C_{mic} values in the soil samples taken at the end of the harvest of the plants at all Zn application doses were found to be lower than the values in the soil samples taken before planting.

Differences were determined between Zn application doses in all Zn application doses and in the with and without sewage sludge parcels ($P<0.05$). This shows that the negative effect of Zn pollution on C_{mic} continues. That is, the Zn removed from the soil by *Brassica juncea*, *Silene vulgaris*, and *Raphanus sativus*, which was used as a hyperaccumulator plant in the experiment, it did not remove the negative effect of Zn remaining in the environment. This probably shows that the destruction of Zn, which is added to the soil with or without sewage sludge, on C_{mic} for 1 year, did not disappear during the cleaning phase by growing hyperaccumulator plants.

2.2.2. Soil respiration

The changes in soil respiration (SR) of soil samples taken at the harvest of hyperaccumulator plants grown in parcels containing increasing levels of Zn added to soils with and without sewage sludge are given in Figure 2. At the end of the statistical evaluations; sewage sludge application ($P<0.01$), changes in the TS in soil samples taken during the harvest periods of plants ($P<0.001$) were found to be significant, while increasing levels of zinc application were found to be insignificant.

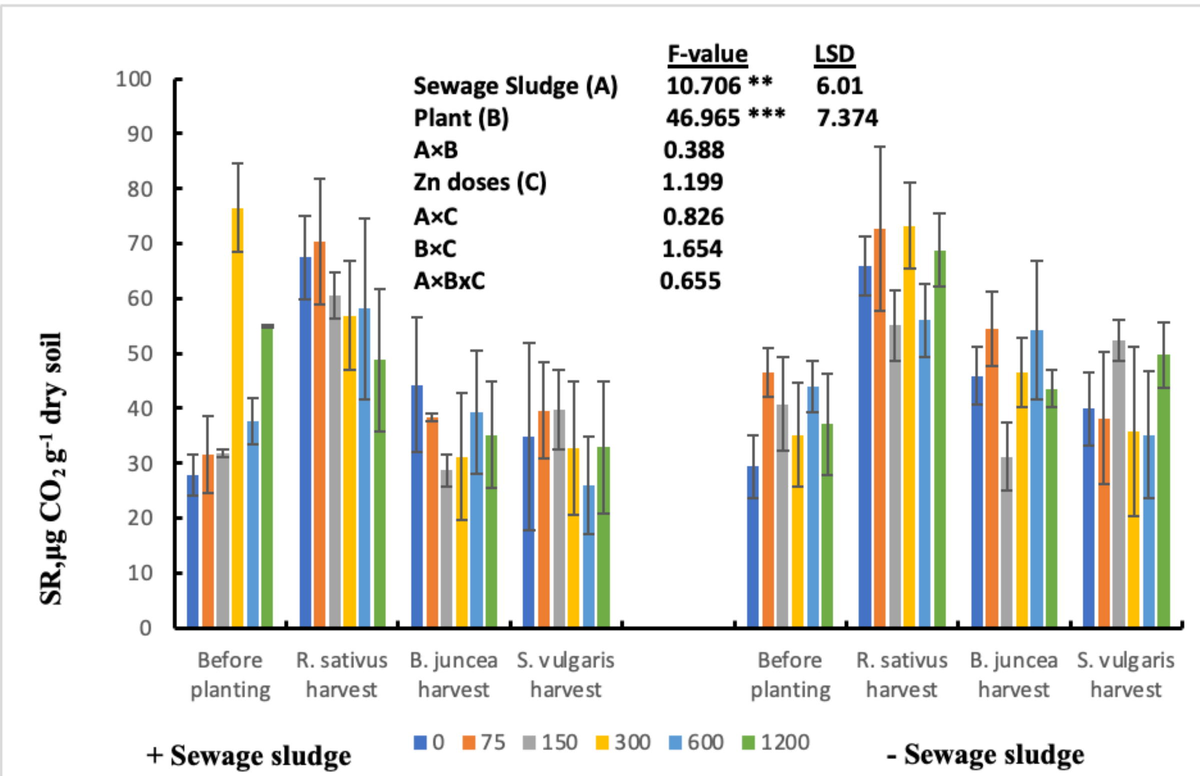

Figure 2. Changes in soil respiration of soil samples taken at the harvest of hyperaccumulator plants by increasing levels of Zn application with and without sewage sludge

Both in sewage sludge applied and unapplied parcels and in all Zn application doses, When the SR level of the parcels where hyperaccumulator plants are grown is compared with the SR level of the soils before planting, it was determined that the presence of plant roots in the environment increased the SR. This is undoubtedly due to the difference in climatic changes of the periods in which the comparison was made and the respiration of plant roots. Especially in plant-grown areas, the source of SR is microbial respiration, as well as CO_2 released as a result of respiration of plant roots.

Significant differences were found in the SR levels determined in the soils from the harvesting periods of the plants in the sewage sludge applied and unapplied parcels with all Zn application doses ($P<0.001$). The highest SR values at all Zn doses were obtained in the soil samples taken after the harvest of *Raphanus sativus* in the sewage sludge applied parcels. This is probably due to root development, root structure and root respiration of hyperaccumulator plants and climatic changes. While *Raphanus sativus* has a tuberous root structure, *Silene vulgaris* and *Brassica juncea* have a pile root structure. In addition, *Raphanus sativus* generated the highest total root biomass among the hyperaccumulator plants used in the experiment (Bayraklı and Kızılkaya, 2007). Therefore, higher SR may be determined due to the fact that *Raphanus sativus*, which has more root biomass, produces more CO_2 as a result of respiration.

In the soil samples taken from the parcels with hyperaccumulator plants, the effects of increasing levels of Zn application doses on the SR were not found to be statistically significant in the parcels with and without sewage sludge applied. In other words, it was determined that the negative effect of low Zn application doses on SR did not occur in the second year of the trial, especially in the trial where no sewage sludge was applied. This situation occurred; i) the removal of Zn by hyperaccumulator plants in the soil during the period (Bayraklı and Kızılkaya, 2007) and the disappearance of the effect of Zn application doses on soil respiration over time ii) It may be due to the reduction of the negative effects of Zn added to soils in biological systems as a result of factors such as adsorption and washing.

2.2.3. Dehydrogenase activities

The changes in dehydrogenase activity (DHA) of soil samples taken at the end of the harvest of hyperaccumulator plants grown in parcels containing increasing levels of Zn added to soils with and without sewage sludge are given in Figure 3. At the end of the statistical evaluations; sewage sludge application ($P<0.01$), changes in DHA content in soil samples taken during the harvest period of plants ($P<0.001$) and their interactions were found to be significant, while increasing levels of zinc application were found to be insignificant.

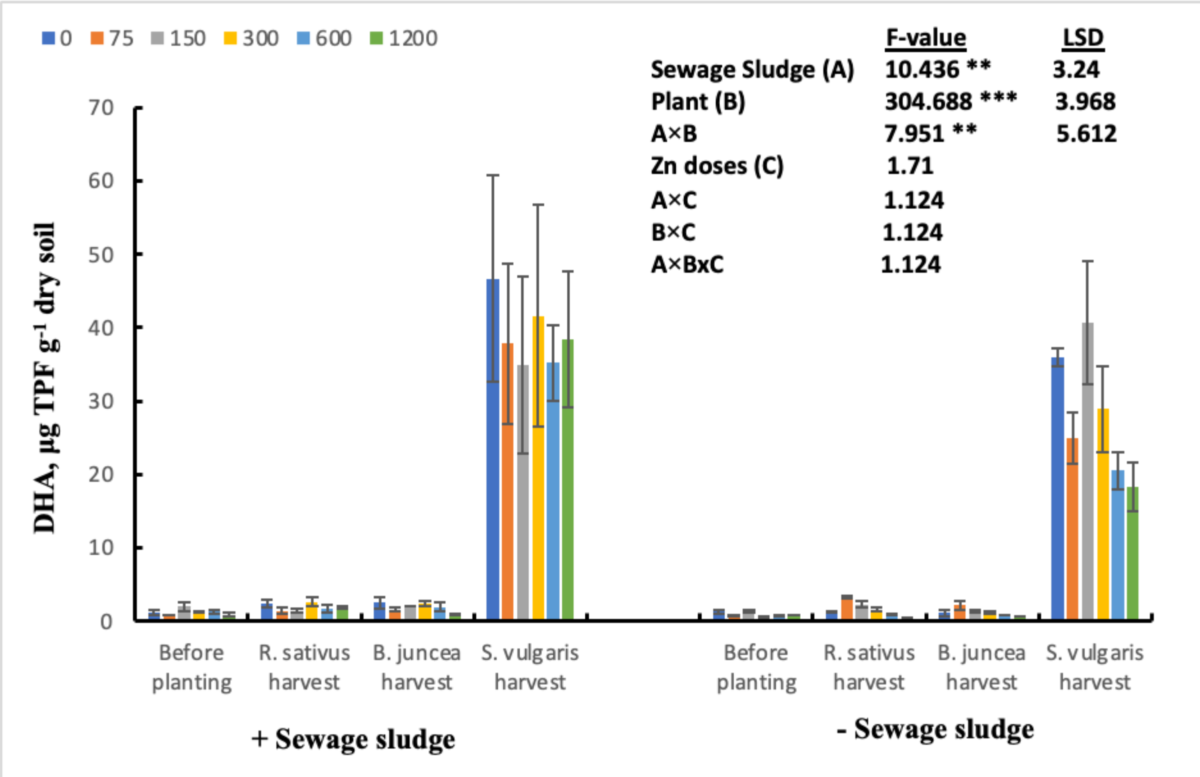

Figure 3. Changes in DHA activity of soil samples taken at the harvest of hyperaccumulator plants by increasing levels of Zn application with and without sewage sludge

The DHA of the soil samples taken during the harvesting period of the hyperaccumulator plants was higher at all Zn application doses in the parcels with the addition of sewage sludge compared to those without the addition of sewage sludge. In addition, no relationship was found between increasing levels of Zn application doses and DHA in both the sewage sludge applied and unapplied parcels. This situation is probably related to the fact that hyperaccumulator plants remove some Zn from the soil and accumulate it in their structures, shading the negative effect of Zn, and the adsorption of the remaining Zn part in the environment by the colloidal systems of the soils. Because, according to the amount of total and labile Zn forms determined in the soil samples taken from the parcels at the end of the harvest, the most common forms in the total are the non-labile forms (Bayraklı and Kızılkaya, 2007). Metals found in soils and entering the food chain by affecting biological systems are metal forms that are predominantly in the soil solution and are completely labile, and metal forms that are weakly bound on colloidal surfaces (Shuman, 1979, 1983, 1988). For this reason, it is thought that significant differences between the doses in DHA could not be determined due to the decrease in Zn in the environment as a result of the bioaccumulation of the hyperaccumulator plants cultivated in the experimental parcels following the first year of the experiment.

Significant differences were found between hyperaccumulator plants at all application doses and in the sewage sludge applied and unapplied parcels ($P<0.001$). The highest DHA values in all Zn doses in the sewage sludge applied and unapplied parcels were determined in the soil samples taken after *Silene vulgaris* harvest, while DHA in other periods was found to be close to each other. This shows that Silene vulgaris is the most effective plant in increasing DHA activity, which decreases as a result of the inhibition of heavy metals.

2.2.4. Urease activity

The changes in the urease activity (UA) in the soils taken at the harvest of the hyperaccumulator plants grown at the end of one year, with the Zn doses applied to the soils together with the sewage sludge and the Zn doses applied without the sewage sludge are given in Figure 4. As a result of the statistical evaluations; sewage sludge application ($P<0.001$) and the changes in the UA activity of the soil samples taken during the harvest periods of the plants and their interactions with each other ($P<0.001$) were found to be significant, but the increasing doses of Zn application and their interactions with each other were found to be insignificant.

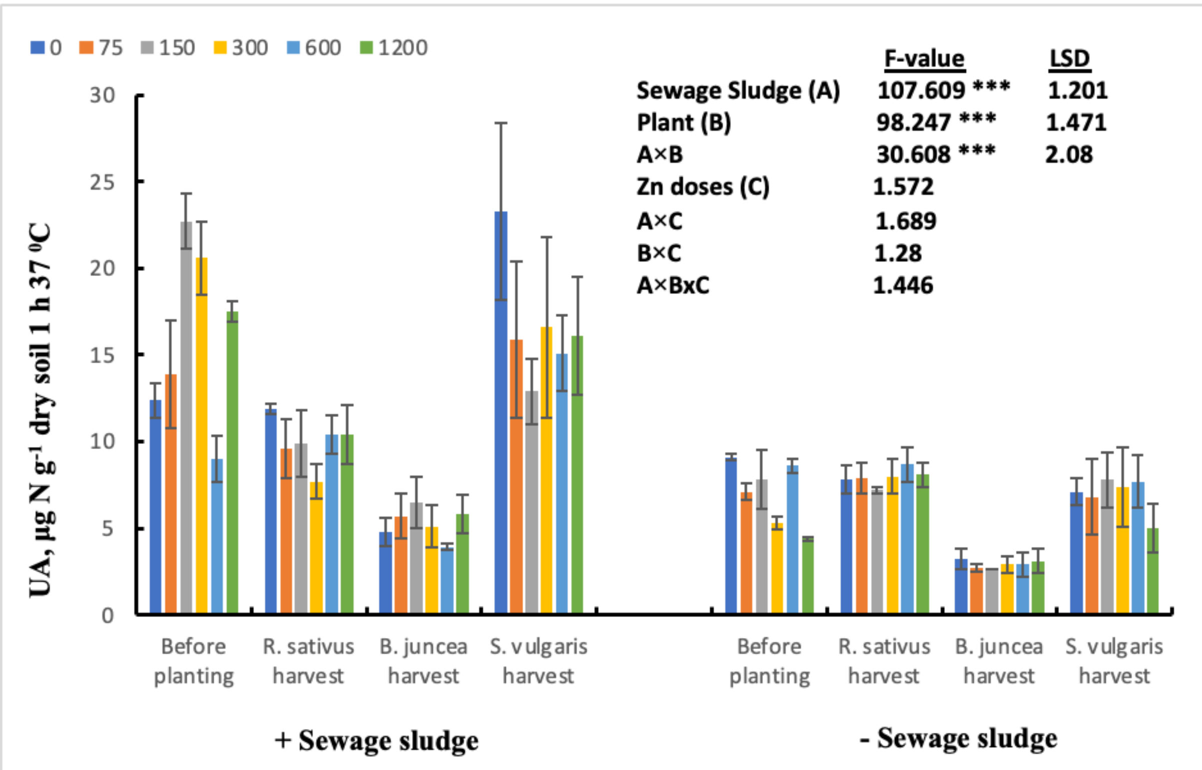

Figure 4. Changes in UA activity of soil samples taken at the harvest of hyperaccumulator plants by increasing levels of Zn application with and without sewage sludge

In all sampling periods and Zn application doses, it was determined that the UA of the parcels with sewage sludge addition was higher than those without sewage sludge addition ($P<0.001$). The reason for this may be that the organic N value of the sewage sludge is high and the urea-N, which acts as a substrate source for urease, increases accordingly (Kızılkaya and Bayraklı, 2005). In addition, the low C/N ratio of the sewage sludge may be due to the increase of UA, which is the origin of microorganisms, due to the increase in microbial activity. As in the first year of the experiment (Bayraklı and Kızılkaya, 2007), the UA obtained in the experiment with sewage sludge applied higher than in the case without sewage sludge, revealing that the effect of sewage sludge continued in the second year.

All of the hyperaccumulator plants grown in parcels with increasing levels of Zn applied with sewage sludge decreased UA in low Zn applications compared to pre-sowing. However, it was determined that UA increased only when *Silene vulgaris* was grown in control and at high Zn doses (600 and 1200 mg kg^{-1}). Hyperaccumulator plants grown in Zn, which was added to soils at increasing levels without the application of sewage sludge, increased the UA of the soils compared to pre-sowing at high Zn doses (600 and 1200 mg kg^{-1}). The increase occurred was mostly in *Raphanus sativus*. It was determined that the negative effect of Zn, which was added to the soils with and without sewage sludge at the levels of 300, 600 and 1200 mg kg^{-1} in the first year of the experiment, on UA disappeared as a result of the hyperaccumulator plants grown in these parcels in the second year of the experiment. This can be interpreted as the elimination of the negative effect of high doses of Zn for UA as a result of the removal of Zn by hyperaccumulator plants.

2.2.5. β-glikosidaz aktivitesi

The changes in the β-glycosidase activity (β-GA) determined in the soils taken at the harvest of the hyperaccumulator plants grown after the first year of the experiment, with the Zn doses applied to the soils together with the sewage sludge and the Zn doses applied without the sewage sludge are given in Figure 5. As a result of the statistical evaluations; The changes in the GA activity in the soil samples taken during the harvest periods of the plants, the application of increasing doses of Zn and their interactions with each other ($P<0.001$) were found to be significant, but the sludge application was found to be insignificant.

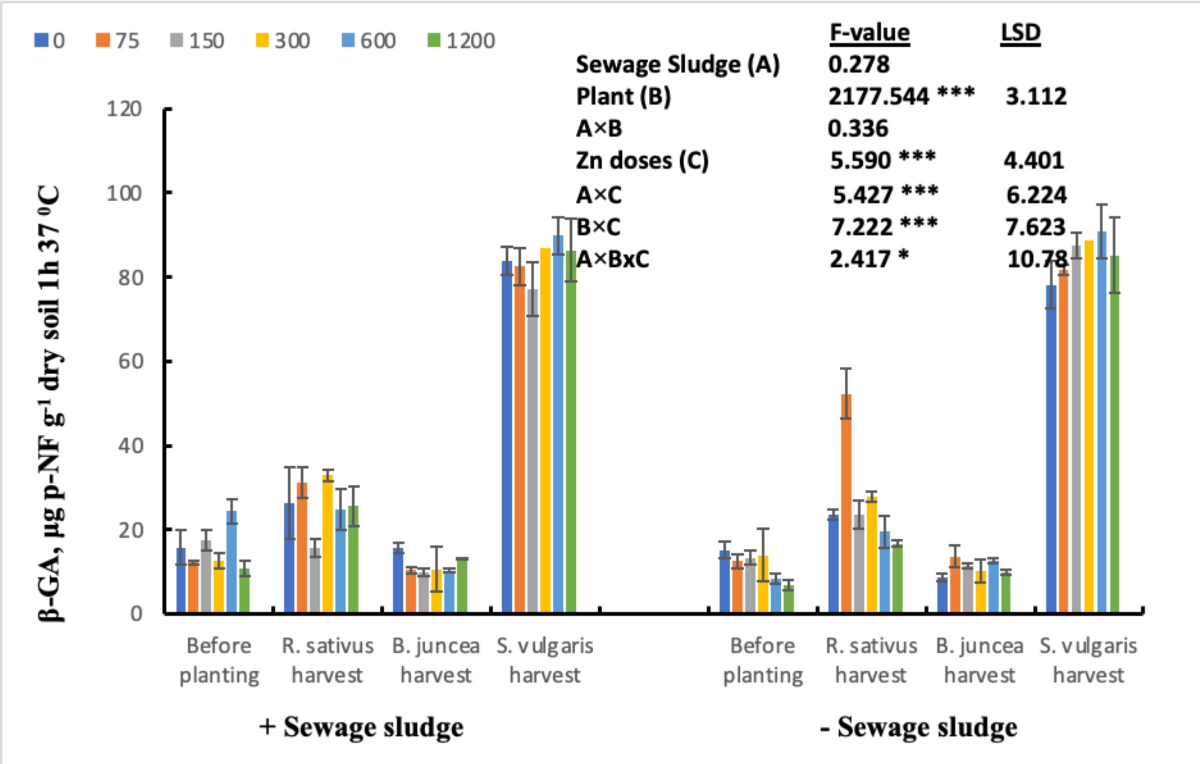

Figure 5. Changes in β-glycosidase activity of soil samples taken at the harvest of hyperaccumulator plants by increasing levels of Zn application with and without sewage sludge

In the soil samples taken at the end of the harvest of the hyperaccumulator plants grown in the parcels where Zn was added to the soil at increasing doses both with and without sewage sludge, β-GA increased significantly with the cultivation of hyperaccumulator plants compared to pre-sowing. It was determined that the increase occurred was highest in *Silene vulgaris*, followed by *Raphanus sativus* and *Brassica juncea*, respectively.

No positive or negative effects of sewage sludge on β-GA were observed in soil samples taken from plots where hyperaccumulator plants were grown. This situation reveals that the negative effect of high Zn dose disappeared in the first year of the experiment for β-GA, an extracellular enzyme that is involved in the C cycle in the soil and provides hydrolysis of cellobiose. Similarly, β-GA in the harvested parcels of hyperaccumulator plants grown in Zn parcels added to the soil without sewage sludge showed significant increases compared to the pre-planting period. Undoubtedly, this situation may be related not only with the Zn that plants remove from the soil, but also with the secretions secreted from plant roots stimulating microbial activity and regulating various physico-chemical properties of soils.

2.2.6. C_{mic}/C_{org} Oranı

The changes in the C_{mic}/C_{org} ratio determined in the soils taken during the harvesting of hyperaccumulator plants by Zn applied in increasing doses to the soils with and without sewage sludge are given in Figure 6. As a result of the statistical evaluations; While the application of sewage sludge to the soil was found to be insignificant, the C_{mic}/C_{org} ratio values ($P<0.001$) in the soil samples taken during the harvest period of the plants, the Zn doses applied at increasing rates ($P<0.05$) and their interactions ($P<0.01$) were found significant.

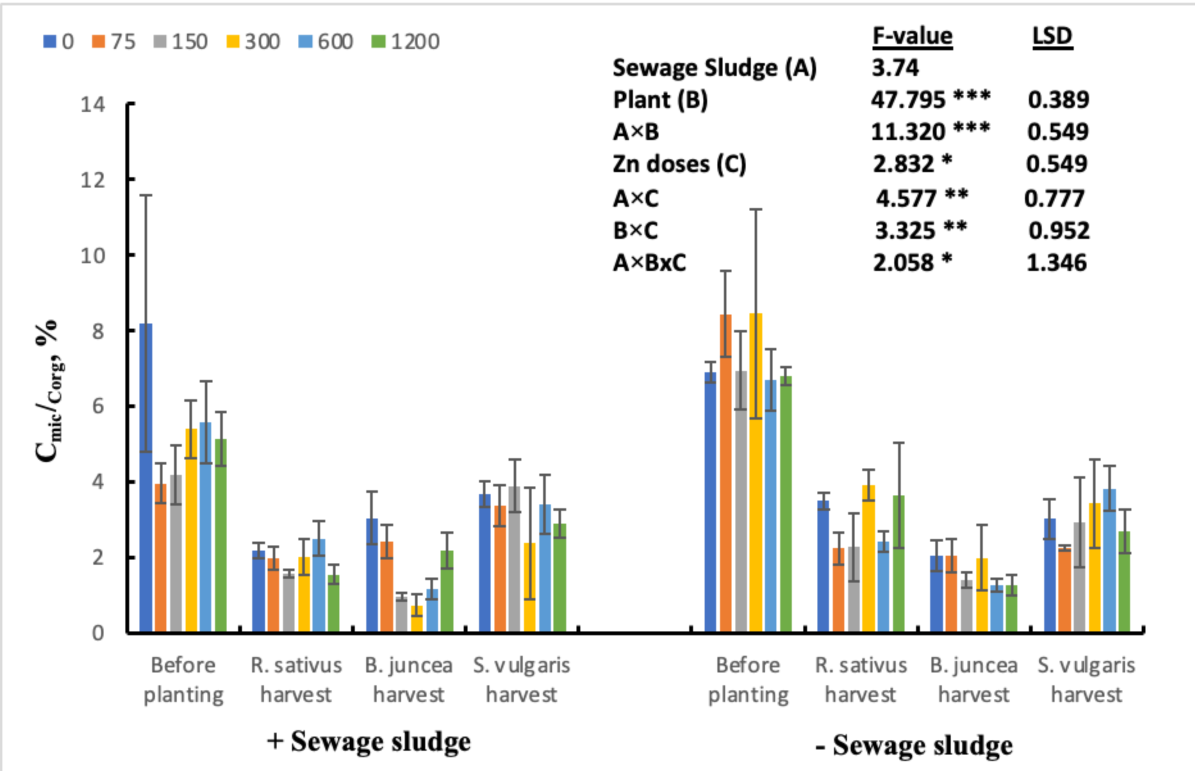

Figure 6. Changes in C_{mic}/C_{org} ratio of soil samples taken at the harvest of hyperaccumulator plants by increasing levels of Zn application with and without sewage sludge

In the soil samples taken at the end of the harvest of hyperaccumulator plants, it was determined that the addition of sewage sludge was not effective on the C_{mic}/C_{org} ratio of the soils at all Zn doses added to the soils at increasing levels. While the C_{mic}/C_{org} ratio was found to be higher in the parcels treated with sewage sludge in the first year of the experiment, the changes in this ratio as a result of growing hyperaccumulator plants in all parcels were not statistically significant. Similarly, sewage sludge applications increased organic C and C_{mic} in the first year of the experiment, and as a result of growing hyperaccumulator plants in the second year of the experiment, higher amounts of organic C and C_{mic} were detected in the sewage sludge applied parcels, similar to the results in the first years. However, the changes in the C_{mic}/C_{org} ratio calculated as a result of these two parameters were found to be insignificant in the 2nd year of the trial. This indicates shows that as a result of the addition of sewage sludge to the soil, the soil organic C level and the microbial biomass C that will decompose this sewage sludge do not increase regularly, there is no increase in C_{mic} as much as the increase in organic C and the suppressive effect of sewage sludge on the microbial population in the soil continues even if hyperaccumulatory plants are grown.

It was determined that the C_{mic}/C_{org} ratio in soil samples taken at the end of the harvest of hyperaccumulator plants decreased compared to before planting in both sewage sludge applied and unapplied parcels and at all Zn application doses. This shows that the presence of plants in the environment increases the C_{mic} more than the increase in the organic C content of the soil and approaches the ideal level of 1-3%. Although *Brassica juncea* was the plant that showed this effect the most at all Zn application doses in the parcels without sewage sludge applied, *Brassica juncea* and *Raphanus sativus* were provided in the sewage sludge applied parcels.

The results show that in the case of growing hyperaccumulator plants, the negative effect of Zn pollution on some biological properties of soils (SR, DHA, UA, β-GA and C_{mic} /C_{org} ratio) partially disappears, while the negative effect on C_{mic} continues. Similar results were found in studies.

Jiang et al. (2010) found increases in soil microbial biomass C (C_{mic}), basal respiration, enzyme activities (urease, β-glucosidase, neutral phosphatase, and arylsulfatase), and the microbial quotient (*q*M = ratio of C_{mic} to C_{org}) after phytoextraction by five crops and concluded that the phytoextraction process had improved the soil quality. In another study; Higher values of biomass C, basal respiration, substrate induced respiration, and β-glucosidase activity were observed in the presence of *T. caerulescens* plants, as compared to unplanted pots. Data confirm the great capacity of *T. caerulescens* to phytoextract Zn from polluted soils and, interestingly, suggest that metal phytoextraction has indeed a beneficial effect on soil biological activity. It was concluded that the revegetation of these metal polluted soils with *T. caerulescens* could help activate their biochemical and microbial functionality (Hernández-Allica et al., 2006). Epelde et al. (2008), reported that in the presence of *T. caerulescens* led to 154%, 115%, 140%, 37%, and 164% increases in the activities of β-glucosidase, arylsulfatase, acid phosphatase, alkaline phosphatase, and urease, respectively. In a short-term Cd and Zn phytoextraction experiment with *Sorghum bicolor x sudanense*, an increase in soil microbial properties (dehydrogenase activity, basal and substrate-induced respiration) was detected in sorghum-planted soils versus unplanted controls (Epelde et al., 2009). The authors concluded that the functioning of the polluted soil was restored as a result of the phytoextraction process, which included both plant growth and heavy metal phytoextraction. In another study; It seemed that the enzyme activity lost could be compensated by *Trifolium repens L*. planting. Plants could increase the content of soil organic matter through root exudates and litter, by stimulating the activity of enzyme. In addition, plants accumulated heavy metals and reduced the bioavailability concentrations in the rhizosphere soil. More importantly, *Trifolium repens L.* planting altered the rhizosphere soil microbial community, the relative abundance of some ecologically beneficial microorganisms was increased, which also contributed to the increase of enzyme activity and available nutrients (Lin et al., 2021). On the other hand Yu et al. (2020), reported that phytoextraction of *Celosia argentea* improved soil metabolic functions by increasing the activities of soil enzymes (urease, invertase, phosphatase and catalase). These findings suggest that Cd phytoextraction using *Celosia argentea* can greatly improve the quality of Cd-contaminated soils with the application of soil conditioners.

3. CONCLUSIONS

In this study, the removal possibilities of Zn added as $ZnSO_7H_2O$ at increasing doses (0, 75, 150, 300, 600 and 1200 µg g^{-1}) were investigated by using *Brassica juncea*, *Raphanus sativus* and *Silene vulgaris* hyperaccumulator plants in sewage sludge treated and untreated loamy soil. According to the results of the biological analysis of the soil samples taken at the harvest of the hyperaccumulator plants, it was determined that there were significant improvements in the biological properties of the soils in the removal of Zn pollution using phytoremediation technology, but the biological properties were far from reaching the initial level in the case of growing a 1-year hyperaccumulator plant. However, since the harvest dates of hyperaccumulator plants grown in Zn-contaminated parcels differ from each other, it is clear that climatic factors also affect biological properties. It is thought that the continuation of these studies in different hyperaccumulator plant, soil and ecological conditions will help to determine the effect of phytoremediation on the biological properties of soils.

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FLAVONOID AND PHENOLIC AMOUNTS OF FIR CONE SYRUP AND MOLASSES

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ABSTRACT

As the world population increases, consumption also increases. This situation paves the way for the conversion of idle products into value-added products and bringing them into production. Today, the importance given to nutritional supplements and functional foods has increased in the concept of nutrition in the field of health. In this context, cone syrup is also included in the classification of foods containing functional components. Today, cone syrup is considered as a functional food against diseases such as asthma and bronchitis. The gum and resin of the fir tree are traditionally utilised by the people in the field of health. In addition to these, syrups and molasses obtained from cones have gained importance recently. In our study, total phenolic acid determination and total flavonoid matter determination of syrup and molasses were carried out by preparing cones collected from fir (Abies ssp.) tree. Total phenolic matter was determined with Folin-Ciocalteu method by calculating gallic acid equivalence. Total flavonoid content was determined by quercetin equivalence using AlCl3 and colorimetric method. The analyses were performed by photometric method using Shimadzu UV 1800 Spectrophotometer. Phenolic acid values obtained from syrup and molasses were calculated as 384.27 mg GAE/g and 304.74 mg GAE/L, respectively, while total flavonoid values were calculated as 48.99 mg KE/g and 43.74 mg KE/L, respectively. Considering the data obtained, it was observed that both functional products were rich in phenolic substances.

Keywords: Fir, Phenolic substance, Flavonoid substance, Molasses, Syrup

INTRODUCTION

There are 48-49 species of fir trees in the Pinaceae family worldwide (Farjon, 2010). These conifers, belonging to the pine family, are seen in many areas in Türkiye. Their moisture requirements are high and their heat requirements are relatively lower. Four species have been identified in Türkiye and they are widely distributed in the Black Sea region. In addition, there are fir types belonging to different species in Uludağ, Mount Ida and Taurus Mountains (Ekim et al. 2000). The purposes of using fir species include sectors such as construction, joinery, furniture, and flooring (Akkaya et al., 2020). In addition to these, it is known that natural products have been used in complementary therapies in recent years worldwide. The trunk parts of the fir tree are used in the aforementioned cases and even used as a substitute for imported pine species and turned into high value-added products. However, most of the fallen cones remain idle. One of the products in these supplements is the use of syrup or molasses of cones with nutraceutical and functional properties. To use natural resources more effectively, it should be examined by experimental studies whether they have bioactive properties especially in the field of health. In the light of this information, it is known that pine fractions contain important bioactive substances (Baser et al., 2002).

In recent years, the importance of cone syrups and molasses has increased with the proving of the bioactive components content of cones, which are generally used as firewood or remain idle in the soil. When the literature is examined, it has been proved that it is rich in polyphenols which have antioxidant properties. Antioxidant substances are secondary metabolites that plants produce by themselves against external threats (Tiring., et. al. 2020). However, when their isolation is provided, they are used as antioxidant-rich nutraceutical products (Yesil Celiktas et al., 2010). Cones, syrup and molasses are especially used against respiratory diseases such as asthma, shortness of breath, bronchitis and cough (Hendek Ertop, M. & İncemehmetoğlu, 2022).

The aim of this study was to determine the total phenolic matter and total flavonoid matter in syrup and molasses of fir cones collected from Kastamonu region. Looking to the molasses products in the market it is seen that they are mostly comprise from grape, carob, mulberry and pomegranate molasses (Tüzün et al., 2020). When scientific studies were examined, it was seen that there are various studies based on these molasses types. For the pinecone syrups, it was observed that there were not pinecone syrups obtained from a specific species, but pine cone syrups have been selling in the markets without any explanation in general. Within the scope of our study, the fact that only syrup and molasses obtained from pinecones from Kastamonu region were analysed makes our study unique.

Folin-Ciocalteu Reagent (FCR) is the most used reagent for total phenol analyses. Polyphenols are called compounds containing more than one phenol group. A link can be established between the determination of phenolic substances in natural products and the determination of this phenol group that provides antioxidant activity and antioxidant capacity (Prior et al., 2005). Folin reagent oxidises phenolic compounds to form a product giving an absorbance at 760 nm (Prior et al., 2005).

As the importance of functional foods increases, it is important that the safety of these foods should be supported more by scientific research. There are many methods to determine and prove the bioactivity of herbal extracts. Among these, the determination of total phenolic matter and total flavonoid matter determination also provides information on whether the extract will have antioxidant activity. In this study, total phenolic matter determination and total flavonoid matter determination examined, and it will be proved whether the product obtained with the data we have obtained will show antioxidant properties in real sense. Analysing the contents of these products obtained by natural methods will make the product used safe as well as having an idea about whether the use of the products will be effective.

MATERIAL AND METHOD

Material Preparation

Traditional method was applied to produce fir syrups and syrups. Fir cones collected in the natural environment were kept in 1 Liter of water with a ratio of 1:2. After soaking in water for a while, 500 g fir cones were boiled by adjusting the extraction time for 4 hours. Clay or sugar are not added during boiling.

The traditional extraction method was used during the preparation of fir cone molasses. For this purpose, 500 g fir cones are kept in 1 Liter of water and then brought to boil. Then, sugar beet is added to the molasses. In addition, citric acid is added to prevent sugar crystallisation and to ensure taste stability. Visual photographs of fir cone solid syrup and fir cone liquid syrup were presented in Figure 1.

Figure 1. Visual photographs of fir cone solid syrup (dark red) and fir cone liquid syrup (brown).

Total Phenolic Substance Determination Method

Folin-Ciocalteu Reagent (FCR) method. Total phenolic matter was determined by Shimadzu UV 1800 Spectrophotometer. The most preferred phenolic compound gallic acid (GA) was used as standard. The results obtained were calculated as gallic acid equivalent. The folin reagent used in the experiment is prepared as 0.5 N. For the calculation of the sample as mg GA/g sample, gallic acid standard stock solution is prepared as standard and other concentrations are prepared by serial dilution. Serial standards are prepared from 1000 ppm GA stock solution as 500; 250; 125; 62.5; 31.25; 15.625 ppm. 10% Na2CO3 is prepared as a colour indicator. After solution preparation, pipetting is started.

Table 1. Pipetting procedure for the determination of total phenolic matter

	BLANK	STANDARD	SAMPLE
Pure water	1400 µl	1360 µl	1360 µl
StandarD		40 µl	
Sample			40 µl
0.5 N Folin Reagent	800 µl	800 µl	800 µl
% 10 Na_2CO_3	800 µl	800 µl	800 µl
The tubes are vortexed and left for 30 minutes.			
Results readed at 760 nm by spectrophotometer.			

Determination of Total Flavonoid Substance

Total flavonoid content is determined by AlCl3 colourimetric method. Based on quercetin (QE) standard, it is analysed according to quercetin equivalence. Total flavanoid content was determined by using Shimadzu UV 1800 Spectrophotometer. The absorbance was made by photometric method at 415 nm.

For the standard, 1000 ppm stock quercetin (QE) standard was prepared, and 6 different standards were obtained by serial dilution as 250; 125; 62.5; 31.25; 15.625 ppm. 2% AlCl3 was prepared as reagent. Pipetting was performed by adding 2% AlCl3, water, sample in certain ratios to the balconies prepared for pure water, standard and sample. It was kept in the dark for 60 minutes. Total phenolic matter concentration was found by reading the results at 415 nm wavelength.

Table 2. Pipetting procedure for the determination of total flavonoid matter

	BLANK	STANDARD	SAMPLE
Pure water	3500 µl	3000 µl	3000 µl
StandarD		500 µl	
Sample			500 µl
% 2 $AlCl_3$	500 µl	500 µl	500 µl
The tubes were vortexed and left for 60 minutes.			
Results readed at 415 nm by spectrophotometer.			

RESULTS AND DISCUSSION

Conifer molasses and syrup is a natural product used by local people in Kastamonu region. Conifer molasses have been used especially to relieve complaints such as upper respiratory tract, lung diseases and chronic cough. In fact, with the COVID-19 outbreak, the use of this type of molasses have been increased. In addition to the widespread production and use of molasses, especially in rural areas, the importance of cone molasses has increased in recent years. The use of this natural product, which is important in this regard, especially in the worldwide epidemic such as Covid-19, has created the necessity to investigate the real effectiveness of these products. There are many plants have been used by making molasses. Carob, mulberry, grape, black mulberry molasses, and pomegranate molasses are among the most demanded molasses. In a study conducted in 2020, antioxidant and total phenolic substance values of different molasses types were examined. It was found that the highest rate was in carob molasses, blackberry and pomegranate molasses were lower than carob, but still could be considered high according to the amount of phenolic substances examined in some herbal extracts, and grape molasses had the last place.

In the present study, the results obtained from fir cones collected from Kastamonu region were analysed by spectrophotometer for the determination of phenolic and flavonoid substances. Phenolic matter values in syrup and molasses were calculated as 384.27 mg GAE/g and 304.74 mg GAE/L, respectively, while total flavonoid values were calculated as 48.99 mg QE/g and 43.74 mg QE/L, respectively. Considering the data obtained, it was observed that both functional products were rich in phenolic substances. In addition, the total amount of flavonoid substances is also quite high.

It is very difficult to achieve stability in natural products obtained from plant products. Because the substances that will show antioxidant properties to be obtained, in other words, secondary metabolites are the materials produced by the plant to protect itself by being affected by environmental factors. Every year, the number of sunny days, humidity, precipitation, soil structure, etc. When things vary, different results may occur in the extracts, molasses, syrup to be obtained. In addition, sometimes even the period or even the time when the plant material is harvested may cause certain differences. However, this situation does not change whether there is a phenolic component or flavonoid component in the interpretation of the analysis made for that material.

CONCLUSIONS

In this study, phenolic and flavonoid ratios of molasses and syrup samples obtained from Abies Nordmanniana, one of the fir species belonging to Kastamonu region and widely used among the people, were determined. The values observed within the scope of the data obtained contain positive results. In addition to proving the existence of products rich in phenolic substances with this study, it is very valuable to evaluate the effectiveness of these products used among the people with scientific studies. The results obtained are only a part of general scientific studies. In addition to these studies, chemical content analyses by high pressure liquid chromatography can be performed to show which phenolic components are more in these products. It can be examined whether any toxicity occurs in terms of long-term and high temperature exposure. It can be also investigated whether the material collected from nature causes any contamination. In addition to all these, in-vivo in-vitro studies can be carried out on diseases that are thought to be good for cone molasses. With these studies, it will be possible to provide a reliable product feature in the return to natural products that have recently increased worldwide.

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ALTERNATIVE OILSEED CROPS IN TURKEY

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ABSTRACT

Despite its sufficient production in many agricultural crops, our country has a large foreign trade deficit, especially in the production of oilseeds and edible oil. Alternative oilseeds are valuable crops that are grown in marginal areas in Turkey and have a crucial contribution to oil production. Alternative oilseed crops cultivated in our country are rapeseed (canola), safflower, sesame, linseed, camelina and cephalaria. While these oilseeds can be successfully grown in Turkey, the markets and supply chains some of them are not necessarily developed. Especially camelina and cephalaria production is too low to be recorded in the statistical database. In 2022, 150.000 tons of rapeseed, 30.000 tons of safflower, 17.366 tons of sesame and 8 tons of linseed were produced in our country. The production of these crops is highly low in Turkey where edible oil consumption is high. Although almost every region of our country is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. In this study, the availability, production and future of alternative oilseed crops in Turkey are considered as a whole.

Keywords: Alternative oilseed crops, Rapeseed, Safflower, Sesame, Linseed, Camelina

INTRODUCTION

In parallel with the increase in the world population, the consumption of foodstuffs is increasing and at the same time the consumption of edible oil is also increasing. This consumption forces producers to increase production on available agricultural area. In addition, the fact that vegetable oils have become the raw material of the energy sector such as biodiesel increases the need for these oils even more (Culpan, 2015).

Edible oils, a staple food, are obtained from oilseed crops. Some of these crops are wild and some of them are cultivated (Arslan and Culpan, 2023). Vegetable or edible oils, which are basic foods, are obtained from oilseed crops. Oilseed crops rich in primary and secondary metabolites (fat, protein, carbohydrates, vitamins etc.) constitute an essential source of raw material for human and animal nutrition as well as for the industrial sector (Yılmaz et al., 2021).

Despite its sufficient production in many agricultural crops, our country has a large foreign trade deficit, especially in the production of oilseeds and edible oil. Alternative oilseeds are valuable crops that are grown in marginal areas in Turkey and have a crucial contribution to oil production. Alternative oilseed crops cultivated in our country are rapeseed (canola), safflower, sesame, linseed, camelina and cephalaria. While these oilseeds can be successfully grown in Turkey, the markets and supply chains some of them are not necessarily developed. Although almost every region of our country is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. In this study, the availability, production and future of alternative oilseed crops in Turkey are considered as a whole.

ALTERNATIVE OILSEED CROPS

Rapeseed (*Brassica napus* L.)

Rapeseed, a crop of Mediterranean origin, is important for human and animal nutrition (Gürsoy, 2019) and biodiesel production. It is the third most produced annual oil crop in the world (71.3 million tons as of 2021), behind soybean and seed cotton. The countries with the highest production are Canada, China, India, Germany and France (FAOSTAT, 2021). According to the data of the Turkish Statistical Institute for the year 2022, 150.000 tons of rapeseed was produced from 41.145 ha and the average seed yield was 3650 kg/ha (Table 1). Rapeseed is intensively cultivated in Thrace region in Turkey and its contribution to the oil production is highly important (Culpan et al., 2022).

Table 1. Rapeseed production in Turkey in the last 10 years

Years*	**Area Harvested (ha)**	**Yield (kg/ha)**	**Production Quantity (tons)**
2013	31.127	3280	102.000
2014	32.133	3420	110.000
2015	35.081	3440	120.000
2016	35.453	3530	125.000
2017	16.520	3640	60.000
2018	37.845	3300	125.000
2019	52.514	3430	180.000
2020	34.989	3470	121.542
2021	37.601	3720	140.000
2022	41.145	3650	150.000

* Turkish Statistical Institute, 2022

Rapeseed cultivation is highly similar to wheat cultivation practices (Tıraş, 2011); it is sown and harvested in almost the same period (September-July). The reasons that make rapeseed valuable are that it winter survival when it enters winter during the rosette period, does not require additional irrigation by being content with natural rainfall, reaches harvest maturity in July at the latest, can be easily harvested with a wheat harvester and yields more than cool climate cereals under dry farming conditions (Arslan, 2016). Due to the 40-50% quality oil in the seeds of rapeseed (Murphy, 1995; Gürsoy and Kolsarıcı, 2017), high oil yield from per hectare is an advantage compared to other spring oilseed crops such as safflower. The most important problem of rapeseed in Turkey is the poor availability of moisture in the soil in September and early October, the time of winter sowing in Central Anatolia and Thrace. This can lead to poor emergence and perhaps to repeat sowing.

Safflower (*Carthamus tinctorius* L.)

Safflower (*Carthamus tinctorius* L.) is a multipurpose oilseed crop that can grow in arid and semi-arid environments because of its tolerance for drought stress (Mosupiemang et al., 2022). *Carthamus tinctorius* L. which belongs to the Asteraceae, is one of the oldest cultivated plants that started to be cultivated 3000 years ago. It contains 25-45% oil in its seeds, has two different types as linoleic (ω-6) and oleic (ω-9), has high quality edible oil, is suitable for biodiesel production, is cultivated in the form of residue and mixture and is considered as animal feed (Arslan et al., 2012; Culpan and Arslan, 2022). On the other hand, drought tolerant and cultivation without irrigation enable especially availability of fallow areas (Arslan and Culpan, 2018). According to the data of the Turkish Statistical Institute for the year 2022, 15.000 tons of safflower was produced from 26.237 ha and the average seed yield was 1140 kg/ha (Table 2). There was a significant increase in safflower cultivation areas until 2014 and 2015 growing seasons, but then a decrease was observed again.

Table 2. Safflower production in Turkey in the last 10 years

Years*	**Area Harvested (ha)**	**Yield (kg/ha)**	**Production Quantity (tons)**
2013	29.292	1540	45.000
2014	44.305	1410	62.000
2015	43.107	1640	70.000
2016	39.571	1470	58.000
2017	27.376	1830	50.000
2018	24.693	1420	35.000
2019	15.860	1380	21.883
2020	15.115	1410	21.325
2021	14.588	1110	14.000
2022	26.237	1140	15.000

* Turkish Statistical Institute, 2022

The most important advantage of safflower is that it does not require much agricultural practices such as irrigation and fertilization. However, since safflower is known as a drought tolerant crop, it is cultivated without irrigation conditions and therefore its seed yield is low. According to the researches, seed yield of safflower increases up to 2 times with irrigation under appropriate growing conditions in dry areas (Öztürk et al. 2009; Arslan and Culpan, 2023). In the arid and semi-arid areas of the Central Anatolia Region, where the wheat-fallow system is widely practiced in Turkey, safflower is the most important alternative oilseed crop that can take place in crop rotation with wheat.

Sesame (*Sesamum indicum* L.)

Sesame is one of the first oil crops to be cultivated and is very important for human nutrition due to its valuable nutrients. It seeds contain 50-60% oil and 25% protein and are sown as spring crops. Sesame oil contains high levels of unsaturated fatty acids such as oleic (40-50%) and linoleic (45-50%) and saturated fatty acids such as palmitic (7-9%), stearic (4-5%) and arachidic (0.4-1.0%) (Bakal and Arıoğlu, 2020).

In 2022, 17.366 tons of sesame was produced from 24.285 ha and the average seed yield was 720 kg/ha (Table 2). While sesame production in Turkey was 40 thousand tons in the early 1990s, it has been below 20 thousand tons in recent years. This is mainly because its seed yield is low and the price of oil is quite expensive. Accordingly, sesame has no contribution to our vegetable oil industry. It is used in cakes, pastries and breads in our country, in addition to tahini production, which takes the crop away from oil production. By introducing varieties with high seed yields in the Aegean, Mediterranean and GAP regions, the oil production potential can be increased if mechanization problems are solved under irrigation conditions (Arslan, 2016).

Table 3. Sesame production in Turkey in the last 10 years

Years*	**Area Harvested (ha)**	**Yield (kg/ha)**	**Production Quantity (tons)**
2013	24.807	620	15.457
2014	26.349	670	17.716
2015	28.088	660	18.530
2016	28.933	670	19.521
2017	28.031	660	18.410
2018	25.985	670	17.437
2019	24.860	680	16.893
2020	25.666	730	18.648
2021	25.486	690	17.657
2022	24.285	720	17.366

* Turkish Statistical Institute, 2022

Linseed (*Linum usitatissimum* L.)

Linseed (*Linum usitatissimum* L.) is a traditional oilseed crop that represents a valuable alternative for fallow areas due to its adaptability to unfavorable soils and its high economic value relative to the high quality of the seed oil (Zanetti et al., 2013). It is grown either for its fiber (fiber flax) or for its oil (oilseed flax) (Hall et al., 2016). Its oil is the best source of the n-3 fatty acid, α-linolenic acid, which constitutes nearly 55 % of its total fatty acids. This value is 5.5 times more than the next best sources of α-linolenic acid (Bloedon and Szapary, 2004).

Linseed is an alternative oilseed crop and has unique drought tolerance; in extreme conditions, it can complete its life cycle in climates in which annual rainfall is only 200 mm (Li and Wang, 2016). Genotype × environment interactions have been shown to be high for linseed (Diepenbrock et al., 1995), and seed yield change significantly between production years, depending on location and climate conditions. Linseed, like safflower that can be used to utilize fallow and poor soil fields in Turkey. It is an important source of vegetable oil that can be utilized especially in conditions where rain and irrigation water is limited and therefore other oil crops cannot be grown (Arslan, 2016). Linseed cultivation has resumed in our country in the last few years and production has reached 8 tons according to 2022 data (TSI, 2022).

Camelina (*Camelina sativa* (L.) Crantz)

Camelina (*Camelina sativa* (L.) Crantz) is ancient oilseed that blongs to *Brassicaceae* family that is grown worldwide (Righini et al., 2019; Schillinger, 2019). Several characteristics of camelina make it an alternative oilseed crop, indeed a potential oilseed crop. In recent years, camelina has started to gain importance in the international arena again and many new researches have been carried out on it (Sevilmiş et al., 2019). Many researchers documented that camelina is drought and heat tolerant (Angelini et al., 1997; Blackshaw et al., 2011). It (*Camelina sativa* (L.) Crantz) is more adaptive to drought conditions than other oil seeds crops such as canola (Raza et al., 2015). The seed yield of camelina ranged from 1177 kg/ha under drought conditions in Saskatchewan to 3012 kg/ha in northern Alberta (Francis and Campbell, 2003). Zubr (1997) reported seed yields of 2600 kg/ha and 3300 kg/ha for spring and winter varieties, respectively. In Turkey, on the other hand, camelina is not cultivated at present. However, with the expansion of the cultivation area in the future, the potential to provide raw materials to the oil industry can be reached.

Cephalaria (*Cephalaria syriaca* L.)

Cephalaria (*Cephalaria syriaca* L.) is an annual plant in the Dipsacacea family. Studies have revealed that the oil content of cephalaria seeds varies between 21-26% and protein content between 14-20% (Çağlar, 1968). Its oil can be used directly as edible oil or mixed with other oils. However, the 7-8% epoxy acid in oil indicates that this oil should not be used as edible oil in this form (Sezgin et al., 2017). It is a crop that is widespread in Anatolia, grows wild in wheat fields and although its growth form is not similar to wheat, it is quite similar in terms of seed structure, size and shape (Boz and Karaoğlu, 2013; Sezgin et al., 2017). Cephalaria is a potential alternative oilseed crop for the future as it is cold and drought resistant. It grows very well in clay and loamy soils. As in camelina, cephalaria can reach the potential to provide raw materials to the oil industry by expanding its cultivation area in the future.

CONCLUSIONS

In 2022, 150.000 tons of rapeseed, 30.000 tons of safflower, 17.366 tons of sesame and 8 tons of linseed were produced in our country. The production of these crops is highly low in Turkey where edible oil consumption is high. Although almost every region of Turkey is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. Our country will be able to make use this advantage ideally and plan and program it with a number of precautions so that it will be able to exert itself in the production of oil seed and vegetable oil and export the production excess. However, the necessary measures and precautions listed below must be taken to unlock this potential and increase production quantities;

1. Long-term planning and sustainable policies should be implemented in the vegetable oil industry and alternative oilseed crops production.
2. In oilseed production, local seed breeding and production should be accelerated and supported by the state.
3. Alternative oilseed crops production should be included in the alternative crop project in fallow areas.
4. Irrigation investments should be accelerated and oilseed crop cultivation should be emphasized in new irrigable cultivation areas.
5. The Ministry of Agriculture should play an active role in seed supply and technical support for alternative oilseeds (rapeseed, safflower and linseed etc.) and should be encouraged to purchase the produced product.
6. In order to eliminate the low seed yield of safflower and to use it more effectively in crop rotation, winter-tolerant varieties should be developed.
7. Measures should be taken to expand mechanization in sesame agriculture.
8. Production of alternative oilseeds should be supported with low interest loans and premium amounts per kg should be increased.

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CONTRACT FARMING IN BUCKWHEAT CULTIVATION

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Abstract

Characteristically, contract farming is a deal between a producer and a buyer regarding cultivation of an agricultural product. In reality, terms and conditions of these contracts can alter dramatically. It can refer to those arrangements involving public firms, government organisations or NGOs and can also refer to private schemes. In this study, we only focus on contract farming on arrangements between farmers and private actors. In recent years, buckwheat has gained increasing notice as a promising functional food, owing to its several human health issues and lack of gluten. This paper examines buckwheat farmer's involvement in contract farming in counties of Gümüşhane province which is in the northeast of Turkey. An empirical analysis of the contract farming regarding the buckwheat farmers and postharvest practice has been provided. The survey was conducted in Kelkit, Köse and Şiran counties as only production places of the buckwheat in the province. Data collected from 30 farmers who are engaged in contract farming model. According to data, issues of the contract farming, marketing channels of the buckwheat and perspectives of the farmers for the buckwheat production with contract farming are addressed. The findings of this study reveal that the contract farming model, mostly eliminates the marketing-related concerns of the farmers in the region. Perceive benefits derivable from beginning to buckwheat contract farming was measured based on the following proposals (the commitments to purchase product, the market guarantee, the compliance with the contract terms, purchasing at the agreed price and timely payment) was evaluated using 5 point likert scale. As a result, buckwheat contract farmers should be monitored by the contract buyer for the continuity of the production.

Keywords: Agricultural production, Buckwheat, Contract farming, Contract farmers, Gümüşhane

INTRODUCTION

Agricultural production is one of Turkey's economic sectors, having about 24.1 million hectare of the farmland and employing 3 million farmers. A fundamental view is that the country population carries on to raise while the farming remains below expectation. World Bank (2023) reveals that agriculture which accounted for there about 25% of the gross domestic product of some developing countries is important to economic growth on the other hand in Turkey it is contributing 7% of the country's gross domestic product (GDP). Crop products include grain and other crop products in the agricultural production of Turkey. According to data from Turkish Statistical Institute (TSI) in Turkey, while total grain production was 33 468 699 tonnes in 2018-2019, this amount was 33 401 704 tonnes in 2019-2020 marketing year and when we look at the area sown, it was 10 779 036 ha and 10 645 742 ha in the relevant years respectively. Turkish Republic (TR) Ministry of Agriculture and Forestry reports that the livelihood of almost all of the population living in rural areas is agriculture. Therefore, the farming in the economy of the countries and creating new alternative livelihood is paramount (Gaffney et al., 2019). Growth at a slow pace in farming can pioneer lacking the skills, technologies, financial services and agricultural policies instability in the country (Collier and Dercon, 2014). As it was clarified by Mishra et al. (2016) growers notice that skills and abilities in developed agricultural production such as the selection of alternative product kinds, off-season crop production and suitable technologies in harvest time are the key to increase profitability. Alternative crop production is an important advantage for a country like Turkey with a wide variety of climatic conditions. Finally, a study by Bozkurt and Kaya (2020) brings to an issue that increasing crop production and profitability mostly are attached to on the improving of land use, climatic, financial, fiscal, monetary policies and new alternative crops.

Buckwheat is not related to cereals such as wheat, barley and rice. It is one of the main alternative products in the crop production pattern in the world and is an important food item due to its components. Buckwheat production has become one of the most developed crop production. In addition to its nutritive effect, foods that protect and improve well-being and reduce the risk of disease are defined as functional foods (Erbaş et al., 2008), and the increase in consumers' interest has led to the supply of such products in the market (Sedej et al. 2011). The buckwheat production is not limited to producing buckwheat for bakery products only, but also produces many by products such as in animal feeding, in the paint industry, in the beekeeping as nectar. Russia's share in world buckwheat production (1 875 067 tons) in 2021 was 919 147 tons, followed by China (502 369 tons) and Ukraine (105 780 tons). Russia accounted 48% of the world's total buckwheat production in 2021 (FAO, 2023). Buckwheat, which is produced in many countries of the world and has begun to take its place in international trade but it is not commercially cultivated in Turkey. Based on the data of Turkish Statistical Institute (2023), there was 1 359 tons of buckwheat production with area sown 8 482 decare under total cereal production. When it is looked at yield of the buckwheat in Turkey, it was 147 kg/daa in 2021 and it was 160 kg/daa in 2022. With this data it could be said that both area sown and yield is relatively low when it is compared with other cereals.

To promote alternative crop production in rural areas, buyers can be encouraged to form collaboration with farmers based on contract agreements. The farmer makes decisions about what to produce, how much and which inputs to use non contract farming. In this case, it will be possible for the capital to dominate in agriculture only if the production decisions are not taken by the farmer. Linking smallholder farmers to markets with the contract farming (CF) is also one solution to overcome high levels of poverty in many underdeveloped areas (Meemken and Bellemare, 2020). As it is stated in Singh and Raj's (2019) study that farmers especially smallholders have a difficulty to participate in the market as the condition of the market is changing every day and large farms can have easy access to enter the big markets. CF has recently encouraged as a key solution to combine small scale farmers into part of a discourse of market-led agricultural development (Vicol et al., 2021). Therefore, contract farming (CF) can be a method that should be applied for the commercialization and industrialization of the buckwheat production in the research area. The CF strives to make a steady supply of products which is very important given market liberalization and globalization too (Saigenji and Zeller, 2009).

This paper is presented as a qualitative work with focus only contract farming and how it helps to promote newly introduced crop like buckwheat in the research area. While many factors inhibiting agricultural production has been introduced in the beginning, the contract farming can be good alternative the promotion of buckwheat and availability of contract farming is important for the local farmers. This paper demonstrates this point qualitatively and also examines the perceived benefits accruable by the farmers in buckwheat contract farming.

MATERIAL AND METHOD

The data used in this study has been provided from face to face interviews that conducted from June to July 2021 in districts of Gümüşhane province. Due to the limited number of buckwheat farmers in Gümüşhane province, no sampling was made in the research area. As a qualitative work with basic on only 30 farmers (total available sample) is presented by solely focusing on contract farming. There are also no farmers served by the traditional and other forms of buyers in the study area, there is only a buyer who is an entrepreneur so survey questions have been designed considering 30 farmers who are engaged to contract farming and this buyer.

Data collection was conducted using structure questionnaire complemented with schedule interview. The question form used a detailed form to have enough information on farmers such as age, sex, education, household size and farming experience. To give a clear picture of perception of buckwheat farmers on the accruable benefits of contract farming, a 5-point Likert scale (1: strongly disagree; 5: strongly agree) was used. This study makes a hypothesis that growers encounter many difficulties while cultivating and selling their products. Our argument is that these difficulties encountered in farming are positively connected to contract farming. The Likert scale can evaluate the degree of farming difficulties (Hoang and Nguyen, 2022).

RESULTS AND DISCUSSION

Farmers’ Descriptive Characteristics

Results in Table 1 point out that nearly all of the contract farmers are male. Agricultural production, which has been going on for years in the research region, constitutes the livelihood of the households and is carried out by men so farming is seen as a male job. This finding agreed with Yisa et al.,’s study (2022) that larger proportion of rice contract farmers in Benue State of Nigeria are male.

The majority of the farmers’ age changes between 51 and above (43.3%). On the other hand, %36.7 of the participants’ age varies between 41 and 50 years. This result shows that the contract farmers are still in their effectual and productive ages. This means the farmers migh be risk takers and enthusiastic in the sustainability of the contract farming model. This intersects with Akanbi et al.’s, (2019) result that the average age of the contract farmers is about 42 years. Table 1 also indicates that the years spent in education are 5-8 years (30%) and this value corresponds secondary school. With this, majority of the farmers are high school (26.7%) or university graduates (26.7%). This finding tells us that the level of education for the farmers is sufficient. Farmers with a high level of education may be more open to change with alternative production methods. It was mentioned in the study by Tambo and Wünscher (2017) that growers with a higher level of education are more willing to tolerate modern agricultural production opportunities such as contract farming that involves risk-taking actions.

On the topic of household size of the farmers it is seen that the household ranges of 0-5 (90%) encompass the majority of the farmers. Large household size can be advantage in agricultural production in rural areas and this might positively affect agricultural production. Because, workforce is one of the important inputs in small-scale farms. Mostly, family workforce is used by smallholders in production or harvesting time. Whereas, the household size of the farmers included in this study is relatively low. This finding is in agreement with Khan et al. (2019), who outlined that potato contract farmers' average household number is 4.37. The years of farming experience vary by a majority between 1-10 years (36.7%) for the contract farmers. Moreover, 23% of the farmers has got farming experience between 11 and 20 years. According to this result, we can say that the farmers have got relatively experience in agricultural production, but they can also get farming experience over the years and this might affect their engagement in contract farming. As it is stated in Ganewo et al.'s study (2022) the more years farmers are in crop production, the better they get involved in terms of contract farming model. So, it was anticipated that buckwheat cultivation experience positively affect engagement with buckwheat contract farming.

Table 1. Descriptive of farmers in terms of characteristics (n=30)

Variables	**Frequency**	**%**
Gender		
Male	29	96.7
Female	1	3.3
Age		
‹ 30	3	10
31-40	3	10
41-50	11	36.7
51 and above	13	43.3
Year of education		
1-4 Primary school	5	16.7
5-8 Secondary school	9	30.0
8-12 Highschool	8	26.7
›12 University	8	26.7
Household size		

‹ 5	27	90.0
6-10	3	10.0
11-15	-	
Experience in farming		
1-10	11	36.7
11-20	7	23.3
21-30	6	20.0
31-40	4	13.3
41 and above	2	6.7

Buckwheat Farmers' Crop Production Pattern (2020)

In Table 2, the crop production patterns of the farmers are shown as average cultivation area, average production amount and average sales price. As mentioned earlier the farmers only produce buckwheat with contract farming model. Unfortunately, there are no buckwheat farmers served by traditional and other form of buyers in the research area. Therefore, no comparative analysis can be established. The full count sample from this study has only 30 farmers who are linked to contract farming. The remaining farmers are producing their crops apart from buckwheat with the traditional method which is non-contract farming. It is seen that the farmers' production patterns are mainly wheat, barley, oat, corn and alfalfa. Considering the average production amounts, the highest production amount is corn with 123 333 kg, followed by wheat with 43 480 kg, alfalfa with 12 319 kg, oats with 11 784 kg and barley with 8 814 kg, respectively. Although the number of potato producers among the total farmers is low, the average amount produced is 26 500 kg. Considering the prices received by the farmers, it is understood that the highest price per kg belongs to dry beans and buckwheat. It is among the results that the unit sales prices of products such as corn, wheat, barley, and alfalfa, which are included in the traditional production pattern, are much lower. According to this finding, it is extremely important to increase the income of the farmers by going out of the traditional production pattern and directing the farmers to crop products with higher economic returns. In this respect, it is necessary to conduct studies to increase the cultivation area of buckwheat as an alternative product and to increase the production amount. In the study by Singh and Raj (2019), the growers earned more by growing basmati under contract farming and on an average increase in gross income under contract farming was found as 9.69%.

Table 2. Buckwheat farmers' crop production pattern

Crop	Production Method (1: Contract Farming, 2: Non-contract Farming)	Average Cultivation area (daa)	Average Total Production (kg)	Average Price (kg/TL)
Dried Bean	2	14.9	1699	23.1
Wheat	2	165	43480.9	1.71
Corn	2	28.6	123333	1.85
Barley	2	34.8	8814	1.67
Oat	2	47.9	11784	1.08
Eincorn	2	10	1000	5
Potatoes	2	10	26500	1.35
Clover	2	25	12319	1.21
Buckwheat	1	23	1082.8	4.46

*The average exchange rates between Turkish Lira (TRY) and the Euro (€) for June 2021 was 1 EUR = 10.3832 TRY.

The Form of Contract Farming in Buckwheat Cultivation

The orientation to alternative products like buckwheat in agriculture is not easy for farmers due to traditional production habits. Generally, the transition of farmers to alternative products is realized through contract farming models with the initiative of private firms or entrepreneurs. Buckwheat production with the contract farming model in Kelkit, Köse and Şiran districts of Gümüşhane province started in 2018 under a private firm. The contract farming experience of the farmers in buckwheat cultivation changes from 1 to 3 years and covers an average of 1.51 years. Contract buyers generally supply growers with inputs to use in production under predefined terms such as fertilizer and pesticides (Dubbert et al., 2021). However, in contrast to widespread production inputs according to the agreement between the farmers and the firm, the buyer only supplies limited inputs in this research. Inputs that the buyer provides to the farmers are generally limited to seeds. Intervention of the buyer in decisions regarding buckwheat cultivation is given in Table 3.

Table 3. Intervention of the buyer in decisions regarding buckwheat cultivation

Statements	Average
Determining the sowing time	3.44
Determining the type and amount of seed to be used	3.41
Determining the type and amount of fertilizer to be used	2.60
Determining the type and amount of pesticide to be used	1.58
Determining the number and amount of irrigation	1.51
Determining the harvest time	3.03

*Likert Scale Mean: (1: None 2: Slightly 3: Neutral 4: Quite 5: Permanent)

To compare the production amount for the buckwheat in the study area, both 2019 and 2020 were considered in this study. As it is seen from Table 4, the total amount of the buckwheat production in 2019 was 32 785 kg, and it was only 27 070 kg in 2020. As 76.66% of the buckwheat farmers who are engaged in the contract farming in 2019, this rate decreased to 40% in 2020.

Table 4. Buckwheat production amount in 2019 and 2020

Crop	2019	2020
Total Buckwheat Production (kg)	32 785	27 070
Contract Farmers (%)	%76.6	%40

Analysis of Farmer's Perception Towards Contract Farming

Knowing how the farmers evaluate the contract farming in terms of production and marketing, is considered important especially for the adaptation of farmers to alternative agricultural products such as buckwheat. For this purpose, the perceptions of the farmers about the contract buckwheat farming model were examined. A 5-point Likert scale was used for the responses of the farmers to the propositions are shown in Table 5. According to the results, the interviewed farmers strongly agree with the propositions such as the buyer's compliance with the commitments to purchase the entire product (4.75), the market guarantee (4.61), the buyer's compliance with the contract terms (4.50), the purchase of the buckwheat at the agreed price (4.43) and timely payment (4.22). These results indicate that for the promotion of new crops like buckwheat; market guarantee, timely payment, price are very important for the local farmers to participate contract farming. This also translates to higher buckwheat income at the farm level. Because, farmers are of the view that they need financial support to buy inputs like fertilizer, seed and harvester or tractor services to enable gain high yield (Yakubu et al., 2022). These results are also consonance with Yisa et al. (2022) who pointed out that contract farming helps farmers' inputs and enhances their earnings (4.87 and 4.77).

The contract farmers reported that they agreed with the proposition "contract farming is more reliable than traditional one" at a moderate level (3.39). In the study of Ruml et al., (2021) it is stated that there is an additional advantage of the agreements between farmers and buyer because of the difficulty of finding buyers for larger amounts in traditional chain. Therefore, working with contract buyer can be an advantage for the local small scale farmers.

There are also some propositions that the farmers have close to the indecisive attitude and agree less. These propositions are as follows; "The buyer makes fair decisions (2.89)", "It is possible to obtain more yield (2.75)", "Contract farming can be more profitable (2.71)", "Higher price in contract farming (2.61)", "Consultancy service in the production time (2.25)", "Lower production cost (2.14)", "Meeting demands of buyer in terms of product quality (2.11)", "I have enough knowledge about contract farming (2.04)", and "The buyer provides credit facilities during the production and harvest time (1.18).

Table 5. Farmers' perceptions towards contract farming

Statements	**Average ***	**Std. Dev.**
The buyer receives the entire product promised in the contract.	4.75	0.4410
There is a market guarantee in contract farming.	4.61	0.4973
The buyer complies with the terms of the agreement.	4.50	0.6383
The buyer buys the buckwheat at the agreed price.	4.43	0.8357
The buyer pays the crop prices on time.	4.22	1.0500
Contract farming is more reliable than non-contract farming	3.39	1.1001
The buyer makes fair decisions when it interferes with production decisions.	2.89	1.4489
It is possible to obtain higher yields in production in contract farming.	2.75	1.1746
Contract farming is more profitable than traditional farming.	2.71	1.1501
A higher price can be obtained in contract farming than in traditional one.	2.61	1.1333
The contract buyer provides continuous consultancy service during production.	2.25	1.2656
The cost of production in contract farming is lower than in non-contract one.	2.14	1.0789
In terms of crop quality, I can meet the demands of the contracted buyer.	2.11	0.7373
I think I have enough knowledge about contract farming.	2.04	1.1380
The buyer provides credit facilities during the production and harvest time.	1.18	0.7724

*Likert Scale Mean: 1-Strongly disagree; 5- Strongly agree

CONCLUSION

Contract farming, which is used as a tool in the industrialization of agriculture, especially in developing countries, is a model that should be evaluated in increasing the production crops in this context. For this reason, it is necessary to analyse the internal dynamics in the current agricultural production system and support the technical infrastructure for the implementation of contract farming. Policies to directly improve the contract environment of both the buyer and the farmer, the specific problems faced by farmers while signing the contracts, and the plans that local authorities will prepare in this context are extremely important. The most important problem faced by the farmers is buckwheat yield. In this respect, technical consultancy to be given to the farmer in the process from planting to harvest will be an important factor in the sustainability of production. In addition, in order to continue buckwheat production with contract farming model, agricultural credits or grants that will support the loss of farmers should be increased and programs specific to buckwheat farmers should be prepared.

The buyer's support in production and post-harvest chain in contract farming will increase the buckwheat productivity. Therefore, strict controls over the buckwheat cultivation create not only sustainability of production with better quality, also the increase the buckwheat productivity. This paper indicates that the production and post-harvest practices on contract farmers should be enhanced by the buyer and also by possible buyers, so farmer's status should not be tied to only a buyer. The awareness level of CF is found to be less in case of farmers. Training and advertisements should be set in a manner which extends knowledge about CF issues among the farmers in particular. In terms of the results of this study we can conclude that the farmers state the same perceptions on four of the top five advantages of CF such as assured purchasing, guaranteed market, reliability of the contractor, guaranteed price and timely payment. In conclusion, CF can progress farmers' welfare and reduce cost of inputs. So, the buckwheat farmers should be controlled by the buyer for the sustainability of the cultivation.

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A GENERAL EVALUATION ON THE MARKET PRICE EFFECT OF GEO-LABELLED PRODUCTS IN TURKEY

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ABSTRACT

Geographical indications (GIs) received by public authorities or NGOs are important tools used to create added value for local values and increase their trade. The GI labelled product market in the world has reached 200 billion dollars annually. The largest share in this market is the European Union (EU) countries, and 17% of the union's food exports consist of GI labelled products. Although there is a wide variety of local products in Turkey, GI registered local values are brought into the economy has been later. The total number of patents registered by the Turkish Patent and Trademark Office in Turkey in 2023 has reached 1341. Initiatives made through e-commerce, which is the marketing channel with the highest growth, in order to increase the competition in the market and the commercial volume of GI registered local products indicate that this market will grow even more in Turkey. In this study; pulses group, fruit-vegetable-pickle group, crispy-pastry-dessert group, cheese group, sausage-salami-red meat group which are registered with GI in Turkey, are discussed. After the GI registration of these products, the price effects in the market were examined and evaluations were made. According to the results, the price premium calculated by comparing the prices of GI-labelled and non-GI-labelled products in selected food products was determined in favour of GI-labelled products. This price premium has been found to be quite high, especially in packaged and processed products (primarily in the pulses and dessert group-dried fruit pulp).

Keywords: Geographical indication, Price, Local products, Türkiye

INTRODUCTION

Human being's relationship with food products has certainly undergone a transformation in recent years. Food was supplied from the local region where it was lived until the 19th century. While basic foodstuffs were included in people's daily food consumption, consumption patterns have changed with globalization. This transformation has reduced the consumption of local food products. However, the production of large quantities of food with a long shelf life by using intensive inputs has started new searches in consumer demands. The fact that issues such as healthy life, negative effects of production on the environment, biodiversity, sustainability, agro-ecological production and circular economy have become the agenda necessitated the revision of production processes and local production. As a result, the demand for local food products is increasing, and the share of these products in the market is also increasing. Local products especially in France constitute approximately 20% of annual food expenditures, and this rate increases by more than 10% each year (Özsoy, 2015).

Geographical indication (GI) is a quality label that shows and guarantees the source of the product, its characteristics and the connection between the characteristics of the product and the geographical area. With this label, it is ensured that products that have gained a certain reputation depending on their quality, traditionality, raw materials obtained from the local area and local qualities (TPO, 2023). In terms of Turkish Patent and Trademark Office (TPO) GIs are registered as a PDO or a PGI. If essential qualities of a product originate from natural and human elements belonging to a certain geographical area, the geographical indications in this case are called "Protected Designation of Origin (PDO)". The attribute of PDO is because of the production is completely linked to a certain geographical area, while in the situation of PGI (Protected Geographical Indication); leastways one of the raw material or production and processing stages of a product is linked to a specific area or location. Food, agriculture, mining, handicrafts, industrial products can be subject to geographical indication registration. In this study, it has been only dealt with pulses group, fruit-vegetable-pickles group, crispy-pastry-dessert group, cheese group and sausage-salami-red meat group.

So far, 1438 local products have been registered in Turkey with the name of origin (PDO) or the sign of origin (PGO). Industrial property rights protection is valid in the country of registration all over the world. In this context, the registration of GIs within the scope of industrial property rights in our country is valid only in the borders of Turkey. In order to obtain protection in different countries, it is necessary to apply for registration within the framework of the legislation of those countries or to apply within the scope of systems providing international protection. Currently, 13 geographical indications have been registered in the EU. These are; Antep Baklava, Aydın Fig, Aydın Chestnut, Bayramiç Nectar, Malatya Apricot, Milas Olive Oil, Taşköprü Garlic, Giresun Chubby Hazelnut, Antakya Kunefe, Suruç Pomegranate, Çağlayancerit Walnut, Gemlik Olive and Edremit Olive Oil.

Previous studies have focused on issues such as the effect of geographical indication on the producer and on the consumer attitude, local development and the environmental effects. Santeramo and Lamonaca (2020) study evaluation of GI label in consumers' decision-making process, determining that the quality and fame of a product originated in a geographical origin. By using GI label, growers and sellers take advantage available connotations linking consumers and geographical indication (Marcoz et al., 2016). Many authors previously have discussed that appropriate marketing strategies can help producer welfare and also support a positive impact on producing GI-labelled products (Anson and Pavithran, 2014). The study has been conducted by Deselnicu et al., (2013) that price premiums differentiate agri-food products with GI-labelled. In the study of Larson (2018) it is said that, producers or retailers give priority to labelled products because these featured products generally have high gross margin and they can take advantage of differentiating themselves from rivals.

To figure out the value of GIs it is important to look at market size and price premiums for GI-labelled products (Jantyik and Török, 2020). In this respect the issue of what appropriate marketing strategies for geographical indication gains importance. At the same time, it can be said that studies on the use of geographical indications as a tool for agri-food products to enter international markets have become popular.

The aim of this study is to consolidate GI-labelled food products registration within the framework of pulses group, fruit-vegetable-pickle group, crispy-pastry-dessert group, cheese group, sausage-salami-red meat group. In the meantime, the market price effect of GI-labelled products has been examined.

MATERIAL AND METHOD

Pulses group, fruit-vegetable-pickle group, crispy-pastry-dessert group, cheese group, sausage-salami-red meat group, which are registered with GI label in Turkey, are evaluated in this study.

To do this, internet search on the related websites was done to see actual prices of the GI labelled food products. This attempt can prevent the possible insufficiency of face to face interviews methods. In the study, it has been endeavoured to get a general look of GI-labelled related food groups (in particular whether they have any different prices comparing with non-GI ones). In order to obtain comparable prices with GI and non-GI products, online shopping web sites were visited. First of all, we gathered data on GI labelled pulses group, fruit-vegetable-pickle group, bakery and pastry products, cheese group, sausage-salami and their closest substitutes.

The following formula has been used when calculating the price premium of geographically indicated products compared to those that do not (Jantyik and Török, 2020).

$$Price\ premium\ (\%) = \frac{Price_{GI} - Price_{NonGI}}{Price_{NonGI}} * 100$$

where $Price_{GI}$ is the price of the identified GI labelled related product and $Price_{NonGI}$ is the price of the identified GI food product's substitute, both determined as per kilogram or in case of liquid products per litre.

On account of finding the GI-labelled food products' closest substitute, the following outlines were used:

- First of all, we investigated for a substitute food product with almost the same geographical location features (e.g., for GI-labelled Bodrum mandarin "Bodrum Mandalinası", we chose mandarin with non-GI which was produced in Bodrum area),
- If the first option did not exist, we investigated for a substitute food product very similar features and different brands (e.g., for GI-labelled Tokat Erbaa pickled grape leaves "Tokat Erbaa Salamura Yaprak", grape leaf which has been grown near Tokat area was chosen).
- In cases where GI- labelled product had original features, and no other food products had an alike characteristic, no substitute food product was considered, and that GI-labelled

food product was not involved the price premium (e.g., no other flour had similar attribution like Aydın Chestnut Flour; especially, there was no other chestnut flour available.

RESULTS AND DISCUSSION

Market Size of Geographically Indicated Products in Turkey

Turkey is located in a geography that has hosted many civilizations with its 12 thousand years of history. It has thousands of local products. Thanks to non-governmental organizations, the geographical indication of local and traditional values is obtained, increasing the added value of these local products and gaining commercial value. 40 % of the 734 geographical indications whose application process is still in progress are owned by the Chambers/Exchanges. Only 13 geographical indications of Turkey are registered in the European Union. But if these local values cannot be promoted and marketed, they will not be worth anything if they are not brought into the economy. Projects such as the Local Products Program carried out within this scope will contribute to the producers' finding new markets and direct access to consumers.

The study based on the market price of the GI-labelled products considering e-commerce. The number of GI-labelled food products current in Turkish food market is very limited, GI-labelled food products available in the big franchises supermarkets in the big cities. The supply of these products is not permanent and they are not generally available to shoppers and are part of the non-GI products. Therefore it was more suitable and reliable to search the market price of the labelled food products on online shopping web sites.

The traditional trade method is now leaving its place to e-commerce with the change of technology and trade perception. With the right software, firms started to save both time and money in this way. It seems that e-commerce and e-export are growing very rapidly around the world. According to the data, by 2027, the world will reach a market of 8 trillion dollars. Considering the export figures in Turkey today, it corresponds to a value of approximately 1 dollar per kilogram. In e-commerce and e-export, an average of 30 to 35 dollars per kilogram corresponds to a figure. E-commerce and e-export pave the way especially for value-added exports (WORLDEF, 2023). Looking at the figures after the pandemic, it is seen that e-commerce has turned into a necessity rather than a choice. It is a fact that Turkey has a very serious advantage and potential in the field of local products. As a result, companies accelerate their e-commerce processes and increase the marketing of featured products such as GI-labelled products. The Union of Chambers and Commodity Exchanges of Turkey and a digital shopping website ensure that local products come to the forefront in terms of traditionality and local quality, with the program “All from Turkey” that supports producers and businesses by performing digital marketing and sales activities. With the support of chambers of commerce and stock exchanges in all provinces of Turkey, from local to national, it aims to expand market opportunities by reaching local producers, SMEs, women's cooperatives, introducing the program and enabling them to meet with the digital economy and e-commerce. At the same time, Turkey's e-commerce volume is supported.

Our observations present that at present, GI-labelled food products have only restricted significance in the Turkish food market in terms of their market share. Only a small number of these products are included in the supermarkets. At the same time, while e-commerce of GI-labelled products has been growing recently, these products have a limited supply. At this point, improvements in e-commerce will give an opportunity for current and future Turkish GI labelled products.

Price Premium on Selected Geographical Indications Products

In the research, the selected of the GI-labelled food products' price premiums in Turkey were studied to get an overview. In order to get real market prices data, we looked at different web pages of big stores operating in Turkey. The most popular GI-labelled food products were chosen as pulses group, fruit-vegetable-pickles group, crispy-pastry-dessert group, cheese group and sausage-salami-red meat group (Table 1). When the GI-labelled products were chosen, a substitute food product was considered with almost the same feature. In case where the GI-labelled food product had some very original features and no other food products had similar characteristics, no substitute product has been included, and that GI-labelled food product wasn't presented in the Table 1. To see significant difference between GI-labelled products and non-GI labelled products some popular e-food marketing sites were investigated. Supermarket web sites, local food products web sites, GI-labelled products web sites,.etc… can be given as examples. The GI-labelled food products which are in the scope of this study were checked in Turkish Patent and Trademark Office site. GI-labelled food products were not separated as PDO and PGI in the study. Both PDO and PGI labelled food products were included as GI-labelled products.

Table 1. GI-labelled products in the research

Pulses Group	**Fruit Vegetable Pickles**	**Crispy Pastry Dessert**	**Cheese**	**Sausage Salami Red Meat**
Akkuş Dried Beans	Amasya Marble Apple	Kayseri Ravioli (Manti)	Diyarbakır Knitting Cheese	Kayseri Sausage (Sucuk)
Kelkit Dried Beans	Silifke Strawberry	İzmit Cotton Candy	Hellim Cheese	Rize Village Roaster (Kavurma)
Mardin Bulgur Wheat	Amasya Flower Okra	Bursa Candied Chestnut	Kars Cheddar Cheese	Kayseri Pastrami (Pastirma)
Kastamonu Einkorn Bulgur	Alanya Avocado	Safranbolu Turkish Delight	Hellim Cheese	Akçaabat Meatball
Karacadağ Rice	Taşköprü Garlic	Gümüşhane Dried Fruit Pulp (Pestil)		

Kastamonu Einkorn Flour	Tokat Erbaa Grape Leaf	Gümüşhane Kome		
Uşak Tarhana	Çubuk Pickle	Antep Baklava		
	Bodrum Mandarin	Erzurum Stuffed Kadaif		
	Antep Dry Eggplant	Maraş Tarhana Crispy		

To calculate the price premium of the selected food products, the market prices of GI and non-GI labelled products were provided from the related websites (Table 2). When the different prices of the products were observed from the different web sites, average prices were taken into consideration. Our investigation was accomplished in e-shopping stores in Turkey. The fundamental reason for this was that these sites had expanded extraordinary in recent years, both in Turkey and across the world, making them reachable to the average shopper almost everywhere. Another important change in recent years was that an impressive part of labelled food products and local food products can be supplied from e-shopping stores. Shoppers can have a better price choice of local food products from everywhere. According to Table 2, there is an impressive difference in the price premium for GI-labelled food products. Overall, the average premium was around Sausage-Salami-Red Meat group had 10.08%, Pulses Group had 36.08%, Fruit-Vegetable-Pickles 44.22%, Crispy-Pastry-Dessert group had 53.54 and the highest was in Cheese group with 79.56%. However, it is significant to note that only products that had a potential direct substitute product were considered in the calculations of the premium prices. In the calculation of the price premium, current prices have been used as the representative prices for both GI-labelled food products and substitute products. It should not be forgotten that prices can change during the promotional offer in the discounts.

It can be seen from Table 2 that while in Antep Dry Eggplant from Fruit-Vegetable-Pickles group there is no difference in the price premium for GI-labelled and non-GI ones; Diyarbakır Knitting Cheese, Gümüşhane Köme, Gümüşhane Pestil (Dried fruit pulp) and Taşköprü Garlic have a higher price premium with 163%, 119%, 100%, and 100% respectively while Rize Village Roaster (Kavurma) has a lower premium for (0.2%). Kelkit Dry Beans, Kastamonu Einkorn Flour, Amasya Marble Apple, Safranbolu Turkish Delight and Maraş Tarhana Crispy had similar price premiums interval (50-70%).

When the online shopping web sites' current data was taken into account, it can be expressed that the proportion of GI-labelled food products is much lower comparing to their substitutes in the Turkish food marketing. Apparently, local sourcing starts progressively important for retails also for customers. Therefore, the role of GI-labelled food products is also increasing in these days (Jantyik and Török, 2020). According to food products groups in this study, packed or processed products (mainly pulses group and pestil- kome), are not represented too much in the market so, price margin between GI-labelled and non- GI is higher. In addition vegetables, fruit, fresh meat do not appear either. Perishability situations may be the reason for this. On the other hand, mainly pastry and cheese group are over-represented in the available online shopping web sites.

It should be mentioned that although local food stores play a key role, they are not acting of the whole food retail market in Turkey. With the GI-labelled food market size and price premium, it can be likely forecasted that if a GI-labelled food products market grows on food varieties, less price-sensitive customers may have a higher portion and price premium of GI-labelled food products. Hence supermarkets, local stores, hypermarkets,…etc. gain importance apart from the online web shops.

As a result, marketing the GI-labelled food products with the higher price premium can be an additional source of income for small-scaled growers, on the other hand additional costs should be taken in the labelling processes (Borowska, 2018). According to Crescenzi et al., (2022)'s research, undeveloped areas endorsing differentiated food products like GI food products experience better efficiency in terms of economic grow than others. With this, GI labelling protect the geographical characteristics of the goods and provide economic benefits like higher price premium for producers (Jena et al., 2015).

Tablo 2. Price premiums of the products included in the research

GI-Labelled Products			
Pulses Group	**Price of GI products (TL/kg)**	**Price of non-GI products (TL/kg)**	**Price premium (%)**
Akkuş Dried Beans	190	144	31.9
Kelkit Dried Beans	200	130	53.8
Mardin Bulgur Wheat	32	25	0.28
Kastamonu Einkorn Bulgur	110	80	37.5
Karacadağ Rice	71	53	33.9
Kastamonu Einkorn Flour	80	47	70.2
Uşak Tarhana	200	160	25
Fruit-Vegetable-Pickles			
Amasya Marble Apple	39	22	77.2
Silifke Strawberry	129	100	29
Amasya Flower Okra	1000	800	25
Alanya Avocado	224	160	40
Taşköprü Garlic	80	40	100
Tokat Erbaa Grape Leaf	83	60	38.3
Çubuk Pickle	90	70	28.5
Bodrum Mandarin	32	20	60
Antep Dry Eggplant	150	150	0
Crispy-Pastry-Dessert			
Kayseri Ravioli (Manti)	250	200	25
İzmit Cotton Candy	288	256	12.5
Bursa Candied Chestnut	1000	700	42.8
Safranbolu Turkish Delight	319	200	59.5

Gümüşhane Dried Fruit Pulp	360	180	100
Gümüşhane Kome	460	210	119
Antep Baklava	600	470	27.6
Erzurum Stuffed Kadaif	240	180	33
Maraş Tarhana Crispy	650	400	62.5
Cheese Group			
Diyarbakır Knitting Cheese	329	125	163
Hellim Cheese	940	636	47.7
Kars Cheddar Cheese	409	319	28
Sausage-Salami-Red Meat			
Kayseri Sausage (Sucuk)	600	499	20
Rize Village Roaster (Kavurma)	900	898	0.2
Kayseri Pastrami	1400	1156	21
Akçaabat Meatball	408	400	2

*The average *exchange rates* between *Turkish Lira (TRY)* and the *US dollar* (USD) for 24th August 2023 is $1= 25.30 TRY

CONCLUSIONS

This study concludes some benefits of GI-labelled food products focusing on price premiums. Price premiums of selected food products have been found to explain the efficiency of these products in the market. These results presented that GI labels are impressive differentiation tools in the local food markets, although their relevance changes across products. For example, GI labelling is the main differentiation for the Fruit-Vegetable-Pickles group (Amasya Marble Apple, Taşköprü Garlic, Gümüşhane Pestil and Köme) and for the cheeses group, but it is low relevance for red meat group and for the dried vegetable group.

In terms of these results some strategic inferences may be derived considering the price premiums: first of all, sellers or producers can benefit of the strength of GI labelling, particularly when they are associated with a positive label image. Retail sector should take advantage the advantages of an e-commerce. Similarly, authorities should promote the development of powerful brand images like GI and encourage effective local labels.

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DESIGN AND IMPLEMENTATION OF AN OFF-GRID SOLAR BASED SEMI-AUTOMATIC DRIP IRRIGATION SYSTEM FOR OLIVE GROVES IN CANAKKALE

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ABSTRACT

Consumption of energy is increasing at a rate of roughly 1% per year, and at a rate of 5% per year in developing countries. At the present time, the increased energy demands, decreasing fossil fuels reserves, and escalating environmental issues such as greenhouse effect and global warming make necessary researchers to survey productive and alternative energy resources. Renewable energy resources play a crucial role in retainable development and environmentally friendly energy resources. Renewable energy resources include many types of energy resource such as hydropower, solar, wind, biomass, hydrogen, geothermal and wave and it is aimed to eliminate dependence on fossil-based fuels through these energy resources. Solar energy is the most conspicuous, free, available, and clean energy for the environment, among all renewable energy resources and has an important position in electricity production worldwide. Solar irradiation can be converted to direct current electricity in care of the use of photovoltaic (PV) technology. Türkiye has an abundant resource in solar energy due to lies in a sunny belt of the earth between and the 26th and 45th east meridians and 36th and 42nd northern parallels in regard of most of other countries. Solar water pumping systems are one of the most featured applications of solar energy systems and are required in recent years in various fields such as desert places, certain metropolitan areas, and especially rural areas. Irrigation is significant for agricultural productivity. To obtain high yield and quality products is possible with an adequate amount of irrigation. For agricultural irrigation, either gasoline-based water pumping systems or electrical water pumping systems are used. Electrical water pumping systems which are extensively seen on operation are single and multi-stage centrifugal pumps, surface pumps, and submersible pumps. Motor types used in mentioned water pumping system can be specified as AC induction motors, and permanent magnet brushed DC motors. Solar water pumping systems are required in rural areas due to the inability of consumers to connect to the utility grid. In this study, the setup of 340W off-grid solar based semi-automatic drip irrigation system necessary to accommodate the energy necessity of submersible pump to use in the irrigating almost 3 acres of olive grove located at Ezine, Çanakkale is fulfilled. The solar irrigation system, which was installed in the spring of 2022, basically consists of 2 PV panels (12 Volt, 340 Watt), 12 Volt DC submersible water pump, a paco switch, and a digital timer. Solar irrigation system is planned without battery group since irrigation is programed between 9.00 and 18.00 hours during the day. Thus, installation cost of system is reduced. Also, the solar irrigation system can be repeatedly operating in 7 days in different time periods with a digital timer. Owing to the installed semi-automatic drip irrigation system, the water needs of the olive trees are met by manual or time adjusted. Therefore, the economical irrigation is realized while agricultural productivity is increased. In addition, the specified irrigation system can be applied in farms, various

agricultural lands, parks, greenhouses, horticultural lands and wherever an efficient, eco-friendly, cost-effective, and sustainable irrigation system is needed.

Keywords: Semi-automatic drip irrigation, Off-grid solar system, Sustainable production, Renewable energy

INTRODUCTION

Energy consumption is increasing at a rate of almost 1% per year in the world, this rate is around 5% per year in developing countries. Briefly, the energy demand is heightening day by day. However, fossil fuels cannot meet this energy demand because of various reasons. The main reasons for this are the reduction of fossil fuels and environmental pollution such as global warming and greenhouse gas emissions (Senthil Kumar et al., 2020).

Expanding the use of renewable energy resources (RES) is a sustainable approach aimed at both reducing the burden on fossil-based energy resources and reducing environmental pollution around the world. Due to the scarcity of fossil-based energy resources, all countries around the world have sought new energy resources such as renewable and nuclear (Rana et al., 2020). Nowadays, the current trend has been switching from fossil-based energy resources to RESs. RESs include many types of energy resource such as hydropower, solar, wind, biomass, hydrogen, geothermal and wave (Yıldırım et al., 2018).

Solar energy is the most notable, free, available, and clean energy for the environment, among all RESs and has a remarkable position in the generation of electricity worldwide. Türkiye has an abundant resource in solar energy due to lies in a sunny belt of the earth between and the 26th and 45th east meridians and 36th and 42nd northern parallels in regard of most of other countries (Kotcioğlu, 2011). Sunlight durations in Türkiye seasonally change and daily sunlight duration is nearly 5 hours in winter, 7 hours in spring, and 11 hours in summer months (Yüksel and Türkboyları, 2018). Therefore, Türkiye has a remarkable solar energy potential and this potential is shown in Figure 1. Atlas's colors show total annually average values. Potential of solar energy is directly proportionate to region and remarkable amount of power could be acquired from solar energy in Türkiye, as can be seen from Figure 1 (Kabalci et al., 2016).

Figure 1. Türkiye's solar energy potential atlas (Kabalci et al., 2016).

Systems of solar energy are constantly finding several application areas. Solar based agricultural irrigation systems are one of the most featured applications of solar energy systems.

Nowadays, gasoline-based water pumping systems or electric water pumping systems are mostly used in agricultural irrigation. Electrical water pumping systems which are extensively seen on operation are single and multi-stage centrifugal pumps, surface pumps, and submersible pumps. Motor types used in mentioned water pumping system can be specified as AC induction motors, and permanent magnet brushed DC motors. Solar water pumping systems are required in rural areas due to the inability of consumers to connect to the utility grid. Thus, off-grid solar based agricultural irrigation systems are required in recent years in various fields such as especially rural areas, desert places and certain metropolitan areas.

Freshwater resources are mostly used by agricultural irrigation sector, not only in Türkiye but all over the world. Agricultural irrigation sector uses about 70% of freshwater resources. Agricultural irrigation is applied on 280 million hectares of estate, which corresponds to 19% of agricultural lands of the world. However, the need for freshwater is increasing worldwide. Therefore, the share of freshwater resources used for agricultural production tends to decrease gradually. Considering changes of climate, the increasing population and requirements of food, it is seen that the importance of freshwater for human life increase day by day. Consequently, more effective and more efficient use of freshwater used in agriculture becomes a necessity. The concept of irrigation refers to how water is transferred to the root area of the tree or crop. There are two types of irrigation systems: surface irrigation (wild) and pressure irrigation. The drip irrigation system and sprinkler irrigation system are included in the pressure irrigation system (Arık and Korkut, 2022).

Drip irrigation system is a micro irrigation technology. This system has a network structure consisting of pipes and drippers. With this system, sufficient amounts of water and nutrients are provided to the root zone of trees or crops in a controlled way. It has been demonstrated that preferring drip irrigation method over other inadequate surface irrigation methods can cut down on the water wasted during irrigation by 20% to 76% and enhance the water productivity of the crop by 15%. However, smallholders do not generally prefer the drip irrigation method today. High capital cost and increased labor requirement of drip irrigation method compared to traditional irrigation methods such as flood and furrow irrigation are seen as the main reasons for this situation. But if smallholders adopt drip irrigation systems, they can save water and increase crop yields (Grant et al., 2022).

Olive trees make a great economic contribution to countries such as food industries, olive oil industries, soap industries, wood productions. Olive tree is known as a traditional rain-fed fruit tree and one of the most drought resistant fruit trees. Olive trees are one of the few suitable trees available for farmers in arid agricultural estate all over the world (Molina-Moral et al., 2022). Although olive trees are resistant to water stress, if it is desired to obtain high yields or increase the amount and quality of olive oil every year, it is necessary to give importance to agricultural irrigation (Sousa et al., 2019). In spite of the fact that olive tree is a drought-resistant species, some phenological stages such as the flowering, fruit set, and oil accumulation periods are the most sensitive to water-stress conditions (Molina-Moral et al., 2022).

In this study, the setup of 340W off-grid solar based semi-automatic drip irrigation system necessary to accommodate the energy necessity of submersible pump to use in the irrigating almost 3 acres of olive grove located at Ezine, Çanakkale, Türkiye is fulfilled. The solar based irrigation system, which was installed in the spring of 2022, consists of 2 photovoltaic (PV) panels (12 Volt, 340 Watt), 12 Volt DC submersible water pump, a circuit breaker, a paco switch, and a digital timer. Solar based irrigation system is planned without battery group since irrigation is programed between 9.00 and 18.00 hours during the day. Thus, installation cost of system is reduced. Also, the solar based irrigation system can be repeatedly operating in 7 days in different time periods with the digital timer. Owing to the installed semi-automatic drip irrigation system, the water needs of the olive trees are met by manual or time adjusted.

MATERIAL AND METHOD

Study Area

Çanakkale which is within the borders of the Marmara Region is a province of Türkiye, located in the northwestern part of the country. Çanakkale has lands in both the Asian continent and the European continent. Çanakkale has 12 districts and the provincial borders of Edirne, Tekirdağ and Balıkesir surround this province. The study is carried out in Ezine district of Çanakkale. The location of Ezine in Türkiye is shown in Figure 2.


Figure 2. Ezine's location in Çanakkale, Türkiye

Ezine is in the transition zone of the Marmara and Aegean Regions. Hence, the climatic characteristics seen in these regions are reflected in Ezine. Winters are mild and rainy. Precipitation is generally in the form of rain. According to meteorological observations, the rainiest months are November, December, March, and April. On the other hand, summers are hot and dry. The hottest months are July and August, with the average temperature between 25°C and 35°C and generally there is not much precipitation during these months. For this reason, agricultural irrigation is a significant need especially in summer. In addition, since Ezine is surrounded by hills in terms of settlement, the average humidity is quite high.

Olive tree is a species that exhibits selectivity in point of climate characteristics. Çanakkale and its nearby districts are among the significant regions of olive and olive oil production in Türkiye. Especially Ezine is one of the places where olives are grown intensively in Çanakkale. The mentioned solar based irrigation study is applied on almost 3 acres of olive grove in Ezine shown in Figure 3.

Figure 3. The olive grove in Ezine where the study is carried out

Design and Realization of the Proposed System

In this study, off-grid semi-automatic drip irrigation of almost 3 acres olive grove in Ezine has been realized with system consisting of solar panels. Overall scheme of the system is shown in Figure 4 and photograph of the solar water pumping system in use is demonstrated in Figure 5, respectively.

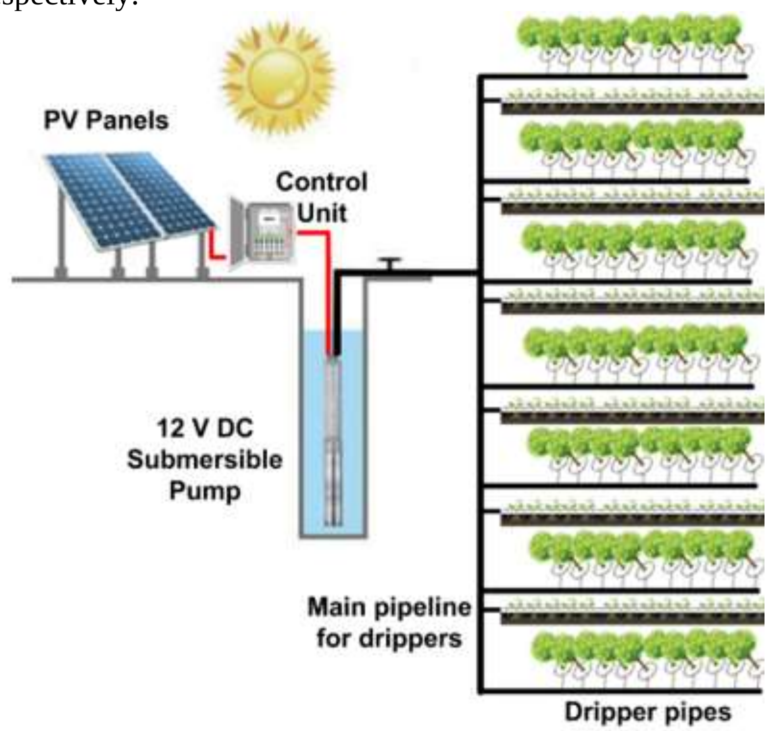

Figure 4. Overall scheme of the proposed solar water pumping system for irrigation

Figure 5. Photograph of the solar water pumping system in use

As seen in Figure 4 and Figure 5, the electrical part of the proposed system consists of 3 main parts. These are PV panels, DC submersible pump and control unit, respectively. Solar irrigation system is planned without battery group since irrigation is programed between 9.00 and 18.00 hours during the day. Thus, installation cost of system is reduced.

In the present system, there are 2 PV panels, each PV panels value is 12 Volt 170 Watt. By connecting these PV panels in parallel, 12 Volt, 340 Watt DC source is obtained. Characteristics of PV panel are expressed in Table 1.

Table 1. Characteristics of PV panel

Name	**Rating**
Panel Maximum Power	205 W
Peak Voltage	21.45V
Peak Current	9.6 A
Open Circuit Voltage	25.92 V
Short Circuit Current	9.9 A

There is an artesian well with a depth of 33 meters in the olive grove where the study is carried out. The water level is about 6 meters below the ground. DC submersible pump is located at 13 meters below the ground and it has been observed that the submersible pump works successfully. Characteristics of DC submersible pump in use are shown in Table 2.

Table 2. Characteristics of DC submersible pump

Name	**Rating**
Voltage	12 V
Power Consumption	130 W
Speed	2850 r/min
Maximum Flow Rate (Qmax)	2 m^3/h
Maximum Head (Hmax)	35 m

The last main unit of the proposed system is the control unit which consists of the circuit breaker, the digital timer and the paco switch. Photograph of the control unit and general electrical connection scheme of proposed system are shown in Figure 6 and Figure 7, respectively.

Figure 6. Control unit of solar water pumping system in use

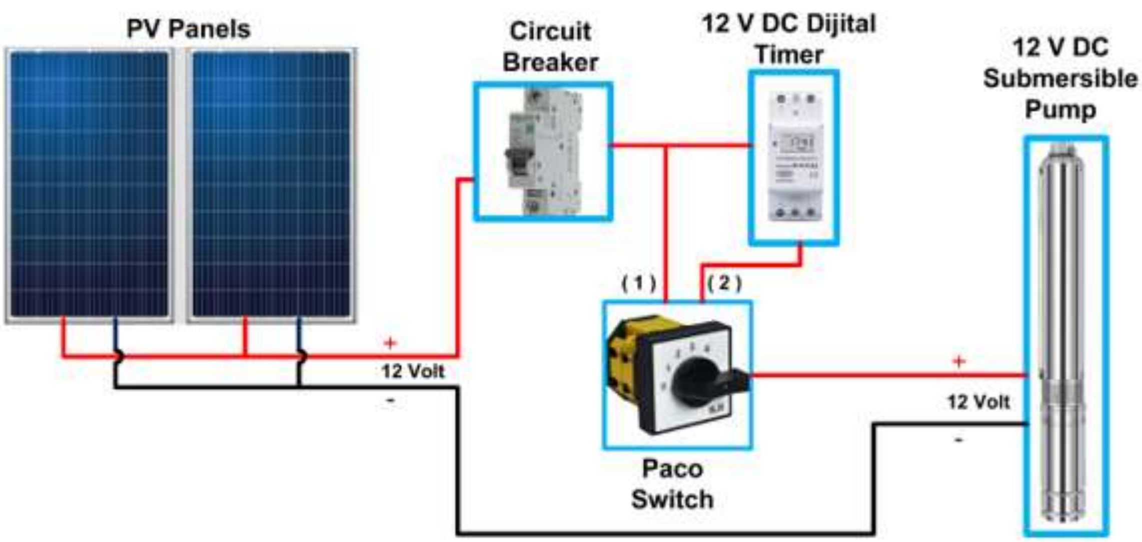

Figure 7. General electrical connection scheme of proposed system

The duties of the elements in the control unit are as follows:

- The circuit breaker in the control unit has two main functions. The first of these is to ensure that the system is completely shut down. In other word, the current from the PV panels is completely cut off. The other duty of the circuit breaker is to protect the whole system by cutting the current flow in case of a possible fault.

- 12 V digital timer in the control unit allows 12 V DC submersible pump to operate between 09:00 and 18:00 every day of the week with a total of 16 different programs. Also, adjustments of the timer are not deleted since the timer has an internal rechargeable battery.
- Paco switch enables the user to stop irrigation, to perform manual irrigation or to perform timed irrigation. If selector of paco switch is in the position 0, the irrigation system does not work. If selector of paco switch is in the position 1, the user can irrigate manually. If selector of paco switch is in the position 2, automatic irrigation can be performed according to the setting on the timer. In addition, the control unit gains a semi-automatic structure as above selections can be made with the paco switch.

As a result, it is determined that 40 olive trees within olive grove are successfully irrigated every day of the week between 09:00 and 18:00 both manually and time adjusted by the proposed system.

CONCLUSIONS

Nowadays, non-renewable energy resources are mostly used to provide energy worldwide. New energy resources are needed because non-renewable energy resources which damage the environment, are decreasing day by day. RESs play a crucial role in retainable development and environmentally friendly energy resources. One of the significant RESs is solar energy. Solar energy can be used in various areas and one of these areas is agricultural irrigation. Türkiye has a significant solar energy potential due to its location. By including solar based irrigation systems in Türkiye's energy policies, energy imports environmental pollutions caused by fossil fuels can be reduced. Also, solar based irrigation systems can be a serious alternative to the AC irrigation system when designed appropriately for the selected field.

In this study, the off-grid solar based semi-automatic drip irrigation system is designed and installed for olive grove irrigation in Ezine district of Çanakkale province. Although the initial investment cost of solar based irrigation systems are high, operating costs are almost non-existent. In order to reduce initial investment cost even more in this installed system, battery group is not included, considering only daytime irrigation. With installed the semi-automatic system, irrigation can be done both manually and time adjusted. After use of mentioned system, it is observed that successful results have been obtained from the solar based drip irrigation system. The product quality and the yield of harvests from the olive grove is increased with regular drip irrigation. Also, it has been concluded that owing to irrigation with the digital timer, human errors and save labor / time can be prevented.

As a result, it is confirmed that the need of energy in the irrigation duration for olive groves can be met from solar energy. The presented study is a suitable solution that can be used to meet other agricultural irrigation needs besides olive groves irrigation. According to the size of the agricultural lands, the need of energy can be met by adjusting the system capacity. Solar based drip irrigation systems are a significant alternative because these systems are more economical and eco-friendly, especially in rural areas where electricity facilities are not sufficient. Therefore, it will be beneficial to introduce these solar based irrigation systems in order to develop both our farmers and our country economically and agriculturally.

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INVESTIGATING THE CHANGES IN THE NUTRIENT COMPOSITION OF BROKEN RICE THROUGH SOLID STATE FERMENTATION USING RUMEN LIQUID

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ABSTRACT

In this study, we investigated the effects of solid-state fermentation using rumen liquid as an inoculant on the nutrient composition of broken rice. The study was conducted on 15 samples, including a non-fermented group and four different fermentation times (1, 3, 5, and 7 days), with three replicates in each group. Broken rice was used as the substrate for fermentation and was ground to 1 mm in the laboratory before being prepared for analysis. Rumen liquid was obtained from 2-year-old cattle fed a roughage-based diet. For inoculation, 1 ml of rumen liquid was used for every 100 g of broken rice. Fermentation was carried out at 38 °C, and the pH was measured in samples that completed the fermentation period. At the end of the study, pH values were found to be low in all fermentation periods except for the first day. Fermentation for three, five, and seven days decreased the pH value significantly compared to the first day ($P<0.001$). Fermentation positively affected the nutrient composition of broken rice. The highest crude protein and ash levels and the lowest crude fiber level were found on the fifth day, and the difference was significant ($P<0.001$). The highest ether extract level was detected on the first day, and the difference was significant ($P<0.001$). In conclusion, fermenting broken rice using rumen liquid in solid culture fermentation improves its nutrient composition. The increased crude protein level and decreased crude fiber resulted in a more nutritious feedstuff. The most effective fermentation period was determined to be five days.

Keywords: Rumen liquid, solid-state fermentation, broken rice, nutrient composition.

INTRODUCTION

Rice (*Oryza sativa* L.) has a large production area worldwide and is primarily used for human consumption (Marco et al., 2013). Broken rice, an agricultural by-product, is obtained during paddy processing (Kotupan & Sommart, 2021). Its nutrient content is similar to rice's (Brestenský et al., 2021). The nutrient content of broken rice varies depending on the type and is reported to be 85-90% carbohydrate, 6-8% crude protein, 1-3% fat, 1-3% crude fiber, and 1-2% ash (Zhao et al., 2020; Nawaz, 2018). Broken rice is used in the diets of poultry (Filgueira et al., 2014; Bala et al., 2017; Naik et al., 2021), ruminants (Kotupan & Sommart, 2021), and livestock such as pigs and horses (Marco et al., 2013; Liu et al., 2016). It is a highly digestible carbohydrate, and low crude fiber content is essential for its nutritional value. Although the crude protein level of broken rice, which has the potential to be used in animal nutrition, is similar to corn, its nutrient composition can be improved by solid-state fermentation to obtain a more nutritious feedstuff.

The solid-state fermentation method involves the metabolic activity of various microorganisms on feedstuffs at a specific moisture content (Mitchell et al., 2000). As a result of the enzymatic activities of microorganisms, feedstuffs become more nutritious (Steudler et al., 2019), improving their nutrient composition (Özlü & Altop, 2023) and increasing their digestibility (Güngör & Erener, 2023). Fermentation studies use bacteria, fungi, yeasts, or combinations as inoculants (Adeyemi et al., 2008; Akinfemi, 2010; Ari & Ayanwale, 2012; Ari et al., 2012). In recent years, rumen liquid has been used as an inoculant in such studies, and it has been reported to affect the nutrient composition of feedstuffs positively (Özlü et al., 2022a; Özlü et al., 2022b; Altop et al., 2022; Koç et al., 2021).

In the present study, rumen liquid was used as an inoculant in solid-state fermentation to investigate its effects on the nutrient composition of broken rice. Additionally, the effects of fermentation on pH were evaluated by measuring the pH of the fermented product.

MATERIAL AND METHOD

Broken rice was used as the substrate for fermentation and was ground to 1 mm in the laboratory before being prepared for analysis. Rumen liquid was obtained from 2-year-old cattle fed a diet of roughage. The rumen liquid was then brought to the laboratory and prepared for analysis in a sterile environment.

The study was carried out in 15 samples, including a non-fermenting group and four different fermentation times (1, 3, 5, and 7 days) and three replicates in each group.

After mixing the substrate and nutrient salt, the fermentation medium was sterilized. The initial pH value was then adjusted using 1 N HCl. For inoculation, 1 ml of rumen liquid was used for every 100 g of broken rice.

Fermentation was carried out at 38 °C, and the pH was measured in samples that completed the fermentation period. The samples were then dried and stored until the analysis period. Dry matter, ash, crude protein, crude fiber, and ether extract analyses were performed according to the method described by Akyıldız (1984).

The data obtained at the end of the research were analyzed using SPSS 21.0 (SPSS Inc., NY, and the USA) statistical package program. Duncan test compared the differences between groups after the ANOVA test for the data variance. Results were considered significantly different at $P<0.05$.

RESULTS AND DISCUSSION

At the end of the study, the pH value was low in all fermentation periods except for the first day. Fermentation for three, five, and seven days decreased the pH value significantly compared to the first day ($P<0.001$). The decrease in pH values in fermentation studies using rumen liquid is consistent with previous research (Özlü et al., 2023; Güngör et al., 2023). Therefore, this decrease is an expected result and indicates that microorganisms are active in the fermentation environment.

Fermentation positively affected the nutrient composition of broken rice. The highest crude protein and ash levels and the lowest crude fiber level were found on the fifth day, and the difference was significant (P<0.001). The highest ether extract level was detected on the first day, and the difference was significant (P<0.001). In fermentation studies using rumen liquid, crude protein levels increase while crude fiber levels decrease, increasing the nutritional value of the feedstuff and a decrease in antinutritional factors (Güngör et al., 2023). The increase in ether extract level is consistent with previous research (Altop et al., 2018). It is thought to be due to lipids produced metabolically by various microorganisms in the rumen liquid. The increase in ash level is similar to that reported by Okpako et al. (2008) and is due to an increase in mineral levels resulting from the use of nutritional salt.

CONCLUSION

In conclusion, fermenting broken rice using rumen liquid in solid-state fermentation improves its nutrient composition. The increased crude protein level and decreased crude fiber resulted in a more nutritious feedstuff. The most effective fermentation period was determined to be five days.

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ANALYSIS OF THE LIVESTOCK FARM'S STRUCTURE AND THE PROFILE OF FARMERS IN ÇANAKKALE

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ABSTRACT

The agricultural sector consists of plant and animal production sectors. Each sector of production has its unique structure and problems. Animal production is an essential sub-sector of agriculture, considering its contribution to human nutrition, supply of raw materials to industry, and employment. First, in identifying the sector's problems, the analysis of the current situation is essential. This study aims to reveal the structure of enterprises and farmers engaged in livestock activities in Çanakkale. For this purpose, a survey study was conducted with 146 farmers in Örtülüce village, which is the village with the highest number of livestock farms in Çanakkale. According to the results, the average age of the farmers is 44 years. 49.3% of the farmers are primary school graduates. Farmers have an average of 24 years of agricultural experience. The average land size of the farms is 63.6 decare. The average number of cattle in the farms is 19 heads. According to this, it can be said that the farms in the village where animal husbandry is most intense in the province of Çanakkale are medium-sized.

Keywords: Farmer, livestock, farm structure, socioeconomic characteristics

INTRODUCTION

Living things need an energy source to continue their existence. From the moment it first appeared on the stage of history, people have lived dependent on an energy source (İnan and etc., 2018). Human civilizations, which developed in pursuit of energy resources in order to meet the need for energy, succeeded in supplying the energy source by producing and relatively under their own control in a way other than using it in time of need by monitoring the agricultural revolution. After the realization of agricultural production, more energy sources than movable amounts and in need of maintenance emerged and people established settlements around agricultural regions (Işık, 2018). This path of energy supply has made it possible to supply energy within certain areas without the need to pursue energy, and thus energy has been more easily obtained and it has become easier to meet basic requirements.

Humanity, which has managed to control energy resources, has started to produce thoughts and ideas thanks to this energy. After these ideas, civilizations were built and humanity jumped into the age and carried out studies in the fields of science and art. Advances in science and art triggered the industrial revolution and forced human civilization to use energy obtained from heat energy (steam technology) rather than energy source, which was based on the strength of energy obtained from agricultural products due to muscle (Günay, 2002). With the increasing production after the industrial revolution, it increased the need for labor force and the world population rapidly, and when the energy needed by the increasing population over time could not be met from coal, the orientation to other fossil resources in nature began.

The use of energy from fossil sources has rapidly increased exponentially and has become the main source of energy that drives human civilization (IEA, 2012). Other greenhouse gases, especially methane gas and carbon dioxide, released after the use of fossil fuels, have also begun to corrode the planet's atmosphere. Our atmosphere, which is affected by these greenhouse gases, has begun to undergo changes and changes in climates have occurred (Abdeshahian et al., 2010; Abdeshahian et al., 2016).

Fossil fuels play both an indirect and direct role in negatively affecting living habitats. Apart from causing the global temperature to rise, it has caused the duration and shape of climates to change. Climate changes have most affected the polar regions of our planet and caused the glaciers to melt. Apart from glaciers, it has increased desertification in continental parts. The increasing rate of desertification has raised the surface temperature. If the use of fossil fuels continues, it is inevitable that these events will reach the point where they cannot be stopped. CO2 gas, which was 280 ppm in our atmosphere in the mid-18th century, was 410 ppm by 2019 (Lindsey, 2020). In order to slow down and stop the pace of this increase, renewable sources should be preferred instead of fossil fuels.

Some of the benefits of using renewable energy sources as a basic energy source are as follows: There are fuel types that can replace each other, import substitution to countries with limited fossil energy resources, much less environmental pollution and production of harmful by-products during energy production than fossil resources, no negative effects on water and air pollution and opening new employment areas. (Ploetz et al., 2020). There is a positive relationship between the use of renewable energy and climate change. It will be healthier for our atmosphere if renewable energy sources are preferred more than fossil energy in energy supply.

The sources of fossil energies are formed as a result of more than one thermodynamic dynamics under the soil layers of organic substances over millions of years. The accumulation of organic wastes continues today. Examples of these wastes are: Garden wastes, plant production residues, animal production waste materials, organic basis wastes of household and workplace origin, food wastes, forest industry wastes, textile and leather industry wastes and treatment plant accumulation wastes. By processing these organic wastes, it is possible to produce energy from them and to benefit from these wastes. The way to benefit from these wastes is through biogas energy. All organisms that survive on Earth continue to produce organic waste. Apart from natural processes, humanity produces tons of organic matter in their lives, especially in farms. In our country, the amount of waste collected by municipalities in landfills in 2018 was calculated as 32.2 tons (Kanat ve Ergüven, 2020).

Tons of waste are generated during the production of plant and animal products. Biogas plants can be used effectively to recycle waste materials of organic origin (Kougias ve Angelidaki, 2018). In biogas plants, certain processes take place using the processing of animal and vegetable wastes consisting of organic substances and the characteristics of the natural structures of these wastes. While the gas and heat generated in the recovered organic wastes are used in electricity production, the coarse substance of these organic wastes can be preferred as organic burnt soil fertilizer.

Obtaining energy with biogas plants provides waste recycling. This energy produced is environmentally friendly and fertilizer and energy production is more affordable than fossil fuels. There is no odor in the burnt soil fertilizer obtained after the animal wastes processed in the biogas plant have passed, and the microorganisms harmful to humans and the environment are largely neutralized. The fact that the energy provided by biogas has versatile outputs has a positive effect on the preference of biogas. In some studies conducted for the coming years, it is predicted that there will be more than 25% of the energy planned to be used on the world in 2050 at a rate exceeding 25 (Hosseini and Wahid, 2016).

Organic fertilizer, which is produced while obtaining energy with biogas technology, can be used in agricultural production by re-participating in the energy cycle of waste and organic substances that have been discarded in an inert form. This example obtained can be shown as a good example of applying the recycling of waste materials effectively in harmony with nature. This study was carried out in Örtülüce village of Biga district of Çanakkale province, where animal production is the most intense. With the survey conducted in Örtülüce village, the opinions of the people of the region about biogas technology and renewable energies were examined as well as socio-economic situation determination.

MATERIAL AND METHOD

Örtülüce village, located in Biga district, is the village with the highest number of livestock farmers in Çanakkale. There are 233 agricultural farmers engaged in animal husbandry in Örtülüce village (Çanakkale Provincial Directorate of Agriculture and Forestry, 2021). The livestock farmers in Örtülüce village, which were chosen purposefully, constituted the population of the research and the sample volume of the said population was determined according to the formula below (Newbold, 1995). While determining the sample volume, the 95% confidence interval 0.05 margin of error was studied and the sample volume was found to be 146.

$$n = \frac{N * \mathrm{p} * \mathrm{q}}{(\mathrm{N}-1) * \sigma^2 \mathrm{p} + \mathrm{p} * \mathrm{q}}$$

$$n = \frac{233(0.5)(0.5)}{(233-1)\cdot(0.02551)^2+(0.5)\cdot(0.5)} = \frac{58{,}25}{0.40} = 145{,}62 \sim 146$$

n = Number of farmers engaged in animal production in the sample

N = Population size

p = population ratio

In the research, numbers and percentages and basic descriptive statistics were used to reveal the socio-economic status, business characteristics, biogas awareness of the farmers.

FINDINGS

Within the scope of the research, 146 farmers were interviewed. 87% of these farmers are men. The youngest of the farmers surveyed is 18 years old and the oldest is 68 years old. The average age of farmers is 44 years. There are 70 farmers under the age of 44. (Table 1).

Table 1. Farmers age

Criteria	Frequency	Percent (%)
Age (Year)		
≤44	70	47.9
>44	76	52.1
Min:18, Max:68, Mean:43,54, Std. Deviation :12,4		

General agricultural experience is minimum 2 years and maximum 50 years. The average experience of 146 farmers was determined as approximately 23.5 years (Table 2).

Table 2. Farmers Experience (Years)

Criteria	Frequency	Percent (%)
General Agricultural Experience (Years)		
≤23	72	49.3
>23	74	50.7
Min:2, Max:50, Mean:23,53, Std. Deviation :12,84		

The highest rate of education of farmers (49.3%) is primary school graduate, while the rate of farmers with high school education and higher is 33.6% (Table 3).

Table 3. Farmers Educational Status

Criteria	Frequency	Percent (%)
Educational Status		
Literate but not graduated	3	2.1
Primary school	72	49.3
Middle School	22	15.1
High school	26	17.8
University	23	15.8

While 84.2% of farmers prefer to use the internet to access the information they need, 15.8% of farmers do not (Table 4).

Table 4. Internet Use in Accessing Knowledge

Criteria	Frequency	Percent (%)
Internet Use in Accessing Knowledge		
Internet user	123	84.2
Not using internet	23	15.8

55.5% of the surveyed farmers have a profession other than farming. While 81.5% of farmers have non-agricultural income (Table 5).

Table 5. Farmers non-farm income status

Criteria	Frequency	Percent (%)
Non-Farm Income Status		
Yes	119	81.5
No	27	18.5

The vast majority of farmers (91.1%) are partners in an agricultural association or cooperative (Table 6).

Table 6. Tarımsal birlik veya koop ortaklığı

Criteria	Frequency	Percent (%)
Tarımsal birlik veya koop ortaklığı		
Yes	133	91.1
No	13	8.9

78.8% of the farmers interviewed attended a meeting on agriculture in the last three years (Table 7).

Table 7. Attendance at an Agricultural Meeting in the Last 3 Years

Criteria	Frequency	Percent (%)
Attendance at an Agricultural Meeting in the Last 3 Years		
Attending the meeting	115	78.8
Not attending the meeting	31	21.2

The annual agricultural income of 83.6% of the farmers is below 50,000 TL (Table 8).

Table 8. Annual Agricultural Income (TRY)

Criteria	Frequency	Percent (%)
Annual Agricultural Income (TRY)		
0 to 10000	30	20.5
10001 to 20000	23	15.8
20001 to 30000	26	17.8

30001 to 40000	24	16.4
40001 to 50000	19	13.0
50001 to 100000	12	8.2
100001 to 150000	10	6.8
150001 to 200000	2	1.4

It was determined that 86.3% of the farmers interviewed within the scope of the study were engaged in cattle farming and 13.7% in sheep and goats. (Table 9).

Table 9. Types of operation

Farming type	Frequency	Percent (%)
Cattle Farming	116	79.45
Sheep and Goats	20	13.7
Cattle + Sheep and Goats	10	6.85
Total	146	100.0

The average number of animals in cattle breeding farmers was determined as 19 heads. The number of cattle breeder of 59.5% of the holdings was below the average and the number of animals of 40.5% was above the average (Table 10).

Table 10. Cattle assets

Cattle asset (Number)	Frequency	Percent (%)
≤19	75	59.5
>19	51	40.5
Min:3, Max:77, Mean:18,95, Std. Deviation:15,72, Total:126		

According to data 30 of the farmers engaged in animal production are engaged in sheep and goats. While 23.3% of the farmers raise more than 57 heads sheep and goats, 76.7% of the farmers raise 57 heads and less sheep and goats, (Table 11).

Table 11. Ovine animal existence

Sheep and Goats asset *(Number)*	Frequency	Percent (%)
≤57	23	76.7
>57	7	23.3
Min:1, Max:250, Mean:57,47, Std. Deviation: 64,22, Total :30		

According to the data, 97.26% of farmers own their own land, and these lands are on average 63.6 decares in size and divided into 9.7 pieces. 15.75% of the interviewed farmers use rented land. The rented lands are on average 60.7 decares and approximately 8 pieces (Table 12).

Table 12. Operating land information

Operating land information	Land tillage rate (%)	Average land size (decares)	Average number of pieces of land (pcs)
Own land	97,26	63,6	9,67
Land treated with rent	15,75	60,7	7,87

CONCLUSIONS

The following conclusions and recommendations were obtained from this study, which examined the socio-economic characteristics of farmers engaged in animal production in Çanakkale. It has been observed that the farmers engaged in animal production in Çanakkale province are at the middle age level, generally primary school graduates, 24 years of general agricultural experience, with an average of 63.6 decares of land, cattle breeders have an average of 19 cattle, and small cattle breeders have a large number of animals of 65 or less. As a result of the work carried out, it should be aimed to increase the education levels of farmers and to increase the number of young farmers. Farmers in the region should be supported with individuals with higher education levels and new conscious and educated farmers should be brought to the region.

Note: This study was produced from the master's thesis.

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YIELD AND YIELD COMPONENTS OF CONFECTION SUNFLOWERS (*Helianthus Annuus* L.) GENOTYPES AND RESPONSE OF DIFFERENT NITROGEN DOSE FERTILIZATION

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ABSTRACT

Sunflower is recognized as one of the most important oil crops worldwide. Sunflower, which is largely used to meet the need for edible oil, is also produced as a snack sunflower.

In this study, sunflower cultivars with different grain colours were compared in terms of yield and yield components under four different nitrogen doses (0, 6, 12, 18 kg/da). While this study aims to lead the knowledge to eliminate the lack of quality standards in our country, it also aims to reveal the effect of nitrogen dose on the yield and yield elements of black and white confectionery sunflowers.

Among the genotypes used in the study, Ahmetbey confectionery cultivar stood out in terms of yield items. The marginal benefit increased as the dose increased compared to the N doses we used. For further studies, it would be appropriate to try a higher dose of fertilization to find the peak point of the N dose. This study and the studies to be carried out will make significant contributions to yield improvement and sunflower agriculture development.

Keywords: Confection sunflower, N doses, Yield traits.

INTRODUCTION

Sunflower is recognized as one of the world's most important oil crops. Today, the sunflower, which is used to a large extent to meet the demand for edible oil, is also produced as a snack sunflower. The USA is now the largest producer of sunflower kernels. Other major producers include Hungary, Argentina, Spain, China, Israel, Turkey and some Eastern European countries such as Moldova. The main world importers of sunflower are Germany, Denmark, the Netherlands, Canada, Mexico, the United Kingdom and Belgium, while the main importers of sunflower nuts are Spain, China, Turkey, Jordan, Canada, Israel, Germany, Mexico and Japan (Fao, 2020).

Sunflower production in Turkey is generally concentrated in Thrace and Marmara regions. On the other hand, sunflower production is mostly carried out in Central Anatolia, Çukurova, and Aegean regions and in small amounts in other regions. However, although the adaptation area of sunflower, which can grow in almost every region of our country under wet and dry conditions, is quite wide, our cultivation areas have remained at the level of 700 - 800 thousand hectares (Tuik 2019).

An ideal sunflower should have characteristics such as grain size of at least 8-9 mm, length of 2.5 cm, internal ratio of 50%, thousand-grain weight of 80 g, oil ratio of less than 30%, high protein and vitamin E (tocopherol) (Pekcan, 2014). Therefore, it is very important to select the right variety for sunflower cultivation. Researchers such as Fick and Miller (1997), Lofgren (1997) and Jovanovic et al. (1998) emphasized the importance of genotype selection. Sunflower production in Turkey has been increasing in recent years. According to 2021 data, Turkey's sunflower production is around 1.5 million tonnes, of which approximately 10% is allocated to sunflower production (Tüik, 2021). Sunflower cultivation in our country has an important place in terms of both agricultural production and economy. In recent years, with the increase in black-shelled sunflower consumption, agricultural production has increased to meet the need. While peasant populations are generally used in white-skinned sunflower cultivation, the use of Chinese hybrid seeds is common in black-skinned sunflower cultivation. The use of peasant populations in sunflower agriculture and the lack of knowledge of our farmers about cultivation techniques lead to the inability to obtain the desired yield in production and also to the lack of a certain quality standard of the products. For this reason, the studies we will carry out in this field are of great importance to deliver quality products to the consumer and contribute to the national economy.

Fertilization is of great importance in terms of yield and product quality in agricultural production. However, excessive, or inadequate fertilization can both reduce yield and have negative effects on product quality. For example, El-sarag (2007) applied different nitrogen doses up to 14 kg/da, Hamadtov (2009) up to 8.6 kg/da and Day and Kolsarıcı (2014) up to 12 kg/da and observed that yield increased in sunflower. However, Nasim et al. (2012) reported a decrease in yield after 18 kg/da nitrogen application. Therefore, it is important to determine appropriate fertilization strategies to obtain both yield and quality products.

In this study, black and white sunflower varieties with different grain colors were compared in terms of yield and yield components under four different nitrogen doses (0, 6, 12, 18 kg/da). This study aims to lead the accumulation of knowledge to overcome the lack of quality standards in our country, while at the same time, it aims to reveal the effect of nitrogen dose on the yield and yield components of black and white sunflower.

MATERIAL AND METHOD

Characteristics of Sunflower Varieties Used

Table 3. Properties of sunflowers used in the study.

Features	Ahmetbey	Somon Beyazı
Type	**confection sunflowers**	**confection sunflowers**
Yield	**Irrigated 360-380 kg/da**	**Irrigated 198,9-304,5 kg/da**
Recommended Planting Areas	**Marmara, Aegean, Central Anatolia and Passage (irrigated)**	**Marmara, Aegean, Central Anatolia and Passage (irrigated)**
Variety Specificity	**Medium-sized, medium-early, black, shiny and long-grained**	**Medium-sized, medium-early, black, shiny and long-grained**
Oil Ratio	**%28**	**%28**
Number of Flowering Days	**63 days**	**73 days**

Location of the Test Area

This research was conducted in 2020 in the Çivril plain, which is a tectonic plain within the borders of İsabey neighbourhood of Çal district, located in the northeastern lands of Denizli province in the Aegean Region. The size of the plain, which is 820 m above sea level and located between 38° 5' north latitude and 29° 36' east longitude, is 63.823 hectares.

When the climate data of Denizli Province between 1957-2019 are examined, the average temperature between April and September varies between 14.6°C and 27.2°C, while the highest temperature was measured between 31.3°C and 34.5°C in June and July. The highest sunshine duration was 10.9 hours in June and the lowest was 9.2 hours in September. The average monthly total precipitation was 42.7 mm in May and 15.2 mm in July. The highest temperature values ranged between 35.8°C and 44.4°C, while the lowest temperature values were between -2°C and 12.6°C (Table 1).

Table 1. Long-term Climate Data of Denizli Province (Measurement Period 1957 – 2019)

Denizli	April	May	June	July	August	September
Mean Temperature (°C)	14,6	19,5	24,2	27,2	26,8	22,5
Average Highest Temperature (°C)	20,8	26,3	31,3	34,5	34,4	30
Average Lowest Temperature (°C)	9,1	13,2	17,4	20,1	19,8	15,8
Average Solarisation Time (h)	6,9	9	10,9	11,8	10,9	9,2
Average Number of Rainy Days	10,1	8,9	5,1	2,1	2	3
Monthly Total Avg. Precipitation (mm)	52,2	42,7	27,2	15,2	10,8	16
Highest Temperature (°C)	35,8	37,8	44,1	43,9	44,4	41,6
Lowest Temperature (°C)	-2	2,7	7,9	12,6	11,6	6,6

Soil Characteristics

According to the results of soil analyses of the test area, the soil was determined as silty clay loam with a clay content of 38.67%, sand content of 16.83% and silt content of 44.50%. The pH value of the soil was alkaline with 8.23 and the salt content was low with 0.020%. The lime ratio was measured as 46.44% and it was determined that it was excessively calcareous. Organic matter content is at low level at 1.93%. The available phosphorus (P) ppm value was low at 5.16 and exchangeable potassium (K) ppm value was very low with 5.6. However, the exchangeable calcium (Ca) ppm value is at a high level with 4685 (Table 2).

Table 2. Soil Analysis Results of the Experimental Field

Soil Texture	Conclusion	Evaluation
Composition	Silty Clayey Loam	-
Clay (%)	38.67	-
Sand (%)	16.83	--
Silt (%)	44.50	-
Ph	8.23	Alkali
Salt (%)	0.020	Unsalted
Lime (%)	46.44	Extremely Calcareous
Organic Matter (%)	1.93	Low
Available Phosphorus (P) ppm	5.16	Low
Exchangeable Potassium (K) ppm	5.6	Very Low
Exchangeable calcium (Ca) ppm	4685	High

Irrigation Method

The water requirement of the land where the experiment was carried out was met by irrigation canals. The furrow irrigation method was used in the experiment field.

Fertilizer Application in the Experiment

The fertilizers used in the experiment were purchased from Agricultural Credit Cooperative. The application of different nitrogen fertilizer doses, which is one of the research factors, was carried out with the following methods. Fertilization was carried out in two different periods. Half of the nitrogen fertilizer was applied just before sowing, while the other half was applied before intermediate hoeing (Çalışkan and Kevseroğlu, 1997; Süzer and Kahraman, 1999).

Ammonium sulphate (AS 21%, N) fertilizer was applied as base fertilizer during the preparation of the soil for sowing, calculated from ammonium sulphate (AS 21%, N) fertilizer as 6 kg/da nitrogen (N) fertilizer to 6, 12 and 18 kg/da nitrogen fertilization plots. In the control plots, no nitrogen fertilizer was applied. In addition, Triple Superphosphate (TSP 43-44%, P_2O_5) fertilizer was calculated and applied as 6 kg/ha of pure Phosphate (P) fertilizer per decare during seedbed preparation before sowing in the experimental field. Potassium sulphate (50%, K_2SO_4) was applied at a rate of 6 kg/ha of pure potassium (K) fertilizer during seedbed preparation before sowing. Urea (46% N) was used as nitrogen fertilizer source in the top dressing. Plots with 0 kg/da N application and 6 kg/da N application were not fertilized. Pure 6 kg/da N was applied to plots with 12 kg/da N application and pure 12 kg/da N was applied to plots with 18 kg/da N application.

Experimental Design

The experiment was carried out by using 2 sunflower cultivars (Somon Beyazı and Ahmetbey), 4 different N doses (0, 6, 12, 18 kg/da) with 3 replications in a randomized block split plots experimental design. Sowing was done with a 4-row pneumatic seeder with an inter-row distance of 70 cm, an over-row distance of 20 cm and a row length of 8 m. The row spacing was hoeed with an inter-row hoeing machine 25 days after sowing and manual thinning and hoeing were carried out 40 days after sowing. Harvesting was done when the moisture content was below 10%.

Grain yield (kg/ha), thousand-grain weight (g), head diameter (cm), husk rate (%), hectoliter weight (g), grain length (mm) and grain width (mm) were analyzed. At the end of harvest, sunflower grain yields obtained from the plots were corrected according to 10% moisture value and converted to yield per decare. The results of the research were analysed by using the Jump package program.

RESULTS AND DISCUSSION

The effects of applied N doses on genotypes are given in Table 4 and mean values for nitrogen doses and genotypes in Table 5.

Table 4. Analysis of variance results of applied nitrogen doses and genotypes

Sources	Df	Mean Square						
		HD	**GY**	**SW**	**SL**	**Hec.**	**TGW**	**HR**
Blok	2	0.101	21.187	0.031	0.241	0.79	1.89	0.012
Nitrogen (N)	3	99.68**	5437.5**	4.539**	7.816**	1.69öd	2611.2**	4.72**
Error 1	6	0.101	34.067	0.083	0.154	0.75	5.41	0.033
Genotype	1	0.96**	25636.8**	7.370**	6.615***	101.68*	560.66**	365.04**
Nitrogen × Genotype	3	1.096**	393.872**	0.130öd	0.294öd	3.87öd	191.72**	1.124**
Error 2	8	0.047	31.532	0.165	0.01	1.13	14.42	0.071

*HD: Head diameter; GY: Grain yield; SW: Seed width; SL: Seed length; Hec: Hectoliter; TGW: Thousand grain weight; HR: Husk rate.

Table 5. Mean values for nitrogen doses and genotypes

N Doses (kg/da)	HD (cm)			GR (kg/da)			SW(mm)		
	Somon Beyazı	**Ahmetbey**	**Mean**	**Somon Beyazı**	**Ahmetbey**	**Mean**	**Somon Beyazı**	**Ahmetbey**	**Mean**
0	12.70 d B	13.73 d A	**13.22 d**	194.73 a B	237.73 d A	**216.2 d**	5.80	7.03	**6.42 c**
6	16.43 c B	17.63 c A	**17.03 c**	204.70 c B	268.83 c A	**236.7 c**	5.93	7.20	**6.56 c**
12	19.46 b A	18.90 b B	**19.18 b**	229.86 b B	305.10 b A	**267.5 b**	7.30	7.96	**7.63 b**
18	23.00 a A	22.93 a A	**22.96 a**	243.63 a B	322.73 a A	**283.18 a**	7.60	8.86	**8.23 a**
Mean	**17,90 b**	**18.30 a**		**218.23 b**	**283.60 a**		**6.66 b**	**6.66 a**	
LSD $_{G(0.05)}$	0.20			5.4			0.38		
LSD$_{ND(0.05}$	0.45			8.24			0.41		
LSD $_{GXND.(0.05)}$	0.41			10.56			ns		

Table 5. Mean values for nitrogen doses and genotypes (continued)

N Doses (kg/da)	SL (Mm)			Hec.			TGW		
	Somon Beyazı	**Ahmetbey**	**Mean**	**Somon Beyazı**	**Ahmetbey**	**Mean**	**Somon Beyazı**	**Ahmetbey**	**Mean**
0	19.80	18.63	**19.22 d**	30.16	24.00	**27.1**	104.3 d A	93.46 d B	**98.9 d**
6	20.43	19.96	**20.20 c**	28.15	24.03	**26.2**	122.66 c A	104.83 c B	**114 c**
12	21.50	20.46	**20.98 b**	28.03	24.60	**26.3**	138.03 a A	121.40 b B	**130 b**
18	22.66	21.13	**21.90 a**	27.00	24.60	**25.8**	144.06 a A	150.73 a A	**147 a**

Mean 21.10 a	**20.05 b**	**28.43 a**	**24.31 b**	**127.28 a**	**127.28 b**
LSD $_{G(0.05)}$ 0.35		0.99		0.35	
$LSD_{ND(0.05}$ 0.33		ns		0.33	
LSD $_{GXND.(0.05)}$ ns		ns		0.71	

Table 5. Mean values for nitrogen doses and genotypes (continued)

N Doses (kg/da)	SR(%)		
	Somon Beyazı	**Ahmetbey**	**Mean**
0	**56.53 b**	**56.53 c**	**52.03 c**
6	**55.66 c**	**55.66 b**	**52.10 c**
12	**56.06 bc**	**56.06 b**	**52.46 b**
18	**57.87 a**	**57.87 a**	**53.93 a**
Mean	**56.53 a**	**56.53 b**	
LSD $_{G(0.05)}$	0.25		
$LSD_{AD(0.05}$	0.28		
LSD $_{GXAD.(0.05)}$	0.50		

*HD: Head diameter; GY: Grain yield; SW: Seed width; SL: Seed length; Hec: Hectoliter; TGW: Thousand grain weight; HR: Husk rate.

++ Lowercase letters indicate the significance levels of nitrogen doses according to genotypes.

+++ Capital letters indicate the significance levels between genotypes at each nitrogen dose.

The difference between the mean values of the mean head diameter of was found to be statistically significant at the level of 0.01% for genotypes, N doses and cultivar x N dose interactions. In terms of nitrogen doses, the highest average head diameter was found in the 18 kg/da N dose application with 22.96 cm and the highest head diameter was found in the Somon Beyazı genotype with 23 cm. The lowest average of the diameter was 13.22 cm in the control treatment of N dose. There was a statistically significant difference between the mean of the mean diameter of the tray obtained in 0, 6, 12 and 18 kg/da N dose applications and it was determined that the diameter of the tray increased as the N dose increased. When the mean head diameter of the genotypes were compared, Ahmetbey had a higher value.

In many studies on nitrogen fertilization in sunflower, like our findings, it was reported that there was a steady increase in the diameter of the canopy in parallel with increasing nitrogen doses (Zubriski and Zimmerman, 1974; Bindra and Kharwara, 1992; Salehi and Bahrani, 2000). Similarly, Aydın (1996), Akhtar et al. (1997), Khot and Patil (2002), Ali et al. (2004), Olowe et al. (2005), Jahangir et al. (2006), Hamadtov (2009), El-Kady et al. (2010), Osman and Awed (2010), Wajid et al. (2012), Seghatoleslami et al. (2012), Albayrak (2014), Yıldız (2014), Pekcan and Esendal (2015), Rasool et al. (2015), Kandil et al. (2017), Öztürk et al. (2017), and Aydoğdu (2019) also concluded that increasing nitrogen doses increased the diameter of the pan compared to control samples.

The difference between the mean values of grain yield was found to be statistically significant at 0.01% for genotypes and nitrogen doses, and at one percent significance level for variety *x* nitrogen dose interaction.

The highest grain yield mean value was obtained from 18 kg/da N dose application of Ahmetbey variety with 322.7 kg/da and the lowest grain yield was obtained from Somon Beyazı variety with 194.7 kg/da. In terms of different nitrogen dose applications, the highest grain yield average value was obtained from 18 kg/da N application with 283.1 kg /da and the lowest grain yield average value was obtained from the control application with 116.2 kg /da.

In the studies, it was found that different nitrogen dose applications increased grain yield compared to the control. This result was supported by Homenauth et al. (1986), Tenebe et al. (1996), Akhtar et al. (1997), Bharambe et al. (1997), Ayub et al. (1998), Mahender et al. (2000), Rameshwar and Giri (2000), Gürsoy (2001), Legha and Giri (2001), Taha et al. (2001), Khot and Patil (2002), Zubillaga et al. (2002), Mojiri and Arzani (2003), Ruffo et al. (2003), Syed et al. (2003), Ali et al. (2004), Kıllı (2004), Özer et al. (2004), Montemurro and De Giorgio (2005), Jahangir et al. (2006), El-Sarag (2007), Gholinezhad et al. (2009), Hamadtov (2009), Abdel-Motagally and Osman (2010), El-Kady et al. (2010), Osman and Awed (2010), Oyinlola et al. (2010), Ardali and Bahrani (2011), Day (2011), Tursun (2011), Ali and Ullah (2012), Wajid et al. (2012), Seghatoleslami et al. (2012), Gül (2013), Salih (2013), Sıncık et al. (2013), Ali et al. (2014), Toosi and Azizi (2014), Yıldız (2014), Ashraf (2015), Pekcan and Esendal (2015), Rasool et al. (2015), Kandil et al. (2017), Al-Haidary (2018), Metwaly et al. (2018) and Aydoğdu (2019).

In contrast to the studies mentioned above, some studies such as Herdem (1999), Mojiri and Arzani (2003), and Nasim et al. (2012) reported that yield decreased with increasing nitrogen dose. For example, in Herdem (1999) study, the highest yield was obtained when nitrogen dose was applied at 12 kg/ha, while decreases in yield were observed at higher nitrogen doses. Similarly, in Mojiri and Arzani (2003) study, the highest yield was obtained when nitrogen dose was applied at 15 kg/da and decreases in yield occurred at higher nitrogen doses. In the study of Nasim et al. (2012), it was stated that the nitrogen dose applied at 18 kg/da did not provide higher yield. Therefore, the effect of nitrogen dose on yield may vary between studies and should be evaluated carefully.

In addition to these findings, some studies reported different results. For example, Bahrani et al. (2011) reported that high nitrogen doses did not cause an increase in sunflower yield. Similarly, Zhang et al. (2012) reported in a meta-analysis that nitrogen fertilizer application did not increase sunflower yield. These different results may be due to the differences in the applied methods, soil properties, plant varieties and environmental factors.

The difference between the mean values of grain width was found to be statistically significant for genotypes and nitrogen doses at 0.01% level of significance, while no statistical difference was found for the cultivar x nitrogen dose interaction. When the difference between nitrogen doses in terms of grain width was evaluated, it was found that the average grain width varied between 6.42 and 8.23 mm. The highest grain width was statistically determined at 18 kg/da nitrogen dose in both genotypes. When the genotype averages were compared, it was determined that Ahmetbey statistically exceeded Somon Beyazı with 7.76 mm. Pekcan and Esendal (2015) and Coşkun (2017) analyzed the effect of different nitrogen doses on grain width and reached similar results. According to the results of these studies, an increase in grain width was observed as the nitrogen dose increased, these results are in parallel with our study.

The difference between the mean values of grain length was found to be statistically significant for genotypes and nitrogen doses at 0.01% significance level, while no statistical difference was found for the cultivar x nitrogen dose interaction. When the genotypes were evaluated in terms of grain length, it was found that Somon Beyazı (21.10) variety was 5% shorter than Ahmetbey (20.05) variety. When the grain lengths were compared in terms of nitrogen dose, the shortest grain length was obtained from Ahmetbey variety with 18.63 mm at 0 kg/da nitrogen application, while the longest grain length was obtained from Somon Beyazı variety with 22.66 mm at 18 kg/da nitrogen application. It was determined that the average grain length decreased statistically as the nitrogen dose decreased. Similar results were also reported by Pekcan and Esendal (2015) and Coşkun (2017), who found that increasing nitrogen dose applications increased grain length compared to the control.

According to the results obtained, no statistically significant difference was observed between nitrogen doses and nitrogen x genotype interactions in the analyses made in terms of hectoliter. However, in the comparison between genotypes, a statistically significant difference was observed at 5% level. It was observed that Somon beyazı variety had higher hectoliter weight than Ahmetbey variety. Studies conducted by Coşkun (2017), Albayrak (2014) and Aydoğdu (2019) show that, contrary to our study, nitrogen dose applications cause an increase in hectoliter weight.

In terms of thousand-grain weight, significant differences were found between nitrogen × genotype interactions, nitrogen doses and genotypes at 0.1% level. In terms of nitrogen doses, the highest thousand-grain weight was determined from 18 kg/da N dose application with 98.9 g, while the highest thousand-grain weight average was reached in Somon Beyazı genotype with 127.2 g. The lowest thousand-grain weight average was determined in the control application of N dose with 93.46 g. There was a statistically significant difference between the thousand-grain weight averages obtained in 0, 6, 12 and 18 kg/da N dose applications and it was determined that the diameter of the table increased as the N dose increased. Many studies have reported an increase in thousand-grain weight in sunflower with increasing nitrogen dose. For example, Allam and Galal (1996), Hamadtov (2009), Gholinezhad et al. (2009), Seghatoleslami et al. (2012) and Day and Kolsarıcı (2014) reported an increase in thousand-grain weight with increasing N doses. Similarly, Nor-Mohammadi and Ehdaie (1980), Gürsoy (2001), Ali et al. (2004), Kıllı (2004), Özer et al. (2004), Hamadtov (2009), El-Kady et al. (2010), Eba and Awed

(2010), Wajid et al. (2012), Seghatoleslami et al. (2012), Yıldız (2014), Pekcan and Esendal (2015), Rasool et al. (2015), Kandil et al. (2017), Metwaly et al. (2018) and Aydoğdu (2019) also showed that increasing nitrogen doses caused an increase in thousand-grain weight compared to control groups. As a result, the above-mentioned sources agreed that thousand-grain weight increased with increasing nitrogen dose in sunflower plants.

There was a significant difference at 0.01% level between nitrogen × genotypes, nitrogen doses and genotypes in terms of husk ratio. In the study, the mean value of the husk ratio of the varieties was found to vary between 57.87% and 47.53%. The highest husk ratio was found in Somon Beyazı genotype at 18 kg/da application. In parallel with our study, as reported by Pekcan and Esendal (2015) and Coşkun (2017), it was determined that the husk ratio increased as a result of different nitrogen dose applications.

In terms of nitrogen doses, the highest thousand-grain weight was determined from 18 kg/da N dose application with 98.9 g, while the highest thousand-grain weight average was reached in Somon Beyazı genotype with 127.2 g. The lowest thousand-grain weight average was determined in the control application of N dose with 93.46 g. There was a statistically significant difference between the thousand-grain weight averages obtained in 0, 6, 12 and 18 kg/da N dose applications and it was determined that the diameter of the table increased as the N dose increased.

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There was a significant difference at 0.01% level between nitrogen × genotypes, nitrogen doses and genotypes in terms of husk ratio. In the study, the mean value of the bark ratio of the varieties was found to vary between 57.87% and 47.53%. The highest bark ratio was found in Somon Beyazı genotype at 18 kg/da application. In parallel with our study, as reported by Pekcan and Esendal (2015) and Coşkun (2017), it was determined that the bark ratio increased because of different nitrogen dose applications.

CONCLUSION

As a result, 18 kg/da N dose contributed positively to yield and yield components in both genotypes for all traits examined. The genotypes used in this study were affected differently by N doses. Among the genotypes used in this study, the Ahmetbey variety stood out in terms of yield components. According to the N doses we used, the marginal benefit increased as the dose increased. For further studies, it would be appropriate to try higher doses of fertilization to find the peak point of the N dose. This study and the studies to be carried out in the future will provide important contributions to the improvement of yield and development of sunflower agriculture.

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THE EFFECTS OF VARIOUS MEDIA STRENGTH AND SUCROSE CONCENTRATIONS IN ADVENTITIOUS ROOT CULTURE OF OKRA 'SULTANI' CV. (*ABELMOSCHUS ESCULENTUS* L.)

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Okra (*Abelmoschus esculentus* L.), one of the important vegetables of the *Malvaceae* family, has attracted attention due to its healing properties in terms of health. *In vitro* adventitious root culture provides a preferred alternative method to produce valuable bioactive compounds. The adventitious roots induced by *in vitro* methods not only support the propagation of medicinally high-value plants but also offer a possible method to harvest valuable bioactive compounds from plants. In the current study, hypocotyls obtained after germination of okra 'Sultani' cv. seeds were used as starting explant material, the effect of MS basic medium at different strengths (¼, ½, ¾, and 1) and the effect of different amounts of sucrose (20, 25, 40 and 50 mg L^{-1}) on adventitious root formation were evaluated in terms of biomass formation. When the results obtained from the study are evaluated, it is thought that okra adventitious root cultures can be used effectively on large scales for different industries such as pharmaceuticals.

Keywords: *in vitro*, root cultures, medium strength, biomass

INTRODUCTION

Plants are high-value natural resources that are used as preventive medicine by humans on a global scale. Highly important bioactive compounds, called plant secondary metabolites, are intensively collected from plants to improve human health and standard of living.

Human activities affect the protection of nature and cause much damage. The development of modern cities, serious air pollution caused by vehicles, a decrease in fertile land due to mass housing construction, and significant consumption of natural resources have increased the difficulties in plant cultivation. In other words, the medicinal activity and effectiveness of bioactive compounds obtained from plants may be weakened due to environmental irregularities and physiological damages, especially factors that prevent their stable production (Beppu et al., 2004).

The increasing global demand for bioactive compounds obtained from medicinal plants has led to the overconsumption of many valuable species, and the natural production of plants has become unable to meet the increasing market needs. Additionally, due to the complex structure and configuration of plant secondary metabolites, artificial chemical synthesis has generally been found to be non-cost-effective. Therefore, accurately obtaining sufficient medicinal herbal ingredients has become a high-priority endeavor for the advancement of the global pharmaceutical industry (Gaosheng and Jingming, 2012).

Secondary metabolite production largely depends on species and variety differences, the growing period, the developmental process, and the physiological state of the plant, and the yield is usually very low (less than 1% of dry weight) (Ramachandra Rao and Ravishankar, 2002). The development of alternative and highly creative strategies for plant breeding is a crucial social and economic challenge, especially for the high-yield production of biologically essential bioactive compounds. Therefore, biotechnological approaches, especially plant tissue culture methods, attract attention as an alternative solution to overcome such difficulties. Biotechnological approaches in plant cell, tissue, and organ culture have been and continue to be intensively investigated in recent years as a promising technology for the cultivation and production of pharmacologically useful plant bioactive compounds (Ramachandra Rao and Ravishankar, 2002; Rahmat and Kang, 2019).

One of the widely used biotechnological approaches in this regard is the use of adventitious root cultures as a propagation strategy. Adventitious roots produced under aseptic conditions in a nutrient medium enriched with appropriate phytohormones provide high growth rates and production of important secondary metabolites. In particular, their rapid growth ability and special ability to produce many secondary plant metabolites have led to the widespread use of organ culture techniques such as adventitious root culture for plants (Murthy et al., 2008). Adventitious root cultures show a higher stability in the production of highly active compounds with faster growth than cell cultures (Sivakumar, 2006). Moreover, the cultivation of plant bioactive compounds with a bioreactor system using adventitious root cultures has emerged as a technology with potential in commercial applications (Paek et al., 2009; Rahmat and Kang, 2019).

Plant roots are an important source of high-value secondary metabolites that may be beneficial to human health (Bais et al., 2001). Adventitious roots are plant roots that may originate from any organ other than the root itself, usually leaves, stem parts, and nodes, and form during normal development or in response to environmental stresses such as injury or mineral deficiency. Adventitious roots serve many important roles for the plant and help the plant survive even in adverse environmental conditions.

Adventitious root formation is a complex molecular process involving numerous endogenous and exogenous physiological factors (Sorin et al., 2005). The adventitious root formation process is divided into four stages: (a) the pre-emergence stage of the root, which includes the molecular and biochemical process changes that occur prior to any cytological formation up to the emergence of primordial roots, (b) the early stage of root development, (c) the root growth stage (weight and volume increase) and (d) the final stage of root configuration (emergence of the first root) (Zhang et al., 2017). The initiation and differentiation process during the physiological stages of rooting can be triggered by changes in endogenous auxin concentrations and external addition of specific auxins (Praveen et al., 2009).

Adventitious roots show high stability, high growth rate, and continuous secondary metabolite production when triggered in an artificial nutrient medium under aseptic conditions with optimum phytohormone addition (Hahn et al., 2003). While these roots show high stability and growth rate, they provide the production of high amounts of alkaloids, terpenoids, and phenols in the cell and tissue spaces, and these compounds can be easily produced with the help of adventitious roots in a nutrient medium supplemented with a low amount of inoculum and an appropriate plant growth regulator (Sivakumar et al., 2006).

Okra (*Abelmoschus esculentus* L.), one of the important vegetables of the *Malvaceae* family, attracts attention due to its medicinal properties. Okra, which is very rich in flavonoids, terpenes, vitamins and steroid derivatives, is not only effective in preventing cardiovascular diseases, but also has physiological activities such as antihyperlipidemia, antioxidant, antidiabetic, anticancer, and is effective in preventing cancer and regulating immunity (Abdel-Razek et al., 2023).

In this study, hypocotyls obtained from 'Sultani' okra seeds germinated *in vitro* were used as starting material, and Murashige and Skoog (MS) basic medium at different strengths (¼, ½, ¾, and 1) and various amounts of sucrose (20, 25, 40 and 50 mg L^{-1}) were used to reveal their effects on adventitious root formation regarding biomass accumulation.

MATERIAL AND METHOD

The present study was carried out in the Tissue Culture Laboratory of Akdeniz University, Faculty of Agriculture, Department of Horticulture.

Explant and Media Preparation, Explants Culturing

Seeds of ‘Sultani’ okra cultivar were used in the study. Seeds of the cultivars were subjected to surface sterilization before being cultured. The seeds were first kept in 70% ethanol solution for 1 minute and then sterilized in 50% sodium hypochlorite solution for 12 minutes, and then, it was rinsed 3 times with sterile distilled water.

Sterilized seeds were cultured in MS (Murashige and Skoog, 1962) medium (MS0) containing 30 g L^{-1} sucrose and 6 g L^{-1} agar, without the addition of plant growth regulators, to germinate under *in vitro* conditions. Cultured seeds were incubated under 24±2 °C temperature and 16/8 hour light/dark photoperiod conditions. For seed germination under *in vitro* conditions, 15 glass jars with a volume of 660 mL were used, and 15 seeds were planted in each jar.

The 7-day-old hypocotyls from the germinated plantlets were used as starting material for the adventitious root culture study. Hypocotyls were cut into 1 cm pieces in a laminar flow and cultured in the combinations of media presented in Table 1 for the initiation of adventitious root cultures. For each cultivar, 90 mm 5 petri dishes/medium were used while 15 hypocotyl explants were cultured in each petri dish and the study was carried out in 3 replications.

Table 1. Media combinations used in the study

Media Codes	**Media Combinations**			
	MS	**IBA (mg L^{-1})**	**Sucrose (g L^{-1})**	**Agar (g L^{-1})**
1	¼	2	30	6
2	½	2	30	6
3	¾	2	30	6
4	1	2	30	6
5	¾	2	20	6
6	¾	2	25	6
7	¾	2	40	6
8	¾	2	50	6
Control	1	-	30	6

Establishing and Propagating Adventitious Root Cultures

Adventitious root cultures were initiated with 2-month-old roots consisting of hypocotyl explants cultured in different combinations of media. Adventitious roots (0.15 - 0.20 g/petri) obtained from hypocotyls of okra cultivar were inoculated into 100 mL conical flasks containing 30 mL of nutrient medium. The medium combinations used in the initiation of adventitious root cultures were used as liquid medium without adding agar for adventitious root propagation. Cultures were shaken at 130 rpm on an orbital shaker under dark conditions at 24±2°C for 4 weeks. Three subcultures with 4-week intervals were conducted in the study, and the weights of the proliferating adventitious roots at the end of each subculture were measured and recorded.

Statistical analysis

The experiments of the current study were conducted in three replicates with a completely random factorial design. The data obtained were made in the JMP package program and the differences between the averages were determined by the 'least significant difference' (LSD) test, and the differences were found to be statistically significant at the $p < 0.05$ level.

RESULTS AND DISCUSSION

In this study, the effects of MS media at various strengths and sucrose at different concentrations on the initiation of adventitious root cultures and biomass accumulation in ‘Sultani’ okra cultivar were evaluated (Figure 1-3).

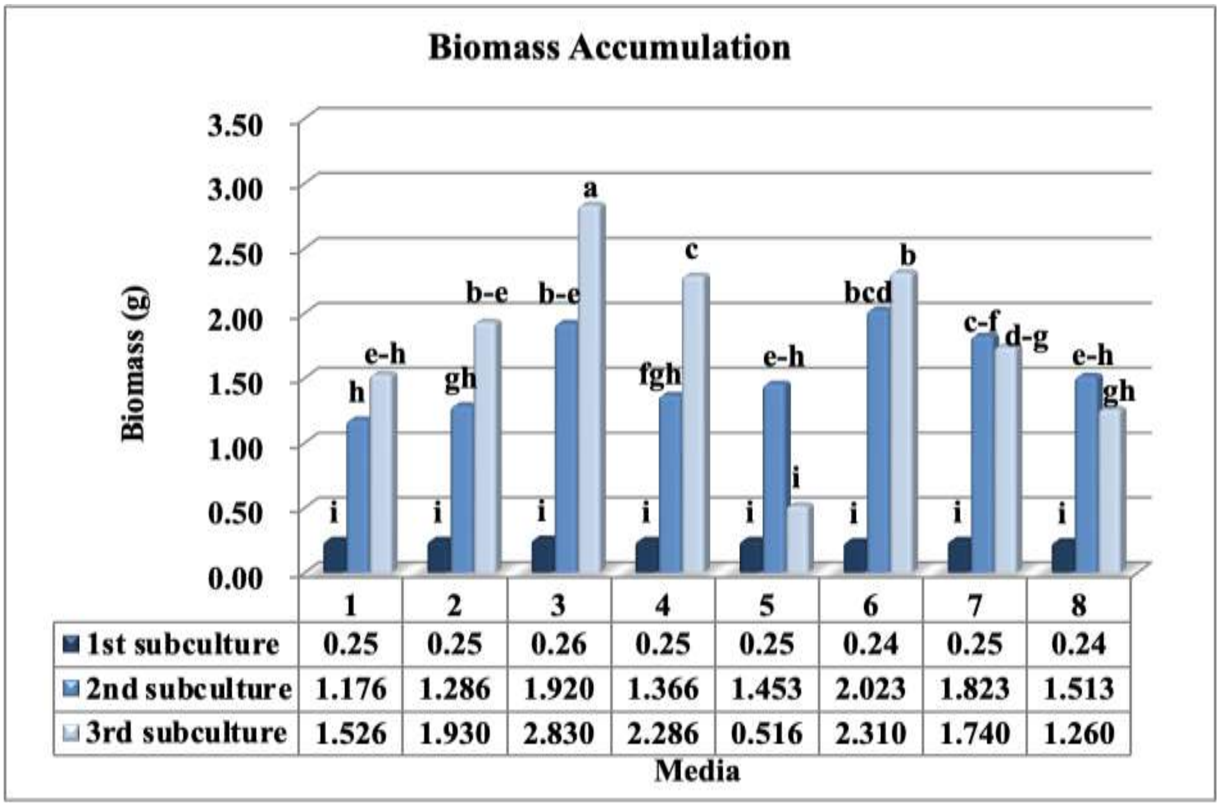

Figure 1. Biomass accumulation in ‘Sultani’ okra cultivar regarding different media and subcultures

(1): Different letters among media and subcultures denote significant differences (LSD test, $p<0.05$).

(2): LSD media* = 0.279; LSD subculture* = 0.171; **(b)** LSD media x subculture* = 0.484

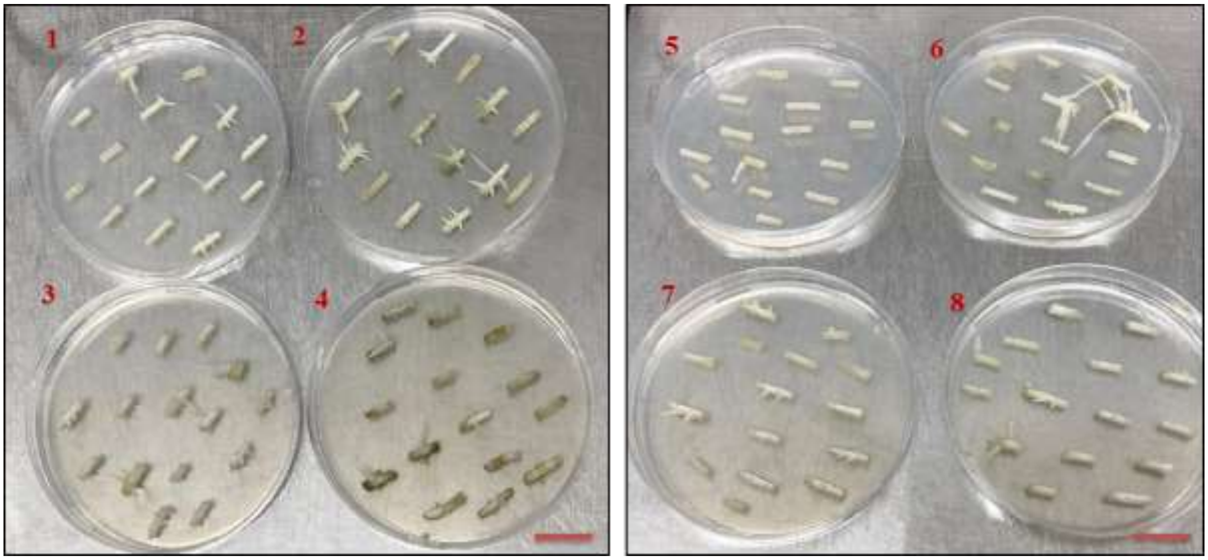

Figure 2. Adventitious root formation from hypocotyls of 'Sultani' okra cultivar in different media

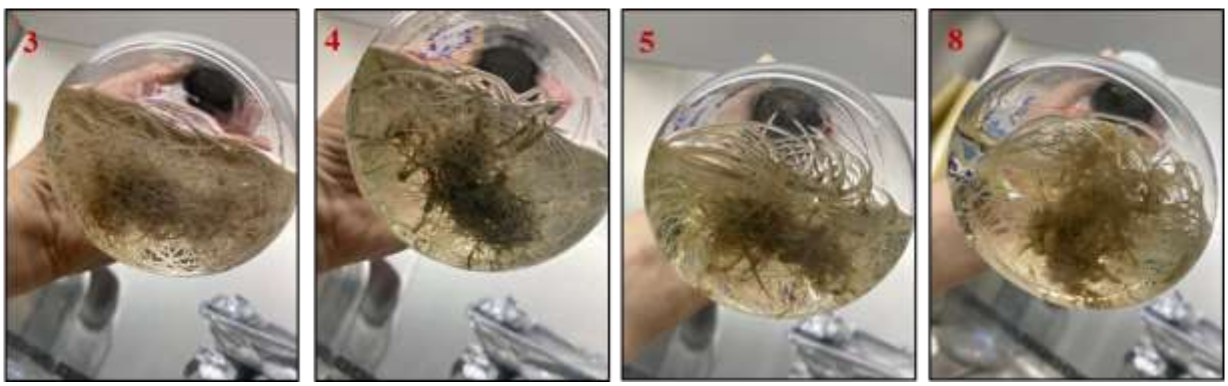

Figure 3. Adventitious root cultures of 'Sultani' okra cultivar in different media

Considering the findings obtained from the study, differences were also determined in terms of the media combinations evaluated. The medium combination (media No. 3), supplemented with 30 g L^{-1} sucrose, where ¾ of the MS strength gave the most positive value in terms of biomass accumulation. The least accumulation in biomass was obtained from medium No. 5, and ¾ MS strength was used in this medium, but 20 g L^{-1} sucrose was added.

Adventitious root formation consists of a complex molecular process involving numerous physiological factors (Sorin et al., 2005). Among various plant cell and organ cultures, adventitious root culture is an alternative and complementary tool to whole plant cultivation to produce high-value phytochemicals, as well as offering the opportunity to produce year-round biomass with low cost and time (Hao and Guan, 2011; Baque et al., 2012; Sivanandhan et al., 2013).

Auxins are among the most widely used plant growth regulators for the stimulation of adventitious roots in various medicinally important plants (Saeed et al., 2017). It has been reported that the initiation of root formation and the differentiation process in rooting can be triggered by the exogenous addition of specific auxins (Praveen et al., 2009; Khanam et al., 2022). The types and concentrations of auxins and their interactions with other components in the culture medium affect the growth of adventitious root cultures, as well as their ability to produce various secondary metabolites (Reis et al., 2011; Amoo et al., 2013). Indole butyric acid, IBA, from synthetic auxins, is among the first plant growth regulators used to increase root formation in plants (Deloso et al., 2020). A previous study reported that IBA has a greater ability to support adventitious root formation than indole acetic acid (IAA), and this may be due to the fact that IBA is partially more stable than IAA (Hartmann et al., 1990; Deloso et al., 2020). It has also been reported that the effectiveness of various auxins in stimulating adventitious roots is variable for different species. For example, one study reported that IBA was more effective than IAA and NAA in stimulating adventitious roots from hypocotyl explants of *Psoralea coryfolia* (Baskaran and Jayabalan, 2009), while it was reported in *Helianthus annuus* (Vesperinas, 1998) and *Antirrhinum majus* (Atkinson et al., 1991) reported that IAA induces adventitious roots. In current study, IBA (2 mg L^{-1}) was used and had a positive effect on adventitious root development.

The effects of various factors affect the success of adventitious root cultures. In addition to the effect of auxins on the stimulation of adventitious roots, the strength of the medium, sucrose concentration, or the type of explant used to initiate root cultures are among the determining factors for success in adventitious root cultures.

In previous studies, it has been reported that the low salt strength of the medium increases root growth by increasing the access of ions to the roots. High salt strength negatively affects root growth, and therefore low water potential prevents the absorption of mineral nutrients and water from the soil (Cui et al., 2010; Silja and Satheeshkumar, 2015; Sivakumar et al., 2006). In a study conducted on *Curculigo orchioides* by Trinh et al. (2021), researchers reported that culture conditions containing ¾ strength MS medium and 4% sucrose addition provided high biomass accumulation and phenolic content of the plant. In another study, Zhang et al. (2020) showed that low salt strength increased root growth in their adventitious root culture study on *Tripterygium wilfordii*. Similarly, in current study, it was found that adventitious root growth and biomass accumulation obtained in ¾ MS medium supplemented with 30 g L^{-1} sucrose.

In vitro plantlets require carbon sources in their artificial media for biological processes such as survival, growth, development, and accumulation of bioactive compounds under aseptic and controlled conditions. Sucrose is the most common carbon source in tissue culture media, as it is the main sugar translocated in the phloem of many plants. As previously reported in many studies, sucrose serves as the building block of a living cell and regulates cellular osmotic potential (Calamar and De Klerk, 2002; Cui et al., 2010). At the same time, the growth rate of biomass is directly related to sugar consumption. In previous studies, it was reported that low sucrose supplementation to the culture medium in cell suspension cultures was not suitable for biomass biosynthesis in *Artemisia absinthium* (Ali et al., 2016) and *Prunella vulgaris* (Fazal et al., 2014) plants. Similarly, in this study, the increase in biomass accumulation of adventitious roots in the medium containing 20 g L^{-1} sucrose was found to be the lowest compared to other medium combinations.

Different explants, including leaves, stems, petioles, roots, and hypocotyl parts, can be used for the induction of adventitious roots *in vitro* in many industrially important plant species (Paek et al., 2009; Sharma et al., 2013; Kawakami et al., 2015; Khan et al., 2015; Khan et al., 2017; Saeed et al., 2017). For this reason, it is thought that the different results obtained in terms of biomass in adventitious root cultures of various plant species may be also due to differences in species, varieties, and explants.

CONCLUSION

Considering the results obtained from the study, reducing the MS medium strength to a certain extent resulted in an increase in the biomass accumulation obtained from adventitious root cultures in okra. While the increase in biomass was achieved by adding 30 g L^{-1} sucrose to the medium, reducing the sucrose rate to 20 g L^{-1} did not yield positive results. Considering that adventitious root cultures are an important alternative method for producing high-value phytochemicals, it is thought that okra adventitious root cultures can be used effectively on large scales in different industries such as the pharmaceutical industry, within the framework of the results obtained.

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FARMERS WHO ARE PARTNERS IN THE AGRICULTURAL IRRIGATION COOPERATIVE IN ANTALYA PROVINCE REVIEW OF IRRIGATION PRACTICES

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ABSTRACT

In arid countries, irrigation is the most important factor in increasing productivity in agriculture. In these countries, the construction of an irrigation facility involving a single enterprise (drilling a well, installing a water pump or motor) can be done by a private firm or a multi-purpose cooperative. In this case, the job of the firm or the cooperative ends after the facility is completed and the cost is paid.

If the irrigation facility requires that many farmers benefit from the same water source (river, pond, canal, artesian, well, etc.), then there is a need to establish an irrigation cooperative in order to regulate both the amount of water each farmer will use and the order of water intake. If these issues are not regulated through cooperatives, it will lead to constant and inexhaustible fights, especially in arid countries where water is scarce and vital. Cooperative is the gathering of economic forces of individuals by means of solidarity in order to do the things that they cannot do alone or that are beneficial for them to do together, in the best way and at a cost price.

In this study, it is aimed to reveal the practices and thoughts of the farmers who are partners in the agricultural irrigation cooperative. In the study, a questionnaire was applied to the farmers in order to determine the common cooperative relations in terms of irrigation management and the data obtained were evaluated in the SPSS package program. The number of partners to be surveyed was determined by using the current information in the irrigation cooperatives in the research area. In the research, a questionnaire was applied to 61 farmers who are partners in the agricultural irrigation cooperative; 43% of this is made up of the farmers in Dosemealti and 57% in Korkuteli district. The survey application was carried out in December 2021. The data obtained from the survey application are summarized in the charts as scale, ratio and percentage values.

Keywords: *Cooperative, Agricultural organization, Demographic characteristics, Irrigation, Antalya*

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INTRODUCTION

Many countries, especially those located in arid and semi-arid regions, face significant water problems. Population growth and financing problems are at the forefront of the factors that create the water crisis. The most important aim of the agricultural sector is to get more products per unit area to feed the rapidly increasing population. The most intense water demand occurs in agricultural uses. Turkey is not among the countries suffering from water scarcity in the light of the worldwide evaluations in terms of water resources. However, considering the increasing water consumption values due to population growth, urbanization and industrialization, it is clear that there is a decrease in renewable fresh water resources in terms of quantity (Süheri ve Topak, 2005).

Among the water user sectors in Turkey, agriculture ranks first as the most water user sector. For this reason, the use of tools and techniques that ensure efficient water use in agriculture should be among the priority targets. If the current use of water in agriculture continues unchanged, it is highly likely that water scarcity will turn into a water crisis in Turkey. Precautions should be taken to ensure that the agricultural sector is affected by a possible water problem at the minimum level and due importance should be given to irrigation management. Therefore, the things to be done in agriculture are as follows: Making limited irrigation, designing irrigation systems according to pressurized irrigation methods, developing alternative water resources (recovery of waste water, directing surface water to areas where water is scarce, developing water-saving irrigation methods and techniques, opportunities to benefit from waste water and drainage water), dissemination of closed pipe systems instead of open systems in order to minimize water distribution losses, re-evaluation of the area, monitoring and evaluation of irrigation water quality, establishing the infrastructure for the transition to price-based pricing, ensuring inter-institutional coordination, giving more importance to farmer education and disseminating the trainings (Çakmak ve ark., 2008).

One of the obligatory options for increasing agricultural production in Turkey, where arable land is at the limit, is to buy more products per unit area. It is a fact that the expected production increase in irrigated agriculture in Turkey has not been achieved today. The factors affecting this situation start from the planning of the resources and continue their effects until the end use area. Problems encountered in this field; organization, personnel, financial opportunities, irrigation application problems, legal problems and business problems (Yurteri, 2011).

When the forms of irrigation management in Turkey are examined, it is seen that there are five types of management: public irrigation management, local governments irrigation management, irrigation union management, irrigation cooperatives management and public irrigation management (Sarı, 2017). In order to obtain the expected benefit from irrigation, the necessary care must be taken during the distribution and use of water. One of the most important organizations that ensure the distribution of water in Turkey, in other words, the delivery of water to the end user, is irrigation cooperatives (Cengiz, 2018).

Cooperative is the gathering of economic forces of individuals by means of solidarity in order to do the things that they cannot do alone or that are beneficial for them to do together, in the best way and at a cost price (Mülayim, 2013). According to the Cooperatives Law No. 1163, the cooperative; It is defined as partnerships with variable partners and variable capital, which are established by real and legal persons in order to provide and protect certain economic interests of their partners, especially their professional and livelihood needs, as having legal personality, through mutual assistance, solidarity and surety with their labor and monetary contributions.

Irrigation cooperatives are organizations formed by those who want to benefit from underground and surface resources, by combining their economic powers, according to Law No. 1163. The aim of irrigation cooperatives is to obtain the water needed by the farmers who want to benefit from water resources in an organized manner for their agricultural production, to make a fair distribution and to ensure its effective use (Ünver, 2016). Irrigation Cooperatives; It was established in 1963 under the name of "Soil-Water Cooperatives" in accordance with the main contract of association prepared by the General Directorate of Soil-Water affiliated to the Ministry of Rural Affairs. Later, these cooperatives became irrigation cooperatives and the Ministry of Agriculture and Rural Affairs had a new main contract of association prepared for irrigation cooperatives. According to the new main contract of association, the purpose of irrigation cooperatives; It has been determined as "to establish agricultural irrigation facilities such as land leveling, field head canals, in-field irrigation and drainage related to the use of water to be taken from irrigation facilities supplied or to be supplied by the state or to be extracted from agricultural fields in any way, or to operate, operate, maintain and have the irrigation facilities established".

Some of the studies conducted in Turkey on irrigation practices, behaviors, participation of producers in irrigation investments and similar issues in agricultural production can be listed as Aydin (2006), Uzunlu (2008), Sayın (2011), Yurteri (2011), Demir (2014), Buyukbas (2015), Unver (2016), Sari (2017), Cengiz (2018), Fisekcioglu (2018), Patlar (2018), Buz (2019), Candan (2020).

In this study, irrigation practices and behaviors of producers who are partners in agricultural irrigation cooperatives in Antalya province were investigated.

MATERIAL AND METHOD

Primary and secondary sources were used in the study. The primary material of the study consisted of the data obtained from the survey application made by face-to-face interview method with the producers who are partners in the Antalya province Agricultural Irrigation Cooperatives. Secondary data were obtained from sources such as national and international research reports, articles, theses, statistical data on similar subjects.

The data obtained from the questionnaires applied to determine the common cooperative relations in terms of irrigation management were evaluated in the SPSS package program. The number of partners to be surveyed was determined by using the current information in the irrigation cooperatives in the research area. The total number of Agricultural Irrigation Cooperatives in Antalya is 76, and the total number of partners is 11,879. In the districts of Döşemealtı and Korkuteli, a survey was conducted with the producers who are partners in the irrigation cooperative. In the research, a total of 61 partners were surveyed; 43% of this includes the district of Dosemealti and 57% of Korkuteli (Table 1). These data are summarized in the tables as scale, ratio and percentage values. In the study; a survey was conducted with the face-to-face interview method in Korkuteli and Dosemealti districts in December 2021 for the producers who are partners of the Irrigation Cooperative.

Table 1. Survey Application by District and Village

County Name	***Person***	***%***	***Village Name***	***Person***	***%***
Dosemealti	26	42.6	Karatas	8	13.1
			Karaveliler	2	3.3
			Kovanlik	9	14.8
			Yagca-Çiglik	7	11.5
Korkuteli	35	57.4	Bozova	10	16.4
			Sulekler	8	13.1
			Yelten	8	13.1
			Yesilyayla	9	14.8
Total	***61***	***100.0***	***Total***	***61***	***100.0***

Information about the Research Area

Antalya province is a center of tourism and agriculture in the south of Turkey, located on the Mediterranean coast, with an area of 20,177 km² and a population of 2,548,308 as of the end of 2020. The province of Antalya is between 29° 20'-32° 35' east longitudes and 36° 07'- 37° 29' north latitudes in the southwest of Turkey. It is surrounded by the Mediterranean Sea in the south and the Taurus Mountains in the north, and borders with Mersin, Konya and Karaman in the east, Isparta and Burdur in the north, and Mugla in the west. The surface area of the province corresponds to 2.6% of Turkey's surface area. Antalya province, located in the west of the Mediterranean region, constitutes 17.6% of the region's surface area.

Antalya province ranks 5th in Turkey in terms of population size, 2nd place with 156,395 farmer families and 9,53 billion TL agricultural production values, ranks 1st with 6.79% of Turkey's plant production value, and is the locomotive of Turkey's agriculture. Agricultural structure, land distribution and distribution of agricultural areas of Antalya province are given in Table 2. In the province of Antalya, 50,667 hectares of open and greenhouse vegetables, 551 hectares of ornamental plants, 74,787 hectares of fruit growing, 184,867 hectares of field crops are cultivated, of the 3,617,072 hectares of agricultural land.

Table 2. Agricultural Structure in Antalya Province (2019)

Number of Districts (unit)		19
Total Number of Neighborhoods (units)		909
Total Number of Farmers (person)		156,395
Number of Farmers Registered with CKS (person)		39,533
Average Size of Farm Registered with CKS (da)		32
Land distribution	***Antalya (da)***	***(%)***
Area	***20,177,000***	
Agricultural Land	3,617,072	17.9

Irrigated Agricultural Land	523,580	2.6
Meadow-Pasture Area	2,044,630	10.1
Forest Area	11,417,020	56.6
Agricultural Land		
Field Crops	1,789,048	49.5
Vegetable (Open Field)	214,643	5.9
Vegetable (Covered)	286,522	7.9
Ornamental Plants	5,944	0.2
Fruit Garden	797,144	22.0
Fallow	523,771	14.5
Total	***3,617,072***	***100.0***

Source: Anonymous, 2022.

Agriculture in Antalya is carried out in 4 agricultural basins and the product variety differs according to the basins (Figure 1).

In the Coastal Aegean Basin, the land starts from sea level and rises inland. Kas district is located in the Coastal Aegean Basin.

In the Coastal Mediterranean Basin, the land structure starts from sea level and rises up to 800-1000 m in the interior. This basin has coast and plateau sections. It is a region where coastal tourism is common. It is the basin with the best market opportunity for agricultural products. As the overseas connection points (port and airport) are in this region, it is in an advantageous position especially for export-oriented agricultural production. Aksu, Dosemealti, Kepez, Muratpasa, Konyaalti, Kemer, Kumluca, Finike, Demre, Serik, Manavgat, Alanya and Gazipasa districts are located in the Coastal Mediterranean Basin.

The Aegean Plateau Basin is a region with a plateau feature, located in the Western Taurus Mountains at an altitude of over 1000 meters topographically, with rich water resources. In this basin, the amount of agricultural land in the total land surface is the highest compared to other sub-regions. Korkuteli and Elmalı counties are located in the Aegean Plateau Basin.

The Lakes Basin has a mountainous terrain that forms the high plateau of the Taurus Mountains. This basin is limited in terms of agricultural land and is covered with forested areas. It starts from 800 meters above sea level and goes up to 1300 meters. Akseki, İbradi and Gundogmus districts are located in the Lakes basin.

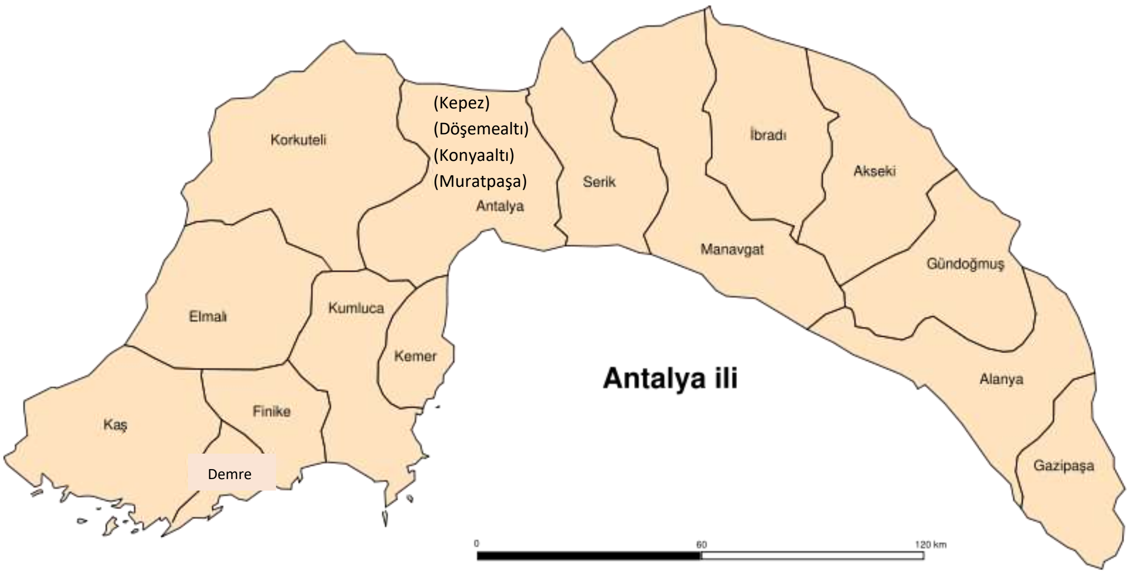

Figure 1. Districts of Antalya Province

2.8% of the 2451 Agricultural Irrigation Cooperatives in Turkey in 2020 are located in Antalya. Although the number of irrigation cooperatives in Antalya in the 2013-2020 period varies in this period, it is 69 in 2020. When irrigation cooperatives are examined by districts in Antalya province, Korkuteli district ranks first, followed by Elmali, Dosemealti and Alanya districts, respectively (Table 3).

Table 3. Irrigation Cooperatives by Districts in Antalya Province (number)

Districts	***2013***	***2014***	***2015***	***2016***	***2017***	***2018***	***2019***	***2020***	***2020 (%)***
Akseki	1	1	1	1	1	1	1	1	1.4
Alanya	8	8	8	8	8	7	7	7	10.1
Demre	3	3	3	3	3	3	3	3	4.3
Dosemealti	8	10				9	9	9	13.0
Elmali	12	13	13	13	13	13	13	13	18.8

Finike	2	2	2	2	3	3	3	3	4.3
Gazipasa	1	1	1	1	1	1	1	1	1.4
Kas	3	4	4	4	4	3	3	3	4.3
Konyaalti	1	2	2	2	2	2	2	2	2.9
Korkuteli	21								
Kumluca	3	3	3	3	3	3	2	2	2.9
Manavgat	4	5	5	6	5	4	3	3	4.3
Serik	0	1	1	1	1	1	1	1	1.4
Total	***67***								

Source: Anonymous, 2022.

Research Results

Demographic Characteristics of the Investigated Farms

The findings regarding the gender, educational status, age and experience of the farmers in the farms surveyed in the research are given in Table 4. Of the 61 farmers participating in the survey, 59 are male and 2 are female farmers. 71% of the farmers are primary school graduates. In the surveyed farms, 73.8% of the farmers are in the 50 and over age group. The average age is 56.

Table 4. Characteristics of Farmers in the Investigated Farms

	Person	***%***
Gender		
Female	2	3.3
Male	59	96.7
Total	***61***	***100.0***
Education Level		
Not Literate	1	1.6
Primary School Graduate	43	70.5
Secondary School Graduate	7	11.5
High School Graduate	7	11.5
College/University Graduate	3	4.9
Total	***61***	***100.0***
Age		
15-49	16	26.2

50 +	45	73.8
Total	***61***	***100.0***
Average age	56	
Average Experience in Agriculture (years)	34	

34% of the farmers participating in the research are engaged in plant production, 23% in animal production, and 43% in both plant and animal production. 5% of the farmers work in agricultural jobs outside the enterprise, 26% work in non-agricultural jobs, and 95% have their own social security (Table 5).

Table 5. Employment Information of the Farmer in the Investigated Farms

	Person	***%***
Production Activity		
Plant Production	21	34.4
Animal Production	14	23.0
Plant and Animal Production	26	42.6
Total	***61***	***100.0***
Off-Farm Agricultural Work		
Yes	3	4.9
No	58	95.1
Total	***61***	***100.0***
Working in Non-Farm Works		
Yes	16	26.2
No	45	73.8
Total	***61***	***100.0***
Social Security		
There is social security.	58	95.1
There is no social security.	3	4.9
Total	***61***	***100.0***

Type of Social Security		
Social Insurance Institution	20	34.5
Bagkur	26	44.8
Pension Fund	4	6.9
Social Security Institution	1	1.7
Social Insurance Institution Pension	7	12.1
Total	***58***	***100.0***

In the research, within the scope of the survey application, the opinions of the farmers on organization were also included. In the survey conducted to determine the partner-cooperative relations of the study, the target group is the irrigation cooperative partners. 14.8% of the producers are agricultural development partners and 39.3% are Agricultural Credit Cooperative partners (Table 6).

Table 6. Organizational Status (N=61)

Become a partner/member in an organization	***Person***	***%***
Agricultural Development Cooperative	9	14.8
Irrigation Cooperative	61	100.0
Agricultural Credit Cooperative	24	39.3
Chamber of Agriculture	20	32.8
Chamber of Commerce	1	1.6
Agricultural Producer Association	2	3.3
Organic Agriculture Association	1	1.6

*** More than one answer has been received.**

Irrigation Practices in Investigated Farms

In this part of the research, the demographic characteristics of the farmers who are partners in the irrigation cooperative, their practices on irrigation, their thoughts, etc. were examined.

In Table 7, the interest, knowledge and satisfaction levels of the farmers regarding irrigation are given. 52.5% of the cooperative partners are highly interested in irrigation. 36.1% of the farmers have high satisfaction with the irrigation application, and 26.2% have a medium satisfaction level. The rate of farmers who state that they have a high level of knowledge about irrigation is 44.3%.

Table 7. Opinions of Farmers on Irrigation in Investigated Enterprises (N=61) (%)

	Very low	***Low***	***Medium***	***High***	***Very high***	***Total***
Level of interest in irrigation	1.6	8.2	31.1	52.5	6.6	100.0
Satisfaction level with irrigation	4.9	23.0	26.2	36.1	9.8	100.0
Knowledge level about irrigation	3.3	8.2	31.1	44.3	13.1	100.0

In the examined farms, 47.5% of the farmers determine the irrigation time by looking at the plant, 34.4% according to their experience, 14.8% by controlling the soil and 11.5% according to the irrigation order (Table 8).

Table 8. How Farmers Determine Irrigation Time in Investigated Plants (N=61)

How to Determine Irrigation Time	***Person***	***%***
By controlling the soil	9	14.8
By looking at the plant	29	47.5
According to their experience	21	34.4
According to the irrigation order	7	11.5

* More than one answer has been received.

The ways of determining the amount of water to be given to the field by the farmers who are partners in the cooperative are shown in Table 9. 62.3% of the farmers stated that they determined the amount of water according to the plant characteristics, and 42.6% according to their experience.

Table 9. How the Producers Determine the Amount of Water to be Given to the Field in Investigated Farms

How to Determine the Amount of Water	***Person***	***%***
Soil feature	9	14.8
Plant feature	38	62.3
Experience	26	42.6
By looking at the moisture in the root zone	1	1.6
With soil moisture meter	1	1.6
Calculating plant water consumption	3	4.9

* More than one answer has been received.

The soil analysis practices of the farmers who are partners in the irrigation cooperative in the examined farms are given in Table 10. 65.6% of the farmers who are partners in the irrigation cooperative did not have a soil analysis. The rate of farmers who have soil analysis done in farms is 34.4%. The reasons for the farmers to have a soil analysis were to learn the fertilizer requirement of the product they will plant (85.7%) and to obtain diesel-fertilizer support (14.3%). 55.7% of the farmers find the soil analysis reliable.

Table 10. Soil Analysis Practices in Investigated Farms

	Person	***%***
Soil Analysis Status		
Yes, I did	21	34.4
No, I didn't	40	65.6
Total	***61***	***100.0***
Reason for Soil Analysis		
Mandatory to get diesel-fertilizer support	3	14.3
To find out the fertilizer needs of the plant	18	85.7
Total	***21***	***100.0***
Reliability of Soil Analysis		
Yes, reliable	34	55.7
No, it's not reliable	9	14.8
No idea	18	29.5
Total	***61***	***100.0***

The way in which the farmers who are partners in the cooperative determine the fertilizer requirement of the soil is shown in Table 11. 82% of the producers estimate the fertilizer need of the soil.

Table 11. How to Determine the Fertilizer Need of the Soil in the Investigated Farms (N=61)

How to Determine the Fertilizer Need of the Soil	***Person***	***%***
According to the results of soil analysis	7	11.5
Estimated	50	82.0
By asking my friends	6	9.8

* More than one answer has been received.

The findings related to the irrigation source in the farms surveyed in the research are given in Table 12. 60.7% of the farmers use drip irrigation. Groundwater is used for irrigation in 88.5% of the farms.

Table 12. Irrigation Source in Investigated Farms

	Person	***%***
Drip Irrigation Usage Status		
Yes, I'm using.	37	60.7
No, I do not use it, but I intend to.	17	27.9
No I do not use; because it is an expensive irrigation method.	4	6.6
There's no need	1	1.6
No, there is no system.	2	3.3
Total	***61***	***100.0***
Groundwater Use		
Yes I'm using.	54	88.5
No I do not use.	7	11.5
Total	***61***	***100.0***

Table 13 shows the findings regarding whether the farmers agree with the idea that "the more I irrigate, the more yield I get" in the farms examined within the scope of the research. 77% of the farmers stated that they did not agree with this idea and stated that water should be given as much as the plant needs. While 67.2% of the farmers knew the harms of giving too much water to the soil, 26.2% stated that they did not know. The rate of farmers who partially know the harms of giving too much water to the soil is 6.6%.

Table 13. State of Participation in Over-Irrigation and Over-Efficiency in the Investigated **Farms**

	Person	***%***
State of Participation in Over-Irrigation-Over-Efficiency Thought		
Yes, with a lot of irrigation, a lot of yield is obtained.	14	23.0
No, water should be given as much as the plant needs.	47	77.0
Total	***61***	***100.0***
Do You Know the Harms of Giving Too Much Water to the Soil?		
Yes, I know.	41	67.2
I partially know.	4	6.6
No, I do not know.	16	26.2
Total	***61***	***100.0***

In the examined farms, 80.3% of the farmers stated that they did not know the quality of the irrigation water, 13.1% stated that they knew, and 6.6% stated that the quality of the irrigation water was not important (Table 14).

Table 14. Information of the Famers on Irrigation Water Quality in the Investigated Farms

Water Quality	***Person***	***%***
Yes, I know its quality.	8	13.1
No, I don't know the quality.	49	80.3
The quality of the water does not matter.	4	6.6
Total	***61***	***100.0***

In the study, the opinions of the farmers about the cost of irrigation in the examined farms were investigated. Of the farmers surveyed, 83.6% stated that the electricity consumed for irrigation was expensive, 55.7% said that they irrigated economically, 67.2% stated that irrigation water was expensive. 63.9% stated that the irrigation fee should be calculated according to the hour (Table 15).

Table 15. Opinions on the Cost of Irrigation in the Investigated Farms

	Person	***%***
Electricity Fee Paid for Irrigation		
Expensive	51	83.6
Not expensive	9	14.8
No idea	1	1.6
Total	***61***	***100.0***
Economic Aspect of Irrigation		
I am irrigating economically	34	55.7
I do not make economically appropriate irrigation.	25	41.0
No idea	2	3.3
Total	***61***	***100.0***
Irrigation Water Fee		
Expensive	41	67.2
Normal	20	32.8
Total	***61***	***100.0***
How to Calculate Irrigation Fee		
Calculated According to Irrigation Hours	39	63.9
It should be calculated according to the irrigation flow	11	18.0
Calculated by Area	9	14.8
No idea	2	3.3
Total	***61***	***100.0***

In the farms examined within the scope of the research, 78.7% of the farmers stated that they irrigate at night, while 21.3% stated that they do not irrigate at night (Table 16).

Table 16. Night Irrigation Situation in Investigated Farms

Night Irrigation	***Person***	***%***
Yes I do.	48	78.7
No, I don't.	13	21.3
Total	***61***	***100.0***

75.4% of the farmers surveyed do not have information on the useful water capacity of the soil (Table 17).

Table 17. Information of the Farmers on the Useful Water Capacity of the Soil in the Investigated Farms

About Useful Water Capacity of Soil	***Person***	***%***
Yes, I have information.	15	24.6
No, I don't know.	46	75.4
Total	***61***	***100.0***

The availability of irrigation water for the farmers at any time in the examined farms is shown in Table 18. While 77% of the farmers buy irrigation water when it is their turn, 14.7% buy it whenever they want. The rate of farmers who cannot get irrigation water whenever they want is 6.6%.

Table 18. Availability of Farmers to Supply Irrigation Water at Desired Time in the Investigated **Farms**

Ability to Supply Irrigation Water	***Person***	***%***
I can take it whenever I want.	9	14.8
I can't get it whenever I want.	4	6.6
I get it when it's my turn.	47	77.0
I get delayed from time to time.	1	1.6
Total	***61***	***100.0***

Drip irrigation (73.8%) is the most suitable irrigation method according to the farmers in the enterprises included in the study, followed by sprinkling (18%) and surface irrigation (8.2%), respectively (Table 19).

Table 19. The Most Appropriate Irrigation Method According to the Farmers in the Investigated Farms

Irrigation Method	***Person***	***%***
Sprinkler irrigation	11	18.0
Drip irrigation	45	73.8
Surface irrigation	5	8.2
Total	***61***	***100.0***

Within the scope of the survey application in the research, the opinions of the producers about the irrigation organization that best meets their needs were determined. While 72.1% of the farmers stated that the organization that best met their irrigation needs was the irrigation cooperative, this was followed by DSI (19.7%) and the Irrigation Association (8.2%) (Table 20).

Table 20. Irrigation Organizations to Meet the Needs of the Farmers in the Investigated Farms

Irrigation Organization	***Person***	***%***
Irrigation Cooperative	44	72.1
Irrigation Association	5	8.2
State Irrigation Management (DSI etc.)	12	19.7
Total	***61***	***100.0***

The opinions of the farmers regarding the water distribution program application of the irrigation cooperative in the examined enterprises are given in Table 21. While 49.2% of the farmers stated that the water distribution program of the irrigation cooperative was determined according to the decision of the managers, 23% stated that it was made according to the products grown, 4.8% according to the water reserves, 4.9% according to the order.

Table 21. Water Distribution Program Implementation of the Irrigation Cooperative According to the Farmers in the Investigated Farms (N=61)

Water Distribution Program Application	***Person***	***%***
According to the number of farmers demanding water	12	19.7
According to the decision of the managers	30	49.2
According to the grown products	14	23.0
According to water reserves	9	14.8
By order	3	4.9

* More than one answer has been received.

The characteristics of the irrigation organization according to the farmers in the examined farms are shown in Table 22. According to this; 59% of the farmers said that the state should supervise and help, and 26.2% said that the democratic participation of the farmers should be essential.

Table 22. Characteristics of the Irrigation Organization According to the Farmers in the Investigated Farms

Irrigation Organization Features	***Person***	***%***
The democratic participation of the farmers should be essential.	16	26.2
The government should supervise and assist	36	59.0
Government should not interfere	1	1.6
Must be public enterprise	8	13.1
Total	***61***	***100.0***

In the research, the status of the farmers receiving technical information support from agricultural engineers in the surveyed farms was examined. 83.6% of the farmers did not receive technical information support from agricultural engineers on irrigation (Table 23).

Table 23. The Status of Obtaining Technical Information Support from Agricultural Engineers for Irrigation in the Investigated Farms

Technical Information Support	***Person***	***%***
Yes, I am getting information.	6	9.8
I rarely get information.	4	6.6
No, I have not received any information.	51	83.6
Total	***61***	***100.0***

In Table 24, the participation of farmers in any seminar or meeting on irrigation and fertilization in the investigated farms was examined. Accordingly, 85.2% of the partners stated that they did not attend a seminar or meeting on irrigation and fertilization.

Table 24. Participation of Farmers in the Meetings on Irrigation and Fertilization in the Investigated Farms

Participation in a Seminar or Meeting on Irrigation and Fertilization	***Person***	***%***
Yes, I joined.	9	14.8
No, I did not participate.	52	85.2
Total	***61***	***100.0***

In the study, the information sources of the farmers on irrigation were examined in the farms where the survey was applied, and the findings are given in Table 25. Friends (47.5%) take the first place among the information sources of farmers on irrigation. This is followed by Agriculture and Forestry Provincial-District Directorates (34.4%), television programs (24.6%), own experience (23.0%) etc. information sources.

Table 25. Information Sources of the Farmers on Irrigation in the Investigated Farms (N=61)

Information Sources	**Person**	**%**
From the Provincial and District Directorates of Agriculture	21	34.4
From private institutions	3	4.9
From printed publications on agriculture	3	4.9
From television programs	15	24.6
From friends	29	47.5
From the internet	5	8.2
according to my own experience	14	23.0

*** More than one answer has been received.**

In the study, the state of being aware of the state's support for irrigation methods was examined. 67.2% of the farmers are not aware of the support on this issue. The rate of farmers who are aware of the irrigation supports but cannot benefit is 24.6% (Table 26).

Table 26. Awareness of the Farmers of the State's Support for Irrigation in the Investigated Farms

About State Support for Irrigation Methods	***Person***	***%***
Yes, I know, I'm using it.	5	8.2
Yes, I know, but I don't use it.	15	24.6
No, I don't know.	41	67.2
Total	***61***	***100.0***

Opinions of farmers to benefit from credit support for irrigation systems in the examined farms are presented in Table 27. 65.6% of the farmers stated that they intend to benefit from credit support.

Table 27. Opinions of the Farmers on Benefiting from the Credit Support for the Irrigation System in the Future in the Investigated Farms

Benefiting from Credit Support	***Person***	***%***
Yes, I'm thinking of using it.	40	65.6
No, I don't intend to use it.	20	32.8
No idea	1	1.6
Total	***61***	***100.0***

In the farms included in the research, who performed the maintenance and repair services for irrigation is given in Table 28. 86.9% of the farmers stated that the maintenance and repair services were carried out by the cooperative they are a partner of, 11.5% of the farmers stated that they carried out the maintenance of the canals in their own land and 1.6% stated that the maintenance and repair was done by the state.

Table 28. Maintenance and Repair Services for Irrigation in the Inspected Enterprises

Maintenance and Repair Services	***Person***	***%***
I maintain the canals on my land myself.	7	11.5
Maintenance and repairs are done by the state.	1	1.6
Maintenance and repair is done by the cooperative.	53	86.9
Total	***61***	***100.0***

CONCLUSION

In this study, irrigation practices and behaviors of producers who are partners in agricultural irrigation cooperatives in Antalya province were investigated.

In the farms examined within the scope of the research, 70.5% of the producers are primary school graduates and the agricultural experience period is 34 years. Crop production is carried out in 77% of the examined farms. 26% of farmers work in non-agricultural jobs. The rate of farmers with social security is 95%. All farmers in the farms are partners in the irrigation cooperative, followed by partnerships in the agricultural credit cooperative and agricultural development cooperative.

In the study, 54% of the farmers stated that they were not satisfied with irrigation. According to 74% of the farmers, the most suitable irrigation method is drip irrigation. According to 72% of the farmers in the examined farms, the irrigation organization that will meet the needs is Irrigation Cooperatives. The rate of farmers who share the opinion that the state should supervise and support the irrigation organization is 59%. In the farms included in the research, 84% of the farmers stated that they did not receive technical support from agricultural engineers on irrigation, 85% did not attend meetings on irrigation, and 67% stated that they were not aware of the state's support for irrigation methods.

In regions where the place of agriculture in the economy is important, rational operation and continuity of irrigation facilities are of great importance in order to develop water and soil resources and increase their contribution to the national economy (Süheri and Topak, 2005). Irrigation methods also play an important role in the effective and economic use of existing resources and ensuring their sustainability. Improper, unplanned and inappropriate management of irrigation networks prevents the expected benefit from these systems. The most important application of increasing irrigation efficiency is to reduce water losses during transmission and application. Thus, as the losses in irrigation networks can be reduced, the amount of irrigated area will also increase (Sarı, 2017).

In order to increase production in the agricultural sector and to ensure rural development, first of all, it is necessary to develop soil and water resources and determine the principles of benefiting from them. In the development of soil and water resources; It is important to establish agricultural infrastructure, rational management of resources and to carry out studies for effective use. On the other hand, in order to ensure the sustainability of utilizing soil and water resources, besides the planning and construction of irrigation investments, it is necessary to rationally operate irrigation networks and ensure full economic and social participation of farmers in irrigation management (Patlar, 2018).

In particular, information training on water management such as soil-plant-water relations, irrigation time, irrigation methods should be provided to water users through applied training and extension programs to be organized by irrigation cooperatives and agricultural stakeholders on agricultural issues.

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INTERDISCIPLINARY WORKING TENDENCIES IN AGRICULTURAL RESEARCH: THE CASE OF ÇANAKKALE ONSEKİZ MART UNIVERSITY

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ABSTRACT

Interdisciplinary work is the coming together of different scientific disciplines for the solution of a problem in all scientific researches and projects and establishing a link between the subjects. As a matter of fact, when the economies and production factors of developed countries are analysed, "high value added production", "technological development" and "innovation" come to the fore as important concepts. This situation points to an interdisciplinary approach in all branches of science.

When we look at the issue from the perspective of agriculture, agriculture in Turkey, as in the whole world, is a sector that constantly maintains its importance in terms of nutrition, its contribution to employment, its share in national income and its role in the balance of payments. On the other hand, rapid population growth, changes in consumption trends and especially the global climate change in agriculture make it increasingly difficult to respond to current needs with existing resources. In addition, considering the marketing problems encountered after agricultural production, obstacles encountered in foreign trade, business management, organisation and extension activities, as well as effective and innovative agriculture in the rapidly digitalising world, production and productivity-oriented studies alone in the agricultural sector are not sufficient, and the need to increase interdisciplinary academic studies is extremely important.

Therefore, working on agricultural studies in cooperation with all fields of engineering, biology, chemistry, mathematics, statistics, econometrics, sociology and anthropology will increase the quality and innovations in researches and increase the chance of application of scientific findings in practice. In this study, interdisciplinary behaviours and tendencies of academicians working in Çanakkale Onsekiz Mart University Faculty of Agriculture in scientific studies and projects were examined.

Keywords: Agriculture, Interdisciplinary Study, Çanakkale.

INTRODUCTION

Agriculture is narrowly defined as plant and animal production. In the broad sense, agriculture is defined as all consecutive activities in the agricultural production and marketing process, such as the processing of agricultural products, the production of inputs and the sale of products to consumers. Based on these definitions, it is possible to divide agricultural activity into three as crop production, animal production, agricultural products and inputs technology (İnan, 2016).

However, today, agricultural activity should be considered not only production-oriented but also processing and technology-oriented. Biological technology is gradually replacing mechanical and chemical technologies, especially in increasing productivity. Although the definition of agriculture is "using seeds and soil", nowadays, plant production can be carried out without soil and even without seeds (Yavuz and Dilek 2019).

Agriculture is an extremely important sector in terms of providing raw materials and labour force to Gross Domestic Product, employment, foreign trade, balance of payments and agriculture-based industry (Tan et al., 2018). Therefore, in terms of economic development and growth, it is imperative that this contribution of agriculture to the economy is not only production-oriented, but also through the production of technology-oriented finished goods that provide high Added Value. This situation clearly demonstrates the importance of agriculture and industry and technology integration and interdisciplinary studies. On the other hand, fragmentation in markets and consumers, individualisation, development of consumption culture, change in consumer wants and needs, increase in consumer education, income and awareness levels have brought about the need for "market orientation" with diversification and differentiation in products and services (Özhan, 2002).

National academies define interdisciplinary research as "a style of research conducted by teams or individuals". Often it may be insufficient to look at a problem from the framework of a single discipline. Interdisciplinary research offers different perspectives on knowledge (Çobanoğlu, 2015). Interdisciplinary work involves collaborative problem solving, complex research problems, borrowing tools and methodologies, and cross-seeding between disciplines and concepts (Gür, 2003). The interdisciplinary approach aims to eliminate the boundaries between scientific disciplines that limit the potential of science to provide solutions (Müller and Merbach, 1984). Today, developed economies are in the stage of transition from low value added production to high value added production by utilising knowledge as a factor of production. The areas where high value added can be realised are the areas where advanced technologies are applied.

In this context, many changes and innovations are needed in agriculture in the globalising world. For example, technological and scientific studies in areas such as new drugs, fertilisers, more nutritious and disease-resistant plants or animals, seed breeding are rapidly increasing in the agricultural sector with current branches of science such as molecular biology and biotechnology and nanotechnology. In 2020 and beyond, some of the groundbreaking agricultural technologies are smart agricultural automation, soil DNA testing, precision agriculture, autonomous tractors equipped with artificial intelligence, and cutting-edge products that require high and cutting-edge technology such as laser railings. In addition, engine oils based on high temperatures with nanotechnology, agricultural tools and machines with light materials, and the construction of animal shelters in more favourable conditions are being built (Akbaş and Özarslan, 2007).

In this context, it will be useful to look at the subject from an economic point of view. Agricultural Economics is a branch of science that seeks solutions to agricultural problems by using the basic principles of economics. In this context, agricultural economists have to know both the technical subjects of agriculture such as field crops, horticultural crops, animal husbandry and evaluation of agricultural products and the subjects of economics such as planning, management, financing, accounting, business administration, marketing, agricultural policy, agricultural law, village sociology, agricultural extension. Agricultural Economics is related to mathematics, ecology, history, geography, politics, sociology, law, behavioural sciences, psychology, physics, chemistry, technology, informatics and even biotechnology, nanotechnology sciences which have a very important place in the development of countries recently. r.

To summarise briefly, as stated in the 11th Development Plan, while increasing food demand, climate change, urbanisation, soil and water resources, agricultural products and producers are under pressure; the development of plant and animal species suitable for the changing climate, the protection of the environment and biodiversity are gaining importance, and the need for qualified labour and technology is increasing in order to meet food demand with less resources (Anonymous, 2019). This is possible by intertwining and supporting each other and it is not possible for quality academic studies to work in isolation from other disciplines.

MATERIAL AND METHOD

The aim of this study is to examine the interdisciplinary working tendencies of academicians working in agriculture-related departments at Çanakkale Onsekiz Mart University. It is thought that the results obtained from the research will contribute to the determination of other stakeholders and academic units with which academicians working in agriculture-related departments cooperate and the difficulties encountered in interdisciplinary studies. The scope of this research is academicians who conduct scientific research on agriculture at Çanakkale Onsekiz Mart University and their tendency to work interdisciplinary.

Primary and secondary data were utilised in the research. In Çanakkale Onsekiz Mart University, 84 academicians working in 9 departments in the Faculty of Agriculture, 40 academicians working in 4 departments in Biga Vocational School, 21 academicians working in 3 departments in Lapseki Vocational School and 3 academicians working in Bayramiç Vocational School, and 81 academicians who accepted to conduct a face-to-face survey from a total of 148 academicians were selected by the census sampling method and the surveys conducted constituted the primary data, the publications made by various institutions and organisations on the subject and the research results conducted by other researchers were used as secondary data.

In the research, basic descriptive statistics were used to reveal the interdisciplinary working tendencies of the academicians participating in the survey. Likert scale questions were used in order to reveal the interdisciplinary working tendencies of academicians working in the field of agriculture, and the non-parametric Chi-Square Test was applied to create cross-tables to investigate the relationships (degree of dependency) between the data and to understand whether there is a relationship between them.

RESULTS AND DISCUSSION

Approximately 68% of the researchers who participated in the research by answering the survey questions were male and 32% were female, and the average age of the academics was 45. The age range of academics varies between 25 and 66 years.

Table 1. Distribution of Academics According to Gender

Gender	Number	Percentage (%)
Woman	26	32,0
Male	55	68,0
TOTAL	**81**	**100,0**

While 69 per cent of the academics participating in the research are involved in an interdisciplinary study, 31 per cent have never been involved in an interdisciplinary study. According to the table, as the age of the researchers increased, the status of being involved in interdisciplinary studies also increased.

Table 2. Effects of age factor on interdisciplinary work

Interdisciplinary Working Status	Frequency	Percentage(%)	Average Age	Standard Deviation
Yes	56	69,1	46,0596	9,10628
No.	25	30,9	40,1600	10,48284
TOTAL	**81**	**100**	**T:2,567**	**P:0.012>0.05**

The distribution of the academicians included in the research according to their titles is given in Table 3. Among the academicians, 33.8% are Professor, 30.00% are Assistant Professor, 10.0% are Associate Professor, 7.5% are Research Assistant, 12.5% are Lecturer and 6.3% are Research Assistant.

Table 3. Distribution of Academics According to Their Titles

Title	Number	Percentage (%)
Professor	27	33,8
Associate Professor	8	10,0
Dr.Lecturer	24	30,0
Dr. Research Assistant	6	6.3
Research Assistant	6	7,5
Lecturer	10	12,5
TOTAL	**81**	**100,0**

As seen in Table 4. 40.7% of the academics in the study have more than 20 years of professional experience, 18.5% have 15-20 years of professional experience, 13,6% have 10-15 years of professional experience, 12.3% have 1-5 years of professional experience, 11,1% have 10-15 years of professional experience and 3.7% have less than one year of professional experience.

Table 4. Distribution of Academicians according to their Professional Experience

Professional Experience	**Number**	**Percentage (%)**
<1	3	3,7
1-5 Years	10	12,3
5-10 Years	9	11,1
10-15 Years	11	13,6
15-20 Years	15	18,5
>20	33	40,7
TOTAL	**81**	**100,0**

The foreign language levels of the academics who contributed to the research are shown in Table 5. From this chart, 51,9% of the academics stated that they have an intermediate level, 44,4% advanced level and 3,7% beginner level foreign language.

Table 5. Foreign Language Level of Academics

Foreign Language Level	**Number**	**Percentage (%)**
Start	3	3,7
Centre	42	51,9
Forward	36	44,4
TOTAL	**81**	**100,0**

The overseas experience of the academics participating in the surveys is given in Table 6. While 82.7% of the academics have experience abroad, 17.3% do not have experience abroad.

Table 6. Academics' Experience Abroad

Experience Abroad	**Number**	**Percentage (%)**
Yes	67	82,7
No.	14	17,3
TOTAL	**81**	**100**

Academics with overseas experience mostly travelled to Italy, Spain, Israel, Japan and Poland for education and USA, Germany, Italy, Kazakhstan and Greece for study. For courses, they mostly travelled to USA, Israel, Czech Republic and Hungary. The purpose of the academic staff travelling abroad is 61% for education, 19% for study and 16% for courses.

Findings Related to Interdisciplinary Working Tendencies

While 69% of the academicians participating in the research have been involved in an interdisciplinary study, approximately 31% have not. When the study subjects of the academicians who have conducted an interdisciplinary study are analysed, animal husbandry ranked first with 33%, followed by Soil with 22%. On the other hand, Molocular Genetics, Field crops, Geographical indications, Variety Development, Ecology and organic chemistry were ranked last with 2%. The other subjects researched were Energy Use with 12%, Agricultural Economics and Irrigation with 10%, Horticulture with 5%, Food with 4% and Mathematical Physics with 7% (Table 7.).

Table 7. Interdisciplinary Study Status and Subjects

Interdisciplinary work	**Number**	**Percentage (%)**
Yes	56	69,1
No.	25	30,9
Total	81	100
Study Subject	**Number**	**Percentage (%)**
Livestock breeding	18	32,5
Soil	13	22,5
Energy Utilisation	7	12,5
Irrigation	6	10,0
Agricultural Economics	6	10,0
Mathematical Physics	4	7,5
Horticulture	3	5,0
Food	3	5,0
Geographical indications	1	2,5
Variety development	1	2,5
Ecology	1	2,5
Molecular Genetics	1	2,5
Field Crops	1	2,5
TOTAL	**56**	**100,0**

While the academics participating in the research ranked the third parties that they can work with in the region in their research projects according to their importance, "Provincial Directorate of Agriculture and Forestry" and "Other Çomü Units" took the first place. According to the available data, other universities, chambers of agriculture, international organisations, private sector, cooperatives and NGOs, municipalities, Çanakkale Chamber of Industry and Commerce, Çanakkale Commodity Exchange and Çanakkale Organised Industrial Zone were ranked last, followed by district governorships.

As seen in Table 8, the majority of the academicians who responded to the questionnaire are prone to inter-institutional cooperation in interdisciplinary studies. However, the fact that the cooperation with the Chamber of Industry and Commerce and the enterprises operating in the Organised Industrial Zone in the province is lower is a negative finding in terms of strengthening the integration between agriculture and industry.

As a matter of fact, in the 10th and 11th Development Plans, it was emphasised that in order to increase Turkey's international competitiveness and its share in world exports, a transformation in the manufacturing industry is essential. In this context, moving to a high value-added structure and increasing the share of high technology sectors are considered as the main objective. Therefore, strengthening the integration between agriculture and industry will not be successful unless it is supported by scientific research and data. In this sense, it is extremely important to carry out studies with the data received from industrial enterprises and in line with their needs in all kinds of researches prepared in the academic sense, in terms of commercialisation and transformation of knowledge into economic value.

Table 8. Preferred Institutions and Organisations in Interdisciplinary Studies

Third Parties	**Score**	**Order of Importance**
Provincial Directorate of Agriculture and Forestry	343	1
Other ÇOMU Units	343	1
Other Universities	342	2
Chambers of Agriculture	333	3
International Organisations	327	4
Private Sector	323	5
Cooperatives and Non-Governmental Organisations (NGOs)	309	6
Municipalities	278	7
Çanakkale Chamber of Industry and Commerce	277	8
Çanakkale Commodity Exchange	273	9
Çanakkale Organised Industrial Zone	261	10
District Governorships	255	11
TOTAL	**3664**	

According to the data we obtained from the academics who contributed to the research questionnaire with their answers on what they understand from the concept of interdisciplinary work, the most supported option was "solving problems that existing disciplines cannot solve alone by cooperating with different disciplines". "Producing high value-added knowledge", "Producing high value-added knowledge", 3. "Addressing problems on a different information plane", 4. "Establishing a link between subjects based on a problematic," 5. "Facilitating the transfer of theoretical scientific results to practice", 6. The relationship between positive sciences and humanities", 7. "Presenting knowledge and its complementary elements with open-mindedness", 8. "Analysing the data held by various institutions", 9. "Rational use of resources" and finally "Increasing the contribution of humanities in solving the problem" (Table 9.).

Table 9. What is Understood from the Concept of Interdisciplinary Work

What he understands	**Score**	**Order of Importance**
Solving problems that cannot be solved alone with different disciplines	383	1
Generation of high value-added knowledge	358	2
Addressing problems on a different level of knowledge	357	3
Establishing a connection between subjects based on a problematic	346	4
Facilitating the transfer of theoretical scientific results to practice	337	5
The relationship between positive sciences and humanities	322	6
To present knowledge and its complementary elements with an open mind	320	7
Analysing the data held by various institutions	319	8
Rational use of resources	317	9
Increasing the contribution of humanities insolving the problem	306	10
TOTAL	**3365**	

The disciplines that academicians who participated in the surveys conducted within the scope of the research are most related to interdisciplinary studies are given in Table 10. When the relationship status of the agricultural sector with other disciplines is investigated, when the data obtained from the academicians are examined, the academicians see the discipline of Ecology as important in the 1st place, followed by Biotechnology in the 2nd place, General Engineering Sciences in the 3rd place, Mathematics in the 4th place, Information and Technology in the 5th place, Chemistry in the 2nd place, Geography in the 7th place, Nanotechnology in the 8th place. Chemistry, 7. Geography, 8. Nanotechnology, 9. Sociology, 10. Physics, 11. Economics and Politics, 12. Law, 13. Psychology, 14. Behavioural Sciences and 14. History is the last discipline that academics consider important.

Table 10. Relationship Status of Agriculture with Other Disciplines

Other Disciplines	**Score**	**Order of Importance**
Ecology	393	1
Biotechnology	380	2
General Engineering Sciences	361	3
Maths	350	4
Informatics and Technology	350	5
Chemistry	344	6
Geography	342	7
Nanotechnology	339	8
Sociology	311	9
Physics	304	10
Economics and Politics	301	11
Law	287	11
Psychology	270	12
Behavioural Sciences	268	13
History	264	14
TOTAL	**3516**	

The difficulties encountered by the academics participating in the survey while conducting interdisciplinary studies are listed in Table 11. According to the table, when the difficulties encountered by academicians while conducting interdisciplinary studies are analysed, the information "Lack of the habit of conducting a holistic study" is ranked in the1st place. "Difficulties in breaking fixed-mindedness" ranked 2nd, "Difficulty in finding sufficient resources, time and experts" ranked 3rd, "Adaptation period to new subjects and terminology" ranked 4th, "Different approaches to complex problems" ranked 5th, "Prejudice against new and different information" ranked 6th, "Conflicts of interest in various fields" ranked 6th. "Conflicts of interest in various fields" 7th, "Professional prejudice" 8th, "Difficulty of working with specialists in other subjects" 9th, "Fear of encountering the risk offailure" 10th.

Table 11. Difficulties Encountered in Interdisciplinary Studies

Challenges of interdisciplinary work	**Score**	**Order of Importance**
Lack of the habit of conducting holistic studies	310	1
Difficulties in overcoming fixed mindedness	298	2
Difficulty in finding sufficient resources, time and expertise	293	3
Adaptation time to new topics and terminology	291	4
Different approaches to complex problems	288	5
Prejudice against new and different information	278	6
Adaptation time to new topics and terminology	276	7
Conflicts of interest in various fields	274	8
Occupational Prejudice	263	9
Difficulty in working with specialists in other subjects	260	10
Fear of facing the risk of failure	245	11
TOTAL	**2133**	

CONCLUSIONS

In our country, where agriculture is very important in terms of social and economic aspects, technology, innovation and R&D-oriented approaches have become widespread instead of production-oriented policies and approaches. As a matter of fact, in countries where agriculture is developed, the contribution of high value-added agricultural products to the economy is much higher. Therefore, the main objective of today's agricultural policies is to increase the qualitative impact of agriculture in addition to its quantitative size in the national economy. One of the most important tools in achieving this goal is interdisciplinary studies.

In this study, it was aimed to examine the interdisciplinary approaches and tendencies of academicians in scientific researches on agriculture by conducting a face-to-face survey with 81 academic staff working in Çanakkale Onsekiz Mart University Agriculture related departments. Survey results were interpreted with frequency tables and score analyses.

According to the results obtained from the research findings, 66.7% of the researchers were male, 33.3% were female and the average age was 45.37 years. Approximately 69% of the academicians participating in the study have participated in an interdisciplinary study, while 31% have never participated in an interdisciplinary study, so the rate of interdisciplinary study increased as the age of the researchers increased. The age range of academicians varies between 25 and 66 years. 33.8% of the academicians are professors, 30.0% are assistant professors, 12.5% are lecturers, 10% are associate professors, 7.5% are research assistants, and 6.3% are research assistants. It was determined that approximately 41% of the academics participating in the study have more than 20 years of professional experience. The majority of the academics have intermediate and advanced foreign language level, and the majority of them have experience abroad. According to the table, as the titles, professional experience and overseas experience of the academicians increased, their involvement in interdisciplinary studies also increased.

From the research findings, it is seen that more than 70% of the academicians have experience in interdisciplinary studies, and interdisciplinary studies are concentrated in the field of animal husbandry and soil. Researchers stated that they cooperate with Provincial Directorate of Agriculture and Forestry, other ÇOMU units and other universities as 3 institutions. In interdisciplinary studies, it is a widely adopted idea that problems that cannot be solved conceptually alone can be solved by co-operating with different disciplines. While ecology, biotechnology and various fields of engineering are the most common areas of interdisciplinary studies, sociology, psychology, history and other behavioural sciences are ranked last.

According to the data obtained from the research findings, the most important difficulty faced by academics while conducting interdisciplinary studies is the lack of a holistic study habit. The fear of encountering the risk of failure is the last information they consider important.

Based on these findings, it is suggested that academic staff working on agriculture should develop academic cooperation between departments in their own units and include institutions and organisations and other stakeholders in their research. On the other hand, all institutions and organisations related to agriculture, NGOs and especially industry and private sector should be cooperated and information should be produced in line with the needs of stakeholders. Another suggestion that can be made within the scope of the results to be obtained from the findings is to develop co-operation with humanities as well as positive sciences. Such an approach will make it easier for research to reach its target audience.

Again, based on the data we obtained, the importance of overseas education and language development of young and newly titled academics should be emphasised and interdisciplinary studies should be encouraged.

In interdisciplinary research, institutional cooperation and especially the analysis of existing institutional records should be targeted, and professional approaches should be adopted to avoid vicious circles such as professionalism and fixed-mindedness.

Strengthening the integration of agriculture, industry and technology will not be successful unless it is supported by scientific research and data. This means that in all kinds of academic researches, it is extremely important to carry out studies in line with the needs of the industry and technology in partnership with the data received from the institutions in order to commercialise the information and turn it into economic value.

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EXAMINATION OF PRODUCER SATISFACTION TOWARDS RICE SUPPORT POLICIES IN EDİRNE PROVINCE

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ABSTRACT

Paddy is an important foodstuff growing in every continent of the world. China, India, Indonesia, and Bangladesh are the leading countries in paddy production in the world. In Turkey, although paddy is grown in all regions, more than half of Turkey's production is takenplace in Edirne. Ipsala town of Edirne province is one of the important paddy production centresof Turkey. However, although paddy production is widespread in Turkey, the current supply cannot meet the demand. Therefore, it is important to encourage paddy support policies to establish the supply and demand balance in the market and to reduce the foreign exchange flowabroad. In this research, face-to-face surveys were conducted by selecting 100 producers producing rice from 17 villages of Ipsala town of Edirne province. Survey results were interpreted with descriptive statistics and Logistic Regression analysis. According to the research findings, almost all paddy producers benefit from the existing support policies and almost all of them state that they are satisfied with the existing policies. However, most of the producers state that policies do not affect their production decisions. In the light of the findings obtained from the research, the producers do not see the supports as money going directly into their pockets, and it is necessary to raise their awareness on increasing paddy production. Within the scope of this research, it can be suggested to policy makers to determine policy tools that will reduce paddy imports, increase producer profits, and reduce production costs.

Keywords: Paddy, Agricultural Subsidies, Producer Satisfaction, Edirne

INTRODUCTION

Paddy is cultivated in every continent of the world. China, India, Indonesia and Bangladesh are the leading countries in the world in paddy production. Although paddy is cultivated in Turkey, most of the time imports are made due to insufficient domestic supply of this product. Therefore, it is important to establish supply and demand balance in the market and to encourage paddy support and policies in order to stop the flow of foreign currency abroad (Yurdakoş 2009). In Turkey, paddy is the plant with the highest cultivation area after wheat, barley and maize in the cereals group and its cultivation areas continue to increase gradually. When paddy cultivation areas are analysed on the basis of provinces, Edirne ranks first with a share of 39.4% in total cultivation areas. Edirne is followed by Samsun (15.7%), Balıkesir (13.1%), Çanakkale (9.0%), Çorum (6.1%), Sinop (3.5%). When paddy production is analysedby provinces, Edirne ranks first with a share of 40.5% in total production. Edirne is followed by Samsun (16.2%), Balıkesir (12.4%), Çanakkale (9.6%), Çorum (6.1%) and Sinop (3.4%). While paddy production value in Turkey was 2,945 million

TL in 2019, it reached 3,777 million TL in 2020. Within the cereals group, paddy ranks fourth after wheat, barley and maize with a share of 6.93% in production value in 2021 (TUİK, 2021).

As in many countries, rice ranks first as a staple food in Turkey. Especially in Far Eastern countries, the consumption of this product is quite high. For this reason, every individual in the world consumes this product. The fact that paddy plant is rich in vitamins increases its consumption (Elmacı, 2012). Paddy is one of the important agricultural products of Turkey. Thrace Region, which has an important potential in this product, is also very important in terms of being a gateway to Europe. Although paddy production is carried out in 31 provinces in our country, the Marmara region is leading in paddy production. İpsala district of Edirne ranks first in paddy production. İpsala district of Edirne Province meets 35.0% of the total paddy production in Turkey (Arda et al., 2015).

In Turkey, agriculture has been supported in various ways especially since the beginning of the Planned Period in 1963. With agricultural support policies in our country, the state aims to meet the demand for sufficient food, to increase productivity in agriculture, to increase the income of the producer, to meet the consumption of the product produced in the market, to develop exports and to keep the economic and welfare level of the society in balance. Supports to agriculture and agricultural policies implemented are in the form of product support, input support, intervention in prices and low-interest credit support. However, it is also a fact that agricultural policies are constantly undergoing changes and reforms. Therefore, although different support policies have been implemented in agriculture since the foundation of the Republic, not enough progress has been made in terms of adequate and stable policies (Güzel, 2012). In order to overcome the problems of paddy production and marketing, direct sales to retailers after completing the processing and packaging processes in the processing units established by the producer organisations will solve the problems of the producers. Therefore, incentivising state policies are needed in this regard (Gaytancıoğlu, 2007). In this study, the level of paddy producers' utilisation of the support policies in practice and their level of satisfaction with these supports were examined.

MATERIAL AND METHOD

Primary and secondary data were used in the research. The data obtained through mutual questionnaires in the centre and 17 villages of İpsala district of Edirne province are primary data and constitute the main material of the research. Statistics published by national and international statistical institutions and other scientific studies on this subject constitute the secondary data of the research.

The total population of the study consists of producers engaged in paddy cultivation in İpsala district of Edirne province. The total population consists of 26.966 producers registered in the Farmer Registration System (ÇKS) in 28 villages of Ipsala district and in the centre. The sample of this research consists of 100 producers selected among the total producers. The sampling was carried out using the following formula with a margin of error of 0.10 and 95% confidence interval. The determined sample volume was distributed proportionally among the villages.

$$n = \frac{N\,p\,(1 - p)}{(N - 1)\,var^2\,px + p\,(1 - p)}$$

n = Number of farmers in the sampleN = Main population size
p = Main population proportion
var ² px = Variance of the main mass ratio
The questionnaires made in the form of mutual conversation with 100 producers were

transcribed and analysed in SPSS programme. In the analyses, firstly, general demographic and socio-economic findings were examined with the help of descriptive statistics and expressed asnumbers and rates. Logistic Regression Method was used to analyse the factors affecting the satisfaction of paddy producers with the supports in practice and their satisfaction with these supports.

Logistic regression, by calculating the estimated values of the dependent variable as probability, allows classification in accordance with probability rules, and can also determine the effect sizes of independent variables that affect the change of the dependent variable (Akgül and Çevik, 2003).

RESULTS AND DISCUSSION

When the policies related to paddy production are analysed, it is seen that Diesel, Fertiliser and Soil Analysis support is in the nature of agricultural input support and is intendedto reduce costs. Cotton and paddy are the crops with the highest diesel support. In these crops,250 TL diesel support is provided per decare. In addition, soil analysis support of 50 TL per sample is provided. Another policy to increase production in paddy is "Differential Payment Support". This support is the payment to the producer of the difference between the target price and the market price determined by taking into account the production and marketing costs of the relevant product. Within the scope of this support, a total of 17 products are subject to support, and the Difference Payment support given to paddy is 10 kurus per kilogram. Anotherpolicy instrument to which paddy is also subject is "Domestic Certified Seed Use Support". In this context, 8 TL per decare of paddy is supported.

In this part of the study, the factors affecting the satisfaction of paddy producers with thecurrent policies were analysed. The independent variables used in regression analyses for this purpose were determined as follows. Education, household population, farming experience (years), amount of land (da), household population (person), agricultural credit usage status, presence of non-agricultural income, frequency of meeting with experts, number of parcels (pcs), production area, livestock, agricultural income, non-agricultural income, keeping business records, internet usage status, organisation status, adequacy of supports.

Analysis of Factors Affecting Satisfaction with Diesel Diesel Support

Satisfaction of paddy producers with diesel oil support was analysed. In the model, the dependent variable was determined as 0: those who are not satisfied with diesel subsidy and 1:those who are satisfied with diesel subsidy (Table 1).

Analysis with Logistic Regression Model (Y= Satisfaction with Diesel Support)

Variables	***Coefficient***	***Std. Error***	***p-Value***	***Slope***
Fixed	-7.25727	3.27064	0.02649**	
Education	0.282032	0.47986	0.55671	0.04812
Household population	-0.217691	0.246322	0.37682	-0.0371422
Farming experience	**0.159364**	**0.0428997**	**0.00020*** **	**0.0271905**
Frequency of consultation with experts	0.402651	0.514908	0.43422	0.0686999
Internet use	1.20767	1.03631	0.24387	0.214769
Number of parcels	0.0515756	0.238826	0.82902	0.00879977
Production area	-0.0144521	0.0111743	0.19589	-0.00246581
Raising livestock	-0.465478	0.788753	0.55509	-0.0869604
Agricultural income	**0.715628**	**0.383813**	**0.06225***	**0.1221**
Having non-agricultural income	0.300921	0.835352	0.71867	0.0547025
Loan utilisation	0.696473	0.819429	0.39535	0.126028
Business record keeping	1.03361	1.00496	0.30371	0.213312
X^2: 34.1864 [0.0006]				

*** : 1% significance level, ** : 5% significance level, * : 10% significance level

According to the results of the analysis, farming experience of the farmers and their income from agriculture as a result of one-year production are the variables that are effective on satisfaction with diesel subsidy. Farming experience is significant for 1% and there is a positive relationship between farming experience and satisfaction with diesel subsidy. As the farming experience increases, the tendency to be satisfied with diesel subsidy also increases. Inother words, one year increase in farming experience increases the tendency to be satisfied withdiesel subsidy by 2.7%. An increase of 10.000 TL in annual income from agriculture increasesthe tendency to be satisfied with diesel oil support by 12,2%.

Fertiliser Support Analysis of Satisfaction

In the model in which satisfaction with fertiliser support was analysed, the dependent variable was determined as 0: those who were not satisfied with fertiliser support and 1: those who were satisfied with fertiliser support (Table 2).

Table 2. Logistic Regression Model (Y=Satisfaction with Fertiliser Support)

Variables	***Coefficient***	***Std. Error***	***P-Value***	***Slope***
Fixed	-10.439	3.61941	0.00392***	
Education	0.314865	0.492505	0.52262	0.059722
Household population	-0.050395	0.248105	0.83904	-0.00955868
Farming experience	**0.171796**	**0.0453167**	**0.00015*** **	**0.0325854**
Frequency of consultation withexperts	0.169669	0.516189	0.74239	0.032182
Internet use	1.82705	1.16258	0.11605	0.356931
Number of parcels	0.00845641	0.246736	0.97266	0.00160397
Production area	-0.0160049	0.0116916	0.17102	-0.00303573
Livestock breeding status	-1.29294	0.799999	0.10606	-0.288535
Agricultural income	**0.902884**	**0.40541**	**0.02594** **	**0.171255**
Non-agricultural income	1.26491	0.90418	0.16183	0.283695
Loan utilisation plan	0.743447	0.821793	0.36564	0.148388
Business record keeping	**1.9443**	**1.0735**	**0.07011***	**0.44322**
X^2: 43.0812 [0.0000]				

*** : 1% significance level, ** : 5% significance level, * : 10% significance level

According to the results of logit analysis; farming experience, income from agriculture, keeping business records have a positive effect on satisfaction with fertiliser support. Farmingexperience is significant for 1% and there is a positive relationship between farming experience and satisfaction with fertiliser support. As farming experience increases, the tendency to be satisfied with fertiliser support also increases. In other words, one year increase in farming experience increases the tendency to be satisfied with fertiliser support by 3.2%.

Agricultural income is significant for 5% and there is a positive relationship between agricultural income and satisfaction with fertiliser support. As agricultural income increases, the tendency to be satisfied with fertiliser support also increases. In other words, an increase inthe level of agricultural income increases the tendency to be satisfied with fertiliser support by17%.

Keeping business records is significant for 10% and there is a positive relationship between keeping business records and being satisfied with fertiliser support. Those who keep business records are 44% more likely to be satisfied with fertiliser support than those who do not keep business records.

Satisfaction Analysis of Difference Payment Support

In the model in which satisfaction with differential payment support was analysed, the dependent variable was determined as 0: those who were not satisfied with differential payment support and 1: those who were satisfied with differential payment support (Table 3).

Table 3. Logistic Regression Model (Y=Satisfaction with Differential Payment Support)

Variables	***Coefficient***	***Std. Error***	***P-Value***	***Slope***
Fixed	-9.06666	3.69702	0.01419**	
Education	0.180292	0.554698	0.74516	0.0249554
Household population	-0.240453	0.273615	0.37951	-0.0332827
Farming duration	**0.172068**	**0.0477372**	**0.00031*****	**0.023817**
Frequency of consultation with experts	0.1977	0.571364	0.72933	0.027365
Internet use	1.61603	1.22597	0.18745	0.242931
Number of parcels	-0.120806	0.271625	0.65650	-0.0167215
Production area	-0.0142905	0.0123629	0.24771	-0.00197804
Raising livestock	-0.78669	0.852429	0.35607	-0.130618
Agricultural income	**1.29127**	**0.453168**	**0.00438*****	**0.178733**
Having non-agricultural income	0.475859	0.947379	0.61546	0.0742355
Loan utilisation plan	0.280346	0.892516	0.75344	0.0399926
Business record keeping	**2.26768**	**1.14119**	**0.04691****	**0.468111**
X^2: 42.6394 [0.0000]				

*** : 1% significance level, ** : 5% significance level, * : 10% significance level

According to the results of logit analysis; farming experience, income from agriculture, keeping business records have a positive effect on satisfaction with differential payment support. Farming experience is significant for 1% and there is a positive relationship between farming experience and satisfaction with differential payment support. As farming experience increases, the tendency to be satisfied with differential payment support also increases. In otherwords, one year increase in farming experience increases the tendency to be satisfied with dieselsubsidy by 2.3%.

According to the results of the analysis, farmers' agricultural income level has an effect on their satisfaction with differential payment support. Agricultural income is significant for 1% and there is a positive relationship between agricultural income and satisfaction with differential payment support. As agricultural income increases, the tendency to be satisfied withdifferential payment support also increases. In other words, an increase in the level of agricultural income increases the tendency to be satisfied with the difference payment supportby 17%.

According to the results of the analysis, keeping business records is the variable that has an effect on farmers' satisfaction with differential payment support. Keeping business records is significant for 5% and there is a positive relationship between keeping business records and being satisfied with differential payment support. Those who keep business records are 46% more likely to be satisfied with differential payment support than those who do not keep business records.

Analysing Satisfaction with Basin Based Support

In the model in which satisfaction with basin-based support was analysed, the dependent variable was determined as 0: those who were not satisfied with basin-based support and 1: those who were satisfied with basin-based support (Table 4).

Table 4. Logistic Regression Model (Y=Satisfaction with Basin Based Support)

Variables	***Coefficient***	***Std. Error***	***P-Value***	***Slope***
Fixed	-8.51486	3.60443	0.01816**	
Education	0.589257	0.569121	0.30049	0.0684706
Household population	-0.098151	0.261345	0.70724	-0.011405
Farming experience	**0.15134**	**0.0452026**	**0.00081*** **	**0.0175855**
Frequency of consultation with experts	0.205405	0.553205	0.71041	0.0238677
Internet use	1.2892	1.12391	0.25135	0.162508
Number of parcels	-0.117956	0.255049	0.64373	-0.0137063
Production area	-0.00655332	0.0111714	0.55746	-0.000761485
Livestock breeding status	-1.20516	0.817298	0.14033	-0.189642
Agricultural income	**0.800717**	**0.420364**	**0.05680***	**0.0930418**
Non-agricultural income	0.942276	0.929426	0.31067	0.14132
Loan utilisation plan	0.896061	0.856171	0.29529	0.116194
Business record keeping	1.10597	0.949434	0.24407	0.173622
X^2: 29.478 [0.0033]				

*** : 1% significance level, ** : 5% significance level, * : 10% significance level

According to the results of logit analysis, farming experience and income from agriculturepositively affect satisfaction with basin-based supports.

Farming experience is significant for 1% and there is a positive relationship between farming experience and satisfaction with basin-based support. As the farming experience increases, the tendency to be satisfied with fertiliser support also increases. In other words, one year increase in farming experience increases the tendency to be satisfied with fertiliser supportby 1.7%.

Agricultural income is significant for 10% and there is a positive relationship between agricultural income and satisfaction with basin-based support. As agricultural income increases,the tendency to be satisfied with basin-based support also increases. In other words, an increase of 10,000 TL in agricultural income increases the tendency to be satisfied with fertiliser supportby 9.3%.

Analysis of Satisfaction with Soil Analysis Support

In the model in which satisfaction with soil analysis support was analysed, the dependent variable was determined as 0: those who were not satisfied with soil analysis support and 1: those who were satisfied with soil analysis support (Table 5).

Table 5. Logistic Regression Model (Y=Satisfaction with Soil Analysis Support)

Variables	***Coefficient***	***Std. Error***	***P-Value***	***Slope***
Fixed	-1.35682	4.57824	0.76695	
Education	0.448899	0.556824	0.42014	0.0203544
Household population	0.0895079	0.381121	0.81432	0.00405856
Farming experience	**0.142728**	**0.0581857**	**0.01417****	**0.00647171**
Frequency of consultation with experts	0.874223	0.909046	0.33620	0.0396399
Number of parcels	0.111524	0.28217	0.69267	0.00505683
Production area	-0.0091985	0.0101613	0.36533	-0.000417088
Livestock breeding status	0.924297	1.2919	0.47433	0.0312756
Agricultural income	-0.378394	0.608943	0.53434	-0.0171575
Non-agricultural income	-0.57737	1.00968	0.56743	-0.0216792
X^2: 29.478 [0.0033]				

*** : 1% significance level, ** : 5% significance level, * : 10% significance level

According to the results of logit analysis; farming experience has a positive effect on satisfaction with soil analysis support.

Farming experience is significant for 1% and there is a positive relationship between farming experience and satisfaction with soil analysis support. As the farming experience increases, the tendency to be satisfied with soil analysis support also increases. In other words, a ten-year increase in farming experience increases the tendency to be satisfied with soil analysis support by 6%.

Analysing the Satisfaction of Certified Seed Use Support

In the model in which satisfaction with seed use support was analysed, the dependent variable was determined as 0: those who were not satisfied with seed use support and 1: those who were satisfied with seed use support (Table 6).

Table 6. Logistic Regression Model (Y=Satisfaction with Seed Use Support)

Variables	***Coefficient***	***Std. Error***	***P-Value***	***Slope***
Fixed	-3.79799	3.70324	0.30509	
Education	0.391409	0.485029	0.41968	0.0332108
Household population	0.0339588	0.306402	0.91175	0.00288138
Farming experience	**0.112115**	**0.0432973**	**0.00961*****	**0.00951292**
Frequency of consultation with experts	0.11108	0.571671	0.84594	0.00942503
Number of parcels	-0.174429	0.257061	0.49742	-0.0148002
Production area	-0.00132322	0.00980229	0.89262	-0.000112275
Livestock breeding status	0.425487	1.19658	0.72215	0.0317776
Agricultural income	0.541671	0.431159	0.20900	0.0459604
Non-agricultural income	0.719063	0.880319	0.41403	0.076912
X^2: 17.3447 [0.0436]				

*** : 1% significance level, ** : 5% significance level, * : 10% significance level

According to the results of logit analysis; farming experience has a positive effect on satisfaction with seed analysis support.

Farming experience is significant for 1% and there is a positive relationship between farming experience and satisfaction with seed subsidy. As farming experience increases, the tendency to be satisfied with seed subsidy also increases. In other words, an increase in farmingexperience by ten years increases the tendency to be satisfied with seed subsidy by 9.5 per cent.

CONCLUSIONS

In the research, a face-to-face survey was conducted with 100 producers selected from 17 villages of Ipsala district where paddy production is carried out. In the analyses, firstly, general demographic and socioeconomic findings were examined with the help of descriptive statistics and expressed as numbers and rates. Logistic Regression Method was used to analyse the factors affecting the paddy producers' satisfaction with the supports in practice and their satisfaction with these supports.

When asked about the policies and supports in practice, 55% of the producers stated that they keep financial records, 4% physical records and 41% other records. On the other hand, when asked about the supports that producers benefit from, all of them benefit from diesel support, fertiliser support, differential payment support and basin-based support. The number of those receiving domestic certified seed use and production support and counselling support is 96. The number of producers receiving soil analysis support is 88. When the producers were asked whether they were satisfied with the supports they benefited from, 71 people stated that they were satisfied with diesel oil support, 68 people with fertiliser support, 76 people with soil analysis support, 74 people with differential payment support, 79 people with basin-based support payments, 81 people with domestic certified seed and fertiliser support and 92 people with consultancy support. Approximately 70% of the producers who benefited from the related supports stated that the supports did not affect their production decision. In addition, 54% of the producers stated that the supports provided were not sufficient.

Logistic regression analyses were performed to determine the factors affecting the satisfaction level of paddy producers with the existing supports. As a result of these analyses, the factors affecting the level of satisfaction with diesel and fertiliser support, basin-based support model, farming experience and income from agriculture, and the criteria of farming experience, agricultural income and keeping records in the enterprise were positively significant for differential payment support. For soil analysis support and certified seed support, farming experience is also positively significant. One year increase in farming experience increases the tendency to be satisfied with diesel oil support by 2.7 per cent. An increase of 10.000 TL in annual income from agriculture increases the tendency to be satisfied with diesel oil support by 12.21%. Keeping business records increases the tendency to be satisfied with fertiliser supportby 44.0%.

As a result, the satisfaction of the producers with the supports can generally be associated with the level of income they obtain from the supports. Therefore, considering that the subsidies given aim to close the supply deficit in paddy, the perception of the producers towards the policy instruments used is not in line with the general policy objectives in this regard. In other words, it can be concluded that the policy instruments of the state are not sufficiently understoodby the producers.

It is possible to list the solution suggestions for the problems obtained from the researchas follows;

1. It is necessary for the producers to adopt the aim of closing the supply deficit in production, rather than the supports that will provide direct income to paddy producers.
2. On the other hand, extension activities should be carried out to improve the awarenesslevels of younger producers.
3. By activating the existing agricultural organisations in the sector, the problems of paddyproducers at the marketing and production stages can be overcome.
4. Organised producers can also be encouraged to establish processing and packaging unitsby using the available rural development funds.
5. Considering the general success of the agricultural policies in practice, the bureaucraticintensity in the support system should be reduced and producers should have easier access to support.

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IMPROVING ROOTING PERFORMANCE OF ANATOLIAN SAGE (*Salvia fruticosa* Mill.) CUTTINGS WITH MICROBIAL FERTILIZATION TREATMENT

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ABSTRACT

Anatolian sage (*Salvia fruticosa* Mill.) is a species distributed in different locations from the northwest to the southwest of Turkey with commercial significance. It can be reproduced both generatively and vegetatively. The successful and desired condition of rooting in sage cuttings depends on various factors, such as the plant species, age of plant, timing for cutting, type of cuttings, plant growth regulators, rooting media and environmental conditions. Microbial fertilizers or biofertilizers are substances containing live microorganisms that accelerate plant growth when applied to the soil and plant surfaces. This study aims to investigate the impact of different microbial fertilizer types and application doses on the rooting of Anatolian sage cuttings (*Salvia fruticosa* Mill.). The experiment is conducted in a randomized complete block design with four replications. The experiment consists of 2 microbial fertilizers x 5 applications x 4 replications. EM.1 and EM.5, commercial microbial fertilizers produced by EM Agriton are used in the study. Rooting ratio, Root number, root length, root quality, fresh root weight and dry root weight are measured in the study. According to the results, EM.1 microbial fertilizer with 1.5 doses showed better root development results than other doses and microbial fertilizers.

Keywords: *Salvia fruticosa* Mill., Microbial fertilizer, Rooting, Cuttings.

INTRODUCTION

Salvia fruticosa Mill. (Anatolian sage) is one of the most important species of the genus *Salvia*. *Salvia fruticosa* Mill. is native to the Mediterranean region, especially Western and Southern Anatolia and Greece. The leaves of the plant carry 1-3% essential oil. The main components of the essential oil are 1,8-Cineol, Champer, Borneol and Thujon. Thujon (5%) is very low in this species (Bayram &Sönmez, 2006).

Sage is a plant that can be reproduced both generatively and vegetatively. In vegetative production, it can be produced by dividing axillary shoots or old plants into parts. The sowing time for seed production is spring or fall (Bayram & Sönmez, 2006).

Seed propagation and growth are generally slow, seed germination problems and large variations may occur both morphologically and in terms of characteristics such as essential oil composition. Propagation by cuttings produces vigorous seedlings and fast production. In addition, each cutting has the genetic characteristics of the main plant with the desired characteristics. During propagation with cuttings, healthy and desired seedlings depend on many factors such as plant species, age of the cutting, cutting time, type of cuttings, plant growth regulators, rooting media and environmental conditions. Growth regulator auxins have a positive effect on root formation. The function of auxin is to initiate root formation and rooting (Sarı & Kaçar, 2019).

Commercial formulations of live microorganisms that provide the plant nutrients necessary for the plant and transform nutrients to absorbable form are called "Microbial fertilizers" (Aydın, 2014).

Microbial fertilizers or biofertilizers are substances containing live microorganisms that can form colonies in the rhizosphere or plant tissue when applied to soil, plant surfaces and accelerate plant growth by increasing the usefulness or amount of primary nutrients for the plant (Okur, 2014).

Medicinal plants are used in many fields, especially in medicine, due to their secondary metabolites. The cultivation of this group of plants, which directly affects human health, has gained importance in recent years in line with the principles of organic agriculture. In particular, it is emphasized that the production material to be used during this production (seedling cultivation) should be made without the use of chemicals. For this purpose, the search for alternatives to chemicals in seedling cultivation continues. It was also observed that there is a problem with the germination of plants belonging to the genus Salvia. In order to find solutions to these problems and at the same time to carry out seedling cultivation with the principles of organic agriculture, it was aimed to carry out a study to investigate the effects of microbial fertilizers on seedling production of Anatolian sage. For that, in this study, it was aimed to investigate the effect of microbial fertilizer types and applications on the cuttings of the Anatolian sage (*Salvia fruticosa* Mill.) plant and to contribute to the propagation for production.

MATERIAL AND METHOD

This experiment was conducted in the greenhouse and laboratory of Aydın Adnan Menderes University (ADU) Faculty of Agriculture, Department of Field Crops. In the study, cuttings were obtained from Anatolian sage (*Salvia fruticosa* Mill.) plants in the medicinal plant experimental area of ADU Faculty of Agriculture Research and Application Farm in October 2017. Two microbial fertilizers (EM.1® and EM.5®) were purchased from the market of EM Agriton BV company and applied to the cuttings.

EM.1® microbial fertilizer contains lactic acid bacteria: *Lactobacillus casei*, *Lactobacillus rhamnous*, *Lactobacillus casei*, Yeast: *Saccharomyces cerevisiae*, *Phototrophic bacteria*: *Rhodopseudomonas palustris*. EM.5® contains *Pseudomonas* spp., *Rhizobium* spp., *Azotobacter* spp., *Bacillus* spp., *Seratia* spp., *Aspergillus* spp.

Anatolian sage cuttings were taken from the plants in the field and prepared in the greenhouse to be about 10 cm in length. Fertilizers were prepared with four different applications of microbial fertilizers: control, half of the recommended dose, 1 times of recommended dose, 1.5 times the recommended dose, and two times the recommended dose. Then, the cuttings were dipped into this mixture kept for 4 hours, and planted in perlite-filled viols. For each treatment, 24 cuttings were used, and the plot was counted as a single viol in the experiment. There were four replications of the experiment for each treatment. The experiment was organized according to the two-factor randomized plots experimental design with four replications. Accordingly, the experiment consisted of 40 viols with two microbial fertilizers x 5 treatments x 4 replications. A total of 960 cuttings were planted for this experiment.

Figure 1. *Salvia fruticose* plants in greenhouse condition.

The following observations were made on the seedlings after removal from viols.

1. Rooting rate (%): Rooting was determined by examining the seedlings in each treatment separately. The seedlings that formed one or more adventitious roots were considered rooted, and the results were calculated as % by counting the number of roots.

2. Root number: The number of developed main roots was determined.

3. Root length (cm): It was determined by measuring the length of the seedlings from the root collar to the tip of the roots.

4. Root quality: The root system of each seedling was determined numerically in 5 separate groups with values ranging from 0 to 4 (Çelik, 1982). In this evaluation; 0=no rooting, 1=poor rooting, 2=moderate rooting, 3=good rooting, 4=very good rooting.

5. Fresh root weight (g): The roots of each seedling were cut from the rooting point, washed, dried with a paper towel, and weighed.

6. Dry root weight (g): Weighed fresh roots were dried in an oven at 70 ^{o}C for 48 hours and weighed.

Statistical analysis of the data obtained was performed according to the SAS package program. The differences between the averages were grouped according to the significance level (5% or 1%) in LSD test.

RESULTS AND DISCUSSION

According to the results, the effect of different fertilizers was found significant in rooting rate (%) and root length (cm) parameters, doses in root length, fresh root weight and dry root weight parameters and lastly fertilizer×dose interactions in fresh root weight, rooting rate and dry root weight parameters. However, there are no significant differences in root number and root quality parameters (Table 1.).

Table 1. Variation analysis results of different microbial fertilizers and doses on *Salvia fruticose* cuttings.

Sources of Variation	DF	Rooting Rate	Root Number	Root Length	Root Quality	Fresh Root Weight	Dry Root Weight
Rep	3	43192	0,34	0,11	0,89	0,56	0,43
Fert.	1	43409*	0,01	39,58**	0,12	0,37	0
Dose	4	0.72	1,11	5,85**	1,9	3,5*	3,15*
Fert x Dose	4	11475**	1,88	1,57	2,03	8,28**	7,63**
Error	27						
Total	39						

(P<0.05)*, (P<0.01)**

According to the fertilizer × dose interaction, the highest rooting rate parameter (%) was obtained from Em1 1.5 doses (80.20%) and the lowest in Em1 ½ doses (46.86%) (Table 2).

The effect of different fertilizer doses on the root number of cuttings was found insignificant. However, the highest number of roots was observed in dose EM1 control (13.43 pieces) and the lowest root number was observed in Em1 dose 2 (9.07 pieces).

It was observed that different fertilizers were significant and Em5 fertilizer (20.61 cm) was better than Em1 fertilizer (15.39 cm) as a result of the comparison of microbial fertilizers for root length (cm). Also, fertilizer doses were significant in root length (cm) and the highest root length was observed in dose 2 (21.10 cm) and the lowest root length was observed in dose ½ (15,39 cm) (Table 2).

Table 2. Effect of different microbial fertilizers and doses on rooting rate, root number and root length values

Fert./Doses	Rooting Rate			Root Number			Root length		
	EM1	EM5	Mean	EM1	EM5	Mean	EM1	EM5	Mean
0	52.05 **bc**	67.71 **ab**	59.88	13.43	10.16	11.79	13.53	20.84	17.18 **BC**
0.5	46.86 **c**	79.17 **a**	63.01	10.61	10.98	10.79	12.30	18.79	15.54 **C**
1	51.04 **bc**	77.08 **a**	64.06	10.93	10.98	10.96	13.43	19.96	16.70 **C**
1.5	80.20 **a**	58.33 **bc**	69.27	10.81	11.77	11.29	17.42	21.53	19.48 **AB**
2	63.52 **abc**	56.25 **bc**	59.89	9.07 b	10.61	9.84	20.26	21.93	21.10 **A**
Mean	58.74	67.71	63.22	10.97	10.90	10.93	15.39 **B**	20.61 **A**	18.00

Although the difference between the different microbial fertilizer doses was insignificant for root quality parameter, the highest average root quality scale value was observed in the 1 dose application (2.56 good). The lowest average root quality scale value was obtained with the ½ dose treatment (2.03 medium). Also, numerically Em5 (2.32 medium) was better than Em1 (2.27 medium) for different fertilizers.

According to the interaction, the highest value for the seedling dry root weight parameter (g) was observed in Em1 1.5 dose (7.25 g) and the lowest value was observed in Em1 ½ dose (2.54 g).

When we evaluated fresh root weight results for fertilizer × dose interactions, the highest value was observed in the 1.5 dose of Em1 (69.26 g), and the lowest value was observed in the ½ dose of Em1 (21.41 g) (Table. 3).

Table 3. Effect of different microbial fertilizers and doses on root quality, fresh root weight and dry root weight values

Fert./Doses	Root Quality			Fresh Root Weight			Dry Root Weight		
	EM1	EM5	Mean	EM1	EM5	Mean	EM1	EM5	Mean
0	2.66	1.80	2.23	29.37 **cd**	31.37 **bcd**	30.37	3.75 **cde**	3.51 **de**	3.63
0.5	1.91	2.15	2.03	21.41 **d**	46.29 **bc**	33.85	2.54 **e**	5.38 **bc**	3.96
1	2.40	2.72	2.56	32.13 **bcd**	47.93 **b**	40.03	3.70 **cde**	5.55 **ab**	4.63
1.5	2.51	2.48	2.50	69.26 **a**	34.69 **bcd**	51.97	7.25 **a**	4.00 **bcde**	5.63
2	2.10	2.19	2.14	46.96 **b**	28.21 **b**	37.59	4.67 **bcd**	3.29 **de**	3.99
Mean	2.32	2.27	2.29	39.82	37.70	38.76	4.39	4.35	4.37

Elkoca et al. (2001) conducted a study on the effect of nitrogen bacteria inoculation on sugar beet growth using eight different bacterial species from *Bacillus* (M-3, M-10, M-13, M-27, M-58, BA-140), *Burkholderia* (BA-7), and *Pseudomonas* (BA-8) genera. The parameter of fresh and dry root weight (g) per plant was examined, and the results showed significant increases with bacterial treatments compared to the control (uninoculated). It was found that significant differences were found among the strains based on all examined criteria. *Bacillus* M-58, *Burkholdria* BA-7, *Bacillus* M-13, and *Bacillus* M-27 proved to be the most effective strains.

Research conducted by Hua et al. (2022) and Zeping et al. (2018) has shown the effectiveness of microbial fertilizers. Their studies suggest that the use of fertilizers containing bacteria with growth regulators, such as secreted indoleacetic acid, can help regulate primary growth and development hormones in rice plants. These can enhance the plant's ability to absorb nutrients from the soil, stimulate root and shoot growth, and influence the formation of essential organs such as roots and shoots.

Çakmakçı et al. (2007) 's research investigated the nitrogen-fixing, phytohormones producing and phosphorus-solubilizing properties of nine plant growth-promoting rhizobacteria on wheat and spinach under greenhouse conditions. The rhizobacteria examined were Bacillus cereus RC18, *Bacillus licheniformis* RC08, *Bacillus megaterium* RC07, *Bacillus subtilis* RC11, *Bacillus* OSU142, *Bacillus* M-13, *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05, and RC14. According to study phytohormone-producing bacteria yielded the highest root weight and root number values but did not affect root length. Nitrogen-fixing and phosphorus-solubilizing bacteria boosted the nitrogen and phosphorus contents in the plants. Under greenhouse conditions, Plant Growth-Promoting Rhizobacteria (PGPR) positively affected the growth and yield of plants.

The studies reviewed here demonstrate the potential benefits of microbial fertilizers and plant growth-promoting rhizobacteria (PGPR) on plant growth and yield. Elkoca et al. (2001) found that inoculating sugar beet with bacterial strains from *Bacillus*, *Burkholderia*, and *Pseudomonas* genera significantly increased fresh and dry root weight compared to the control. Similarly, the present study found that the use of Em1 microbial fertilizer significantly increased fresh and dry root weight, with the 1.5 dose yielding the highest values. Hua et al. (2022) and Zeping et al. (2018) reported that the incorporation of bacterial growth regulators can enhance the capacity of plants to absorb nutrients, promote root and shoot growth, and impact key organ development. Çakmakçı et al. (2007) research demonstrated that PGPR can positively affect the growth and yield of wheat and spinach, with phytohormone-producing bacteria yielding the highest root weight and root number values, and nitrogen-fixing and phosphorus-solubilizing bacteria boosting the nitrogen and phosphorus contents in the plants. Our findings are consistent with these studies, highlighting the potential benefits of microbial fertilizers and PGPR in promoting plant growth and yield.

CONCLUSIONS

For improving the dry root weight and fresh root weight of sage cuttings, it is recommended to use 1.5 doses of Em1 microbial fertilizer. This dosage has shown a significant positive impact on the dry root and fresh root weight parameters. To enhance the rooting rate of sage cuttings, applying a 0.5 dose of Em5 microbial fertilizer can be advised. This dosage has been found to significantly affect the rooting rate parameter. In terms of root number, it is suggested not to use any microbial fertilizer (0 doses) as it yielded the best results. Applying other doses may not have the same positive impact on root numbers. If the aim is to increase root length in sage cuttings, it is recommended to use 2 doses of Em5 microbial fertilizer. This dosage has shown a significant effect on the root length parameter.

These suggestions are based on the specific findings of the study and can help optimize the general root quality of sage cuttings by using specific doses of Em1 and Em5 microbial fertilizers. However, it is essential to consider other factors such as soil conditions, climate, and specific requirements of the sage plants when implementing these recommendations. It is recommended to conduct further research and experimentation to validate these suggestions in different growing conditions.

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INDUSTRIAL AND BIOTECHOLOGICAL USES OF β MANNANASE ENZYME

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ABSTRACT

Enzymes are organic molecules involved in the realization of chemical reactions in all living cells. The importance of enzymes, which are involved in almost every aspect of our lives, is increasing day by day. Enzymes are involved in almost all stages of the biosynthesis and biodegradation process (coding, folding, and functionality) faster than expected and with high precision. In every process from a single-celled organism to a complex living organism, protein enzymes produced by living cells are involved in the decomposition of organic and inorganic substances, as well as in the formation or destruction of biomass. Ecological and efficient alternatives to industrial processes increase interest in the use of microorganisms and enzymes as biocatalysts. In addition, enzymes are more environmentally friendly by reducing the use of other chemicals and have advantages such as higher product quality, energy saving, and biodiversity conservation, making their use indispensable in many areas of industry. Mannans are essential components of hemicellulosic fragments of softwoods such as chestnut, tulip poplar, and cypress and are found in plant tissue. The degradation of heteromannan polysaccharide, which is included in the structure of the plant cell wall, is carried out through synergistic activation of enzymes that hydrolyze mannans. There are two enzymes of major importance that degrade heteromannan polysaccharide. These enzymes are endo-β 1,4 Mannanase (EC.3.2.1.78) and exo-β 1,4 Mannosidase (EC.3.2.1.25). β Mannanase enzyme facilitates the digestion of plant material by breaking down the mannan in plant cell walls. In this way, the nutrients contained in plant-based foods are absorbed more effectively by the human or animal digestive system. β mannanases can be synthesized by actinomycetes, animals, plants, bacteria and fungi. Used as an industrial enzyme, mannanases are involved in many industrial fields because they are active in a wide pH and temperature range. The application areas of β-mannanases include animal feed, food, bio-refinery, textile, detergent, paper and pulp. The aim of this study is to provide information about the use of β-mannanase enzyme in industrial and biotechnology fields and to provide a resource for future studies.

Keywords: Enzyme, β Mannanase, Mannanase, Industrial Enzymes, Heteromannan

INTRODUCTION

Enzymes are protein molecules that catalyze biochemical reactions in cells. Enzymes, which have many important metabolic functions in cells, have entered our lives for different purposes (Wiseman 1987). Chemical molecules catalyzed by enzymes and participating in the reaction with enzymes are called substrates. The enzyme reacts only with the substrate compatible with the three-dimensional structure of its active site. Therefore, an enzyme acts only on a certain type of substrate (Erkaya 2006). Enzymes, which have important metabolic functions in the cell, are proteins that catalyze biochemical reactions.

Enzymes used as industrial enzymes are expected to have some capabilities. These are longevity and high resistance, the ability to use the substrate involved in the reaction correctly even if the environment in which it will show activity is different from the intracellular conditions, and the ability to work without degradation in high temperature, low temperature, acidic or basic conditions used in the industrial environment. Considering these demands, enzymes need to be regulated to meet the needs (Gray et al., 2000). Generally used enzymes: Constant kinetics, optimum pH and temperature, resistance of the enzyme to solvents, reaction and substrate specificity, cofactor requirement, molecular weight and subunit structure.

Due to the gradual development of enzyme technology, the diversity of the usage areas of the products, and the high economic value of the products, various research in the field of biotechnology related to industrial enzymes is becoming more important (Solomon El et al., 1996).

Products produced for biotechnological purposes include lactic acid, insulin, antibiotics, bioethanol, and enzymes. Although all of these products have important areas of use, enzymes are the most important biotechnological product groups in food and other industries. Enzymes were previously isolated from plant and animal sources by various extraction methods. However, due to the increasing need for enzymes, new production techniques have emerged. One of these methods is the production of enzymes with natural or recombinant strains. One of the enzymes that can be produced by this method is β-mannanases. β-mannanases are used in different fields such as animal feed improvement, cleaning products, the paper industry, and coffee production. β-mannanases, which have a wide range of applications, are produced by yeasts, molds, and bacteria. (Koç, C. Y. 2021).

In recent years, there has been a growing interest in the development of biorefinery processes for the conversion of lignocellulosic wastes into second-generation biofuels and value-added products with potential industrial applications (Menon and Rao 2012; Paone et al., 2020). Lignocellulose is the primary structural component of woody and non-woody (e.g. grass) plants and is composed of cellulose, hemicellulose, and lignin (Ragauskas et al., 2006; Sanderson 2011). Lignocellulosic wastes are used in pulp and paper, food, and agriculture (Moreira et al., 2008; Saini et al., 2015). Among lignocellulosic wastes, agro-industrial residues have received special attention as sustainable feedstocks for biorefinery due to their abundance and cost-effectiveness (Anwar et al., 2014; Rodrigues et al., 2020). Reports indicate that cellulose and hemicellulose account for more than 50% of the dry weight of agro-industrial wastes (Sanchez et al., 2009; Rodrigues et al., 2020).

Hemicellulose, the second most abundant carbohydrate biopolymer on earth, is a member of various types and structural polysaccharides found in plant cell walls (Scheller et al., 2010; Van Dyk et al., 2012). These structural polysaccharides are generally composed of monomer sugar compounds: d-xylose, d-mannose, d-glucose, d-galactose, l-arabinose, 4-O-methyl-d-glucuronic acid (Scheller et al., 2010; Yamabhai et al., 2016). Xylan and mannan are the most abundant structural polysaccharides of heteropolymeric hemicellulose content in hardwood and softwood, respectively (Moreira et al., 2008; Malgas et al., 2015; Chauhan et al., 2017). Mannan is also found in the endosperm wall of seeds (coconut, coffee, and locust bean) and as a non-starch replacement component in the coffers (konjac, ivory nut, and guar) (Yamabhai et al., 2016; Saittagaroon et al., 1983; Rodrıguez-Gacio et al., 2012). Mannan has been reported as a cell wall component of several seaweed species belonging to the Chlorophyta and Rhodophyta phyla (Stiger-Pouvreau et al., 2016).

Mannans

Mannans are a series of non-crystalline hemicelluloses found in many living organisms such as bacteria, yeast, fungi, and plants. Mannans are found in the cell walls of different cell and tissue types in plants. (Meier and Reid, 1982) Currently, mannans have gained increasing interest in both academic and industrial research based on the growing demand for efficient use of renewable resources for sustainable development. This is not only because of the important role of these polysaccharides in plant cell wall formation (Whitney et al., 1998; Handford et al., 2003) but also because they are abundant sources of untapped raw materials that can be used in many fields. Thanks to this advantage, they are preferred in many industrial fields such as biofuel production, food and feed industries, cleaning, and many more (Yamabhai et al., 2014).
Function and classification of mannans

β-mannans

They have backbones composed of β-1,4-linked mannose residues, and β-1,4-linked mannosyl and glucosyl residues are likely to be randomly distributed in the backbones of heterogeneous mannans (McCleary et al., 1985). In addition, mannose residues in the main chain may be grafted by α-1,6-linked galactoses forming branched mannans. According to the main chain composition and side chain reaction, these mannans are classified into four main groups: pure mannans, glucomannans, galactomannans, and galactoglucomannans.

These mannan species mainly function as seed storage and/or structural components (Matheson 1990). In legumes, galactomannan as a seed storage component occupies about 30% of the dry weight of the seed (Buckerridge 2010). In the Type III primary cell wall of many ferns, structural mannans function as cellulose cross-linked hemicelluloses, a role that xyloglucans and glucuronoarabinoxylans have in other plants (Rodriguez-Gacio et al., 2012). In conifers, galactoglucomannan is the main structural mannan found in the secondary cell wall (Rowell et al., 2005). In addition to their function as storage and structural saccharides, mannans have an important role as signaling molecules in plant cell differentiation (Benova-Kakosova et al., 2006).

Pure mannan

It is usually found in the seed endosperm of non-legume plants such as ivory walnut (Phytelephas macrocarpa) and tomato (Solanum lycopersicum) (Toorop et al., 1998; Moreira, 2008). This type of mannan is also found in the seeds of pinho cuiabano (Schizolobium amazonicum) belonging to the Leguminosae family (de O Petkowicz et al., 2001). In pure mannan, the linear chain contains only β-1,4-linked D-mannosyl residues (Figure 1). This mannan is insoluble in water and therefore acts mainly as a structural hemicellulose in these plant tissues and plays an important role in resistance to mechanical damage (Toorop et al., 1998; Moreira, 2008). Pure mannan extracted from ivory nuts can form two different types of crystals: mannan I (also called mannan A) and mannan II (also called mannan B). The main differences between the two fractions are solubility, degree of polymerization (DP) (Aspinall et al., 1953; Meier, 1958), and morphology (Meier, 1958; Charrier and Rouland, 2001). Mannan I is highly crystalline with a low molecular weight. This mannan polymer is soluble in 6% (wt/wt) sodium hydroxide solution (Chanzy et al., 1984; Chanzy et al., 1987). In contrast, mannan II is less dense and crystalline and is characterized by a high molecular weight (Mackie and Sellen, 1969). It is insoluble even in high concentrations of sodium hydroxide solution (Chanzy et al., 1984; Millane and Hendrixson, 1994).

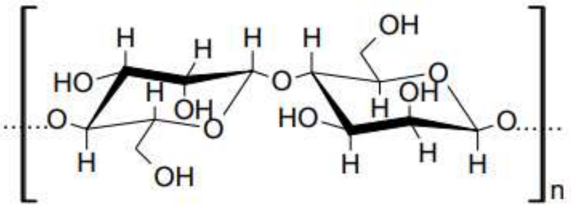

Figure 1. Pure mannan structure

Galactomannan

It is a water-soluble polysaccharide and the main hemicellulose in the seeds of legume plants but is also found in the seeds of several non-legume plants such as Cocos nucifera and Elaies guineensis (Nishinari et al., 2007). In these plant seeds, galactomannan not only provides energy for seed germination but also has the function of preventing seeds from drying out by retaining water, preventing denaturation of proteins important for germination (Dey, 1978; Moreira, 2008). In galactomannan, the backbone is formed by β-1,4-linked D-mannosyl substituted by α-1,6-linked galactosyl as a side group (Figure 2). This mannan is water soluble and its solubility is affected by the number of hydrophilic galactoses attached to the main chain. Three of the most commercially important galactomannans are carob galactomannan from the carob or locust bean tree (Ceratoniasiliqua), guar gum extracted from guar beans (Cyamopsistetragonoloba or Cyamopsispsoraloides) and tar gum isolated from the tara tree (Caesalpiniaspinosa). Carob galactomannan, known as carob gum (E-number: 410), is extracted mainly from the seed endosperm of the carob tree, which is native to the Mediterranean region. Normally, 100 g/kg seed (pod weight) contains roughly 320-400 g/kg of highly purified carob galactomannan. Carob gums are used in the food industry, e.g. in ice cream and ketchup. Typical carob galactomannan has a mannose/galactose substitution level of 4:1 (McCleary and Matheson, 1975) and is viscous and relatively stable in different pH solutions. Guar gum (E-number: E412), the main alternative to carob galactomannan, is isolated from the seeds of the guar tree, a tree grown in abundance in northwest India, Pakistan, and the USA. World production of guar is about 150,000 tons per year, with about two-thirds originating in Pakistan. Commercial guar is widely used for coating/hardening paper in cattle feed and gum production, as it is cheaper than carob. More recently, this gum has found use in hydraulic fracturing of oil (fracking). Guar gum has a mannose: galactose substitution level of 2:1 (McCleary and Matheson, 1975). Tara gum (E-number: E417) is obtained from the tara bush, which grows mainly in Ecuador, Peru, and East Africa. This mannan is widely used in the food industry to control ice crystal growth in frozen desserts and to improve the gel structure of meat-based products. The viscosity of tara gum is similar to that of locust bean and guar gum in cold solution but has higher viscosity in heated solution compared to these two types of galactomannan. Typically, the mannose: galactose ratio of tara gum is 3:1 (Dea, 1993).

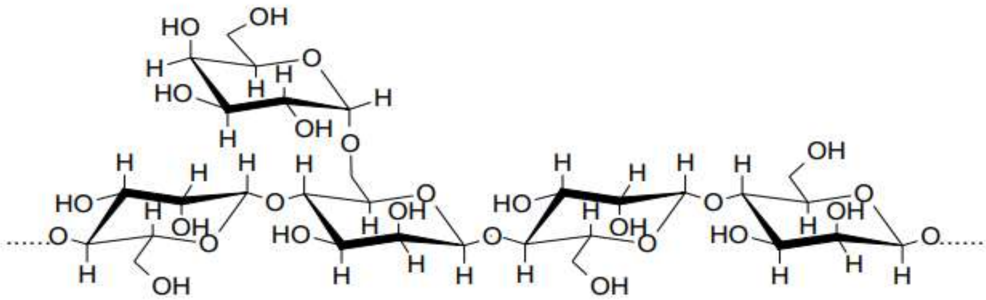

Figure 2. Galactomannan structure

Glucomannan

It is found as a minor structural hemicellulose in hardwoods and grasses (Scheller and Ulvskov, 2010). Glucomannan and xylan have been proposed to form a polysaccharide network closely related to cellulose. Glucomannan is a linear chain of D-mannosyl and D-glucosyl residues linked by β-1,4-bonds and frequently acetylated (Figure 3). These polysaccharides are soluble in water with a mannose: glucose ratio ranging from 4:1 to 1:1 (Meier and Reid, 1982) and a DP value higher than 200. In the konjac plant, about 60-80% of the tuber consists of glucomannan, which acts as the main storage carbohydrate. As Konjac glucomannan is water soluble and abundant, it is widely used in the food industry and pharmaceutical research. Konjac glucomannan (E number: 425) has a mannose: glucose ratio of approximately 1.5:1 (Dey and Dixon, 1985). Generally, 5-10% of the backbone residues of konjac glucomannan are linked to acetyl groups by ester bonds (Maekaji, 1978). These extra chemical modifications significantly affect konjac glucomannan properties such as solubility and gelation. For example, at high temperatures, the higher degree of acetylation weakens the network structure of konjac glucomannan gel (Penroj et al., 2005).

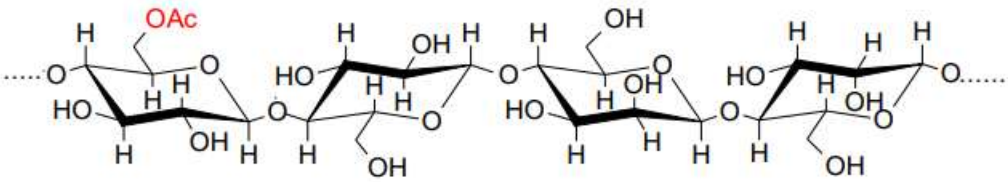

Figure 3. Acetylated glucomannan structure

Galactoglucomannan

It is the most complex mannan. Its backbone consists of β-1,4-linked D-mannosyl and D-glycosyl residues decorated with α-1,6-linked galactosyl side chains (Figure 4). Usually, backbone mannopyranose residues are acetylated at the hydroxyl groups of the C2 or C3 positions and the acetylation ratio is estimated to be 1:3-4. In general, acetylated galactoglucomannan extracted from the lignified secondary wall has a DP range between 100 and 150 and two groups can be distinguished. The first group is rich in galactose (5-8% w/w dry weight) and water-soluble, with a ratio of mannose:glucose: galactose residues of 3:1:1. The second group is poor in galactose (10-15% w/w dry weight) and aqueous alkali-soluble with a ratio of mannose:glucose: galactose residues of 3:1:0.1 (Timell, 1967). Galactoglucomannan is an abundant hemicellulose in many softwood species such as Norway spruce (Picea abies), which contains about 10-20% O-acetylated galactoglucomannan (Timell, 1967; Willför et al., 2005). Economically important Norway spruce is grown in the Scandinavian countries and is mainly used for timber or in the pulp and paper industry. Spruce galactoglucomannan, one of the most characterized and extensively studied galactoglucomannans, has a mannose:glucose: galactose ratio of 3.5-4.5:1:0.5-1 (Sundberg et al., 2000; Hannuksela and du Penhoat, 2004; Hannuksela et al., 2004). In addition to softwoods, it is found in other tree species, e.g. Populus monilifera, and also in club moss (Willför et al., 2008), blackberries (Cartier et al., 1988) and ferns (Bailey and Pain, 1971).

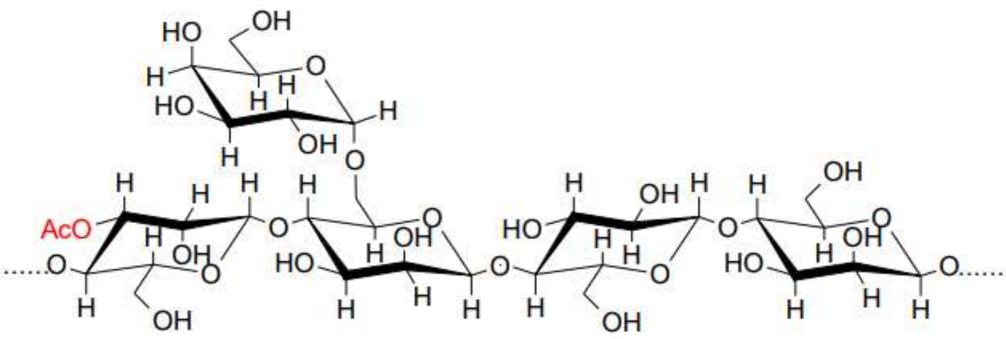

Figure 4. Acetylated galactoglucomannan structure

Mannan's biosynthesis and degradation

Mannan and other hemicelluloses are synthesized in the Golgi complex and then deposited in secretory vesicles and transferred to the plasma membrane for eventual delivery to the cell wall (Buchanan et al., 2000). Mannan synthesis can be divided into two main steps: backbone elongation and side-chain substitution. In the first step, elongation of the β-1,4-linked backbone of pure mannans, glucomannans, and galactoglucomannans is mediated by the cellulose synthase-like A (CSLA) family (Liepman et al., 2005; Goubet et al., 2009; Liepman and Cavalier, 2012; De Caroli et al., 2014). Recently, CSLD gene products have also been reported to be involved in pure mannan synthesis in Arabidopsis (Verhertbruggen et al., 2011; Yin et al., 2011). While glycosyl residues are believed to be iteratively attached to the non-reducing end of the elongating polysaccharide chain, an alternative route suggested that the reducing end of the elongating chain may also accept the addition of glycosyl residues (York and O'Neill, 2008). In the second step, galactosyltransferases add single α-1,6-galactopyranosyl residues to the backbone residues of β-1,4-mannans or β-1,4-glucomannans (Edwards et al., 2002).

Mannans are known to be involved in various biological processes involving cell wall degradation (Buckerridge, 2010). For complete digestion of mannans, the degrading enzyme system usually includes endo-β-1,4-mannanases (EC 3.2.1.78), endo-β-1,4-mannosidases (EC 3.2.1.130), exo-β-1 4-mannosidases (EC 3.2.1.25), β-glucosidases (EC 3.2.1.21), α-galactosidases (EC 3.2.1.22) and acetyl-mannan esterases. Endo-β-1,4-mannanases, exo-β-1,4-mannosidases, and β-glucosidases are mainly involved in the hydrolysis of the mannan backbone into oligosaccharides and monosaccharides. Other enzymes, such as acetyl-mannan esterases and α-galactosidases, endo-β-1,4-mannanases (del Carmen Rodríguez-Gacio et al., 2012), as exemplified in the degradation of O-acetyl-galactoglucomannan (Figure 5).

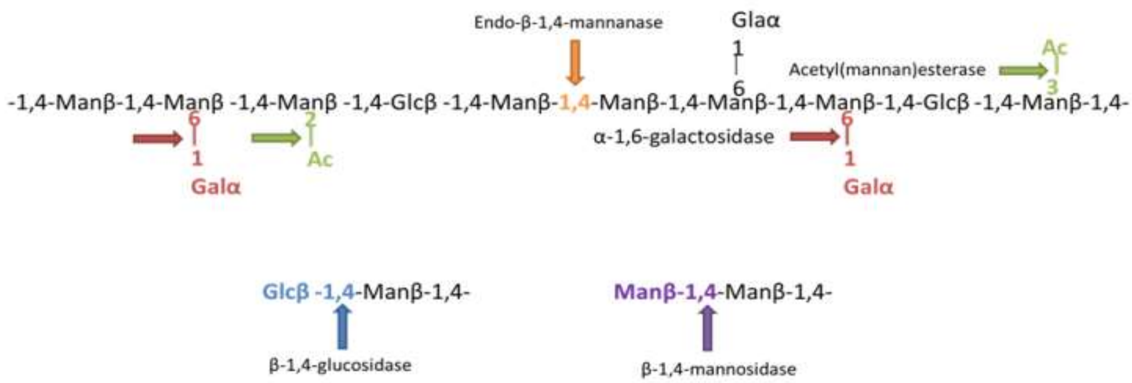

Figure 5. Overview of the enzyme portfolio required for the complete degradation of O-acetyl-galactoglucomannan.

β-Mannanase

In many biotechnological applications of mannan, the aim is to completely degrade polymers to monosaccharides. This degradation can be accomplished by chemical methods or by enzymatic hydrolysis. During the enzymatic hydrolysis process, endo-β mannanases (E.C. 3.1.2.78) play a crucial role in the digestion of mannan backbones. Mannanases from various organisms, including bacteria, fungi, plants, and animals (Larsson et al., 2006; Jagtap et al., 2012; Liu et al., 2012; Wang et al., 2014), have been classified into three GH families: GH5, GH26, and GH113 in the CAZy database (Cantarel et al., 2009). These families are categorized into clan A, and as a result, all mannases share one (β/α)8 barrel-folding structure and two Glu residues. Moreover, they all retain GHs, and therefore all could potentially function as transglycosylases. To date, GH mannanases with transglycosylation activity were found in GH5 and GH113, but not in GH26 (Couturier et al., 2013). Some mannanases show exo-acting activity in addition to endo-acting activity (Setati et al., 2001; Katrolia et al., 2013).

β-Mannanase Function

Mannan is composed of β-1,4-linked D-mannose (and/or glucose) and α-1,6-linked galactose as a backbone and side chain, respectively. Figure 6 shows the effect of B mannanase on various mannan species (Chauhan et al. 2017; Chauhan et al., 2012). Due to the complexity of the structure of mannan, complete hydrolysis of this biopolymer is achieved by the synergistic action of four enzymes: β-mannanase (EC3.2.1.78), β-mannosidase (EC3.2.1.25), β-glucosidase (EC 3.2.1.21) and α-galactosidase (EC3.2.1.22) (Yamabhai et al., 2016; Malgas et al., 2015). Among these enzymes, β-mannanase has received significant industrial interest as it catalyzes the cleavage of β-1,4 linkages in mannan into biologically active mannooligosaccharides. These mannooligosaccharides are then hydrolyzed by three other mannan deconstruction enzymes. Potential industrial applications of β-mannanase are listed in Table 1.

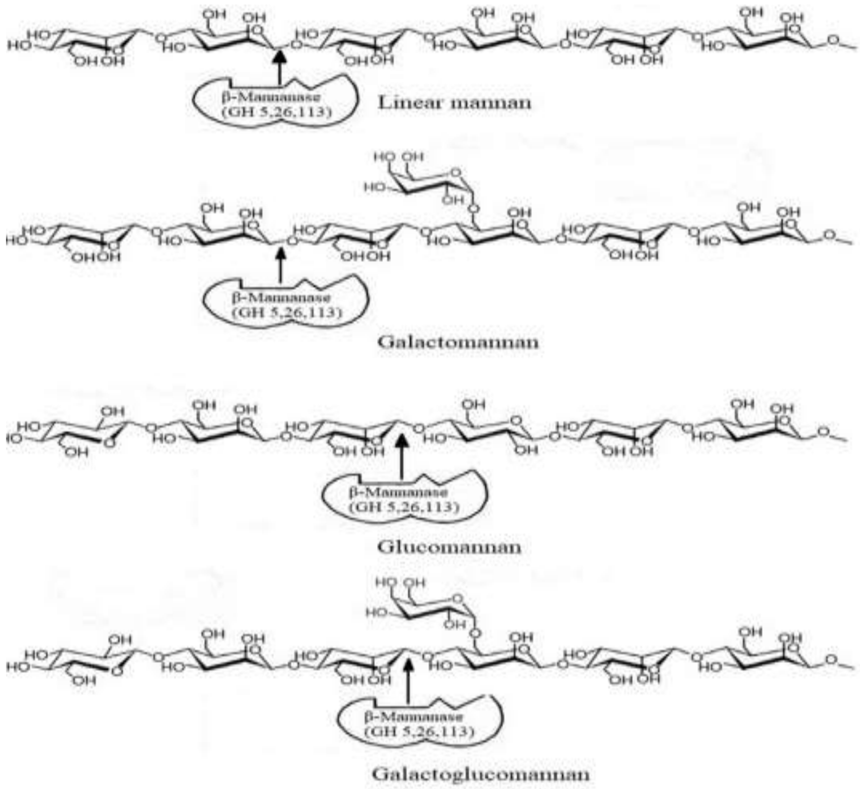

Figure 6. Effect of mannanase enzyme on various mannan species Yeoman et al. (2010).

Table 1. Industrial uses and applications of β-mannanase Mohapatra, B. R. (2021).

Endüstri	**Potansiyel Uygulama Alanları**
Biorafinery	Facilitating efficient enzymatic degradation of lignocellulosic waste into sustainable products
Detergent	Application as an additive for the effective removal of sticky gum stains from fabrics
Feed	Improving the palatability and digestibility of animal feed
Food processing	Reducing the viscosity of coffee extract during instant coffee production n oil extraction, softening and clarification of fruits and vegetables
Oil and gas	Increasing the recovery of oil and gas during the drilling process
Medicines	Use as a therapeutic agent for irritable bowel syndrome, acute diarrhea in children and as a prebiotic
Pulp and paper	To enable biological bleaching of paper and softwood pulp
Textile	Reducing the viscosity of printing paste used in textile processing
Waste water treatment	Control slime and biofilm formation by preventing bacterial adhesion to surfaces

Table 2. Some sources of mannanase

Organism	**Source**
Bacillus subtilis HM7	(Leerawatthanakun, et al., 2022)
Klebsiella pneumoniae SS11	(Singh et al., 2019)
Streptomyces species Alg-S25	(Mohapatra 2021)
Lactobacillus casei HDS-01	(Zhao et al., 2020)
Rhizopus microsporus	(Li et al., 2020)
Penicillium aculeatum APS1	(Bangoria et al., 2021)
Lichtheimia ramosa	(Xie et al., 2019)
Aspergillus sulphureus	(Chen et al., 2007)
Aspergillus terrus	(Huang et al., 2007)
Aspergillus kawachii IFO 4308	(Liu et al., 2020)
Aspergillus niger CBS 513.88	(Tang et al., 2019)
Trichoderma longibrachiatum RS1	(Ismail et al., 2019)
Bacillus sp. R2AL2A	(Kim et al., 2018)
Paenibacillus curdlanolyticus,	(Pason and Ratanakhanokchai, 2006)
Paenibacillus polymyxa,	(Han et al., 2006)

Utilization of β-Mannanase Enzyme in Industrial and Biotechnology Fields
Utilization of Mannanase in the Biorefinery Industry

The efficient use of renewable lignocellulosic biomass as second-generation biofuels has become a global endeavor for sustainable energy systems and environmental reasons (Lin et al., 2013). In addition, more research is also focusing on increasing the value of waste or residual materials through the biorefinery concept (FitzPatrick et al., 2010). Various biorefinery techniques are estimated to greatly reduce the amount of biowaste generated worldwide, as many of them have the potential to be converted into a wide range of value-added products.

Different lignocellulosic plants have a variable composition of macromolecules, but the main components average in the following order: glucan > lignin > xylan > mannan >arabinan > galactan. The mannan content is about 5% (Lavoie et al., 2011; Wolf et al., 2012), except in conifers or softwoods, which contain more mannan (~10%) than xylan. Lignocellulosic biomass is therefore the most suitable feedstock for biofuel production. After cellulose, mannans from softwood are important sugar sources for biofuel production.

The application of mannan-degrading enzymes in biotechnology has gained significant interest over the last decade based on the growing demand for efficient utilization of renewable bio-resources for sustainable development (Do et al., 2009).

Bioconversion of biomass to monomeric sugars and subsequent fermentation to products such as ethanol can be efficiently achieved through hydrolysis using multiple enzymes (Van Dyk and Pletschke, 2012). For softwood, mannan-degrading enzymes constitute an important group of enzymes that can be used both in the pretreatment step and for the production of second-generation biofuels for the complete release of all sugars and potentially for health production. Endo-β1,4-mannanases or β-mannanases are the main enzymes for the complete degradation of mannan (Rodriguez-Gacio et al., 2012).

In a 2015 study by Yoo, H. Y. et al. 2015, an extracellular endo-β-1,4-D-mannanase was produced by Streptomyces sp. It was purified 8.5-fold in 43.4% yield using CS147 (Mn147) and Sephadex G-50 column. The production of sugars such as mannose, mannobiose, and other oligosaccharides shows a clear pathway for the utilization of cellulosic biomass, and fermentable sugars can be converted into bioethanol or biopolymers by biorefinery. The study has shown that Mn147 is attractive in the search for potential bioindustrial applications.

Utilization of Mannanase in the Detergent Industry

The applications of microbial enzymes in the detergent industry are well known. The most commonly used enzymes are proteinases, lipases, amylases, and cellulases (Srivastava and Kapoor, 2014). Recently, alkaline β-mannanases, which show stability against detergent components, have been increasingly used as stain-removal enhancers in certain laundry departments. β-mannanase hydrolyzes different mannan-based materials such as guar gum, glucomannans, galactomannans, and others (Liao et al., 2014). Available as thickeners and due to their gel texture, guar gums are found in an increasing number of consumer products, including barbecue sauce, ice cream, salad dressing, makeup, and hair styling (Mudgil et al., 2014). These gums act as glue, adhering to soil particles and making it difficult to remove dirt. β-mannanases effectively hydrolyze these gums and remove them from the fabric, thus preventing dirt from sticking to the fabric.

ManSS11, a β-mannanase enzyme isolated from a novel Klebsiella pneumoniae strain SS11, was used to perform washing performance experiments (Singh et al., 2019). Analysis of the hydrolyzed products at the end of the washing process shows that β-mannanase has 80.5 ± 1.07% better cleaning power (cleaning power) in removing the strain fixed with locust bean gum + powder compared to the case where detergent alone was used alone, which shows only 30.6 ± 0.86% detergent. Similarly, another study investigated the cleaning power of β-mannanase from Bacillus sp. CFR1601 was tested on a cotton cloth stained with chocolate ice cream and tomato sauce (Srivastava and Kapoor, 2014). The detergent combined with β-mannanase shows greater stain removal than the detergent alone. We also checked the stability and compatibility of the isolated β-mannanase with different commercially available laundry detergents. The enzyme retains 89.0-100% of its original activity for up to 1 hour at 37°C in the presence of different detergents in the following order: Wheel > Surf > Excel> Ariel > Tide > Rin. This suggests that enzyme performance is influenced by the components present in the detergent, as enzyme stability varies with the different detergents tested. David et al. (2018) investigate the optimization of the co-production of protease and β-mannanase from a Bacillus nealsonii strain PN-11, along with the potential of both of these enzymes as additives to detergents. The enzymes show good compatibility with detergents and detergent performance improves on different types of stains when β-mannanase or protease is used. However, dye removal is much more efficient when both β-mannanase and protease are used in combination.

There are opportunities for geographical and quantitative expansion of the use of β-mannanases in detergents. In developing countries, β-mannanases have not found widespread use, even though these countries are dusty and hot and therefore require frequent laundry. In the West, especially in the United States, β-mannanase has found its way into commercial household detergent preparations. Novozymes, a Danish company, markets Mannaway, a laundry detergent containing β-mannanase. This can be used effectively to remove mannan-based stains. Adding β-mannanase to detergents improves the stain-removal ability of the detergent and prevents other particles from sticking to the fabric during the washing process.

Purabrite is another commercial β-mannanase marketed by another United States company, Genencor. For decades, detergent formulators have faced the fundamental challenge of developing products with superior cleaning performance at competitive prices. Genencor claims that Purabrite meets these requirements. Purabrite is available in both liquid and granular form. The granular form, incorporating the patented Enzoguard® coating, is a proprietary technology of Genencor. These granules are safer to handle than poly powders and have enhanced properties for easy mixing and storage.

Use of Mannanase in Feed Industry

Poultry nutritionists are continuously striving to improve the production performance of bird species, which is essential for successful farming. Several strategies have been implemented to increase commercial poultry production over the last two decades, such as introducing environmentally controlled shaping, improving feed formulation (Saeed et al., 2019; Saeed et al., 2018 a, b; Yatao et al., 2018), the application of probiotics (Sun et al., 2016) and the use of herbal medicinal plants as an alternative source of antibiotics. Dietary enzymes are biologically active proteins that facilitate the breakdown of complex nutrients into smaller molecules for further digestion and absorption (Thacker, 2013). There are a large number of enzymes derived by fermentation from microbes such as fungi and bacteria and used in poultry and their benefits in improving feed efficiency and growth. There are different classes of enzymes commonly used in poultry diets, including phytase, carbohydrases (β-mannanase, xylanase, α-galactosidase, cellulase, pectinase, and α-amylase), and proteases. The biological activities of various in-feed enzymes in promoting growth and improving feed efficiency in poultry have been well reported (Bedford and Schulze, 1998; Choct, 2007).

For successful commercial poultry production, it is imperative to identify factors that inhibit nutrient digestibility. Non-starch polysaccharides (NSPs) such as hemicellulose, cellulose, and pectin can reduce nutrient digestibility in poultry (Choct, 2007). Among NSPs, β-mannanes are a group of hemicelluloses found in many ingredients used in poultry feed such as soybeans and other legume seeds. It is found in the plant's cell walls in the form of galactomannans and glucomannans. β-mannan is found in many feedstuffs including palm kernel meal, soybean meal, copra meal sesame meal, and other legume feeds (Dierick, 1989). As protein sources, soybean meal, and whole soybeans are the most important feed ingredients in poultry diets, and most poultry feed contains β-mannan. However, β-mannan has been found to have detrimental effects on animal performance and health. The negative effects of β-mannans on poultry performance were attributed to the low digestibility of the nutrients and high intestinal viscosity, which negatively affects the immune response and microbial proliferation in the gut, as well as growth and carcass characteristics (Alagawany et al. al., 2015, 2017). In an early study, Patel & McGinnis (1985) found that egg weight, egg production, and feed intake were significantly reduced by β-mannan in laying hen diets. Previously published literature has shown that the enzyme β-mannanase can improve innate immunity and promote a non-productive energy depletion response of the innate immune system (Zhang & Tizzard, 1996; Duncan et al., 2002).

Hydrolysis of Coffee Extract

Instant coffee offers consumers a combination of convenience and high-added value. The main residue produced during instant coffee production is used coffee grind (SCG), which consists mainly of polysaccharides such as galactomannan and cellulose. These polysaccharides do not dissolve during the extraction process and therefore remain as insoluble solids (Figure 4). In this context, the application of β-mannanase seems to be a suitable strategy to solubilize/hydrolyze the remaining solids, thus increasing the yield of soluble solids of instant coffee. Jooste et al. (2013) applied different carbohydrase enzymes to increase the solubility of residual solids produced during coffee production. Among the enzymes tested for the hydrolysis of SCG, the highest increase in soluble solids yield was obtained with the use of β-mannanase (Man 1). Combining β-mannanase (Man1) with other enzymes shows an additive rather than synergistic effect, indicating that β-mannanase is mainly responsible for the highest soluble solids yield. Similarly, Favaro et al. (2020) also show that a large amount of carbohydrates in SCG can be hydrolyzed by β-mannanase. When a commercial cellulase cocktail is added to β-mannanase, the hydrolysis yield is further increased (56%), showing the promise of increasing soluble coffee processing. Baraldi et al. (2016) compared enzymatic and thermal processes during instant coffee production. The roasted coffee is first extracted with water at 125˚C and the spent coffee is then processed by enzymatic hydrolysis at 50˚C (with the aid of an enzyme cocktail containing β-mannanase) or thermal hydrolysis at 180˚C. The yield of enzymatic hydrolysis (18%) is lower than the yield of thermal hydrolysis (28%). However, instant coffee produced through enzymatic hydrolysis has a lower amount of undesirable compounds such as furfural, acetaldehyde, and 5-HMF, and less energy is consumed in the process. These findings suggest that the enzymatic procedure is a viable alternative to thermal hydrolysis for instant coffee production.

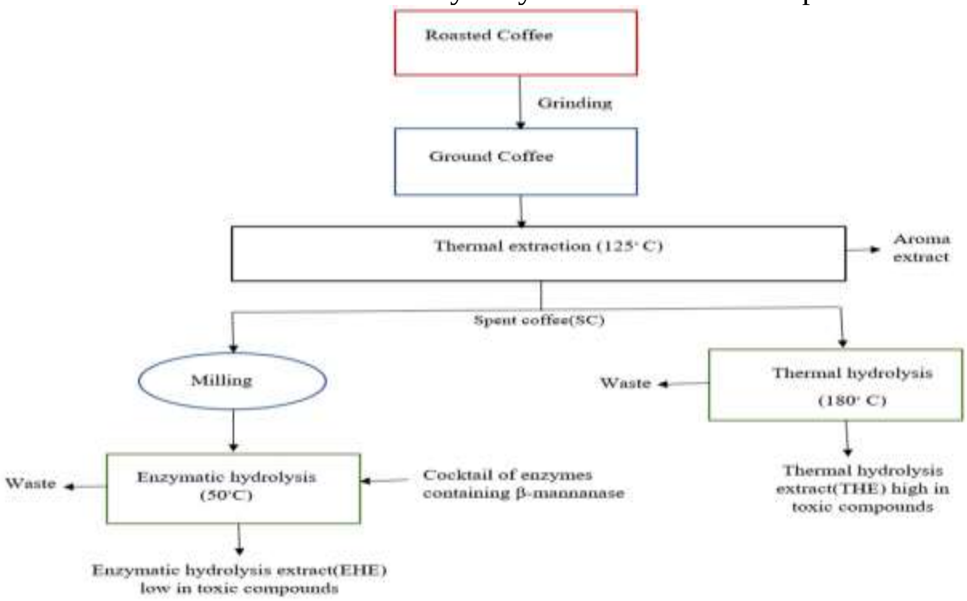

Figure 7. Comparison of soluble coffee production by enzymatic and thermal hydrolysis. Modified from Baraldi et al. (2016).

Use of Mannase in the Pharmaceutical Industry

D-Mannose derived from beech and birch hydrolysates is commercially available as an excipient. The production of fast-dissolving tablets using mannose (Parisi et al., 2002) takes advantage of the high solubility of mannose; it contributes to the structure-forming and rapid dissolution properties of the tablets. Ingestion of mannose is a popular remedy for urinary tract infections, but there is little current scientific literature supporting this treatment regimen. However, there are many mannose products on the market and this sector represents a significant demand for sugar. Currently, mannose is obtained from the chromatographic separation of sulfite-spent liquids (Alam et al., 2000). Mannan-rich substrates such as palm kernel and copra bagasse represent low-cost alternative sources of mannose. The mannose enrichment of animal feeds containing these flours using a crude, commercial A. niger enzyme preparation has been described (Fu et al., 2006). The method involves the addition of a combination of mannan-degrading enzymes and cellulases, presumably to carry out hydrolysis. However, aqueous extraction of the hydrolysate and chromatographic purification of mannose or manno-oligosaccharides represent a more cost-effective option for obtaining these compounds.

Depending on the chemical composition of the mannan-containing substrates, a range of mannan-degrading enzymes, cellulases and even proteases would be required to effectively hydrolyze these substrates. However, hydrolysis of palm kernel press cake with different enzyme combinations has provided little evidence of synergism between hydrolytic enzymes (Heikkila, 1986). The combination of cellulase and β-mannanase/p-mannosidase preparations did not increase the mannose yield relative to the β-mannanase/p-mannosidase preparation alone, implying that glucans or cellulose does not block the access of mannan-degrading enzymes to the substrate. Increasing β-mannosidase activity relative to β-mannanase increased mannose yields, especially when high solid loadings were used (Yokomizo, 2009). The addition of an α-galactosidase to the β-mannanase/β-mannosidase cocktail did not significantly increase the mannose yield. Since the addition of a protease preparation did not increase mannose yield, proteins present in the substrate did not appear to restrict the enzymes' access to polysaccharides (Jorgensen et al., 2010).

Utilization of Mannanase in the Oil and Gas Industry

Another important application of guar gum and subsequently mannanase is the addition of guar gum to increase the viscosity of fracturing fluids used during stimulation of oil/gas wells. Fracturing fluids are pumped into wells and the high pressures cause the bedrock to fracture. Fluid-containing proppant, usually a suspension of sand, flows into the fractures. For the well to start producing, the fluid needs to be pumped out, the viscosity of which needs to be reduced to facilitate pumping. The remaining sand supports open the fractures that allow the oil or gas to flow. -Mannases and galactosidases act as enzyme crackers that hydrolyze galactomannans in the fracturing fluid. Thermostable enzymes are of particular interest in this application due to geothermal gradients in deep oil wells (Comfort et al., 2004). In addition, premature hydrolysis of galactomannanes at the earth's surface is prevented when hyperthermophilic enzymes are used because the environment only reaches optimum temperatures for enzyme activity deep in the well (Kelly et al., 2002). Due to the extreme temperatures in the wells (>80 ◦C), it is unlikely that many wild-type fungal mannan-degrading enzymes will find use in this application. Enzymes capable of operating at high temperatures have been isolated from Thermotoga maritima, Thermotoga neapolitana, and Rhodothermus marinus (Politz et al., 2000; Parker et al., 2001).

Utilization of Mannanase in the Pulp and Paper Industry

Bleaching is an important step to remove the color of pulp for papermaking. In ancient times, methods such as sunlight, soaking in streams, etc. were used to bleach pulp (Toren and Blanc 1997), followed by chemical-based processes. The chemical-based multi-stage bleaching commonly used in industry today involves repeated treatment of the pulp with an alkaline solution and calcium hypochlorite (Hintz 2001). Strict environmental concerns have made it necessary to make industrial processes environmentally friendly. Some alternative bleaching methods such as long-term cooking and hydrogen peroxide/ozone-based delignification are available, but their implementation requires process modifications and is expensive on a large scale (Singh et al., 2016). Several studies have investigated the replacement of chemical-based bleaching processes with enzymatic processes. The most important enzymes in this regard are hemicellulolytic and ligninolytic enzymes (Barneto et al., 2011). Hemicellulolytic enzymes help remove hemicelluloses deposited on the fiber surface, which facilitates the release of lignin with a smaller amount of chemicals (Dawood and Ma 2020). The two main enzymes of this class are xylanases and mannases (Kansoh and Nagieb 2004).

Although enzymes can be used for a variety of purposes in the papermaking process, such as pitch control, de-inking, and crumb removal, their maximum potential lies in the biobleaching of pulp. Several authors have reported that effective pulp biobleaching using microbial enzymes results in a significant reduction in chemical use and better quality paper production (Mahfouz et al., 2020; Saibu et al., 2020). Since crude enzymes can be used for biobleaching of pulp, their application in industries is economically feasible (no need for purification) (Sridevi et al., 2017); moreover, such enzymes are likely to have a longer shelf life than purified enzymes. However, studies are still needed to find stable enzymes or to improve the stability of existing enzymes, to increase their potential, and to make them suitable for commercial application.

Utilization of Mannanase in the Textile Industry

The textile industry uses two-thirds of the dyestuff production (Riu et al., 1998). Large volumes of water and chemicals are consumed during the wet processing of textile products. The chemical agents used in the textile industry are very diverse and range from inorganic compounds to polymers and organic products (Mishra et al., 1993; Juang et al., 1996). The variety of commercial dyes used in the textile industry is more than 100,000 and the annual production of these dyes is over 7 x 105 tons (Zollinger 2022). Due to their chemical structure, textile dyes are highly resistant to discoloration when exposed to light, water, and different chemical oxidizing agents (Poots et al., 1976; McKay 1979). In addition, decolorization of most of these dyes is very difficult to achieve due to their synthetic origin. Moreover, it is not possible to degrade or decolorize all of the different categories of textile dyes using physical or chemical methods. Enzymatic methods are preferred over physico-chemical methods for the treatment of textile dye-containing wastes, as sometimes the breakdown products of these dyes can be much more toxic (Spadaro et al., 1994).

β-mannanases can be used in combination with detergents to clean or prepare fibers in the textile and cellulosic processing industries. To prepare the material for garment manufacturing, cellulosic material is processed in several stages: burning, de-sizing, cleaning, bleaching, dyeing, and finishing (Mojsov, 2011). The application of β-mannanase can be useful in the biological cleaning and desizing of cellulosic fibers, thus preparing the material for good response in subsequent dyeing operations.

Bio-scouring is the process by which the fabric is cleaned of impurities such as pectin, hemicellulose, wax, and mineral salts through enzymatic action (Bristi et al., 2019). These impurities render the raw cotton hydrophobic and thus interfere with aqueous chemical processes such as dyeing and finishing. It is therefore important to remove these impurities so that the fabric can be dyed.

Conclusion

β-mannanases are used in a wide range of industries such as feed, detergents, biorefineries, and textiles. The production and utilization of β-mannanases is increasing due to the growing awareness of their benefits and the incorporation of enzyme engineering and gene manipulation techniques. There is now an urgent need to create β-mannanases at low costs that are more in line with the demands of the industrial sector to replace the use of harmful chemicals in the industrial sector with environmentally friendly biocatalysts. The government and higher levels need to encourage this change to produce cleaner industrial products and reduce the risk of environmental pollution.

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AGRICULTURE OF THE FUTURE: AGRICULTURAL TRANSFORMATION WITH UNMANNED AERIAL VEHICLES

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ABSTRACT

The agriculture and livestock sector is developing more and more every day with technological innovations. One of these innovations is unmanned aerial vehicles (UAV) technology. Unmanned aerial vehicles are used to facilitate agriculture and livestock activities, increase productivity and provide more control to farmers. In the agricultural sector, UAVs are used in many areas such as monitoring agricultural areas, controlling plant health, and application of pesticides. By scanning the vegetation in agricultural lands, UAVs detect plant diseases or harmful insects, thus providing early warning to farmers. Agricultural pesticides can also be applied more effectively with UAVs. UAVs make the spraying process more efficient by providing controlled spraying of the drug towards the target. In addition, UAVs can determine the irrigation needs of agricultural areas. By measuring the humidity level of the agricultural area, it determines the water need and optimizes the irrigation processes. In this way, water resources are used more efficiently and water savings are achieved. In the livestock sector, UAVs provide many benefits. It is used especially in large farms for the control, counting and health monitoring of animals. UAVs can detect animals in large areas, count and check the status of animals. Besides, it can track the location of animals and help them find them back in case of loss. The use of UAVs in the agriculture and livestock sector is especially important for saving manpower. The difficult and dangerous labor that people have to do can be done more safely and quickly with UAVs. In addition, UAVs provide more efficient management of agricultural and livestock activities. Thanks to UAVs, it is possible to access more accurate and timely information. However, there are some difficulties with the use of UAVs. The use of UAVs without the rules and permits governing their flight can lead to various security risks. In addition, the cost of UAVs is a factor limiting their use. The cost of UAVs can be quite high, which may limit the use of drones for small family farms. With regulatory regulations and affordability, UAVs can become an integral part of the agriculture and livestock industry in the future.

Keywords: Unmanned aerial vehicle, agriculture, livestock

INTRODUCTION

People have to produce both animal and plant products to meet their nutritional needs. Agricultural activities are as old as human history. Approaches aiming to increase production in the previous periods have been transformed into production approaches focused on economic profitability, then into quality production perspective and finally into production activities based on sustainable environment and natural resource protection. (Friha et al., 2021). In parallel with technological developments, increases in production amounts have occurred. Agricultural activities have been carried out especially in areas with suitable land conditions and access to water resources. Agriculture is a science that covers a wide range from production to marketing, from quality to protection, from breeding to natural resource utilisation. Agricultural activities are carried out not only to meet nutritional needs but also to create employment and as part of economic activities. Fertilisation, spraying, irrigation, seed selection, mechanisation, labour force are among the important subjects of agriculture. (Zhang and Kovacs, 2012).

The agricultural sector, which seems to be lagging behind in adopting technology, has started to benefit from the advantages and conveniences offered by artificial intelligence technology in the field of rural development. The digital transformation that emerges with the advancement of technology leads to various changes in productivity and employment in agricultural activities on a global scale. According to a report by the Food and Agriculture Organisation of the United Nations, to meet the food needs of the world population in 2050, 70% more food needs to be produced than has been produced since 2006. In order to meet consumer demand, the interest in cereal consumption and sustainable agricultural processes is increasing day by day (Yusuf et al., 2018).

The need to minimise losses by increasing the productivity of the agricultural sector in relation to the growing world population has reached a high point. In response to this demand, drones and other support systems have paved the way for multi-disciplinary work. The raw images imaged by the drone are processed with the help of various programmes or image algorithms (Radoglou-Grammatikis et al., 2020).

Precise imaging techniques provide useful information on plant growth and yield estimation parameters that can be read accurately and estimated with high reliability. By using remote sensing methods, the total number of plants per unit area, the development values of plants subjected to different fertilisations and different issues such as these can be solved quickly. These methods, which save time and money, also help to create data sets that can be used in the coming years (Gnädinger and Schmidhalter, 2017). Drone systems used in agricultural research are shown in Figure 1.

Figure 1. Drone systems used in agricultural research (Teke et al., 2016)

Drone

The growth in the aviation sector with the development of technology has led to many positive developments in the aviation production sector. With the importance given by countries to air defence at the military level, "Unmanned Aerial Vehicles (UAV)" have emerged for various purposes. The use of Unmanned Aerial Vehicles dates back to ancient times and the first unmanned aerial vehicles used were balloons (Kahveci and Can, 2017). However, the first real UAV application suitable for today's usage area started in 1916 when Elmer Speryy demonstrated the gyro-stable flight of the "Curtiss" aircraft of the US Navy (Çetinkaya and Koç, 2023). With the investments made by countries in this field and the use of such aircraft in civil aviation activities, unmanned aerial vehicles have started to have an impact on economic indicators and have created their own sector (Yeşilay and Macit, 2020)

Unmanned aerial vehicles (UAVs) are small aerial vehicles in terms of size and weight, which do not have a human on board for control or travel purposes, and which can fly by remote control or a pre-loaded flight programme. The International Civil Aviation Organisation (ICAO) classifies unmanned aerial vehicles in two categories: military and civilian. According to this classification; UAVs used for armed purposes such as reconnaissance and attack are classified as military; UAVs used for scientific, imaging or hobby purposes are classified as civilian (ICAO (International Civil Aviation Organization), 2011).

The armament of balloons in the early 1900s and the subsequent unmanned flight of small aircraft are considered to be the basis for the use of UAVs and "armed unmanned aerial vehicles (UAVs)". However, after the international political ban on the use of these UAVs for military purposes, the production and development of UAVs and UCAVs stopped. However, technological developments in aeroplanes and aviation have improved the use of UAVs primarily for military purposes, and then, with the confidence in UAVs, UAVs have started to be used in many different fields. With the increase in civilian use, the UAV sector has started to create its own market and economy (Kahveci and Can, 2017).

UAVs are defined as "drone" or "UAV/UAS (Unmanned Aerial Vehicle/Systems)" in the international literature, and they actually mean the same thing except for certain technical features (Kahveci and Can, 2017).

In general, UAVs are classified into two categories: lighter-than-air and heavier-than-air (Figure 2). UAVs used extensively in agricultural areas are fixed-wing and multi-rotor UAVs. In addition, lighter-than-air UAVs are also used by being attached to a rope fixed to the edge of the field (Bozdogan and Yarpuz Bozdogan, 2017). Lighter-than-air and heavier-than-air UAVs are shown in Figure 2.

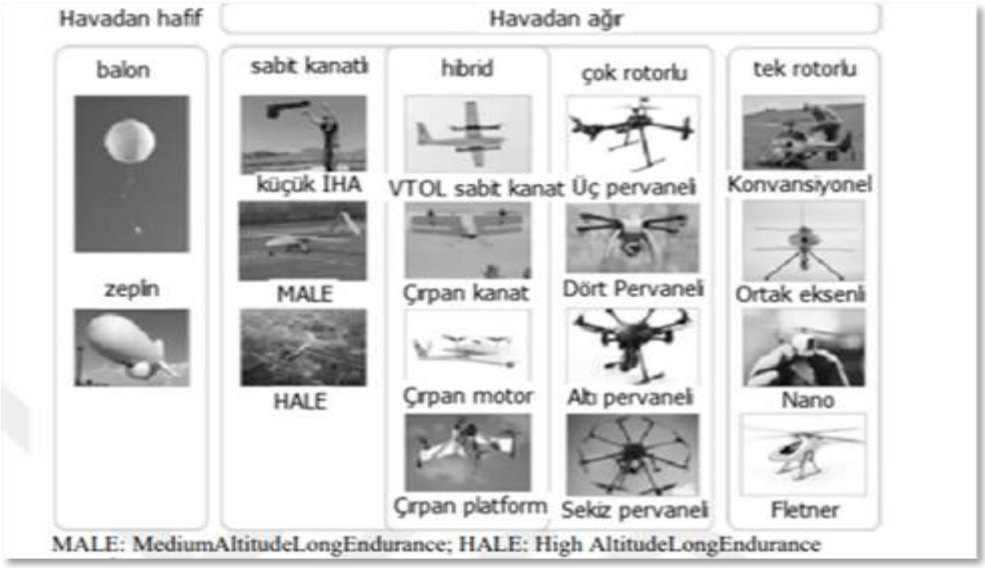

Figure 2. Airborne light and airborne heavy UAVs (Bozdogan and Yarpuz Bozdogan, 2017)

In terms of ease of use and cost, the most widely used UAVs are rotary wing type UAVs. They have different engine numbers, airframe and propeller structures depending on the type of operation, flight duration and flight weight. Although not common in commercial use, they are available in 1-engine (monocopter), 2-engine (bicopter or coaxial copter) or 3-engine (tricopter) designs. The most preferred designs in commercial use are generally 4-motor (quadcopter), 6-motor (hexacopter) and 8-motor (octocopter) structures (Villi and Yakar, 2022).

A drone is a vehicle with four or more propellers in the UAV category, capable of vertical take-off and landing, and capable of remaining stable in the air. Drone has started to be used in agricultural applications in recent years. Studies are carried out for applications such as crop growth monitoring, plant species separation, crop yield determination, automatic harvesting, drought, disease, agricultural pests, etc. damage detection, fruit-vegetable and soil moisture classification, area management, organisation of agricultural activities, agricultural insurance with drone (Tan et al., 2015)

Drone Software Architecture

Drone software exists on three basic backbones. These are system software, user software and a cloud-based control platform. The drone software architecture template is shown in Figure 3.

Figure 3. Drone software architecture template (Inan and Karci, 2021)

The embedded software works as a CPU, manages the hardware, monitors the drone telemetry and partially analyses the data received from the drone sensors.

The operating system allows users to run the firmware partition.

Web and cloud interfaces allow accessing the operating system from remote drone control systems (user applications and cloud control stations) and transferring collected data from embedded software to the cloud or mobile devices.

The cloud-based control platform is used for data processing, storage and analysis. It also enables autonomous reaction actions of a drone. The cloud partition is a must when it comes to complex processes such as, for example, 3D mapping, computer vision, pattern recognition. The cloud-based control platform includes:

- Stream data processor.
- Raw data and big data warehousing.
- Data analysis and machine learning.
- Drone control module.
- Interfaces for communicating with the drone.

A conventional drone consists of standard propellers, pusher propellers, motors, landing gear, electronic speed and stability controls, flight control panel, receiver, transmitter, GPS module, battery and camera components (Figure 4.).

Figure 4. Drone parts (Anonymous, 2022)

1. Standard Propellers, 2. Thruster Propellers, 3. Motors, 4. Landing Gear, 5. Electronic Speed Controls, 6. Flight Control Panel, 7. Receiver, 8. Transmitter, 9. GPS Module, 10. Battery, 11. Camera

Photo Sensors Used in UAVs for Agricultural Purposes

In agriculture, decreasing equipment costs of image processing and computer vision applications, increasing computational capabilities, and increasing interest in quality food evaluation methods draw attention to artificial intelligence technologies (Mahajan et al., 2015). Drones come to the forefront with the advantages of more efficient, precise and reproducible studies in small areas. Drones have recently become an important part of the agricultural sector with the widespread understanding of precision agriculture. With a single flight, hundreds of decares of land can be scanned and data can be obtained. By solving the problem of not being able to take images in cloudy weather, which is one of the disadvantages of satellites, it has made it easier to access data under much more flexible conditions (Türkseven et al., 2016).

The use of unmanned aerial systems (UAS) as sensing and communication platforms is also a breakthrough technology with significant potential in precision agriculture. It is recognised as a low-cost alternative technique for environmental monitoring with high spatial and temporal resolution and low cost of image acquisition. Nowadays, the use of UAVs in agriculture is increasing to assist farmers with monitoring and decision support in agriculture. UAV is used in various agricultural applications such as irrigation, fertilisation, pesticides, weed management, etc. Moreover, the combination of UAV technology with new 3D remodelling techniques has enabled plant-by-plant monitoring of crop growth parameters (Hassler and Baysal-Gurel, 2019; Jang et al., 2020; Manfreda et al., 2018).

By utilising UAVs, remote sensing system based plant monitoring techniques, it is necessary to take images with cameras in order to be used in issues such as disease agent, pest detection, determination of their damage rates, weed flora detection, determination of water stress, determination of harvest time and yield estimation (Koger et al., 2003; de Castro et al.,, 2012). The features of the sensors of the cameras used in these images are extremely important. Visual sensors that provide the closest image to the real image, Lidar sensors that allow us to carry the vagetation to the three-dimensional environment, thermal sensors that distinguish objects on the basis of temperature difference, and multispectral and hyperspectral sensors that can 6 measure infrared wavelength reflections are used. In particular, sensors using infrared wavelength reflections are widely used in remote sensing studies (Türkseven et al., 2016).

Atmospheric sensors such as temperature, pressure, wind, humidity, chemical sensors such as gas, location sensors such as ultrasound, infrared, radio frequency, GPS, microphone etc. sensors can be used in the drone (Metin Özgüven et al., 2022).

Previous generations of UAV platforms were limited to a smaller spectral bandwidth, equipped with only red-green-blue (RGB) sensors or a filter connected to the RGB sensors to obtain manipulated infrared information (Yeom et al., 2019). Recently, UAV platforms with sensors covering the red edge and near infrared (NIR) bands have become available for agricultural applications, allowing plant indices originally developed for conventional remote sensing sensors to be applied to UAV data. Recently, UAV platforms with sensors covering the red edge and near infrared (NIR) bands have been used for agricultural applications (Demir and Başayiğit, 2020).

As remote sensing technologies develop, areas in the field that are diseased or have problems under any stress factor can be quickly identified and specific controls can be carried out for these areas. Thus, unnecessary use of chemical pesticides is prevented, less chemical waste is left to the environment and the maximum product is obtained from the unit area (Mogili and Deepak, 2018).

Developments and innovations in drone technology from unmanned aerial vehicles in agricultural activities attract attention. With drone applications in agriculture, operations such as field mapping, dimensioning, positioning and pesticide spraying are carried out very easily. With the help of drone, farmers can detect a number of problems that are not visible from the ground in their fields from the air and quickly intervene in problematic areas in a timely manner. In addition, farmers can take images of the products in the field from the sky at certain time intervals with the help of drones and analyse whether the products in the field are properly irrigated, the development and yield of the products. A spraying drone is shown in Figure 5.

Figure 5. A drone spraying (Aydogan, 2018)

Use of Unmanned Aerial Vehicles in Agriculture

Product monitoring is very critical in agricultural applications. Today, in many countries, studies on agricultural fields, regulation activities, analysis of yield parameters and land consolidation projects are carried out for sustainability. There is a great need for remote sensing techniques to obtain, calculate and evaluate these studies accurately. Remote sensing methods are developing day by day and are used in wide areas. Drone-based remote sensing is an element of smart agriculture, helping to collect diagnostic information in a timely and efficient manner. UAVs, processed with modern software and controlled by the operator, are recognised as a new remote sensing option in the agricultural field. Emerging sensor technology makes common sensors previously used in satellite systems accessible to UAVs. Precision agriculture practitioners and researchers have started to utilise the available technology for different reasons. According to the results of these studies, data can be obtained in a short period of time and the spatial resolution is better compared to satellite imagery. Drone-based smart agriculture applications equipped with detailed remote sensing sensors are of great importance for sustainability (Milics, 2019).

There is a growing interest in deploying hyperspectral cameras on UAVs to scan agricultural fields at regular intervals in order to increase knowledge of crop and soil conditions and optimise crop productivity. The use of UAVs offers a number of facilities for optimising cropping and checking the condition of arable land. However, hyperspectral cameras bring with them technical challenges, especially related to the lack of spatial information between frames, making it a challenging task to obtain real-time results "off-board" of the captured frames after the end of the flight. The results of the research carried out for this purpose have shown that the developed UAV control has positive results in terms of enabling real-time analysis of the acquired data and the reliability of the results (Guerra et al., 2019).

UAVs capable of vertical and short-range take-off and landing are widely used and have the capacity to cover large areas. These UAVs can be equipped to carry a range of equipment, from NDVI sensors to near full spectrum infrared cameras and different agricultural sensors. Today's modern and advanced technology is crucial for collecting and processing data that is difficult to access in supportable and real-time from the field. Drones are seen as an important technology that will help farmers save time while carrying out agricultural activities in the future. This technology is expected to play a major role in the agricultural areas of the future by enabling agricultural operations to be carried out more effectively (Malveaux et al., 2014).

In recent years, the agricultural sector is becoming a high-tech industry where new companies and investors are involved. Rapid technological progress is not only increasing farmers' production capacities but also developing robotics and automation technologies. Farmers are now using autonomous aerial vehicles and robots for harvest harvesting to increase their production efficiency (Güzey et al., 2020; Zude-Sasse et al., 2016). In a study, it was aimed to carry out the harvest collection process of apples with predetermined targets in agricultural land by means of autonomous unmanned aerial and ground vehicles in communication to minimise time (Güzey et al., 2020). The autonomous harvesting tool is shown in Figure 6.

Figure 6. Autonomous harvesting vehicle (Güzey et al., 2020)

Mogili and Deepak (2018), used drones to prevent serious diseases among farm workers from manually sprayed pesticides. This approach has been recognized as a promising development, especially in rural areas where small areas are the main source of income and any disease can significantly affect household well-being.

Bendig et al., (2014), estimated the fresh and dry biomass of summer barley using plant height from plant surface models in a field experiment with 18 varieties and two nitrogen applications. Super high resolution, very temporal (1 cm/pixel) CSMs are derived from red, green, blue (RGB) images captured from small unmanned aerial vehicle (UAV). At the end of the study, a high correlation was found between pH of CSMs and fresh biomass (R^2 = 0.81) and dry biomass (R^2 = 0.82).

Roy and De (2022), in their research, developed it to monitor plant pests and diseases based on IoT and UAV, which tried to solve the correlation between pests/diseases occurrence and weather parameters. In the study, rotary devices based on solar perception and UAV were used to obtain solar energy for the Yangtze River Region of China. As a result of the study, the researchers stated that it is feasible to monitor pests and diseases through aerial imaging using low-altitude remote sensing and UAV, and further analysis of climate change will provide some precautionary measures.

UAVs are potential remote sensing platforms for precision agriculture. However, nitrogen status needs to be estimated early enough in the growing season to be useful for in-season management. In a study conducted on potato crops, variations in nitrogen status were analysed. By calculating the NDVI values of the images obtained using UAVs, it was found that all nitrogen treatments could be distinguished in the images. Since there is different chlorophyll content per leaf area, differences between nitrogen treatments were observed (Hunt et al., 2018).

A study has been carried out for vine disease detection using a deep learning segmentation approach in an Unmanned Aerial Vehicle. The methodology of the study is based on the combination of visible and infrared images obtained from two different sensors. The infrared images, which enable the fusion of information from the two sensors, helped to develop a new image registration method to align the visible and the image. The proposed method achieved more than 92% and 87% detection at vine level and leaf level, showing promising perspectives for computing (Kerkech et al., 2019).

Zhu et al. (2010), developed a software for precision spraying of an Unmanned Aerial Vehicle (UAV) used for agricultural spraying. They carried out tests with various aircraft. The results showed that the spray system has the sensitivity to increase the spraying efficiency for grain spraying.

Sahin and Yildirim (2011), a civilian UAV was developed specifically for the afforestation of a cedar forest in Gölbaşı, Ankara, using a fixed wing unmanned aerial vehicle. A model aircraft was modified and an indigenously designed and manufactured seed dispersal system was mounted on the UAV. Two test flights were carried out at altitudes of 9 and 6 metres. According to the results of the test flights, the seed distribution reached a width of 45 and 30 metres respectively, the density of the seed distribution for both tests was consistent with the conditions suitable for seed germination, and according to these results, the UAV proved to be a versatile, economical, safe and highly effective tool in afforestation studies.

Doering et al. (2014), created a special design platform for the design of multiple unmanned aerial vehicles in precision agriculture applications. They tried to determine the positive and negative characteristics of the drones by performing various tests.

In his article, Rokhmana (2015), discussed some practical experiences of using Unmanned Aerial Vehicle-(UAV) based platform for remote sensing. They wrote that some information is needed for land preparation, cadastral boundary, vegetation monitoring, phytosanitary and stock valuation to support precision agriculture mapping. According to the researcher, UAV-based remote sensing system; It should be cost-effective, fast in production, easy to use by local personnel, and good geometry accuracy.

Yallappa et al. (2017), designed and produced a 6-engine drone by using technology on rice and in order not to harm human health in the field conditions where people have difficulty in spraying in India, where agriculture is a very important area. This drone, which has a spraying area of 1300 mm, has a capacity of 5 liters and is powered by 2 battery groups with a capacity of 8000 mAh. They underlined that the drone they produced is very useful in field trials, especially in areas where people cannot reach it, and that it is a fast application and that it can be applied quickly and at low cost without people coming into contact with chemicals that are harmful to human health. They emphasized that a drone with at least 15 liters and 30 minutes of flight time would be more efficient for such applications, for this purpose, where the capacity of 5 liters is insufficient in field applications.

Wang et al. (2019), reported that bird damage is a major problem for grapes worldwide. For the control of bird damage, they proposed a new Unmanned Aerial Vehicle (UAV) drone equipped with a system to disrupt bird psychology with a loud noise. They carried out a study on this in vineyards in the southeast of Australia. As a result, they found that the harmful birds were flying away for a long time in a radius of 50 meters. They stated that while one UAV is sufficient for 25 hectares of vineyard area to protect vineyards, more than one UAV is needed to more effectively protect a large vineyard area.

Mogili and Deepak (2018), conducted a study on the application of drone systems in precision agriculture. By incorporating drone technology into precision agriculture applications, productivity increases, especially in spraying operations; They stated that the spraying processes are carried out very quickly and in a healthy way, and that the spraying process is carried out very easily and effectively in regions where manpower is scarce. Product damage detected by multispectral cameras was determined by GPS coordinates, and spraying was carried out successfully with a drone.

Goswami et al. (2019), on maize genotypes, the genotypes before and after rime were examined. High resolution images of genotypes were obtained by using remote sensing methods. The obtained images were tried to be determined by machine learning methods and different algorithms. Different algorithms were compared with each other and the algorithm that best explained the stress factor was determined. Based on the results of the study on the corn plant, the stress factors could be determined by remote sensing methods and the stress factors were explained numerically.

Gnadinger and Schmidhalter (2017), studied the number of plants using the remote sensing method and the evaluation of plant growth indices after different fertilization applications. According to the results obtained, it was emphasized that remote sensing methods are an effective method in converting plant growth performances into numerical dimensions.

Candiago et al (2015), examined the use of multi-band indices for precision farming applications, as well as the use of UAV imagery to analyze vegetation. They reported that the information collected with multispectral sensors could be useful in grape and tomato cultivation studies. High resolution UAV data and photogrammetric techniques have the potential to collect multi-frame images and evaluate different vegetation index. As a result of the research, they stated that technologies in precision agriculture applications are a fast, reliable and cost-effective resource for the evaluation of products.

Demir et al. (2016), toned digital photographs of vinegar, wild lettuce and lettuce as part of their research at Erciyes University. By simply using digital camera data and image processing techniques, the researchers found that the plants could be sufficient for comparison without the use of a colorimeter based on the collected data.

Altaş (2017), processed the images obtained by UAVs in sugar beet cultivation in Tokat province with the help of MATLAB program and aimed to detect leaf spot and diseases in the plant. The researcher compared the severity of the disease. In addition, he determined that the results of the study using image processing techniques were sensitive that could not be determined by observation and gave the exact value of the diseased areas.

Doğan and Yıldız (2019), thanks to developments in parallel with today's technology, remote sensing methods have gained a new dimension. From these methods, it is possible to perform more than one application at the same time with IHA technology. In these applications, the UAV offers advantages such as being able to fly at the desired height and obtaining clear photos. By using a multispectral camera that can be mounted on the UAV, the plant patterns can be clearly grouped thanks to the featured images. In addition, it was stated that it could give an idea about crop yield and plant health status. Researchers have examined that they can detect the product pattern by using colors such as red, green and infrared obtained from the cameras mounted on the UAV. As a result, they stated that classification can be made in plant pattern using multispectral camera.

Shin and Kadıoğlu (2019), stated that UAVs have developed with technology and have more usage areas in agricultural activities. Image processing techniques such as weed detection and phytosanitary detection can be used with UAVs and control methods are being developed. In addition to the detection processes with UAVs, the researchers mentioned the importance of both in terms of environmental health and in terms of determining the product patterns in a healthier way, thanks to studies such as fertilization and spraying. As a result, it has been reported that studies can be conducted on the importance of weed detection with UAV.

Su et al (2019), focused on the spatio-temporal monitoring of winter wheat inoculated with yellow rust inoculation at various levels throughout the entire growing season in their study for the spatial-temporal monitoring of wheat yellow rust using UAV multispectral images. They designed a custom workflow to acquire time-series five-band (visible infrared) aerial images with a multi-band camera and UAV. The researchers concluded that it would be an important guide for future early spatial temporal yellow rust monitoring at farmland scales.

Castaldi et al. (2017), used UAV multispectral images to classify maize and weeds. Application of classification results in cornfield weed management has resulted in a reduction in herbicide use without harmful crop yield implications.

Use of Unmanned Aerial Vehicles in Animal Husbandry

Unmanned Aerial Vehicles (UAVs), also known as drones, are being extensively researched to improve efficiency in agricultural production. Applications include monitoring fields, pastures and livestock, identifying/controlling potential problems as well as locating these problems in the field (Gómez-Candón et al., 2014). The popularisation of drones is mainly due to the consolidation of technologies such as Global Positioning System (GPS), embedded microelectronics, miniature autopilot systems, mobile communication equipment, compact high-resolution digital cameras and high-power batteries. This makes drones low cost, safe and easy to use (Guo et al., 2018).

One of the most important and challenging tasks in animal monitoring is cattle counting. Soares et al. (2021), The use of Unmanned Aerial Vehicles (UAVs) has been extensively investigated to improve the efficiency of agricultural production and animal monitoring. In this study, they proposed a method to detect and count cattle in aerial images acquired by UAVs based on Convolutional Neural Networks (CNNs) and a graph-based optimisation to remove duplicate animals detected in overlapping images. Their results show that the proposed method is very competitive, outperforms the state-of-the-art in detecting duplicated animals and significantly reduces the computational cost of the overall counting task. A drone used in animal counting is the DJI Mavic Pro. It is shown in Figure 7.

Figure 7. A drone DJI Mavic Pro used in animal census (Soares et al., 2021).

With the advent of deep learning and especially convolutional neural networks (CNNs), it has become more effective to extract relevant information from aerial imagery. Despite technological advances in drone, imaging and machine learning technologies, the application of UAVs for cattle monitoring is far from being comprehensively studied, with many research gaps still persisting. In this context, Barbedo et al. (2019), They set three objectives in their study: (1) to determine the highest achievable accuracy in detecting animals of the Canchim genus that are visually similar to the Nelore genus (Bos taurus indicus); (2) to determine the ideal ground sample distance for animal detection; (3) to determine the most accurate CNN architecture for this particular problem. The results revealed that many CNN architectures are robust enough to reliably detect animals in aerial imagery even at far from ideal conditions, demonstrating the feasibility of using UAVs for cattle tracking. Images from animal detection using UAVs are shown in Figure 8.

Figure 8. Images from animal detection using UAVs (Barbedo et al., 2019)

Monitoring the welfare of cattle and sheep on large pastures can be time-consuming, especially if animals are scattered over large areas of semi-natural pasture. Automated equipment allows continuous monitoring and can provide more information than manual monitoring. Electronic identification ear tags can recognise visits to specific points. Collars with positioning (GPS) units can assess animals' movements and habitat selection, and to some extent their health and welfare. Digitally determined virtual fences, rather than traditional physical fences, have the potential to contain livestock in a predefined area using sound signals in combination with weak electric shocks, although some individuals may have difficulties responding as intended, potentially resulting in reduced animal welfare. Remote technology, such as drones equipped with cameras, can be used to count animals, determine their location and study their behaviour. Drones can also herd and move animals. "Sensors on devices with 'cloud' server connectivity offer great opportunities, but need to be explored further. Possibilities to save battery power by reducing signal sampling and connection intervals without compromising sensitivity and authenticity need to be explored (Herlin et al., 2021).

A feasibility study was conducted on remotely sensed imagery (using datasets from satellites, manned aircraft and UAVs) and deep learning techniques to detect, count, identify and characterise the posture of individual cows in pasture production systems. With these techniques, we focussed on: (1) automatic detection of cattle locations and animal counting; (2) cow postures such as standing, grazing or lying down; and (3) individual cow identification. Detection, identification and posture monitoring of cattle is possible to some extent with remotely sensed imagery. However, high accuracy in cattle detection cannot be achieved on every platform. UAVs are the most suitable and can accurately detect, identify and monitor their posture. Aerial photographs and VHR satellite imagery can only detect and potentially count cattle. Future developments in VHR satellite imagery are promising as they can provide higher spatial resolutions (pixels smaller than 30 cm), which improves the detection of cattle. Satellite data have the advantage that they can be made available almost daily and cover large areas, which is not possible with UAV technology. Current results show that UAV imagery gives the highest accuracy (>95%) in cattle detection due to the high spatial resolution of images with a pixel size of a few centimetres. It shows that drones with RGB cameras are the best use for detecting and identifying cattle in pastures. Based on experiments with Holstein dairy cows it seems possible to detect, identify and monitor the behaviour of individual cows and therefore to gain more information about cattle in terms of flexibility and efficiency in the investigated production systems. These results make camera-mounted drones a promising new technology for monitoring extensive beef production systems (Mücher et al., 2022).

Li et al. (2022), In a study, they proposed a novel robotic herding system based on autonomous barking drones. They developed a collision-free sliding mode-based motion control algorithm that navigates a network of barking drones to efficiently gather a herd of animals when they are highly dispersed and drive them to a designated location. Simulations using a dynamic animal herding model based on Reynolds' rules have shown that the proposed drone herding system can efficiently herd thousands of animals with a few drones.

The contribution of this paper is the proposal of the first prototype of herding a large herd of livestock by autonomous drones. A proposed drone herding system is shown in Figure 9.

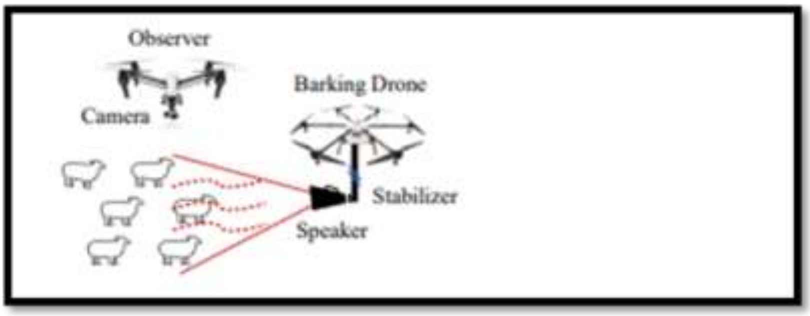

Figure 9. A proposed drone herding system (Li et al., 2022)

Consumer groups are putting pressure on modern farmers to be more efficient, with a focus on better animal welfare. Herding puts farmers' lives at risk, includes stress from farm dogs, and the risks are neglected if not done frequently and intelligently. To adapt mathematical models of shepherding to the new dimension, the behavioural and physiological response of twelve Dorper sheep (Ovies aries) to a drone was studied. The model aims to make it possible for artificial intelligence to enhance the autonomy of farmers and pilots shepherding from the sky. The sheep quickly and positively acclimatised to the drone initiating a ram's flock, regardless of drone speed. The results show that stimulation of sheep auditory awareness during aerial herding leads to altered sheep responses (Yaxley et al., 2021). The expected close response of the sheep to the presence of a Sky Shepherd is shown in Figure 10.

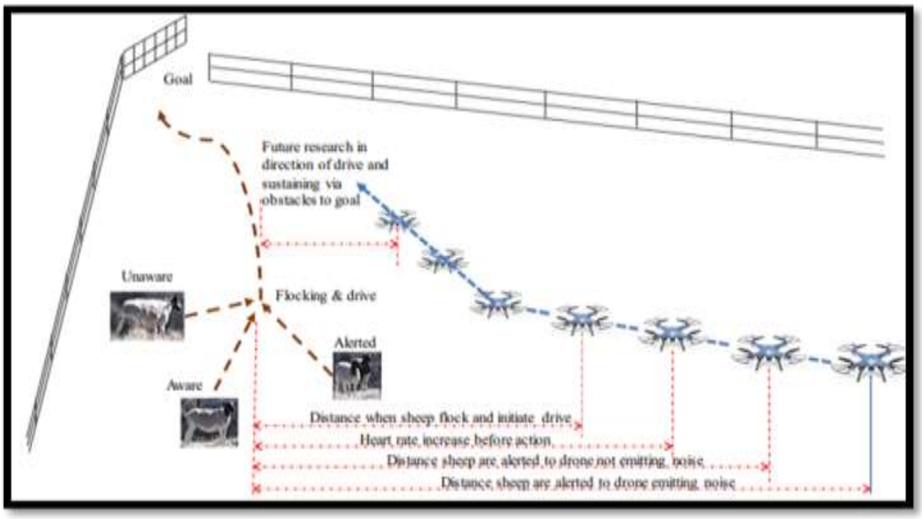

Figure 10. The expected close reaction of the sheep to the presence of a Sky Shepherd (Yaxley et al., 2021)

Conclusion

Although many image processing techniques have recently been developed by researchers for the detection of animal behaviour, further elaboration and enhancement of these techniques with different sensors would greatly contribute to the development of automated management systems that can detect animal behaviour and decide the best solution or instant warning alarm in unusual situations. However, the creation of such a management system is only possible if teams from different research fields such as physiology, zoology and technology are formed.

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EFFECTS OF THE PLANT-GROWTH-PROMOTING RHIZOBACTERIA (PGPRS) ON EXPRESSION OF SALT STRESS RELATED GENES IN TOMATO PLANTS UNDER DROUGHT STRESS CONDITIONS

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ABSTRACT

Climate change, hunger, and food insecurity are among the issues that the agriculture sector is dealing with today. During the critical stages of flowering and seed development, tomato plants are vulnerable to drought stress, and elevated carbon levels also result in yield losses. A decline in tomato productivity, an increase in disease, and a fall in fruit quality will all result from the drought. As a result, emerging biotechnological interventions should focus on enhancing plant yield and stress tolerance. The importance of NAC and NHX genes and the benefits of plant growth–promoting rhizobacteria (PGPR) in improving abiotic stress resistance is widely understood. The potential of a group of SINAC and SINHX genes in the control of drought stress tolerance in the presence of a bacterial strain (113-Bacillusis megaterium) in Solanum lycopersicum is the subject of the present study. In this study, the expression level of 4 SINAC genes and 4 SINHX genes was assessed using the real-time PCR technique. In general, in studied genes, in leaf tissues, expression increased at different levels and times of drought stress compared to the control sample. Also, the inoculation of B. megaterium in the leaf tissue has caused an increase in the relative expression of both genes compared to the control samples and also compared to the samples that were only exposed to drought stress. The results indicated that the transcript accumulation of mentioned genes has been regulated under different levels of drought stress. Once naturally tolerant candidate SINAC and SINHX genes have been discovered and the nature of their correlation with drought stress has been known, transgenic technology can be used to build inherent tolerance in future crops.

Keywords: NAC transcription factor, *NHX* family genes, real-time PCR, gene expression, tomato, drought stress

Introduction:

According to the latest statistics of FAOSTAT (2022), tomato, as one of the most important garden crops, produced over 13 million tons in Turkey in 2020. Also, this product was produced over 251 million tons throughout the world this year. Important nutrients like phenols, flavones, carotenoids, vitamin C, and vitamin A, powerful antioxidants, and minerals like potassium, phosphorus, calcium, iron, and folic acid are present in tomato. Thus, it is frequently consumed both fresh and processed (Tomas et al., 2017). Turkey, for instance, produces tomatoes with a fourth of its total horticultural production (FAOSTAT 2020). The agriculture industry has progressively suffered as a result of recent global climate change. In this regard, it is crucial to focus on thorough research to counter these changes on a global scale (Mahato, 2014). As immobile organisms, plants are subject to a variety of biotic and abiotic stresses that have a detrimental impact on their growth, development, and yield (Lippmann et al., 2019). Plants have created a variety of defense mechanisms to deal with different challenges, including modifications to gene expression and cell metabolism as well as adjustments to plant growth, development, and performance (Akula Ramakrishna et al., 2011). Drought stress is one of the most significant and prominent abiotic stresses in the world today (Xu and Zhou, 2005). This type of abiotic stress is effective in the plant when soil moisture hits critical levels and atmospheric factors like air heat and solar radiation are the root of ongoing water loss. All plants have efficient defensive mechanisms to endure drought stress, however, these defense mechanisms function differently in various species (Xu and Zhou, 2005). Broadly speaking, plants have been shown to have five defense mechanisms against drought stress: the unfolded protein response (UPR), heat shock response (HSR), epigenetic controls, ROS homeostasis, and the regulations in which hormones are involved (Zhao et al., 2020). Genetic screening of plants to find stress-resistant species and develop them is one of the best approaches to dealing with all sorts of stress (Ermawati et al., 2021).

One of the most significant plant-specific TF families is the NAC (NAM, ATAF, and CUC) domain protein family. No apical meristem (NAM), ATAF1-2, and cup-shaped cotyledon (CUC) are three proteins that share a DNA-binding domain and from which it initially got its name [Aida et al., 1997; Souer et al., 1996]. Typically, NAC proteins have a varied transcription regulatory region at the C-terminus and a conserved NAM domain at the N-terminus (Ooka et al., 2003). Nearly 160 amino acids (aa) residues make up the N-terminal *NAC* domain, which was split into different subdomains (Ooka et al., 2003). Some Subdomains were highly diverse and may give NAC TFs functional variety, whereas some others were often largely conserved (Puranik et al., 2012; Ooka et al., 2003). The C-terminal transcription regulatory regions, in contrast, exhibit high levels of divergence and serve as functional domains by regulating a variety of transcriptional activation activities (Puranik et al., 2012; Ooka et al., 2003). Furthermore, several NAC TFs have transmembrane domains at their C-terminal ends that aid in anchoring to the plasma membrane or endoplasmic reticulum (Seo et al., 2008).

Researchers have identified and classified five subdomains for NAC (A to E). Subdomain A is involved in protein dimerization or heterodimerization. E and B subdomains are responsible for diversity in the function of NAC proteins. The presence of D and C subdomains are necessary for DNA interaction (Puranik et al., 2012). The NAC transcription factor family is one of the efficient genes whose function has been established in the tolerance of diverse biotic and abiotic stressors in plants (Shao et al., 2015). Additionally, studies have shown that this large gene family plays an important role in controlling the synthesis of the secondary cell wall (Zhong et al. 2010), the formation of the stem apical meristem (Aida et al. 1997), embryo development (Duval et al. 2002), and flower growth (Sablowski and Meyerowitz 1998) over the years. The study of this gene family in the past years has attracted the attention of researchers due to the significant role they play in the life of plants and their location so far in many plants such as Arabidopsis (Ooka et al., 2003), rice (Nuruzzaman et al., 2010, pear (Ahmad et al., 2018), tomato Li et al. (2022, etc.) has been identified. Also, the effective role of the large *NAC* family against plants with a variety of biotic and abiotic stresses has been investigated in many research, for example, the role of *NAC* in tomato in drought stress (Jian et al., 2021), aluminum, salinity (Wang et al., 2017) and pathogen attack (Du et al., 2022) have been investigated.

Na+/H+ antiporters, also known as NHXs, which serve as secondary ion transporters for H+ exchange and Na+ or K+ transport across the plant membrane during stressors, are among other genes that have a substantial impact on how the plant reacts to various stresses. Tian). SOS1-like*NHX*, which is found on the cell membrane, and the second category, known as IC-NHE/*NHX*, which contains a multitude of isoforms, are the two primary groups into which NHXs have so far been classified by scientists. According to research by Rodriguez-Rosales et al. (2009) and Leidi et al. (2010), NHXs are involved in the regulation of internal pH and cell development. Different NHX isoforms have so far been shown to have a favorable impact on plants that have experienced a variety of abiotic challenges, such as salinity stress, ionic stress, and nutrient shortage stress (Brini and Masmoudi, 2012).

Beet researchers Kloepper and Schroth discovered in 1981 that rhizobacteria in the soil accelerate beet development by altering the roots and also making the plant more resistant to plant diseases. After further research, these helpful rhizobacteria were termed plant growth-promoting rhizobacteria (PGPR) a few years later, in 1981. Based on where each PGPR acts on the plant cell, Martinez-Viveros proposed classifying PGPRs in 2010. This gives them the names Epgpr and iPGPR, respectively, depending on whether they have an extracellular or intracellular action. Numerous researchers have so far looked into how PGPR affects various plants in various environments. The impact of PGPR, for instance, has been researched so far on tomato production growth, fruit quality, resistance to water stress (Tahiri et al., 2022), salinity stress (Nseri et al., 2022), drought stress (Calvo-Polanco et al., 2016), and Verticillium dahliae stress (Bhattacharyya and Jha., 2012). Cakmakci et al. have also conducted other experiments on the impact of PGPRs on potato, wheat, corn, peas, corn, and cucumber (2006). They can be regarded as biological control agents in biotic and abiotic challenges, effective in enhancing production efficiency, and as biofertilizers in sustainable agriculture due to the great strengths that have been demonstrated in PGPR thus far (Freitas et al. 2007; Yildirim et al. 2011).

This study examines the potential role of a collection of *SINAC* and *SINHX* genes in the regulation of drought stress tolerance when a bacterial strain (113-*Bacillusis megaterium*) is present in *Solanum lycopersicum*.

Materials and Methods

Plant Selection and Inoculation: The study used *Solanum lycopersicum* MSC-50 variety. A selected group of these plants were inoculated with *Bacillus megaterium*, a type of PGPR. The objective of this step was to observe how the plant responds to the PGPR treatment.

Induction of Drought Stress: After the inoculation, drought stress conditions were created. This was done by applying three different concentrations of Polyethylene Glycol (PEG), a commonly used substance to mimic drought stress in lab settings. The PEG treatment was administered at two distinct time points: 2 hours and 12 hours after the PGPR inoculation. The doses of PEG and their effects on the plants were detailed in Tables 2 and 3.

Sampling and Tissue Collection: The plants were systematically sampled by collecting both leaves. To maintain the cellular integrity of the samples, they were pulverized using liquid nitrogen. This step was crucial for accurate subsequent analysis.

Sample Preservation: The pulverized samples were stored in Falcon tubes at a temperature of -80 degrees Celsius. This temperature control was essential to ensure the preservation of the biological and biochemical characteristics of the samples.

3.1. Leaf Samples & Treatments

Table 1. Leaf Sample Treatments

Applied Dose of PEG	Samples
(0.25 mM PEG) 31 g of PEG per liter – 1116 g of PEG was used for 36 liters. (36 pots - 1 liter per pot)	**MC:**Control group of MSC-50 tomato variety with no application
	MP1-2h: PEG-treated samples (2 hours)
	MP1-12h: PEG-treated samples (12 hours)
	MBC: Untreated control sample of MSC-50 variety inoculated with 113-*B. megatrium*
	MP1B-2h: PEG-treated samples (2 hours) included with 113-*B. megatrium*
	MP1B-12h: PEG-treated samples (12 hours) included with 113-*B. megatrium*
(0.50mM PEG) 50 g of PEG per liter – 1500 g of PEG was used for 30 liters. (30 pots - 1 liter per pot)	**MP2-2h**: PEG-treated samples (2 hours)
	MP2-12h: PEG-treated samples (12 hours)
	MP2B-2h: PEG-treated samples (2 hours) included with 113-*B. megatrium*
	MP2B-12h: PEG-treated samples (12 hours) included with 113-*B. megatrium*
(0.75mM PEG) 65.5 g of PEG per liter – 1179 g of PEG was used for 18 liters. (24 pots - 750 ml per pot)	**MP3-2h:** PEG-treated samples (2 hours)
	MP3-12h: PEG-treated samples (12 hours)
	MP3B-2h: PEG-treated samples (2 hours) included with 113-*B. megatrium*
	MP3B-12h: PEG-treated samples (12 hours)

	included with 113-*B. megatrium*

RNA Isolation

RNA isolation was accomplished following a modified version of Bray's (1988) method. About 300 mg of the sample was weighed and placed in Eppendorf tubes, followed by the addition of an extraction solution comprising 50 mM Tris (pH 9), 150 mM LiCl, 5 mM EDTA, and 5% SDS. After vortexing and centrifugation, the upper phase was combined with a phenol chloroform isoamyl alcohol mixture. Subsequent centrifugation separated the supernatant, half of which was treated with 10M LiCl and incubated at +4°C. After centrifugation, the upper phase was discarded, and the remaining supernatant was treated with ethanol, centrifuged, and dried. The pellet was then dissolved in DEPC-treated water.

Purification of RNAs from DNA

RNA purification involved the use of DNase I RNase Free (Thermo) to eliminate genomic DNA from total RNA following the manufacturer's guidelines. The procedure included treating 1 µg of RNA with DNase I and specific reagents, incubating at 37°C for 30 minutes, and subsequently at 65°C for 10 minutes. The quality of the RNA was assessed through 1% agarose gel electrophoresis. The resulting DNase-treated RNAs were stored at -20 degrees Celsius for subsequent steps.

cDNA Synthesis

The cDNA synthesis process utilized a Thermo Fisher cDNA kit according to the manufacturer's instructions. Sample analysis employed BiO1D software, using 500 ng of RNA based on observed mRNA bands from gel electrophoresis. A 12 µL solution containing 500 ng RNA, 1 µg oligo(dT)18, and dH2O was heated at 65°C for 5 minutes, followed by rapid cooling on ice. In a separate Eppendorf tube, a mixture of 5X reaction buffer, RiboLock RNase Inhibitor, dNTPs, and RevertAid Reverse Transcriptase RNA was prepared. Then, 8 µL of this mixture was added to each sample. Incubation occurred at 42°C for 1 hour, followed by a 5-minute step at 70°C and cooling on ice. The generated cDNAs were partitioned into separate Eppendorf tubes and stored at -20°C to maintain stability.7.

Gene Sequence Identification

The nucleotide sequences of the genes whose expression will be analyzed were obtained via the Solgenomicsdatabase(https://solgenomics.net/organism/Solanum%20lycopersicum/view) and similar genes were searched using the NCBI and its Blast tool. The Gene ID of the genes studied in this research can be found in Appendix 1.

Designing Specific Primers for *NAC* and *NHX* Genes

The sequences of *SlNAC* and *SlNHX* genes of Arabidopsis was extracted from the TAIR database and in order to find the similar sequences of tomato, using the blast tool on the solgenomics database and the prepared sequences were re-checked for certainty in NCBI and the specific primers were designed in the Eurofins genomics database (https://eurofinsgenomics.eu/en/ecom/tools/pcr-primer-design/). Appendix 2 contains the primer sequences list used in this study. Each primer was evaluated for effectiveness with cDNA produced using standard PCR equipment, and the results were verified on a 1% agarose gel.

Real-Time PCR Test

The Real-Time PCR analysis was performed using a LightCycler 480 II machine from Roche. The RealQ Plus 2x Master Mix Green qPCR Master Kit was utilized with the actin gene as the reference. Peak profiles were established for each gene in the samples, and Ct (Cycle Threshold) values were generated from these profiles. The 2-ΔΔCT method was employed to calculate relative expression values based on Ct values.

Results

Expression Profiles of *SlNAC* Genes in Tomato Leaves

The relative expression profile revealed that the *SlNAC37* gene was significantly upregulated following PEG treatment across all concentrations tested. Notably, after 12 hours of exposure to MP1, the upregulation was evident in comparison to the control group. The application of PGPR strain 113-Bacillusis megaterium further augmented the expression of *SlNAC37*. In conditions of MP2, the gene exhibited a transient downregulation at the 2-hour mark, followed by an upregulation after 12 hours. Meanwhile, under MP3 conditions, a moderate upregulation was recorded both at 2 and 12 hours post-treatment (Fig. A2).*SlNAC40* gene expression saw an upsurge post-PEG treatment, with both 2-hour and 12-hour intervals showing increased transcript abundance relative to the control. Moreover, the presence of PGPR strain 113-Bacillusis megaterium was found to positively regulate *SlNAC40* gene expression in tomato leaves (Fig. A3).For both the *SlNAC43* and *SlNAC45* genes, PEG treatment resulted in a marked increase in transcript abundance at 2 and 12-hour intervals when juxtaposed with the control sample. The introduction of PGPR strain 113-Bacillusis megaterium further modulated the gene expressions, underscoring the combined effects of PEG-induced drought stress and PGPR treatment on the genes' activity in tomato leaves (Fig. A4 & Fig. A5 respectively).

Expression Profiles of *SlNHX* Genes in Tomato Leaves

The *SlNHX1* gene displayed an upregulation in its transcript levels both at 2 and 12-hour marks, in comparison to the control sample. Furthermore, the presence of PGPR strain 113-Bacillusis megaterium distinctly influenced the *SlNHX1* gene's expression patterns (Fig. A6).Similar to *SlNHX1*, *SlNHX2* gene also manifested an elevated expression profile at both the 2-hour and 12-hour intervals following PEG treatment. The influence of PGPR strain 113-Bacillusis megaterium on the gene was evident, bolstering its expression in the tomato leaves (Fig. A7).The *SlNHX3* gene showcased an upregulation in its transcripts at the 2 and 12-hour post-PEG treatment intervals. The inclusion of PGPR strain 113-Bacillusis megaterium further amplified the gene's expression, signifying the synergistic effects of drought stress and PGPR treatment (Fig. A8).Observations for echoed the patterns seen in other *SlNHX* genes, with the transcript *SlNHX4* abundance escalating at both intervals after PEG treatment. The addition of PGPR strain 113-Bacillusis megaterium further augmented the gene's expression, emphasizing the role of both drought stress and PGPR in modulating its activity (Fig. A9).

4. Discussion

Tomato cultivars responded to water restriction with a significant proportional fall in yield in semi-arid climate circumstances such as Turkey, also Water stress made plants more vulnerable to pathogenic diseases such as viruses, bacteria, and fungi (Celebi 2014). It is now widely known that several genes, including transcription factors (TFs) that help plants endure adverse conditions, regulate drought tolerance. These genes continue to be prospective genomic candidates for widespread crop breeding (Joshi et al., 2016). Also, globally, drought stress has an impact on plant development and productivity, and *NHX* genes, are well known for increasing drought tolerance in transgenic plants. Several plants have well-defined; nevertheless, nothing is known about *NHX*s in tomato plant (*S. lycopersicum*).

4.1. Expression Profile of *SlNAC* Genes

In the current study, the expression profile of the tomato NAC gene family was systemically examined. Numerous researche have shown that NAC Transcription factors are present in a wide variety of plant species. and their ability to play a role in controlling plant growth, development, and stress responses (Puranik et al., 2012). Up until this point, this family appeared to be one of the biggest TFs. It was reported that Arabidopsis, rice, grape, apple, maize, chickpea, cassava, sesame, pears, and buckwheat have 117, 151, 79,180, 152, 71, 96, 87, 185, and 80 NAC genes (Ooka et al., 2003; Nuruzzaman et al., 2010; Wang et al., 2013; Shiriga et al., 2014; Ha et al., 2014; Hu et al., 2015; Zhang et al., 2018; Ahmad et al., 2018; Liu et al., 2019).

According to previous studies in the Solanaceae family, a considerable number of NAC genes were overexpressed under drought stress in S. lycopersicum (Al-Abdallat et al., 2015), S. tuberosum (Singh et al., 2013), S. muricatum (Yang et al., 2021), and sweet potato (Yan et al., 2021), and under other abiotic stresses such as S. lycopersicum under Aluminum stress (Jin et al., 2020) or in the development process in S. melongena (Wan et al., 2021). This trend was consistent with the result of the present study where overexpression of a huge number of NAC genes under drought stress and PGPR inoculation has been approved.

Gene expression patterns can typically offer crucial clues for gene activity. Consequently, the expression levels of the 4 SlNAC genes in the leaf of S. lycopersicum were determined using qRT-PCR data. A higher or lower expression level of the studied SlNACs under different conditions (drought stress and PGPR treatment) in the leaf tissue, compared to the control samples was found. These SlNACs demonstrated tissue- and stress-specific expression patterns. These genes may play significant roles in tomato stress tolerance. NAC genes in leaf samples including SlNAC37, SlNAC40, SlNAC43, and SlNAC47 were highly expressed in all doses of drought stress treated samples, indicating that they may be involved in particular drought tolerance system in S. lycopersicum. The specific roles of the tomato SlNAC genes will require further investigation in the future.

4.2. Expression Profile of *SINHX* Genes

For many plants, including A. thaliana (Yokoi et al., 2002), rice (Basu et al., 2014), wheat (Yarra, 2019), sweet beet (Wu et al., 2019), cotton (Ma et al., 2020), and other plants, the importance of NHX gene families under drought and salt stresses have previously been discovered. However, the functionality of NHX genes in S. lycopersicum under drought stress using PGPR has not been studied yet. In this investigation, the genomic expression of four NHX genes in S. lycopersicum was examined. According to the research papers that have been mentioned earlier, the expression level of NHX genes changed significantly in drought and salt-stress-treated samples. Those results are completely consistent with the results of the SINHX gene expression profile in the present study.

Sodium-proton antiporters in tomato plants (S. lycopersicum) facilitate Na+/H+ and K+/H+ exchanges. This contributes to stress tolerance as well as K+ nutrition. NHXs have also been found to increase salinity tolerance in leaves (Zhang and Blumwald, 2001). There was another research which was done by Rodrguez-Rosales et al. (2008) in this regard with the same approach. The SINHXs may also be a part of the responses to drought, according to the expression pattern for different genes and tissues. The tissues' diverse expression patterns suggested that the NHX gene family offers options to breed this plant and overcome the functional restriction imposed by the original gene under drought stress. According to previous studies, it is known that there are many NHX protein isoforms present in tomato plants. Based on a study carried out by Rodríguez-Rosales et al. (2009) the majority of the NHX genes were activated by salt stress in the leaves of tomato (lycopersicon esculentum). It shows that NHX genes play a crucial role and have different functions in the defense system of S. lycopersicum in different tissues.

Regarding the effect of different durations of exposure to drought stress, it is reported that the expression level at different durations of PEG treatment was highly variable in leaf and root tissues of tea (Camellia sinensis) (Paul et al., 2021). This fold change variation was exactly what was observed in this study.

4.3. Plant growth-promoting rhizobacteria

The influence of plant growth-promoting rhizobacteria (PGPR) in bolstering host resilience during abiotic stress periods is well-documented, yet the molecular impact on tomato plants (S. lycopersicum), which frequently face drought conditions in Turkey, remains largely underexplored. Bacillus megaterium was found to stimulate tomato growth under both normal and salt-stressed environments. Regardless of the conditions, B. megaterium notably boosted the development of tomato plants, leading to more robust roots, shoots, and leaves (Nascimento et al., 2020). This study revealed that the inoculation of tomato seedlings with B. megaterium under normal conditions significantly increased the root and shoot dry weight, resulting in a pronounced augmentation in the overall dry biomass of the tomato plant. Similar observations were recorded under stress conditions, where B. megaterium-inoculated tomato seedlings displayed a substantially higher root and shoot dry weight, leading to an increase in total dry biomass compared to non-inoculated plants. These findings were complemented by the observed elevated NAC and NHX expression levels in PGPR-treated samples exposed to PEG, underpinning the beneficial role of PGPR in supporting tomato plants during drought stress.

While *B. megaterium* boosted expression levels of specific genes involved in the repair of damaged photosynthetic equipment and the preservation of redox equilibrium, it lowered the production of ROS and ethylene. Additionally, *B. megaterium* dramatically changed the metabolic profile to fix salinity-induced physiological disturbances in tomato (*l. esculentum)* (Akram et al., 2019). An observed increase in drought tolerance in this study is completely consistent with the higher level of expression in *NAC* and *NHX* genes in the leaf samples treated with X bacteria in this study.

Yang et al., (2022) also reported that *B. megaterium* could efficiently increase the tolerance of tomato (*S. lycopersicum*) under biotic stresses by affecting a number of functional resistance genes. In addition, Samaras et al. (2021) provided the same result in the transcription pattern of defense-related genes when this genus of rhizobacteria was inoculated into this plant. This rhizobacterium had the same impact as these two previous studies on *NAC* and *NHX* genes in this study.

5. Conclusion

The data obtained from this study will provide essential information for the functional characterization of these genes in tomato under drought stress. In general, we can see that Differential gene expression in NAC and NHX genes were considerable in leaf samples.Also a notable increase in the expression of almost all of investigated SINAC genes has been seen in the leaf specially in 12 hour . This increase in expression at the highest level of PEG has been more considerable than other doses. Also, PGPR inoculation had a positive effect on increasing the expression of the mentioned genes, especially in the second and third doses of PEG. In relation to four SINHX genes, an increase in expression has been seen due to exposure to drought. This increase in expression in samples inoculated with PGPR has increased more in the second and third doses and time has a considerable effect on level of expression .

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Appendix 1:

Table 2. Gene IDs

Gene Name	**Ensembl Gene ID**
SlNAC37	Solyc04g079940
SlNAC40	Solyc05g009840
SlNAC43	Solyc05g055470
SlNAC45	Solyc06g008360
SINHX1	Solyc06g008820.2
SINHX2	Solyc04g056600.2
SINHX3	Solyc01g067710.2
SINHX4	Solyc01g098190.2

Appendix 2:

Table 3. Primer Sequences

Primer	Sequence
LeActinF	GCCGGGCGTGATCTTACTGA
LeActinR	AGCTACTCCTGGCGGTCTCC
SlNAC37F	AATGGTGGGACAGCGAGTCA
SlNAC37R	CGGGTCCTAAACGCGCATAA
SlNAC40F	TGTTGGGCGGTATTCCTGCT
SlNAC40R	AACCCGTCCATCCCATTGCT
SlNAC43F	TGTAGCTGCACCTCCTGGTT
SlNAC43R	TGGAGCACTCGCCAATCAGT
SlNAC45F	TGACCCATGGGACCTTCCAG
SlNAC45R	TGTCTTTCCCTGTGGCTTTCCA
SlNHX1F	GCGTCGAGCACCATCTTAGG
SlNHX1R	TCACGGTCAGTAGAGTGCCT
SlNHX2F	CTCCTGCTCCTCGTTCTCCA
SlNHX2R	AAGGACCTGGGTGAAGCTGT
SlNHX3F	GCGAGGGCTGCTAATGTGTT
SlNHX3R	TGACTGCAAAGCAAGGGCAA
SlNHX4F	TGGTGGGCTGGTTTAATGCG
SlNHX4R	TTGGGTGTGGCCAAATCTCG

Appendix 3: Results:

Expression Profile of *SlNAC* Genes in leaves of tomato plants

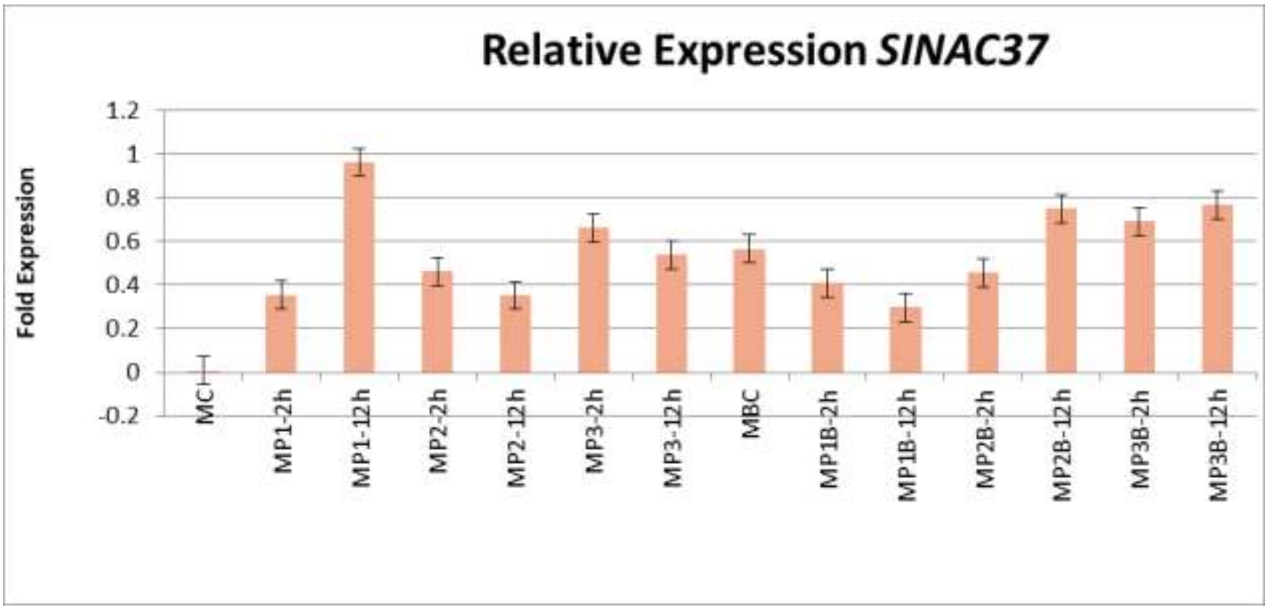

Figure 1. The Relative expression profile of *SlNAC37* in leaves

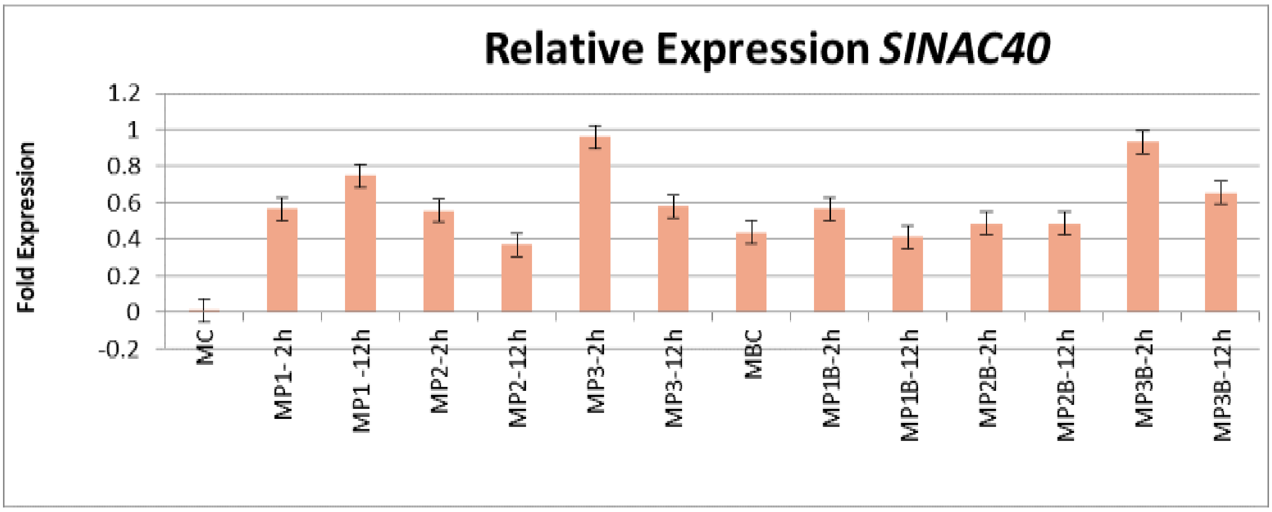

Figure 2. The Relative expression profile of *SlNAC40* in leaves

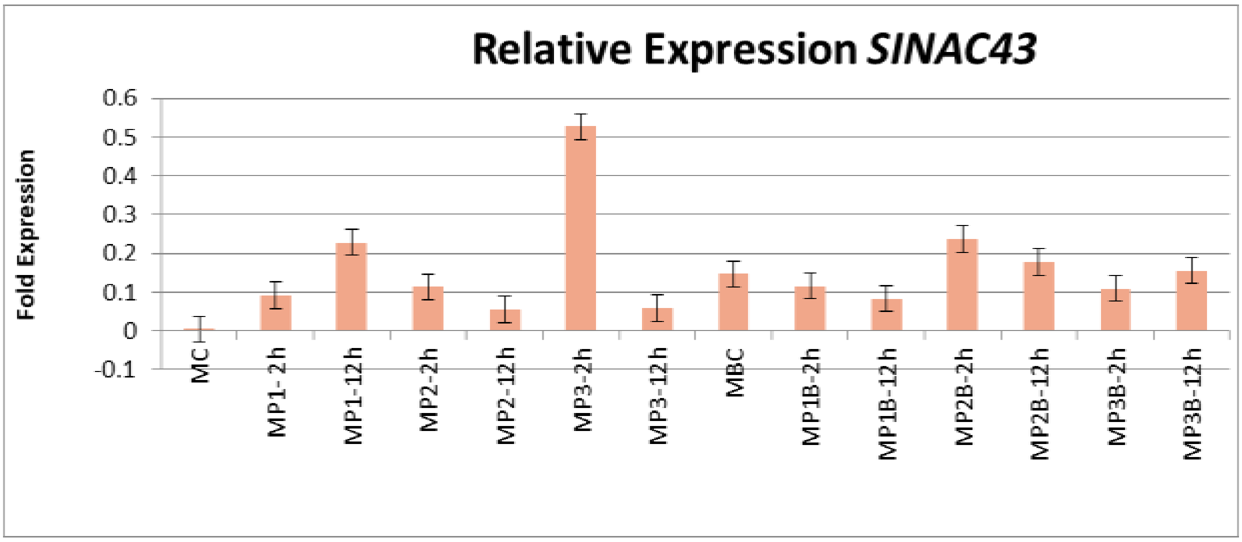

Figure 3. The Relative expression profile of *SlNAC43* in leaves

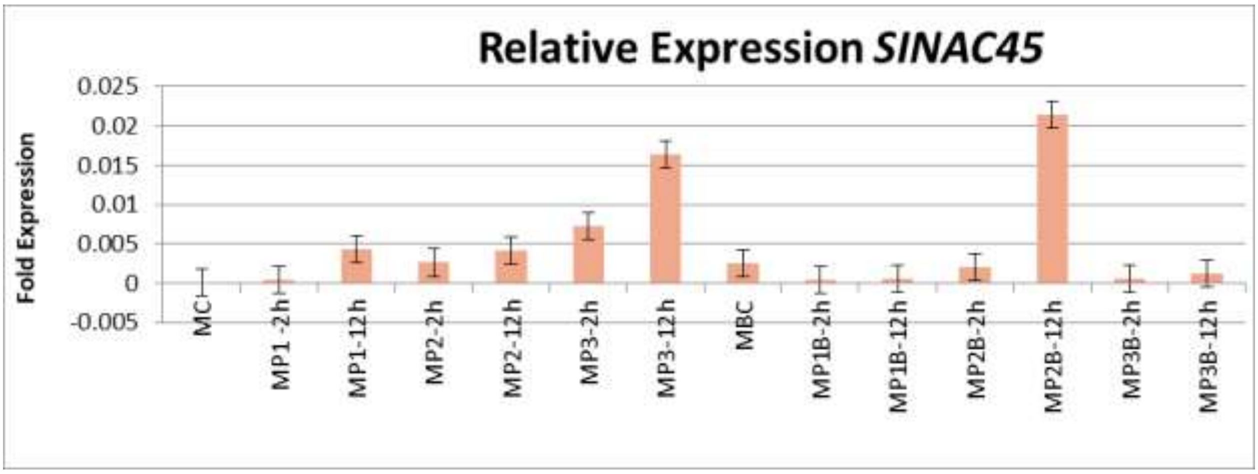

Figure 4. The Relative expression profile of *SlNAC45* in leaves

Expression Profile of *SlNHX* Genes in leaves of tomato plants

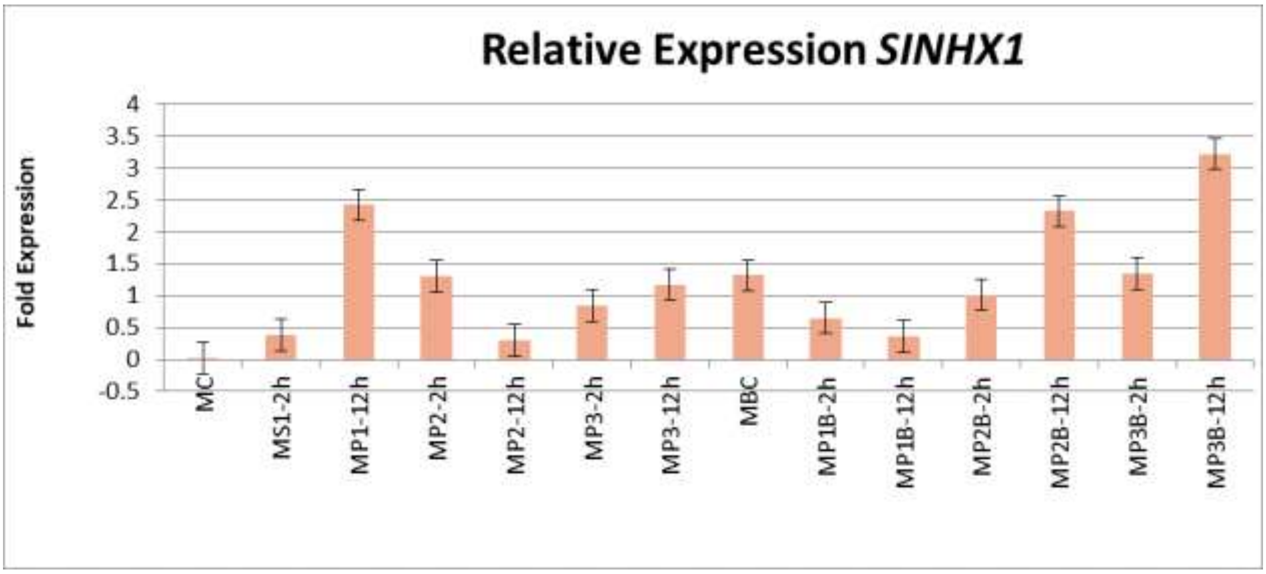

Figure 5. The Relative expression profile of *SlNHX1* in leaves

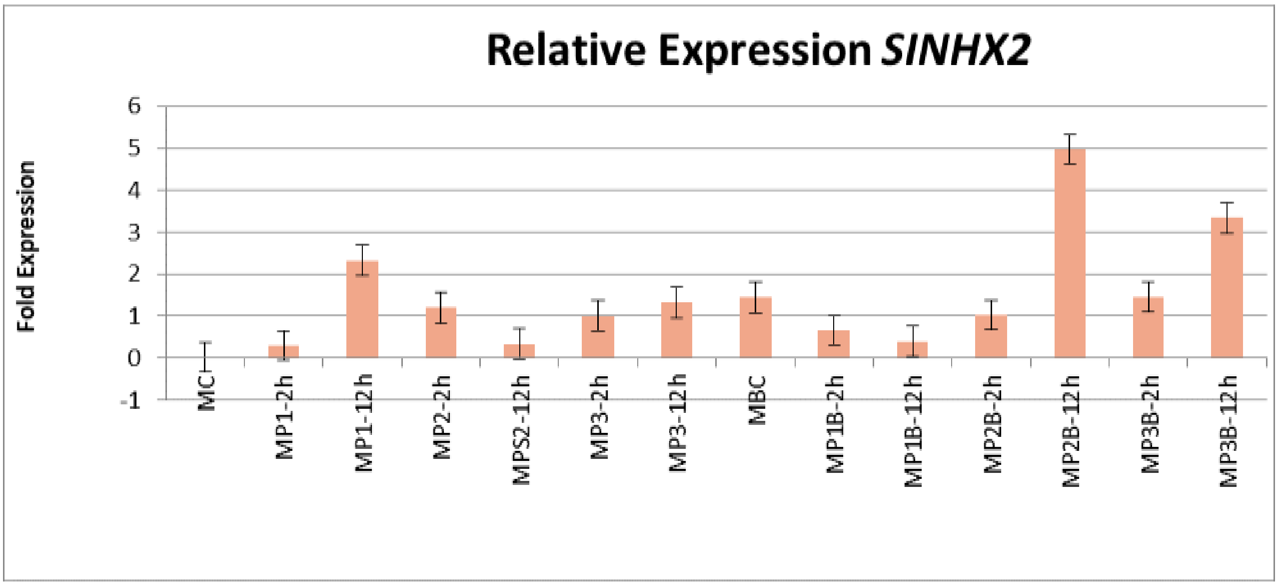

Figure 6. The Relative expression profile of *SlNHX2* in leaves

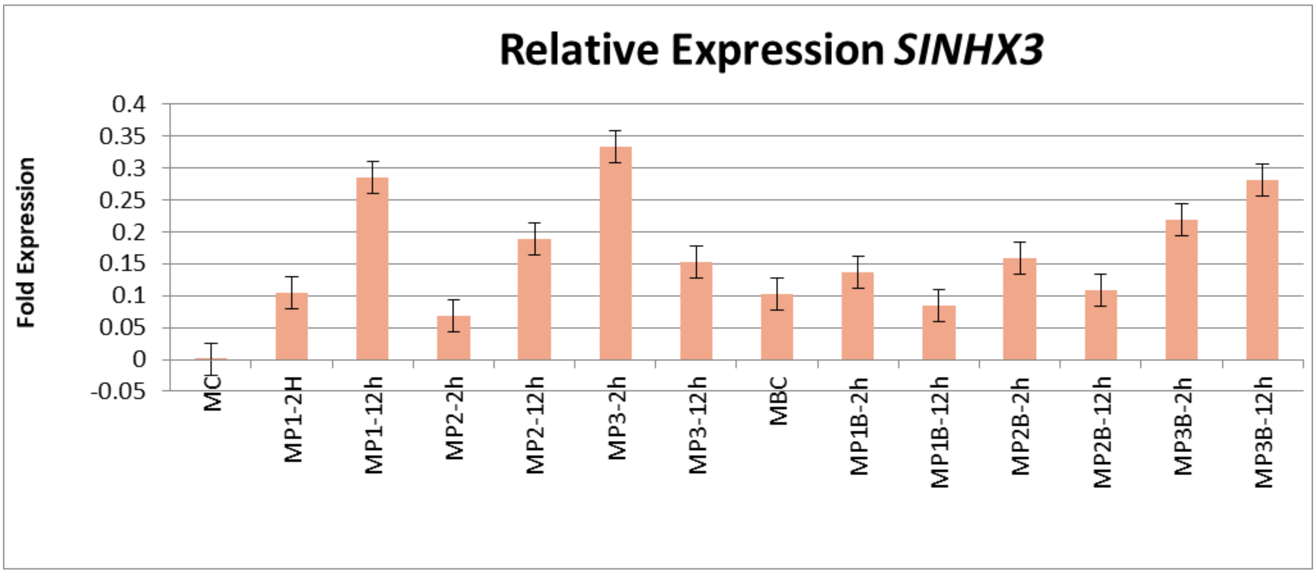

Figure 7. Relative expression profile of *SlNHX3* in leaves

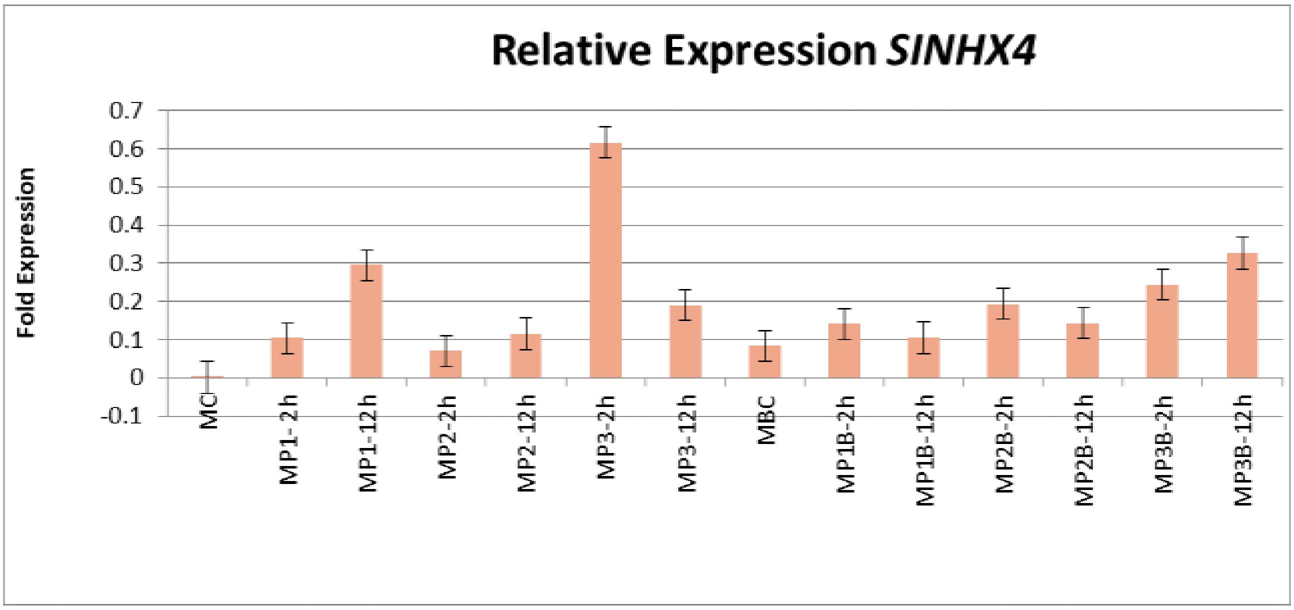

Figure 8. The Relative expression profile of *SlNHX4* in leaves

PHENOLOGICAL DEVELOPMENTAL STAGES AND FRUIT QUALITY PROPERTIES OF DIFFERENT BLUEBERRY CULTIVARS GROWN UNDER SOILLESS CONDITIONS

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ABSTRACT

Blueberry is a type of berry fruit that stands out with its antioxidant properties and that production and consumption is increasing rapidly in the world. Soilless blueberry growing provides high unit area yield compared to soil cultivation. In addition, yield and fruit quality increase with a controlled cultivation process made in computer-controlled automation systems. Furthermore, the use of less water and fertilizer compared to soil-based agriculture ensures sustainability in production. The most important factors affecting success in soilless blueberry growing are cultivar, system planning and plant nutrition management and all these factors should be planned according to the ecology. Highbush blueberry cultivars (southern and northern) are popular in our country. It is necessary to reveal the adaptations of the cultivars according to the regions both in open air and under greenhouse conditions.

In this study, some plant growth parameters and fruit quality properties of three different southern highbush blueberry cultivars were investigated in soilless cultivation in Antalya ecological conditions. 'Misty', 'Biloxi' and 'Star' cultivars were used in the study. In the study, acidic cocopite was used as growing medium. The nutrient solution was applied at pH=4.5, EC=0.8 dS/m throughout the vegetation and irrigation schedule was applied according to solar radiation. Morphological and phenological properties (leaf width, length, flowering and first harvest date) and pomological properties (fruit width, length, weight, total soluble solid content, color L, a*, b*, C° and h* values) were recorded. As a result of the study, it was observed that 'Misty' was the earliest cultivar in terms of both flowering and harvest date. This cultivar was followed by 'Biloxi' and 'Star' cultivars. The highest fruit weight and soluble solid content were determined in 'Star' cultivar, while the lowest was determined in 'Misty' cultivar.

Keywords: Southern highbush blueberry, subtropical conditions, earliness, fruit quality.

INTRODUCTION

Blueberries, which belong to the berry fruit group, systematically belong to the genus *Vaccinium* and there are three types: highbush blueberries (*Vaccinium corymbosum* L.), rabbiteye blueberries (*Vaccinium ashei* Reade synm: *Vaccinium virgatum* Ait) and lowbush blueberries (*Vaccinium angustifolium* Ait.) (Çelik ve Ağaoğlu, 2013). Highbush blueberries are the most widely cultivated type of blueberries in the world and they are divided into two groups as southern and northern varieties according to their chilling requirements. Southern highbush varieties need 150-800 chilling hours; northern highbush varieties need 800-1200 chilling hours; and rabbiteye varieties need 300-700 chilling hours (Retamales ve Hancock, 2012).

Commercially cultivated blueberry varieties started in the 1900s with the varieties bred in the USA and Canada, and the breeding of highbush blueberry varieties of southern origin, which have low chilling requirements in terms of earliness, continues today (Retamales ve Hancock, 2012).

As a matter of fact, earliness is provided with this variety. In the first studies, the 'Star' variety was introduced as an early earliness variety (Lyrene and Sherman, 2000), and in 2008, the 'Rebel' southern origin highbush blueberry variety, which matures earlier than this variety, was bred (NeSmith, 2008). In a study of 'Bluecrop', 'Duke', 'Brigitta', 'Gulfcoast', 'O'Neal', and 'Blue Ridge' highbush cultivars in eastern China, where temperatures of 25, 30, 35, and 40°C were tested, plant growth declined drastically with increasing temperature (Zheng vd. 2017).

Among the southern highbush varieties, Emerald, Jewel, Misty, Misty, Primadonna, Scintilla, Sebring, Sharpblue, Snowchaser, Springhigh, Springwide, Sweetcrisp varieties have a chilling period of less than 300 hours; Abundance, Farthing, Palmetto varieties 300-400 hours; Biloxi, Camelia, O'Neal, Rebel, Southern Belle, Star varieties 400-500 hours; Bluecrisp, Gupton, Legacy, Santa Fe varieties 500-600 hours; Arlen, Bladen, Reville varieties 800-900 hours (Retamales and Hancock, 2012). Therefore, the first condition of regional variety adaptation is to provide the requirement of chilling (Retamales and Hancock, 2012).

Blueberry cultivation in Turkey started in the 2000s and was first practiced with conventional methods in the acidic soils of the Black Sea region. In recent years, blueberry cultivation with soilless growing has become widespread, in particular in the Mediterranean region. Soilless blueberry production, which was first realized in Antalya, is increasing day by day. As a matter of fact, 2610 tons of soilless blueberries were produced on 1740 decares of area in Antalya in 2022 (Antalya Directorate of Provincial Agriculture and Forestry, 2023). According to TUIK (2021), the total area of blueberry production in Turkey is 6613 decares and 4300 tons. As a matter of fact, half of the total production in Turkey is supplied from Antalya province. In particular, the fact that the yield in production from Antalya province is 2-3 tons/da (Antalya Directorate of Provincial Agriculture and Forestry, 2023) shows the efficiency of both climate and soilless production.

In Antalya, which has altitude and low altitude areas, southern and northern blueberry varieties can be grown. However, variety adaptations need to be carried out according to the regions. Carter et al. (2002) stated that chilling capacity and cold resistance are cultivar-specific traits; therefore, it is necessary to determine the performance of cultivars according to regions.

The aim of this study was to investigate southern highbush blueberry cultivars in terms of some growth criterias under soilless conditions in Antalya, one of the southern provinces of Turkey.

MATERIAL AND METHOD

The research was carried out between 2022-2023 in the Akdeniz University Research and Application Areas (36°54'0.98 "N 30°38'53.49 "E). Two-year-old seedlings of Star, Biloxi and Misty varieties were used as material in the research and variety attributes are given below.

Biloxi: The plants have upright and vigorous growth habits and have approximately 400-500 hours chilling requirement. The fruits of the variety, which has the need for cross-pollination, are of medium size, high quality, suitable for fresh consumption, and stand out with their taste thanks to the balance of soluble solid content and acid ratio. It has early flowering and early fruit ripening feature.

Misty: It is one of the earliness blueberry varieties and has a chilling period of 300 hours. It can be used as a pollinator in many blueberry orchards. It is the most popular variety in California due to its medium-sized fruits, regular fruit yield every year, high yield and fast growth. Yield and quality increase when planted with at least 2 different varieties.

Star: It is a southern highbush variety. It was obtained in 1981 from O'Neal x Fla.80-31 hybridization. It is one of the earliness varieties with approximately 400 hours chilling requirement. Fruit shape and size are similar to O'Neal. Star pedicel marks are small and dry, fruits are very firm and have a pleasant sweet taste with a little acidity. It has medium-high resistance to *Phytophthora cinnamomi, Botryosphaeria corticis* and *B. dothidea.*

The seedlings were planted in 30-liter pots containing acidic coccopite (ABI GLOBAL FZ CO, ABI PERU S.A.C) on 1 December 2022. Plant nutrient solution was applied according to Voogt et al. (2014) and fertigation was done with a automation system. Irrigation management during vegetation was planned according to solar radiation (Pozo vd. 2014).

In the study, some morphological and phenological observations and pomological analyzes were carried out on the plants. The experiment was planned as randomized plot design with 3 replications and 2 plants in each replicate. In the study, statistical analyzes were performed in the SAS package program and the averages were compared through LSD test.

RESULTS AND DISCUSSION

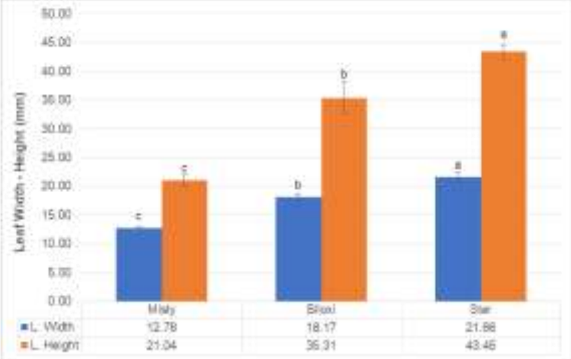
MORPHOLOGICAL AND PHENOLOGICAL PROPERTIES

In the study, flowering and harvest dates showed significant differences among cultivars (Table 1). The earliest flowering started in Misty variety, followed by Biloxi and Star varieties. It was determined that 50% flowering occurred in Misty variety on March 16. Star variety showed only 5% flowering on the same date. The earliest harvest was recorded in Misty variety on March 14. This was followed by Biloxi variety on April 18, 2023 and Star variety on May 01 (Table 1).

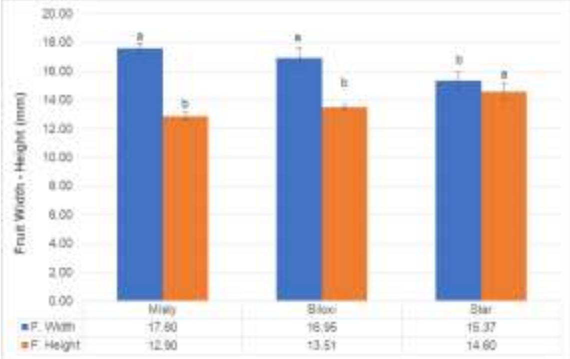
Table 1. Flowering and first harvest date of different blueberry varieties

Variety	Flowering rate on March 16, 2023 (%)	First Harvest Date
Misty	50	March 14, 2023
Biloxi	10	April 18, 2023
Star	5	May 01, 2023

In the research, both leaf width and leaf length values among the vegetative traits differed according to the cultivars. The highest values in terms of both criteria were determined in Star cultivar, followed by Biloxi and Misty cultivars (Figure 1a).



(a)



(b)

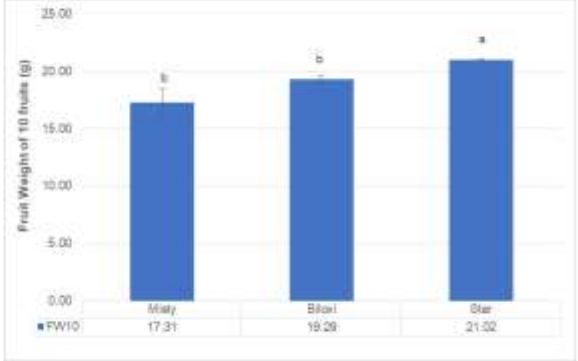
Figure 1. (a) Leaf width and length; (b) fruit width and length (mm) in different blueberry varieties.

POMOLOGICAL PROPERTIES

In the experiment, the effect of blueberry cultivars on fruit width and length characteristics was found to be statistically significant (Figure 2). The highest fruit width values were determined in Misty and Biloxi varieties, while the lowest values were recorded in Star variety. A different situation was observed in terms of fruit length. The highest fruit length was obtained in Star variety. Misty and Biloxi had similar values in terms of fruit length (Figure 1b).

In terms of fruit weight, the highest weight of 10 fruits was determined as 21.02 g in Star variety. This was followed by Biloxi and Misty varieties, respectively. Therefore, Star variety stood out in terms of fruit length and fruit weight (Figure 2a). Fruit SSC values of blueberry varieties showed statistically significant differences. The highest SSC was determined in Star variety with 16.61%, followed by Biloxi variety with 15.51%. The lowest SSC was determined in Misty variety with 13.81% (Figure 2b).

Fruit color brightness (L) values also differed among the varieties. While the highest L value was determined in Misty variety, the other two varieties showed similar values. Therefore, brighter fruits were observed in Misty variety (Figure 3a). There were no statistical differences between the varieties in terms of fruit color a and b values (Figure 3b and Figure 3c). Chroma value, which is the intensity of fruit color, also did not show statistical difference, and quantitatively it can be said that Star variety has more intense fruits (Figure 3d). No statistical differences were observed in terms of fruit color angle value (h^o) and these values varied between 237.32 and 262.56 (Figure 3e).



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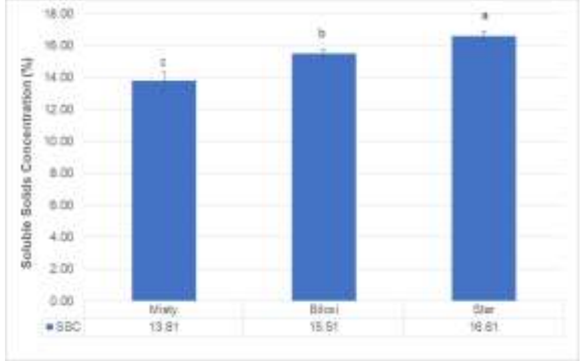
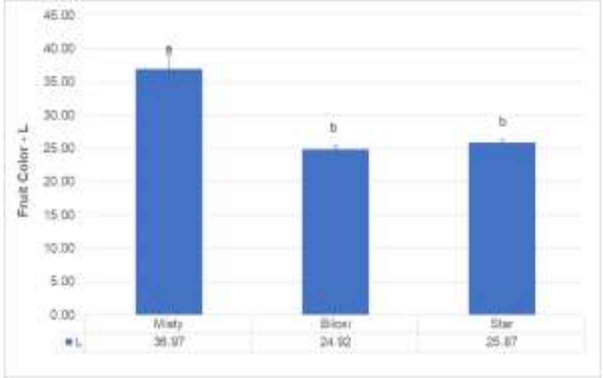


(b)

Figure 2. (a) Weight of 10 fruits (g); (b) SSC (%) in different blueberry cultivars.



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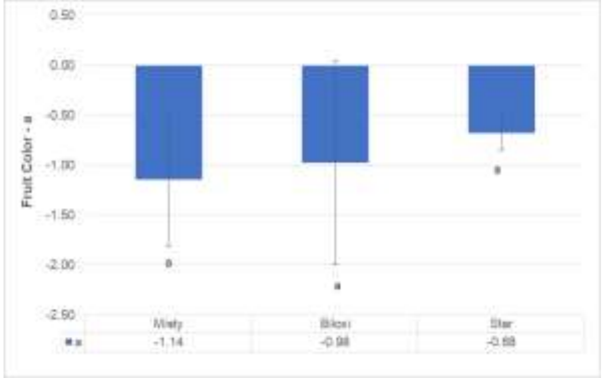

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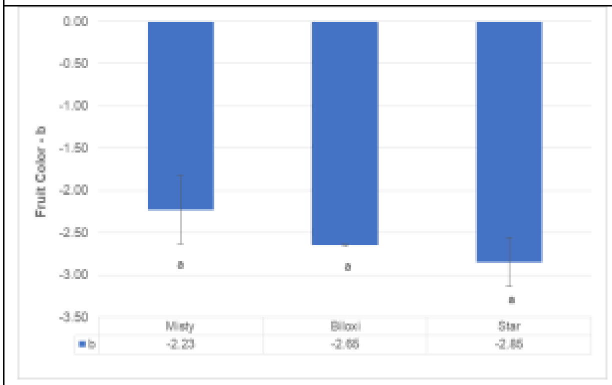

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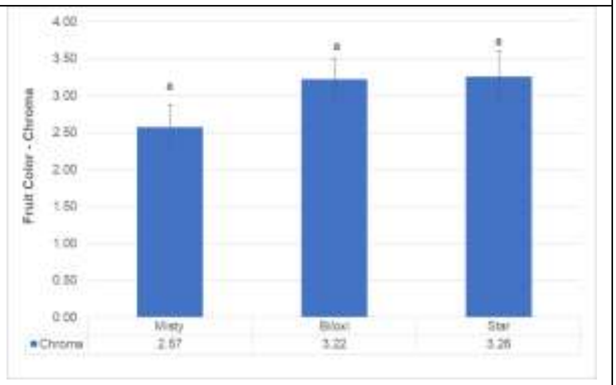
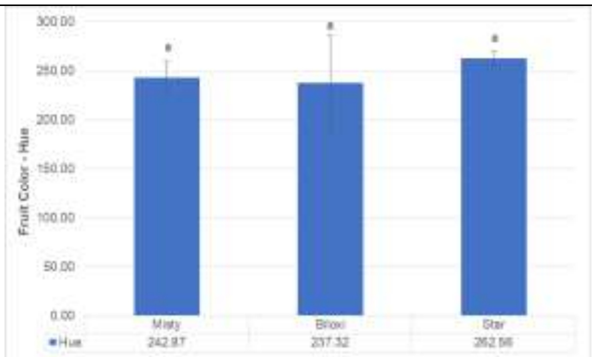


(d)



(e)

Figure 3. Fruit color (a) L* value; (b) a* value; (c) b* value; (d) C* value; (e) h⁰ value in different blueberry cultivars.

Our research findings showed that there were significant differences in terms of earliness among southern tall blueberry cultivars. The earliest variety was Misty, followed by Biloxi and Star varieties. When the fruit sizes of the varieties were evaluated, Misty and Biloxi varieties came to the forefront in terms of fruit width; Star variety came to the prominence in terms of fruit length and fruit weight. Fruit SSC values are one of the most important taste component criteria among the varieties. In this regard, the highest SSC was determined in Star variety with 16.61%. This was followed by Biloxi (15.51%) and Misty (13.81%) varieties. In terms of fruit color, only brightness index showed significant variation. The brightest fruits were obtained in Misty variety.

In the previous studies, the findings obtained for both northern and southern varieties were similar in terms of many criteria. Although there are significant differences among the varieties in terms of earliness, the first harvest dates vary according to the location, since earliness is also affected by climatic factors (Baptista et al. 2006; Ciordia et al. 2006; NeSmith, 2006). In this regard, Çelik (2018) conducted a study on southern highbush ('Misty', 'Ozarkblue', 'O'Neil', 'Jubilee' and 'Sharpblue') and rabbiteye ('Climax', 'Powderblue', 'Tifblue' and 'Austin') blueberry varieties and reported that the earliest variety was Misty, similar to our findings. 'Misty' variety was flowered on January 7 and harvested on May 16. In terms of fruit size, 'O'Neil', 'Tifblue' and 'Climax' had the largest fruits. It was determined that southern highbush blueberry cultivars can be used for early harvesting, while rabbiteye blueberry cultivars ('Climax' and 'Powderblue') can be used to extend the harvest period until the end of August. Ciordia et al. (2006) reported that fruit ripening of southern highbush cultivars (Avonblue', 'Flordablue', 'Misty', 'Reveille', 'Sharpblue' and 'Sunshine Blue') under protected conditions in Spain started in May. In our findings, Misty variety started harvesting in mid-April. Baptista et al. (2006) reported that the southern highbush cultivars 'Georgiagem', 'Cape Fear' and 'O'Neal' are promising for extending the fruit production period under protected conditions.

Our findings on fruit quality criteria were similar to some studies in many aspects. It is reported in the studies that particularly fruit quality criteria show significant differences according to the varieties (Ciordia et al. 2006; Çelik, 2020). Çelik et al. (2020) reported that the fruit width was between 14.22-20.43 mm and fruit weight between 1.76- 4.22 g on blueberry cultivars ('Chandler', 'Patriot' and 'Brigitta') grown with conventional methods; Ateş and Çelik (2016), were determined the fruit length (7.77-12.58 mm), fruit width (9.65 -19.50 mm), number of berries per 250 g cup (50.00-165.67 berry), and SSC content (7.00% -14.67) of some northern highbush blueberry cultivars ('Toro', 'Brigitta', 'Darrow', 'Patriot', 'Bluecrop', 'Bluegold', 'Chandler' and 'Bluejay') in their study. Ozeki and Tamada (2006), in Japan, under greenhouse conditions, southern highbush cultivars with low chilling requirements ('Bladen', 'Blue Ridge', 'Cape Fear', 'Cooper', 'Duplin', 'Flordablue', 'Georgiagem', 'Magnolia', 'Misty', 'O'Neal', 'Reveille', 'Sharpblue' and 'Summit') and northern highbush ('Weymouth') and rabbiteye ('Climax') cultivars, they recommended the southern cultivars 'Blue Ridge', 'Flordablue', 'Georgiagem', 'Misty' and 'Sharpblue' in terms of harvest time, yield, fruit size and taste. Suzuki et al. (1998) used 'Bluecrop' and 'Northland' cultivars of highbush varieties in soilless culture under greenhouse conditions and found correlative relationships between fruit weight and number of seeds, flowering and harvest dates and length of growth period.

In addition to cultivar, plant nutrition management practices also have significant effects on fruit quality attributes in blueberries (Wilber and Williamson, 2008). Wilber and Williamson (2008) found that in 'Star' blueberry variety, plant growth and yield increased with increasing fertilizer rate, while in 'Misty', increasing fertilizer rate negatively affected plant growth and fruit set due to root environment diseases. In addition, Bolanos-Alcantara et al. (2019) investigated the effect of nitrate/ammonium ratio (50/50, 30/70, 10/90) and electrical conductivity (EC 1.0, 1.5, 2.0 $dS.m^{-1}$) on Biloxi cultivar grown in soilless culture in protected. As a result of the research, it was reported that the value of SCC varied between 14.4-15.44 °Brix depending on the treatments.

Based on the hierarchical clustering and PCA – Biplot analysis (Figure 4) results it was concluded that all fo these three varieties are very different from each other. However, it was also found that the Biloxi and Star varieties are more close to each other. The main characteristics of Misty was found to be fruit color L and fruit color b. Moreover, fruit color hue, fruit height and fruit weight characteristics were found to be superior for Star cultivar. The Biloxi varieties was also found to be superior in terms of fruit color C, leaf width, leaf height and SSC.

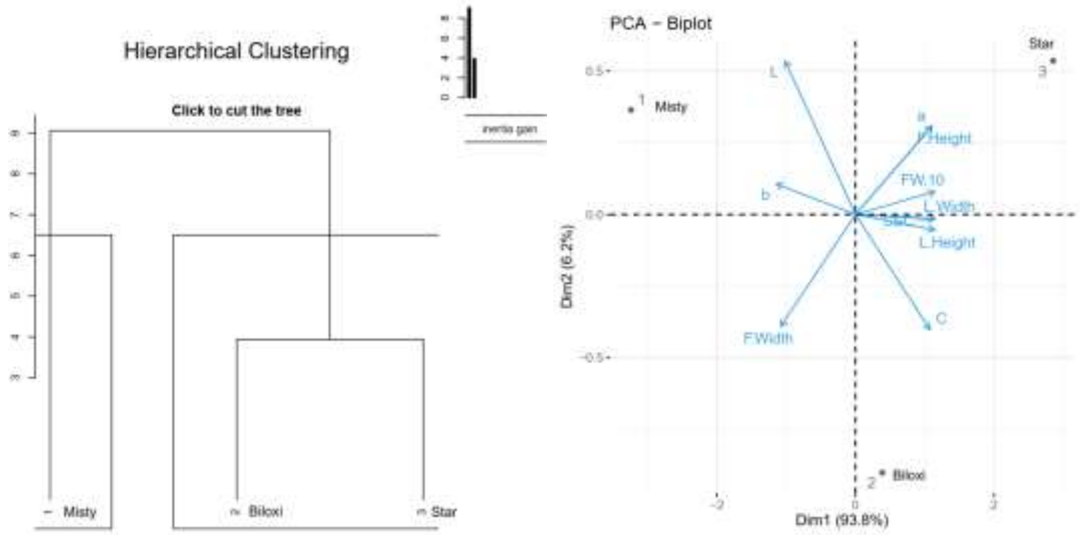


Figure 4. Hierarchical clustering (left) and PCA – Biplot analysis (right) of different varieties

CONCLUSIONS

As a result of our research, it was determined that southern highbush blueberry varieties such as Misty, Star and Biloxi can be easily grown in low altitude areas of Antalya and that these varieties are advantageous in terms of earliness. In addition, compared to conventional cultivation, the number of plants per unit area is higher with soilless production, less water and fertilizer requirement, as well as the advantages of promoting a controllable sustainable growing, it is necessary to expand the cultivation of southern highbush blueberry with soilless production in subtropical conditions.

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SECONDARY METABOLITES OF *ACTINOMYCETES* AND THEIR USES IN AGRICULTURE

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ABSTRACT

In recent years, due to the increase in agricultural production and the disadvantages of chemicals, alternative control studies have been focused on in agriculture. In this study, especially soil microorganisms *Actinomycetes* bacteria play an important role. Their multifunctional activities, including nutrient cycling, soil quality and crop productivity, as well as plant health, make them an environmentally friendly alternative not only for agriculture but also for humanity. Therefore, this study includes the importance of *Actinobacteria* as microbial biopesticides and biofertilizers, and highlights the future needs of using these bacteria in sustainable agriculture. These bacteria are characterized as a unique group due to their similarity to fungi due to their morphological structure in the form of mycelium. However, they are closer to bacteria because they are unicellular and do not contain chitin and cellulose in their cell walls. Among the microbial secondary metabolites produced by *Actinomycetes*; antibiotics, vitamins, pigments, toxins and substances effective in symbiosis, Biosurfactants, enzymes (amylase, cellulase, chitinase, pectinase), pheromones (*Streptomyces werraensis* LD22), compounds that affect the immune system, receptor antagonists and agonists, pesticides, antitumor agents, and plant and animal growth regulators and hormones. These secondary metabolisms are generally controlled by a group of genes in DNA. Of over 23,000 known microbial secondary metabolites, 42 % are produced by *Actinomycetes*, 42 % by fungi and 16 % by other bacteria. In this study, plant growth and yield promoting activities of *Actinomycetes* in important cultivated plants, their antifungal, antibacterial, herbicidal, insecticidal properties and their licensed preparations in the market are included. So future research should focus on number and quality of commercial products improvement. The lack awareness for farmers to use biopesticides and biofertilizers is a challenge to be taken up for an implementation for large scale of biopesticides and biofertilizers application in agriculture. Currently, the development of synthetic biology and nano-biotechnology advance provide opportunities to develop microbial and bioactive biofertilizers with a broad spectrum of application in different agro-systems. Therefore, research efforts should focus on these subjects.

Keywords: *Actinobacteria,* agriculture, biofertilizer, biopesticide, *Streptomyces*

INTRODUCTION

Actinomycetes, known also as *Actinobacteria*, is the bacteria group with the largest number of species living in soil and water ecosystems. It is estimated that one gram of soil contains 109 cfu (colony forming units), of which 107cfu are *Actinobacteria* (Harir et al. 2018).

According to the most recent classification, Salam et al. (2020), rearranged 425 genera of the phylum Acninomycetes into 6 classes, 46 orders and 79 families, including 16 new orders and 10 new families. *Actinomyces, Nocardia,* and *Streptomyces* are three main genera of *Actinomycetes.* Among these genera, *Actinomyces* are anaerobic while the other two genera are aerobic. The largest and most agriculturally important members of this large group are species within the *Streptomyces* genera, 95% of the total *Actinomycetes* isolated, followed by *Nocardia* (2%), then *Micromonospora* (1%), and the remainder includes *Thermomonospora*, *Actinoplanes*, *Microbispora*, and others (Arifuzzaman et al., 2010). *Actinomycetes* are an interesting group of microorganisms that resemble fungi due to their mycelial structure and the spores they produce, but are considered bacteria because they are single-celled and do not contain chitin and cellulose in their cell walls. Some species in this group are used in many areas of agricultural production. They produces several volatile substances like geosmin responsible of the characteristic "wet earth odor" and also exhibit diverse physiological and metabolic properties, for example the manufacture of extracellular enzymes (Barka et al., 2016; Sharma et al., 2014).

In recent years, there are many chemicals licensed and put on the market for various purposes in agricultural areas, and their number is increasing day by day. However, these may have different negative effects on plants, the environment and human health. Additionally, many phytopathogenic microorganisms have become resistant to some pesticides, making it necessary to develop new non-chemical, sustainable agents to prevent this serious situation. Currently, many scientists working in different disciplines all over the world are trying to discover new natural products of plant and microbial origin. Many plants and microorganisms produce different bioactive secondary metabolites that can potentially be used as effective alternatives to various chemical pesticides in the agricultural pharmaceutical industry (Elshafie and Camele, 2022). The largest group of microorganisms in nature capable of producing this type of metabolites are species within the *Actinomycetes* group. Of over 23,000 known microbial secondary metabolites, 42% are produced by *Actinomycetes*, 42% by fungi and 16% by other bacteria. In this study, microorganisms in this group, their metabolites and their possibilities of use in agriculture are included (Rani et al. 2021).

1. Usage of *Actinomycetes* metabolites in plant diseases

Actinomycetes produce a large number of natural products that fully meet the needs of the plant for its development, while suppressing its diseases (Sharma et al., 2017). To date, *Actinobacteria* are known to produce approximately more than 20,000 secondary metabolites; of these, about 13,700 are considered biologically active products and about 10,000 are considered antimicrobials (Berdy, 2012). More than 10,000 diverse bioactive compounds have been obtained from *Actinomycetes* so far (Martins et al., 2019). Metabolites from *Actinobacteria* have a wide range of activities, including antifungal and antimicrobial (Barka et al., 2006; Cao et al., 2020 ; Hamdali et al., 2008; de Lima Proc'opio et al., 2012 ; Guerrero-Garz'on et al., 2020 ; Passari et al., 2015; Ser et al., 2016). Some antimicrobial effects of *Actinomycetes* metabolites in agricultural applications are summarized in Table 1.

Table 1. Some antimicrobial metabolits of *Actinomycetes* and their targeted pathogens.

Organisms	Compounds	Target pathogen
Streptomyces sp. HAAG3–15	Azalomycin B	*F. oxysporum f. sp. cucumerinum*
Streptomyces thermocarboxydus isolate BPSAC147	1H-pyrazole – 1,3 benzothiazole – 7-epi-transsesquisabinene – Cedrane – Azulene derivatives – Piperoidene – Ethyl iso–allochaolate	*F. oxysporum*
Streptomyces sp. MR14	Not determined	*Fusarium moniliforme*
Streptomyces (MG788011, MG788012)	Not determined	*Botrytis cinerea*
Streptomyces strains PC 12, D 4.1, D 4.3 and W1	Chitinase/ hydrogen cyanide (HCN)	*Pyricularia sp.*
Streptomyces lavendulae SPS-33	2–Methyl-1–butanol 3–methyl-1–butanol Pyridine and Phenylethyl alcoho	
Nocardiopsis dassonvillei strain MB22	Chitinase	*Bipolaris sorokiniana*
Streptomyces vinaceus strain RCS260	Cellulase	*Colletotrichum gloeosporioides*
Streptomyces violaceoruber strain BS-26	Not determined	*Colletotrichum capsici*
Streptomyces sp. CB-75	Type I polyketide synthase (PKS-I) and non-ribosomal peptide synthetase (NRPS)	*Colletotrichum gloeosporioides, C. musae*
Streptomyces samsunensis UAE1, *Streptomyces cavourensis* UAE1 and *Micromonospora tulbaghiae* UAE1	ß-1,3-glucanases and chitinase / Siderophores	*Lasiodiplodia theobromae* (Dieback in Mango)

2. Usage of antiobiotics originating from *Actinomycetes* in plant diseases

Antibiotics are today mostly used in the treatment of various infections in humans, animals and plants. It is also used in animal nutrition, food storage, as a research material to ensure selectivity in biochemical and culture environments, as a preservative in agriculture or to accelerate growth. *Streptomyces* have a large share in the production of antibiotics used today. The producers of approximately 55-65% of the 10 000 known antibiotics are members of the Streptomyces genus. Streptomycin, the first bacterial antibiotic discovered in 1943, was obtained from *Streptomyces griseus* (Barka et al., 2016; Harir et al., 2018). According to mathematical modeling, 97% of the antibiotics produced by this genus have not yet been discovered (De Lima et all, 2012). Kasugamycin (Umezawa et al., 1965), a discovered bactericidal and fungicidal metabolite produced by *Streptomyces kasugaensis*, has excellent toxicological properties. Kasugamycin inhibits protein biosynthesis in microorganisms but not in mammals (Table 2).

Table 2. Some antibiotics produced by different *Actinomycetes* and their applications

Organism	Antibiotic produced	Disease
S. griseus	Faeriefungin	Asparagus root diseases
S. malaysiensis	Malayamycin	Blotch of wheat
S. griseochromogenes	Blasticidin S	Broad range of plant diseases
S. hygroscopicus	Gopalamycin	Brown rust of wheat
S. padanus	Fungichromin	Damping-off of cabbage
S. violaceusniger YCED9	Nigericin and guanidylfungin A	Grass seedling disease
S. humidus	Phenylacetic acid	*Phytophthora* blight of pepper
S. violaceusniger	Tubercidin	*Phytophthora* blight of pepper
S. melanosporofaciens EF-76 and FP-54	Geldanamycin	Potato scab
Streptoverticillium rimofaciens	Mildiomycin	Powdery mildew
Streptomyces sp. KNF2047	Neopeptin A and B	Powdery mildew of cucumber
S. kasugaensis	Kasugamycin	Rice blast disease
S. cacaoi var. Asoensis	Polyoxin B and D	Rice sheath blight
S. hygroscopicus	Geldanamycin	Root rot of pea geldanus
S. hygroscopicus var. Limoneus No. T-7545	Validamycin	Sheath blight of rice

3. Usage of *Actinomycetes* spp. isolates in plant diseases

The largest and most agriculturally important members of this large group are species within the genus *Streptomyces.* Antifungal and antibacterial species are generally included in this genus. *Streptomyces* constitute 95% of the total *Actinomycetes* isolated. Other genera members generally include human and plant pathogenic species (Table 3).

Table 3. *Actinobacteria* generas and their features

Phylum	Order	Important genera	Special Features
Actinobacteria (Actinomycetes)	*Actinomycetales*	*Actinomyces*	Filamentousi branching, some human pathogens
		Corynebacterium	Human pathogens
		Frankia	Symbiotic nitrogen fixers
		Gardnerella	Human pathogens
		Mycobacterium	Acid-fast, human pathogens
		Nocardia	Filamentousi branching, opportunistic pathogens
		Propionibacterium	Propionic acid producers
		Streptomyces	Filamentousi branching, many produce antibiotics

Among the *Actinomycetes*, the use of *Streptomyces* species for biocontrol purposes is more documented (Gonzalez-Franco and Hernandez, 2009). *Streptomyces* species, especially *Fusarium* spp. (Cao et al., 2020; Kaur et al., 2019), *Botrytis cinerea (El-Shatoury et al., 2020),* Ceratocystis fimbriata (Li et al., 2020a, 2020b), Colletotrichum capsici (Thilagam and Hemalatha, 2019; Qi et al. (2019) as well as the rice bacterial pathogen. In another notable study, Nimaichand et al. (2013) isolated *Streptomyces hudungensis* from a limestone deposit and showed that it had antagonist activity against rice (*Helminthosporium oryzae*, *Pyricularia oryzae*, *Fusarium oxysporum* and *Curvularia oryzae*). Tamreiha and et al. (2019) also obtained similar results and reported the promising biocontrol activity of *Streptomyces* MBRL

201T against 6 rice fungal pathogens. To date, many researchers have investigated the effects of *Streptomyces* isolates as biocontrol agents against anthracnose disease in various plants such as cucumber, sweet potato, capsicum and banana (Table 4) (Cao et al., 2020; Passari et al., 2019; Kaur et al., 2019; El-Shatoury et al., 2020; Boubekri et al, 2022).

Table 4. Some antimicrobial *Actinomycetes* spp. and their targeted pathogens.

Organisms	Host	Target pathogen
Streptomyces viridodiasticus	Lettuce	*Sclerotinia minor*
S. violaceusniger G10	Banana	*Fusarium oxysporum* f. sp. *cubense*
Streptomyces sp. KH-614	Rice	*Pyricularia oryzae*
Streptomyces sp. AP77	Porphyra	*Pythium porphyrae*
Streptomyces sp. S30	Tomato	*Rhizoctonia solani*
S. halstedii	Red Pepper	*Phytophthora capsici*
Streptomyces spp. 47W08, 47W10	Pepper	*Phytophthora capsici*
S. violaceusniger XL-*2*	Many plants	*P. chrysosporium, P. placenta, C. versicolor, G. trabeum*
S. ambofaciens S2	Red Pepper	*Colletotrichum gloeosporioides*
Streptomyces sp.	Sugarbeet	*Sclerotium rolfsii*
S. hygroscopicus	Many plants	*Colletotrichum gloeosporioides* and *Sclerotium rolfsii*
Streptomyces sp.	Sun flower	*Sclerotinia sclerotiorum*
Streptomyces sp.	Sweet pea	*Oidium* sp.
S. vinaceusdrappus	Rice	*Curvularia oryzae, P. oryzae, Bipolaris oryzae, F. oxysporum*
Streptomyces sp. RO3	Lemon	*Penicillium digitatum, Geotrichum candidum*
S. sporoarveus RDS28	Many Plants	*R. solani, Fusarium solani, F. verticillioides, A. alternata, Botrytis cinerea S. aurantiogriseus*
S. aurantiogriseus VSMGT1014	Rice	*Rhizoctonia solani*
Streptomyces sp.	Maize	*Aspergillus* sp.
S. violaceusniger YCED9	Grass	*Rhizoctonia solani*

S. lavendulae HHFA1	Onion	*Erwinia carotovora* subsp. *carotovora*, *Burkholderia cepacia*
Streptomyces sp.	Soybean	*Xanthomonas campestris pv. glycines*

4. Usage of antiobiotics originating from *Actinomycetes* in weeds

The use of antibiotics derived from *Actynomecetes* dates back to ancient times. Approximately 60% of new insecticides and herbicides derived from them have been discovered in the last 10 years (Harir et al., 2018). (Table 5).

Table 5. Some antibiotics produced by *Actinomycetes* used against some weeds.

Bioherbicides	Biocontrol	*Streptomyces* strains
Anisomycin	Inhibitor efffect of weeds such as common crabgrass and broad-leaved weeds and barnyardness	*Streptomyces* sp.
Bialaphos	Control of annual and perennial grassy weeds	*S. viridochromogenes*
Carbocyclic coformycin and hydantocidin	Control of several weeds	*S. hygroscopicus*
Herbicidines and herbimycins	Several weeds	*S. saganonensis*
Phthoxazolin, hydantocidin, and homoalanosin	Control of several weeds	*Streptomyces* sp.

5. Licensed Plant Protection Products including *Actynomycetes* spp.

Increasing environmental problems are increasing the importance of biocontrol agents for plant protection day by day. Attempts to discover and use natural products in the ecosystem have increased significantly globally. One of these initiatives is to enable the use of Actynomyces bacteria, which are readily available, adapted to natural life, appear to be an effective and sustainable selection, to provide a significant biocontrol effect in the fight against plant pathogens. Table 6 lists microbial pesticides containing *Actinomycetes* spp. registered in the world., Table 7 lists Some microbial pesticides containing *Actinomycetes* metabolites licensed world (Ebrahimi-Zarandi et al., 2022).

Table 6. Some microbial pesticides containing *Actinomycetes* bacteria metabolites licensed worldwide.

Licensed Plant Protection Product	Active Substance	Country of License	Target Pathogen
Actinovate, USA	*Streptomyces lydicus* WYEC 108	Canada, USA	Soil pathogens (*Pythium, Fusarium, Phytophthora, Rhizoctonia, Verticillium*), downy mildew, powdery mildew, *Botrytis, Alternaria, Postia, Geotrichum, Sclerotinia.*
Mycostop,	*Streptomyces* K61	Europe, Canada, USA	*Alternaria* , *R. solani* , *Fusarium, Phytophthora*, *Pythium*
Mykocide	*S. colombiensis*	G. Korea	Downy mildew, gray mold, brown patch
Safegrow	*S. kasugaensis*	G. Korea	*Rhizoctonia sp., Rhizoctonia* spp in turfgrass
Actofit	*S. avermitilis*	Ukraine	Insecticide (Colorado Potato beetle, mites
Bactophil	*S. albus*	Ukraine	Seed diseases
Incide	*S. atrovirens*	India	Insecticide
Actin	*S. atrovirens*	India	Fungicide (many fungi)

Table 7. lists Some microbial pesticides containing *Actinomycetes* metabolites licensed world.

Biocontrol Metabolite and commercial name	Organism	Country	Target diseases
Blasticidin, SBLA-S	*S. griseochromogenes*	USA	*Pyricularia oryzae*
Kasugamycin, Kasumin, Kasurab-valida-sumi	*S. kasugaensis*	Ukrayna	*Cercospora* sp. in sugarbeet; leaf blight in celery; *Venturia* spp. in apple and apricot; Phytophthora sojae in soybean root rot
Streptomycin, Agrimycin, Paushak, Cuprimicin 17, AAstrepto 17, AS-50, Dustret, Cuprimic 100 and	*S. griseus*	India, USA, New Zeland, China,Ukrain,	Bacterial rots, cancer, and other bacterial diseases, (*Xanthomonas oryzae,*

500		Canada	*X. citri*, and *Pseudomonas tabaci* in all hosts)
Avermectine (Nematicide)	*S. avermitilis*	-	*Wucheria bancroftii*
Phytomycin, Mycoshield, Cuprimic 100, 500, S. rimosus	*S. rimosus*	-	Fire Blight (*Erwinia amylovora*), *Pseudomonas* and Mycoject *Xanthomonas* spp., and mycoplasmas
Validamycin, Validacin, Valimun, Valida, Sheathmar Dantotsupadanvalida, Mycin Hustler	*S. hygroscopicus*	Cotton and Vegetables	*Rhizoctonia* in Rice, Strawberry, Potato, Tobacco,
Polyoxorim, Endorse, PolyoxinZ, Stopit, Polyoxin	*S. cacaoi var. asoensis*	-	*Rhizoctonia spp. in field crops. Sphaerotheca spp. and other powdery mildews, Botrytis cinerea, Sclerotinia sclerotiorum, Corynespora melonis, Cochliobolus miyabeanus, Alternaria alternate, Rice Sheath Blight (R. solani), apricot and apple canker and Helminthosporium spp. in rice.*
Natamycin, Delvolan	*S. natalensis* and *S. chattanoogensis*	In many countries	*Fusarium oxysporum'un* neden old. *b*azal çürkülükler
Abamectin (Avermectins) Agri-Meck Avid,	*S. avermitilis*	In many countries	In Many Insects, Mites, Ants
Polynactin, Mitecidin	*S. aureus*	Japan	*Tetranychus cinnabarinus, Tetranychus urticae, P. ulmi* in fruits
Milbemycine Milbeknock, Koromite, Mesa,	*S. hygroscopicus subsp. aureolacrimosus*	-	Citrus red spider, leafworms in tea and eggplant

6. Usage of *Actinomycetes* spp. as biofertilizer

Biofertilizers of microorganism origin in agricultural production are preferred products because they are environmentally friendly, highly productive and do not have any harmful effects on plant products, soil and other living things. The plant needs organic fertilizers and biofertilizers as well as mineral nutrients that can be supplied directly or indirectly when chemical fertilizers are used to increase organic carbon in the soil and support sustainability in the field and horticultural crops. The term biofertilizer refers to a wide range of products containing living microorganisms, including bacteria, fungi, algae. Upon application, these microorganisms help fix atmospheric nitrogen or dissolve/mobilize soil nutrients as well as secrete plant growth promoting substances [4]. Today, biofertilizers and biopesticides can replace traditional inorganic fertilizers and synthetic pesticides, among a variety of other products. *Actinomycetes* group bacteria are the most preferred group in microbial fertilizers used to increase growth and yield in plants. Table 8 shows the *Actinomycetes* bacterial species used as microbial fertilizer and their mechanism of action.

Table 8. *Actinomycetes* bacterial species used as microbial fertilizer and their mechanism of action.

Species	Host and ability to affect plant development	Food intake
Streptomyces sp.	Clover	Nutrient intake
Streptomyces sp.	Rice, chickpea, sorghum	Plant growth promotion, grain yield, root development
Streptomyces sp.	Pea	Nodulation
Streptomyces sp.	Bean	Increase plant growth
Streptomyces sp.	Wheat	Nutrient intake, plant development
S. atrovirens, *S. griseoviridis,* *S. lydicus,* *S. olivaceoviridis* *S. rimosus,* *S. rochei, S. viridis*	Wheat	Auxin/IAA / gibberellin and cytokinin synthesis
Streptomyces sp.	-	Gibberellin biosynthesis

S. igroscopicus	-	ACC deaminase
Streptomyces sp.	*Terfezia leonis* (mushroom)	Siderophore, IAA, gibberellic acid
Streptomyces sp.	Water	Gibberellic acid, IAA, abscisic acid, kinetin and benzyladenine
S. aurantiogriseus	Rice	IAA production
Streptomyces sp.	Soil	IAA and siderophore production
Streptomyces sp.	-	Synthesis of B-1,3-Glucanase, IAA, and HCN
S. rochei, S. carpinensis, *S. thermolilacinus*	-	Siderophore, IAA synthesis and phosphate solubility

CONCLUSIONS

In this review, the biocontrol and plant growth regulatory effects of *Actinomycete* bacteria are discussed. When the studies carried out to date are examined, the biocontrol aspects of *Actinomycetes* bacteria in the field of plant protection and their application in sustainable agriculture have made a great contribution to agricultural production and will continue to do so in the future with the development of new isolates and techniques. Despite the potential of *Actinomycetes* to produce a large number of secondary metabolites and perform a wide variety of activities, very few have been formulated as biofertilizers, and many have difficulty reaching the market. However, to exploit the full potential of *Actinobacteria* in agriculture, future research should focus on optimizing the selection method by ensuring the stability of the selected strains. On the other hand, more studies are needed to obtain and develop metabolites of *Actinobacteria* that may be useful in plant production. The obtained isolates should also be designed for targets such as salinity reduction, drought, and antibiotic resistance. Additionally, efforts need to be made to transfer technology from the laboratory level to industry. For example, most existing biopesticide and biofertilizer products generally have limited shelf lives, the majority being of poor quality. Therefore, future research should also focus on quality improvement. The lack of awareness of farmers about the use of biological products, increasing the use of these products in agriculture is a challenge to be overcome. Currently, advances in synthetic biology and nano-biotechnology offer opportunities for the development of microbial and/or bioactive biofertilizers with a wide range of applications in different agricultural systems. Therefore, research efforts for the development of biopesticides and biofertilizers should focus on these options.

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ALTERNATIVE OILSEED CROPS IN TURKEY

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ABSTRACT

Despite its sufficient production in many agricultural crops, our country has a large foreign trade deficit, especially in the production of oilseeds and edible oil. Alternative oilseeds are valuable crops that are grown in marginal areas in Turkey and have a crucial contribution to oil production. Alternative oilseed crops cultivated in our country are rapeseed (canola), safflower, sesame, linseed, camelina and cephalaria. While these oilseeds can be successfully grown in Turkey, the markets and supply chains some of them are not necessarily developed. Especially camelina and cephalaria production is too low to be recorded in the statistical database. In 2022, 150.000 tons of rapeseed, 30.000 tons of safflower, 17.366 tons of sesame and 8 tons of linseed were produced in our country. The production of these crops is highly low in Turkey where edible oil consumption is high. Although almost every region of our country is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. In this study, the availability, production and future of alternative oilseed crops in Turkey are considered as a whole.

Keywords: Alternative oilseed crops, Rapeseed, Safflower, Sesame, Linseed, Camelina

INTRODUCTION

In parallel with the increase in the world population, the consumption of foodstuffs is increasing and at the same time the consumption of edible oil is also increasing. This consumption forces producers to increase production on available agricultural area. In addition, the fact that vegetable oils have become the raw material of the energy sector such as biodiesel increases the need for these oils even more (Culpan, 2015).

Edible oils, a staple food, are obtained from oilseed crops. Some of these crops are wild and some of them are cultivated (Arslan and Culpan, 2023). Vegetable or edible oils, which are basic foods, are obtained from oilseed crops. Oilseed crops rich in primary and secondary metabolites (fat, protein, carbohydrates, vitamins etc.) constitute an essential source of raw material for human and animal nutrition as well as for the industrial sector (Yılmaz et al., 2021).

Despite its sufficient production in many agricultural crops, our country has a large foreign trade deficit, especially in the production of oilseeds and edible oil. Alternative oilseeds are valuable crops that are grown in marginal areas in Turkey and have a crucial contribution to oil production. Alternative oilseed crops cultivated in our country are rapeseed (canola), safflower, sesame, linseed, camelina and cephalaria. While these oilseeds can be successfully grown in Turkey, the markets and supply chains some of them are not necessarily developed. Although almost every region of our country is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. In this study, the availability, production and future of alternative oilseed crops in Turkey are considered as a whole.

ALTERNATIVE OILSEED CROPS

Rapeseed (*Brassica napus* L.)

Rapeseed, a crop of Mediterranean origin, is important for human and animal nutrition (Gürsoy, 2019) and biodiesel production. It is the third most produced annual oil crop in the world (71.3 million tons as of 2021), behind soybean and seed cotton. The countries with the highest production are Canada, China, India, Germany and France (FAOSTAT, 2021). According to the data of the Turkish Statistical Institute for the year 2022, 150.000 tons of rapeseed was produced from 41.145 ha and the average seed yield was 3650 kg/ha (Table 1). Rapeseed is intensively cultivated in Thrace region in Turkey and its contribution to the oil production is highly important (Culpan et al., 2022).

Table 1. Rapeseed production in Turkey in the last 10 years

Years*	Area Harvested (ha)	Yield (kg/ha)	Production Quantity (tons)
2013	31.127	3280	102.000
2014	32.133	3420	110.000
2015	35.081	3440	120.000
2016	35.453	3530	125.000
2017	16.520	3640	60.000
2018	37.845	3300	125.000
2019	52.514	3430	180.000
2020	34.989	3470	121.542
2021	37.601	3720	140.000
2022	41.145	3650	150.000

* Turkish Statistical Institute, 2022

Rapeseed cultivation is highly similar to wheat cultivation practices (Tıraş, 2011); it is sown and harvested in almost the same period (September-July). The reasons that make rapeseed valuable are that it winter survival when it enters winter during the rosette period, does not require additional irrigation by being content with natural rainfall, reaches harvest maturity in July at the latest, can be easily harvested with a wheat harvester and yields more than cool climate cereals under dry farming conditions (Arslan, 2016). Due to the 40-50% quality oil in the seeds of rapeseed (Murphy, 1995; Gürsoy and Kolsarıcı, 2017), high oil yield from per hectare is an advantage compared to other spring oilseed crops such as safflower. The most important problem of rapeseed in Turkey is the poor availability of moisture in the soil in September and early October, the time of winter sowing in Central Anatolia and Thrace. This can lead to poor emergence and perhaps to repeat sowing.

Safflower (*Carthamus tinctorius* L.)

Safflower (*Carthamus tinctorius* L.) is a multipurpose oilseed crop that can grow in arid and semi-arid environments because of its tolerance for drought stress (Mosupiemang et al., 2022). *Carthamus tinctorius* L. which belongs to the Asteraceae, is one of the oldest cultivated plants that started to be cultivated 3000 years ago. It contains 25-45% oil in its seeds, has two different types as linoleic (ω-6) and oleic (ω-9), has high quality edible oil, is suitable for biodiesel production, is cultivated in the form of residue and mixture and is considered as animal feed (Arslan et al., 2012; Culpan and Arslan, 2022). On the other hand, drought tolerant and cultivation without irrigation enable especially availability of fallow areas (Arslan and Culpan, 2018). According to the data of the Turkish Statistical Institute for the year 2022, 15.000 tons of safflower was produced from 26.237 ha and the average seed yield was 1140 kg/ha (Table 2). There was a significant increase in safflower cultivation areas until 2014 and 2015 growing seasons, but then a decrease was observed again.

Table 2. Safflower production in Turkey in the last 10 years

Years*	**Area Harvested (ha)**	**Yield (kg/ha)**	**Production Quantity (tons)**
2013	29.292	1540	45.000
2014	44.305	1410	62.000
2015	43.107	1640	70.000
2016	39.571	1470	58.000
2017	27.376	1830	50.000
2018	24.693	1420	35.000
2019	15.860	1380	21.883
2020	15.115	1410	21.325
2021	14.588	1110	14.000
2022	26.237	1140	15.000

* Turkish Statistical Institute, 2022

The most important advantage of safflower is that it does not require much agricultural practices such as irrigation and fertilization. However, since safflower is known as a drought tolerant crop, it is cultivated without irrigation conditions and therefore its seed yield is low. According to the researches, seed yield of safflower increases up to 2 times with irrigation under appropriate growing conditions in dry areas (Öztürk et al. 2009; Arslan and Culpan, 2023). In the arid and semi-arid areas of the Central Anatolia Region, where the wheat-fallow system is widely practiced in Turkey, safflower is the most important alternative oilseed crop that can take place in crop rotation with wheat.

Sesame (*Sesamum indicum* L.)

Sesame is one of the first oil crops to be cultivated and is very important for human nutrition due to its valuable nutrients. It seeds contain 50-60% oil and 25% protein and are sown as spring crops. Sesame oil contains high levels of unsaturated fatty acids such as oleic (40-50%) and linoleic (45-50%) and saturated fatty acids such as palmitic (7-9%), stearic (4-5%) and arachidic (0.4-1.0%) (Bakal and Arıoğlu, 2020).

In 2022, 17.366 tons of sesame was produced from 24.285 ha and the average seed yield was 720 kg/ha (Table 3). While sesame production in Turkey was 40 thousand tons in the early 1990s, it has been below 20 thousand tons in recent years. This is mainly because its seed yield is low and the price of oil is quite expensive. Accordingly, sesame has no contribution to our vegetable oil industry. It is used in cakes, pastries and breads in our country, in addition to tahini production, which takes the crop away from oil production. By introducing varieties with high seed yields in the Aegean, Mediterranean and GAP regions, the oil production potential can be increased if mechanization problems are solved under irrigation conditions (Arslan, 2016).

Table 3. Sesame production in Turkey in the last 10 years

Years*	Area Harvested (ha)	Yield (kg/ha)	Production Quantity (tons)
2013	24.807	620	15.457
2014	26.349	670	17.716
2015	28.088	660	18.530
2016	28.933	670	19.521
2017	28.031	660	18.410
2018	25.985	670	17.437
2019	24.860	680	16.893
2020	25.666	730	18.648
2021	25.486	690	17.657
2022	24.285	720	17.366

* Turkish Statistical Institute, 2022

Linseed (*Linum usitatissimum* L.)

Linseed (*Linum usitatissimum* L.) is a traditional oilseed crop that represents a valuable alternative for fallow areas due to its adaptability to unfavorable soils and its high economic value relative to the high quality of the seed oil (Zanetti et al., 2013). It is grown either for its fiber (fiber flax) or for its oil (oilseed flax) (Hall et al., 2016). Its oil is the best source of the n-3 fatty acid, α-linolenic acid, which constitutes nearly 55 % of its total fatty acids. This value is 5.5 times more than the next best sources of α-linolenic acid (Bloedon and Szapary, 2004).

Linseed is an alternative oilseed crop and has unique drought tolerance; in extreme conditions, it can complete its life cycle in climates in which annual rainfall is only 200 mm (Li and Wang, 2016). Genotype × environment interactions have been shown to be high for linseed (Diepenbrock et al., 1995), and seed yield change significantly between production years, depending on location and climate conditions. Linseed, like safflower that can be used to utilize fallow and poor soil fields in Turkey. It is an important source of vegetable oil that can be utilized especially in conditions where rain and irrigation water is limited and therefore other oil crops cannot be grown (Arslan, 2016). Linseed cultivation has resumed in our country in the last few years and production has reached 8 tons according to 2022 data (TSI, 2022).

Camelina (*Camelina sativa* (L.) Crantz)

Camelina (*Camelina sativa* (L.) Crantz) is ancient oilseed that blongs to *Brassicaceae* family that is grown worldwide (Righini et al., 2019; Schillinger, 2019). Several characteristics of camelina make it an alternative oilseed crop, indeed a potential oilseed crop. In recent years, camelina has started to gain importance in the international arena again and many new researches have been carried out on it (Sevilmiş et al., 2019). Many researchers documented that camelina is drought and heat tolerant (Angelini et al., 1997; Blackshaw et al., 2011). It (*Camelina sativa* (L.) Crantz) is more adaptive to drought conditions than other oil seeds crops such as canola (Raza et al., 2015). The seed yield of camelina ranged from 1177 kg/ha under drought conditions in Saskatchewan to 3012 kg/ha in northern Alberta (Francis and Campbell, 2003). Zubr (1997) reported seed yields of 2600 kg/ha and 3300 kg/ha for spring and winter varieties, respectively. In Turkey, on the other hand, camelina is not cultivated at present. However, with the expansion of the cultivation area in the future, the potential to provide raw materials to the oil industry can be reached.

Cephalaria (*Cephalaria syriaca* L.)

Cephalaria (*Cephalaria syriaca* L.) is an annual plant in the Dipsacacea family. Studies have revealed that the oil content of cephalaria seeds varies between 21-26% and protein content between 14-20% (Çağlar, 1968). Its oil can be used directly as edible oil or mixed with other oils. However, the 7-8% epoxy acid in oil indicates that this oil should not be used as edible oil in this form (Sezgin et al., 2017). It is a crop that is widespread in Anatolia, grows wild in wheat fields and although its growth form is not similar to wheat, it is quite similar in terms of seed structure, size and shape (Boz and Karaoğlu, 2013; Sezgin et al., 2017). Cephalaria is a potential alternative oilseed crop for the future as it is cold and drought resistant. It grows very well in clay and loamy soils. As in camelina, cephalaria can reach the potential to provide raw materials to the oil industry by expanding its cultivation area in the future.

CONCLUSIONS

In 2022, 150.000 tons of rapeseed, 30.000 tons of safflower, 17.366 tons of sesame and 8 tons of linseed were produced in our country. The production of these crops is highly low in Turkey where edible oil consumption is high. Although almost every region of Turkey is suitable for the production of major and alternative oilseed crops, the increasing vegetable oil deficit is a major problem. Our country will be able to make use this advantage ideally and plan and program it with a number of precautions so that it will be able to exert itself in the production of oil seed and vegetable oil and export the production excess. However, the necessary measures and precautions listed below must be taken to unlock this potential and increase production quantities;

9. Long-term planning and sustainable policies should be implemented in the vegetable oil industry and alternative oilseed crops production.

10. In oilseed production, local seed breeding and production should be accelerated and supported by the state.

11. Alternative oilseed crops production should be included in the alternative crop project in fallow areas.

12. Irrigation investments should be accelerated and oilseed crop cultivation should be emphasized in new irrigable cultivation areas.

13. The Ministry of Agriculture should play an active role in seed supply and technical support for alternative oilseeds (rapeseed, safflower and linseed etc.) and should be encouraged to purchase the produced product.

14. In order to eliminate the low seed yield of safflower and to use it more effectively in crop rotation, winter-tolerant varieties should be developed.

15. Measures should be taken to expand mechanization in sesame agriculture.

16. Production of alternative oilseeds should be supported with low interest loans and premium amounts per kg should be increased.

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A GENERAL EVALUATION ON THE MARKET PRICE EFFECT OF GEO-LABELLED PRODUCTS IN TURKEY

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ABSTRACT

Geographical indications (GIs) received by public authorities or NGOs are important tools used to create added value for local values and increase their trade. The GI labelled product market in the world has reached 200 billion dollars annually. The largest share in this market is the European Union (EU) countries, and 17% of the union's food exports consist of GI labelled products. Although there is a wide variety of local products in Turkey, GI registered local values are brought into the economy has been later. The total number of patents registered by the Turkish Patent and Trademark Office in Turkey in 2023 has reached 1341. Initiatives made through e-commerce, which is the marketing channel with the highest growth, in order to increase the competition in the market and the commercial volume of GI registered local products indicate that this market will grow even more in Turkey. In this study; pulses group, fruit-vegetable-pickle group, crispy-pastry-dessert group, cheese group, sausage-salami-red meat group which are registered with GI in Turkey, are discussed. After the GI registration of these products, the price effects in the market were examined and evaluations were made. According to the results, the price premium calculated by comparing the prices of GI-labelled and non-GI-labelled products in selected food products was determined in favour of GI-labelled products. This price premium has been found to be quite high, especially in packaged and processed products (primarily in the pulses and dessert group-dried fruit pulp).

Keywords: Geographical indication, Price, Local products, Türkiye

INTRODUCTION

Human being's relationship with food products has certainly undergone a transformation in recent years. Food was supplied from the local region where it was lived until the 19th century. While basic foodstuffs were included in people's daily food consumption, consumption patterns have changed with globalization. This transformation has reduced the consumption of local food products. However, the production of large quantities of food with a long shelf life by using intensive inputs has started new searches in consumer demands. The fact that issues such as healthy life, negative effects of production on the environment, biodiversity, sustainability, agro-ecological production and circular economy have become the agenda necessitated the revision of production processes and local production. As a result, the demand for local food products is increasing, and the share of these products in the market is also increasing. Local products especially in France constitute approximately 20% of annual food expenditures, and this rate increases by more than 10% each year (Özsoy, 2015).

Geographical indication (GI) is a quality label that shows and guarantees the source of the product, its characteristics and the connection between the characteristics of the product and the geographical area. With this label, it is ensured that products that have gained a certain reputation depending on their quality, traditionality, raw materials obtained from the local area and local qualities (TPO, 2023). In terms of Turkish Patent and Trademark Office (TPO) GIs are registered as a PDO or a PGI. If essential qualities of a product originate from natural and human elements belonging to a certain geographical area, the geographical indications in this case are called "Protected Designation of Origin (PDO)". The attribute of PDO is because of the production is completely linked to a certain geographical area, while in the situation of PGI (Protected Geographical Indication); leastways one of the raw material or production and processing stages of a product is linked to a specific area or location. Food, agriculture, mining, handicrafts, industrial products can be subject to geographical indication registration. In this study, it has been only dealt with pulses group, fruit-vegetable-pickles group, crispy-pastry-dessert group, cheese group and sausage-salami-red meat group.

So far, 1438 local products have been registered in Turkey with the name of origin (PDO) or the sign of origin (PGO). Industrial property rights protection is valid in the country of registration all over the world. In this context, the registration of GIs within the scope of industrial property rights in our country is valid only in the borders of Turkey. In order to obtain protection in different countries, it is necessary to apply for registration within the framework of the legislation of those countries or to apply within the scope of systems providing international protection. Currently, 13 geographical indications have been registered in the EU. These are; Antep Baklava, Aydın Fig, Aydın Chestnut, Bayramiç Nectar, Malatya Apricot, Milas Olive Oil, Taşköprü Garlic, Giresun Chubby Hazelnut, Antakya Kunefe, Suruç Pomegranate, Çağlayancerit Walnut, Gemlik Olive and Edremit Olive Oil.

Previous studies have focused on issues such as the effect of geographical indication on the producer and on the consumer attitude, local development and the environmental effects. Santeramo and Lamonaca (2020) study evaluation of GI label in consumers' decision-making process, determining that the quality and fame of a product originated in a geographical origin. By using GI label, growers and sellers take advantage available connotations linking consumers and geographical indication (Marcoz et al., 2016). Many authors previously have discussed that appropriate marketing strategies can help producer welfare and also support a positive impact on producing GI-labelled products (Anson and Pavithran, 2014). The study has been conducted by Deselnicu et al., (2013) that price premiums differentiate agri-food products with GI-labelled. In the study of Larson (2018) it is said that, producers or retailers give priority to labelled products because these featured products generally have high gross margin and they can take advantage of differentiating themselves from rivals.

To figure out the value of GIs it is important to look at market size and price premiums for GI-labelled products (Jantyik and Török, 2020). In this respect the issue of what appropriate marketing strategies for geographical indication gains importance. At the same time, it can be said that studies on the use of geographical indications as a tool for agri-food products to enter international markets have become popular.

The aim of this study is to consolidate GI-labelled food products registration within the framework of pulses group, fruit-vegetable-pickle group, crispy-pastry-dessert group, cheese group, sausage-salami-red meat group. In the meantime, the market price effect of GI-labelled products has been examined.

MATERIAL AND METHOD

Pulses group, fruit-vegetable-pickle group, crispy-pastry-dessert group, cheese group, sausage-salami-red meat group, which are registered with GI label in Turkey, are evaluated in this study.

To do this, internet search on the related websites was done to see actual prices of the GI labelled food products. This attempt can prevent the possible insufficiency of face to face interviews methods. In the study, it has been endeavoured to get a general look of GI-labelled related food groups (in particular whether they have any different prices comparing with non-GI ones). In order to obtain comparable prices with GI and non-GI products, online shopping web sites were visited. First of all, we gathered data on GI labelled pulses group, fruit-vegetable-pickle group, bakery and pastry products, cheese group, sausage-salami and their closest substitutes.

The following formula has been used when calculating the price premium of geographically indicated products compared to those that do not (Jantyik and Török, 2020).

$$Price\ premium\ (\%) = \frac{Price_{GI} - Price_{NonGI}}{Price_{NonGI}} * 100$$

where $Price_{GI}$ is the price of the identified GI labelled related product and $Price_{NonGI}$ is the price of the identified GI food product's substitute, both determined as per kilogram or in case of liquid products per litre.

On account of finding the GI-labelled food products' closest substitute, the following outlines were used:

- First of all, we investigated for a substitute food product with almost the same geographical location features (e.g., for GI-labelled Bodrum mandarin "Bodrum Mandalinası", we chose mandarin with non-GI which was produced in Bodrum area),
- If the first option did not exist, we investigated for a substitute food product very similar features and different brands (e.g., for GI-labelled Tokat Erbaa pickled grape leaves "Tokat Erbaa Salamura Yaprak", grape leaf which has been grown near Tokat area was chosen).
- In cases where GI- labelled product had original features, and no other food products had an alike characteristic, no substitute food product was considered, and that GI-labelled food product was not involved the price premium (e.g., no other flour had similar attribution like Aydın Chestnut Flour; especially, there was no other chestnut flour available.

RESULTS AND DISCUSSION

Market Size of Geographically Indicated Products in Turkey

Turkey is located in a geography that has hosted many civilizations with its 12 thousand years of history. It has thousands of local products. Thanks to non-governmental organizations, the geographical indication of local and traditional values is obtained, increasing the added value of these local products and gaining commercial value. 40 % of the 734 geographical indications whose application process is still in progress are owned by the Chambers/Exchanges. Only 13 geographical indications of Turkey are registered in the

European Union. But if these local values cannot be promoted and marketed, they will not be worth anything if they are not brought into the economy. Projects such as the Local Products Program carried out within this scope will contribute to the producers' finding new markets and direct access to consumers.

The study based on the market price of the GI-labelled products considering e-commerce. The number of GI-labelled food products current in Turkish food market is very limited, GI-labelled food products available in the big franchises supermarkets in the big cities. The supply of these products is not permanent and they are not generally available to shoppers and are part of the non-GI products. Therefore it was more suitable and reliable to search the market price of the labelled food products on online shopping web sites.

The traditional trade method is now leaving its place to e-commerce with the change of technology and trade perception. With the right software, firms started to save both time and money in this way. It seems that e-commerce and e-export are growing very rapidly around the world. According to the data, by 2027, the world will reach a market of 8 trillion dollars. Considering the export figures in Turkey today, it corresponds to a value of approximately 1 dollar per kilogram. In e-commerce and e-export, an average of 30 to 35 dollars per kilogram corresponds to a figure. E-commerce and e-export pave the way especially for value-added exports (WORLDEF, 2023). Looking at the figures after the pandemic, it is seen that e-commerce has turned into a necessity rather than a choice. It is a fact that Turkey has a very serious advantage and potential in the field of local products. As a result, companies accelerate their e-commerce processes and increase the marketing of featured products such as GI-labelled products. The Union of Chambers and Commodity Exchanges of Turkey and a digital shopping website ensure that local products come to the forefront in terms of traditionality and local quality, with the program “All from Turkey” that supports producers and businesses by performing digital marketing and sales activities. With the support of chambers of commerce and stock exchanges in all provinces of Turkey, from local to national, it aims to expand market opportunities by reaching local producers, SMEs, women's cooperatives, introducing the program and enabling them to meet with the digital economy and e-commerce. At the same time, Turkey's e-commerce volume is supported.

Our observations present that at present, GI-labelled food products have only restricted significance in the Turkish food market in terms of their market share. Only a small number of these products are included in the supermarkets. At the same time, while e-commerce of GI-labelled products has been growing recently, these products have a limited supply. At this point, improvements in e-commerce will give an opportunity for current and future Turkish GI labelled products.

Price Premium on Selected Geographical Indications Products

In the research, the selected of the GI-labelled food products’ price premiums in Turkey were studied to get an overview. In order to get real market prices data, we looked at different web pages of big stores operating in Turkey. The most popular GI-labelled food products were chosen as pulses group, fruit-vegetable-pickles group, crispy-pastry-dessert group, cheese group and sausage-salami-red meat group (Table 1). When the GI-labelled products were chosen, a substitute food product was considered with almost the same feature. In case where the GI-labelled food product had some very original features and no other food products had similar characteristics, no substitute product has been included, and that GI-labelled food product wasn’t presented in the Table 1. To see significant difference between GI-labelled products and non-GI labelled products some popular e-food marketing sites were investigated. Supermarket web sites, local food products web sites, GI-labelled products web sites,.etc… can be given as examples. The GI-labelled food products which are in the scope of this study were checked in Turkish Patent and Trademark Office site. GI-labelled food products were not separated as PDO and PGI in the study. Both PDO and PGI labelled food products were included as GI-labelled products.

Table 1. GI-labelled products in the research

Pulses Group	**Fruit Vegetable Pickles**	**Crispy Pastry Dessert**	**Cheese**	**Sausage Salami Red Meat**
Akkuş Dried Beans	Amasya Marble Apple	Kayseri Ravioli (Manti)	Diyarbakır Knitting Cheese	Kayseri Sausage (Sucuk)
Kelkit Dried Beans	Silifke Strawberry	İzmit Cotton Candy	Hellim Cheese	Rize Village Roaster (Kavurma)
Mardin Bulgur Wheat	Amasya Flower Okra	Bursa Candied Chestnut	Kars Cheddar Cheese	Kayseri Pastrami (Pastirma)
Kastamonu Einkorn Bulgur	Alanya Avocado	Safranbolu Turkish Delight	Hellim Cheese	Akçaabat Meatball
Karacadağ Rice	Taşköprü Garlic	Gümüşhane Dried Fruit Pulp (Pestil)		
Kastamonu Einkorn Flour	Tokat Erbaa Grape Leaf	Gümüşhane Kome		
Uşak Tarhana	Çubuk Pickle	Antep Baklava		
	Bodrum Mandarin	Erzurum Stuffed Kadaif		
	Antep Dry Eggplant	Maraş Tarhana Crispy		

To calculate the price premium of the selected food products, the market prices of GI and non-GI labelled products were provided from the related websites (Table 2). When the different prices of the products were observed from the different web sites, average prices were taken into consideration. Our investigation was accomplished in e-shopping stores in Turkey. The fundamental reason for this was that these sites had expanded extraordinary in recent years, both in Turkey and across the world, making them reachable to the average shopper almost everywhere. Another important change in recent years was that an impressive part of labelled food products and local food products can be supplied from e-shopping stores. Shoppers can have a better price choice of local food products from everywhere. According to Table 2, there is an impressive difference in the price premium for GI-labelled food products. Overall, the average premium was around Sausage-Salami-Red Meat group had 10.08%, Pulses Group had 36.08%, Fruit-Vegetable-Pickles 44.22%, Crispy-Pastry-Dessert group had 53.54 and the highest was in Cheese group with 79.56%. However, it is significant to note that only products that had a potential direct substitute product were considered in the calculations of the premium prices. In the calculation of the price premium, current prices have been used as the representative prices for both GI-labelled food products and substitute products. It should not be forgotten that prices can change during the promotional offer in the discounts.

It can be seen from Table 2 that while in Antep Dry Eggplant from Fruit-Vegetable-Pickles group there is no difference in the price premium for GI-labelled and non-GI ones; Diyarbakır Knitting Cheese, Gümüşhane Köme, Gümüşhane Pestil (Dried fruit pulp) and Taşköprü Garlic have a higher price premium with 163%, 119%, 100%, and 100% respectively while Rize Village Roaster (Kavurma) has a lower premium for (0.2%). Kelkit Dry Beans, Kastamonu Einkorn Flour, Amasya Marble Apple, Safranbolu Turkish Delight and Maraş Tarhana Crispy had similar price premiums interval (50-70%).

When the online shopping web sites' current data was taken into account, it can be expressed that the proportion of GI-labelled food products is much lower comparing to their substitutes in the Turkish food marketing. Apparently, local sourcing starts progressively important for retails also for customers. Therefore, the role of GI-labelled food products is also increasing in these days (Jantyik and Török, 2020). According to food products groups in this study, packed or processed products (mainly pulses group and pestil- kome), are not represented too much in the market so, price margin between GI-labelled and non- GI is higher. In addition vegetables, fruit, fresh meat do not appear either. Perishability situations may be the reason for this. On the other hand, mainly pastry and cheese group are over-represented in the available online shopping web sites.

It should be mentioned that although local food stores play a key role, they are not acting of the whole food retail market in Turkey. With the GI-labelled food market size and price premium, it can be likely forecasted that if a GI-labelled food products market grows on food varieties, less price-sensitive customers may have a higher portion and price premium of GI-labelled food products. Hence supermarkets, local stores, hypermarkets,…etc. gain importance apart from the online web shops.

As a result, marketing the GI-labelled food products with the higher price premium can be an additional source of income for small-scaled growers, on the other hand additional costs should be taken in the labelling processes (Borowska, 2018). According to Crescenzi et al., (2022)'s research, undeveloped areas endorsing differentiated food products like GI food products experience better efficiency in terms of economic grow than others. With this, GI labelling protect the geographical characteristics of the goods and provide economic benefits like higher price premium for producers (Jena et al., 2015).

Tablo 2. Price premiums of the products included in the research

GI-Labelled Products			
Pulses Group	**Price of GI products (TL/kg)**	**Price of non-GI products (TL/kg)**	**Price premium (%)**
Akkuş Dried Beans	190	144	31.9
Kelkit Dried Beans	200	130	53.8
Mardin Bulgur Wheat	32	25	0.28
Kastamonu Einkorn Bulgur	110	80	37.5
Karacadağ Rice	71	53	33.9
Kastamonu Einkorn Flour	80	47	70.2
Uşak Tarhana	200	160	25
Fruit-Vegetable-Pickles			
Amasya Marble Apple	39	22	77.2
Silifke Strawberry	129	100	29
Amasya Flower Okra	1000	800	25
Alanya Avocado	224	160	40
Taşköprü Garlic	80	40	100
Tokat Erbaa Grape Leaf	83	60	38.3
Çubuk Pickle	90	70	28.5
Bodrum Mandarin	32	20	60
Antep Dry Eggplant	150	150	0
Crispy-Pastry-Dessert			
Kayseri Ravioli (Manti)	250	200	25
İzmit Cotton Candy	288	256	12.5
Bursa Candied Chestnut	1000	700	42.8
Safranbolu Turkish Delight	319	200	59.5
Gümüşhane Dried Fruit Pulp	360	180	100
Gümüşhane Kome	460	210	119

Antep Baklava	600	470	27.6
Erzurum Stuffed Kadaif	240	180	33
Maraş Tarhana Crispy	650	400	62.5
Cheese Group			
Diyarbakır Knitting Cheese	329	125	163
Hellim Cheese	940	636	47.7
Kars Cheddar Cheese	409	319	28
Sausage-Salami-Red Meat			
Kayseri Sausage (Sucuk)	600	499	20
Rize Village Roaster (Kavurma)	900	898	0.2
Kayseri Pastrami	1400	1156	21
Akçaabat Meatball	408	400	2

*The average *exchange rates* between *Turkish Lira (TRY)* and the *US dollar* (USD) for 24th August 2023 is $1= 25.30 TRY

CONCLUSIONS

This study concludes some benefits of GI-labelled food products focusing on price premiums. Price premiums of selected food products have been found to explain the efficiency of these products in the market. These results presented that GI labels are impressive differentiation tools in the local food markets, although their relevance changes across products. For example, GI labelling is the main differentiation for the Fruit-Vegetable-Pickles group (Amasya Marble Apple, Taşköprü Garlic, Gümüşhane Pestil and Köme) and for the cheeses group, but it is low relevance for red meat group and for the dried vegetable group.

In terms of these results some strategic inferences may be derived considering the price premiums: first of all, sellers or producers can benefit of the strength of GI labelling, particularly when they are associated with a positive label image. Retail sector should take advantage the advantages of an e-commerce. Similarly, authorities should promote the development of powerful brand images like GI and encourage effective local labels.

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THE SIGNIFICANCE AND APPLICATION OF SINGLE CELL RNA SEQUENCING (scRNA-seq) TECHNOLOGY IN PLANT BIOTECHNOLOGY

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ABSTRACT

Abiotic and biotic stress factors (such as nutrient and water resources, pathogens, natural disasters, and soil conditions) are important factors affecting plant growth and development, and it is not always possible to obtain high plant yields with only agricultural activities. The use of sequencing technologies to analyze genetic variation and metabolic regulation has an important role in improving knowledge of plant development processes and response to stimuli. Today, the use of high genomic technologies is widely adopted in plant breeding, conservation of biodiversity, and improvement of crops against stress factors. The emergence of transcriptomic, proteomic, or metabolomic approaches has made important contributions to the discovery of mechanisms in complex biological processes in plants. Single cell RNA sequencing (scRNA-seq) technology is the sequencing of a single genome for genomic or transcriptomic information that can reveal heterogeneity between cell populations. The scRNA-seq methodologies overcome the challenges of mass gene expression of whole tissue, allowing high resolution identification of individual cells, discovery of new cells, and comparison of cell identity in plants. The scRNA-seq workflow includes the separation of target cells from tissue, isolation of cell, RNA extraction, cDNA synthesis, single-cell sequencing, expression profiling, and cell type identification. In recent years, scRNA-seq analysis has become an important technique in gene profiling studies under different environmental conditions, gaining importance in determining cell type-specific gene expression against various stress stimuli. In this study, the recent situation of scRNA-seq technology in plant breeding and the research carried out using scRNA-seq technology is presented.

Keywords: abiotic stress, biotic stress, genomics, single-cell RNA-sequencing

INTRODUCTION

Biomass from plants is an important sustainable resource required for energy and material production. To achieve more sustainable bioenergy and biomaterial production in the future, the understanding of how to develop plant raw material production to adapt to changing environmental conditions needs to be improved significantly (Cole et al., 2021). Abiotic and biotic stress factors (such as nutrient and water resources, pathogens, natural disasters, and soil conditions) are important factors affecting plant growth and development, and it is not always possible to obtain high plant yields with only agricultural activities. In plants, different cell types have biologically distinct roles in the development and adaptation to the changing environment (Shaw et al., 2020). Therefore these cell types should be studied intensively.

The genomic era has significantly contributed to the study of DNA in plants, enabling detailed studies to explore the mechanisms underlying complex biological processes through the use of omics technologies such as RNA (transcriptomics), protein (proteomics), or metabolite (metabolomics) (Cole et al., 2021). The development of genome sequencing technologies to analyze genetic differentiation and metabolic regulation has an important role in improving knowledge of plant development processes and response to stimuli (Tang and Tang, 2019; Bawa et al., 2022). Today, the use of high genomic technologies is widely adopted in plant breeding, conservation of biodiversity, and improvement of crops against stress factors. The emergence of transcriptomic, proteomic, or metabolomic approaches has made important contributions to the discovery of mechanisms in complex biological processes in plants.

The assessment of the transcriptome in a particular type of cell can be revealed by high-throughput transcriptomics techniques such as DNA microarray or RNA sequencing (RNA-seq) (Shaw et al., 2020). Clarifying how plants respond to biotic and abiotic stress factors, functional annotation of genes, and applications that contribute to the production of biomaterials can be identified as the three main topics of RNA sequencing (RNA-seq) technology in plants (Cole et al., 2021). In this study, the recent situation of scRNA-seq technology in plant breeding and the research carried out using scRNA-seq technology is presented.

SINGLE CELL RNA SEQUENCING (scRNA-seq) WORKFLOW

Single cell RNA sequencing (scRNA-seq) technology is the sequencing of a single genome for genomic or transcriptomic information that can reveal heterogeneity between cell populations (Jovic et al., 2022). The cells/tissues of plants are morphologically, biochemically, and physiologically specialized and heterogeneity between cell populations should be revealed. The comparison of the transcriptomics of individual cells is achieved by single cell RNA sequencing technology. The scRNA-seq technology enables studies in various plants such as comparison of cell identity, high resolution identification of individual cells, discovering the new cells, and is advantageous over traditional sequencing technology as it overcomes the difficulties in mass gene expression of the whole tissue (Trapnell, 2015; Bawa et al, 2022).

The scRNA-seq workflow includes (Figure 1);

- the separation of target cells from tissue,
- the isolation of single cell,
- the extraction of RNA,
- cDNA synthesis by reverse transcriptase,
- single-cell sequencing,
- expression profiling,
- the identification of cell type (Bawa et al., 2022).

The cell isolation step of scRNA-seq is the most significant step for this technology due to isolation depending on the plant and its tissue type. Enzymatic cell wall digestion or manual isolation can be used for single-cell isolation in plants (Seyfferth et al., 2021; Bawa et al, 2022). Bioinformatics tools are needed to analyze plant single cell data, especially single cell RNA sequencing (scRNA-seq) data (Yuan et al., 2018). In the literature, it is stated that bioinformatic tools used to analyze data from animal cells can also be used in plants (Iqbal et al., 2020). These tools can be listed as follows; FastQC, Trimmomatic, TrimGalore, scPipe, CellRanger, Hisat2, STAR, HTSeq, ascend, dropEST, DropletUtils, Scanpy, Scater, Seurat, Monocle, and RaceID (Iqbal et al., 2020; Malhotra et al., 2022).

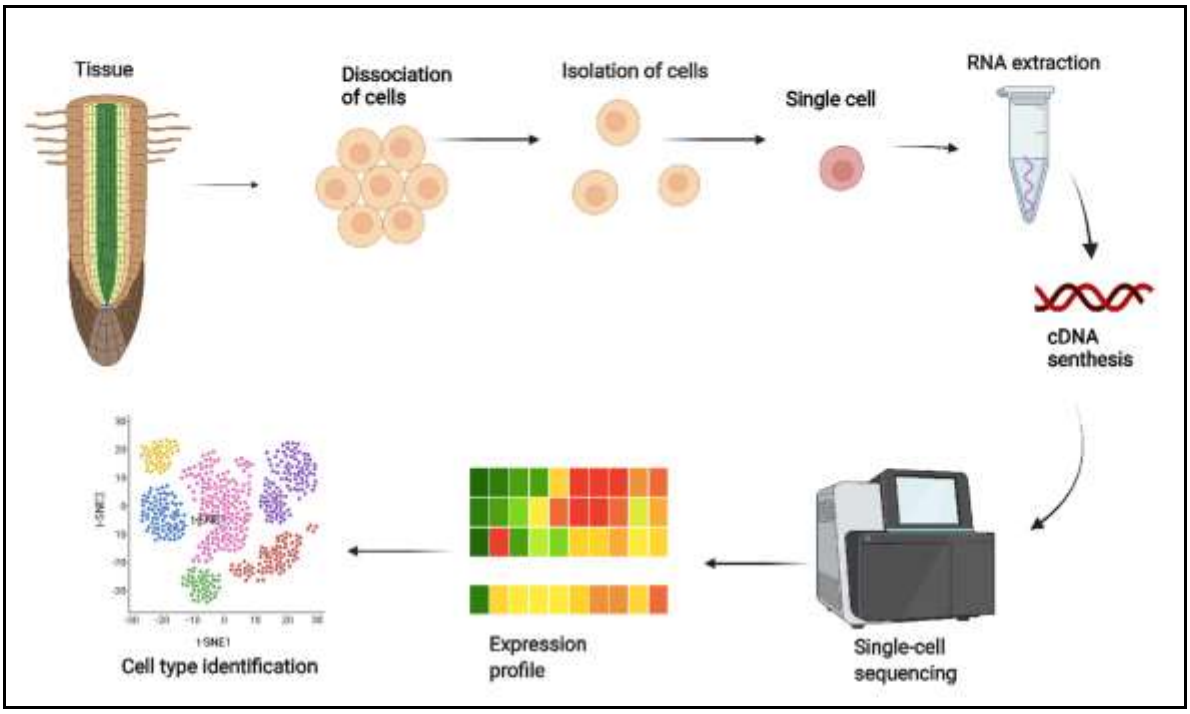

Figure 1. The workflow the single cell RNA sequencing (scRNA-seq) (Bawa et al., 2022)

SINGLE CELL RNA SEQUENCING (scRNA-seq) STUDIES IN PLANTS

The highly increased application of scRNA-seq in various plants has shown that it has an important role in plant research. In recent years, thanks to the important developments in ScRNA-seq technology, cellular and molecular differentiation of plant stem cells can be determined at the single cell level. Moreover, scRNA-seq analysis has become an important technique in gene profiling studies under different environmental conditions, gaining importance in determining cell type-specific gene expression against various stress stimuli (Yuan et al., 2018; Bawa et al., 2022). The scRNA-seq provides transcriptomic analysis of individual cells in an unbiased, high-throughput, and high-resolution manner in plants (Bawa et al., 2022).

Searching the Web of Science Core Collection (WOS) by "single cell RNA sequencing (scRNA-seq)" and "plant" criteria revealed that 38 articles were published during 2019-2023. As a result of the Web of Science (WOS) analysis, we see that the original and/or review articles using scRNA-seq technology in plants are published in prestigious journals with high impact factors. China, the USA, and Australia were the countries that contributed the most to the single cell RNA sequencing (scRNA-seq) studies in plants 2019-2023. In Table 1, some studies on plants in the last 5 years are given. The first scRNA-seq studies in plants have recently been performed on a series of Arabidopsis stem cells (Table 1) (Cole et al., 2021). The scRNAseq technology can be used to profile the development of important plant tissues not only in plant roots but also in cotyledons, leaves, flowers, pollen, and seed endosperm (Liu et al., 2020; Xie et al., 2020; Cole et al., 2021; Bawa et al., 2022; Thibivilliers et al., 2023). The use of scRNAseq technology in plants started with *Arabidopsis* and continued in tomato, rice, maize, moss, and some woody plants (Tang and Tang, 2019; Cole et al., 2021; Zhu et al., 2022) (Figure 2).

Table 1. Some single cell RNA sequencing (scRNA-seq) studies in plants last 5 years

Species	Reference
Arabidopsis thaliana	Denyer et al., 2019
	Jean-Baptiste et al., 2019
	Ryu et al., 2019
	Shulse et al., 2019
	Zhang et al., 2019
	Liu et al., 2020
	Gala et al., 2021
	Graeff et al., 2021
	Hou et al., 2021
	Liu et al., 2022a
	Liu et al., 2022b
	Procko et al., 2022
Zea mays	Kim et al., 2021
	Marand et al., 2021
	Ortiz-Ramirez et al., 2021
	Li et al., 2022
	Cao et al., 2023
Oryza sativa	Xie et al., 2020
	Wang et al., 2021
	Liu et al., 2021a
Nicotiana attenuata	Kang et al., 2022
Solanum lycopersicum	Tian et al., 2020
Populus spp.	Conde et al., 2021
	Xie et al., 2022
Nepeta tenuifolia	Zhou et al., 2022
Gossypium bickii	Sun et al., 2023
Gossypium hirsutum	Zhang et al., 2023a
	Qin et al., 2022
Dimocarpus longan	Zhang et al., 2023b
Arachis hypogaea	Liu et al., 2021b
Physcomitrella patens	Kubo et al., 2019

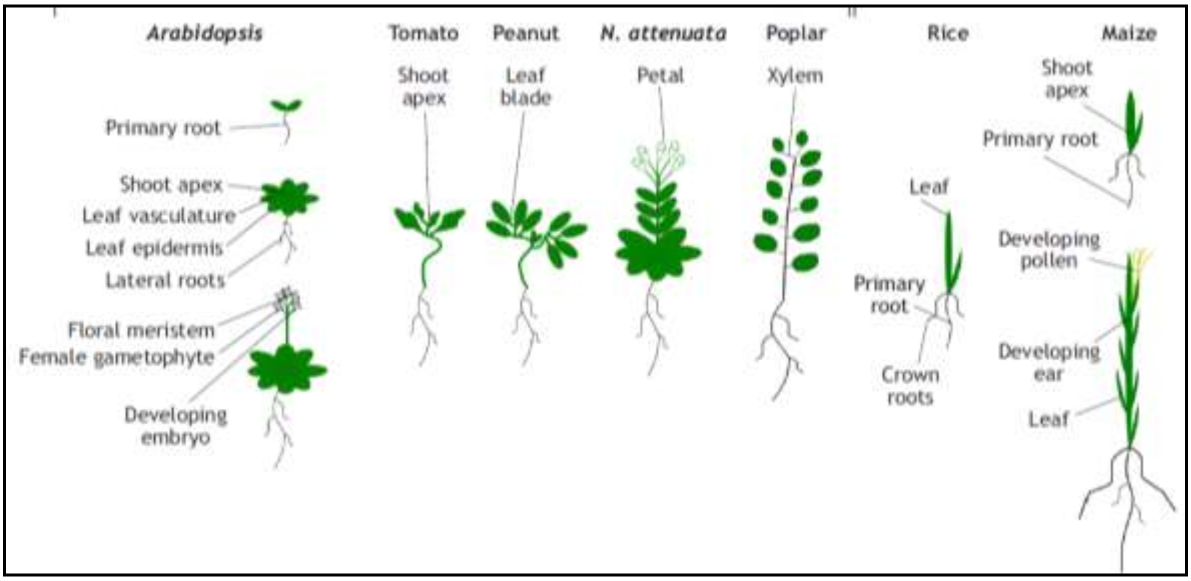

Figure 2. Some plant species have been studied with scRNA-seq (Zhu et al., 2022)

CONCLUSION

Single cell RNA sequencing (scRNA-seq) research in life sciences provides significant and valuable information. The molecular aspects of plant diseases, the interaction between host and pathogens, the determination of cell types, the relationships among cell lineages, the evolution of plant diseases, and cell differentiation are some of the valuable contributions of the scRNA-seq technology (Tang and Tang, 2019). In the future, more detailed characteristics of individual plant cells may be determined using a combination of spatial transcriptomics, DNA methylation, chromatin accessibility, and proteomic studies with the scRNA-seq technology (Sun et al., 2023). The phenotypic and/or characteristic changes in the individual cell that are caused by abiotic or biotic stress factors can be revealed by the sc-RNA-seg technology. Therefore gathered information can be used by the various plant research areas (Zhou et al., 2022). In light of the literature review, we can say that the scRNA-seq technology has great potential in plant research and will continue to develop and its disadvantages will be eliminated over time.

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RETROTRANSPOSON-BASED MOLECULAR MARKERS: AN EFFICIENT TOOL FOR GENETIC DIVERSITY ASSESSMENT IN CROP PLANTS

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ABSTRACT

Plant genetic diversity is the main resource of agricultural breeding. Genetic diversity enables plants to adapt to environmental changes and increase their resilience. To cope with the effects of factors such as climate change, it is important to obtain plants with high genetic diversity. Genetic diversity also increases the ability of plants to fight new diseases and pests. Retrotransposons are mobile elements in the genome and cause genetic variation. Therefore, retrotransposon-based molecular markers are powerful and widely used tools to better understand plant genetic variation. These markers are used to analyze genetic differences between plant species, subspecies, and populations by detecting variations in specific regions of retrotransposons. The use of retrotransposon-based molecular markers offers important applications in many areas such as plant breeding studies, conservation of species, management of genetic resources, and monitoring of ecosystems. Inter-primer binding sequence (IPBS), sequence-specific amplified polymorphism (SSAP), retrotransposon-based insertion polymorphism (RBIP), inter retrotransposon amplified polymorphism (IRAP), and retrotransposon-microsatellite amplified polymorphism (REMAP) are the commonly used retrotransposon-based molecular markers. This study focuses on the use of retrotransposon-based molecular markers that reveals the genetic diversity of crop plants.

Keywords: Breeding, Genetic Diversity, iPBS, Molecular Markers

INTRODUCTION

The concept of biodiversity constitutes the main source of many disciplines like biotechnology, agriculture, medicine, and industry (Yılmaz et al., 2022). Biological diversity is a whole formed by genes, species, ecosystems (living environments), and ecological events (Selim et al., 2015). Ecological diversity can be defined as the different geographical conditions and soil structures, topographic conditions, climatic conditions, and the relationships between all living things in that region (Çetiner, 2010; Doğan et al., 2010). The disappearance of biological factors and the loss of abiotic factors negatively affect the ecological diversity. In addition to these factors, unfavorable conditions in processes such as water circulation, soil formation, and energy flow affect the diversity negatively (Doğan et al., 2010). Genetic diversity is a basic unit of biodiversity. The diversity in an organism's genetic material is the underlying cause of intra- and inter-species variability. Genetic variation assures the endurance and adaptability, fitness, and evolutionary flexibility necessary for organisms to survive. Genetic fitness is a vital survival mechanism for many organisms due to rapid changes in environmental conditions (Ashry, 2013).

Molecular markers are efficient tools for studying the biodiversity and genetic variation studies. DNA molecular markers basically detect variation in the nucleotide sequence at a particular locus in the genome (Kalendar et al., 2011). The DNA markers directly help identifying genetic variations independent of many factors such as developmental stage, habitat conditions, tissue and organ type (Shi et al., 2023). Molecular markers are considered the most effective technique to evaluate and categorize different sources of germplasm (Jiang et al., 2023). They are also indispensable tools of agricultural genetics by discover DNA fingerprints of varieties and determining genetic differences, as well as determining genus and species phylogeny, selection of lines containing genes with desired characteristics for breeding studies, and creation of linkage maps (Sipahi and Yumurtacı, 2020). Important reasons for using molecular markers can be listed as; accelerating the transfer of desired genes between varieties and species, enabling the transfer of new genes from related wild species, enabling genetic analysis of characters controlled by more than one gene, revealing genetic relationships between plants that cannot be crossed with each other in hybridization studies, facilitating and accelerating gene cloning (Aksu and Şahin Çevik, 2015). This study focuses on the use of retrotransposon-based molecular markers that reveals the genetic diversity of crop plants.

RETROTRANSPOSON BASED MOLECULAR MARKERS

Transposons or transposable elements (TEs) are mobile DNA fragments that have the ability to replace in the genome, change the amount of DNA in the genome and cause mutations. TEs perform the transposition process in the genome by a mechanism called transposition (Karaman and Karlık, 2022). Transposons are found in the genome of almost all organisms. For example, they can make up 90% of the plant genome, 54% of the human genome, and 50% of the mammalian genome (Koçak et al., 2020; Yushkova and Moskalev, 2023). They contribute to genetic diversity through both splice site polymorphism and minor structural rearrangement. TEs are divided into two groups based on the transposition mechanism and mode of propagation; Retrotransposons (class I elements) and transposons (class II elements) (Vershinin et al., 2003).

Retrotransposons (REs) can produce high numbers of copies during evolution and are an important component of plant genome structure (Usai et al., 2020). Transposons (TEs) are DNA sequences that have the ability to migrate within the genome. The cut-and-paste mechanism without an RNA intermediate is used by Tes and they have an open reading frame encoding tansposase enzyme and terminal inverted repeats (TIRs). The enzyme named as transposase is responsible for removing the transposon and placing it in a new location (Yushkova and Moskalev, 2023). The dynamism and distribution of various transposon groups has led to their widespread use as molecular markers (Retrotransposon-based molecular markers) (Kalendar et al., 2011). Several molecular markers based on retrotransposon insertion polymorphisms such as IRAP, REMAP, S-SAP, iPBS, and RBIP have been previously developed (Monden et al., 2014) (Figure 1).

RBIP (Retrotransposon-based Insertion Polymorphism)

In this method, first primer binds to the flanking genomic region at the LTR-RT insertion and second primer binds to the LTR-RT at regions of the 3' or 5' ends of the LTRs, and then this provide detection of retrotransposons in the genome. One of the disadvantages of this method is its high cost compared to other methods. Secondly, RBIP requires knowledge of the sequences of the 5' and 3' flanking regions of LTR-RT insertions. Another limitation of RBIP is wide size range (about 3-5 Kbp) compared to standard PCR. RBIP marker can be a codominant marker when three primers used for detection both the presence and absence of TE insertion, and also can be dominant marker when only two side primers are used (Alzohairy et al., 2014; Alzohairy, 2016).

Searching the Web of Science (WOS) by "RBIP" criteria revealed that some articles were published in pea, jatropha, pear, mango, sweet potato, and coffee during 2010-2021 (Table 1). Meng et al., (2021) used the RBIP molecular marker method to determine intraspecific genetic diversity in sweet potato plants. The results of this study show that the RBIP marker can be used for cultivar identification, genetic diversity assessment and linkage mapping, but that sweet potato germplasms do not diversify well evolutionarily. In Hamon et al., (2011), SSAP, REMAP, and RBIP markers were applied to 182 accessions from 31 *Coffea* species and one *Psilanthus* accession. According to the combined results of markers, the usefulness of markers in estimating *Coffea* genetic diversity and timing of the speciation/differentiation events is reported.

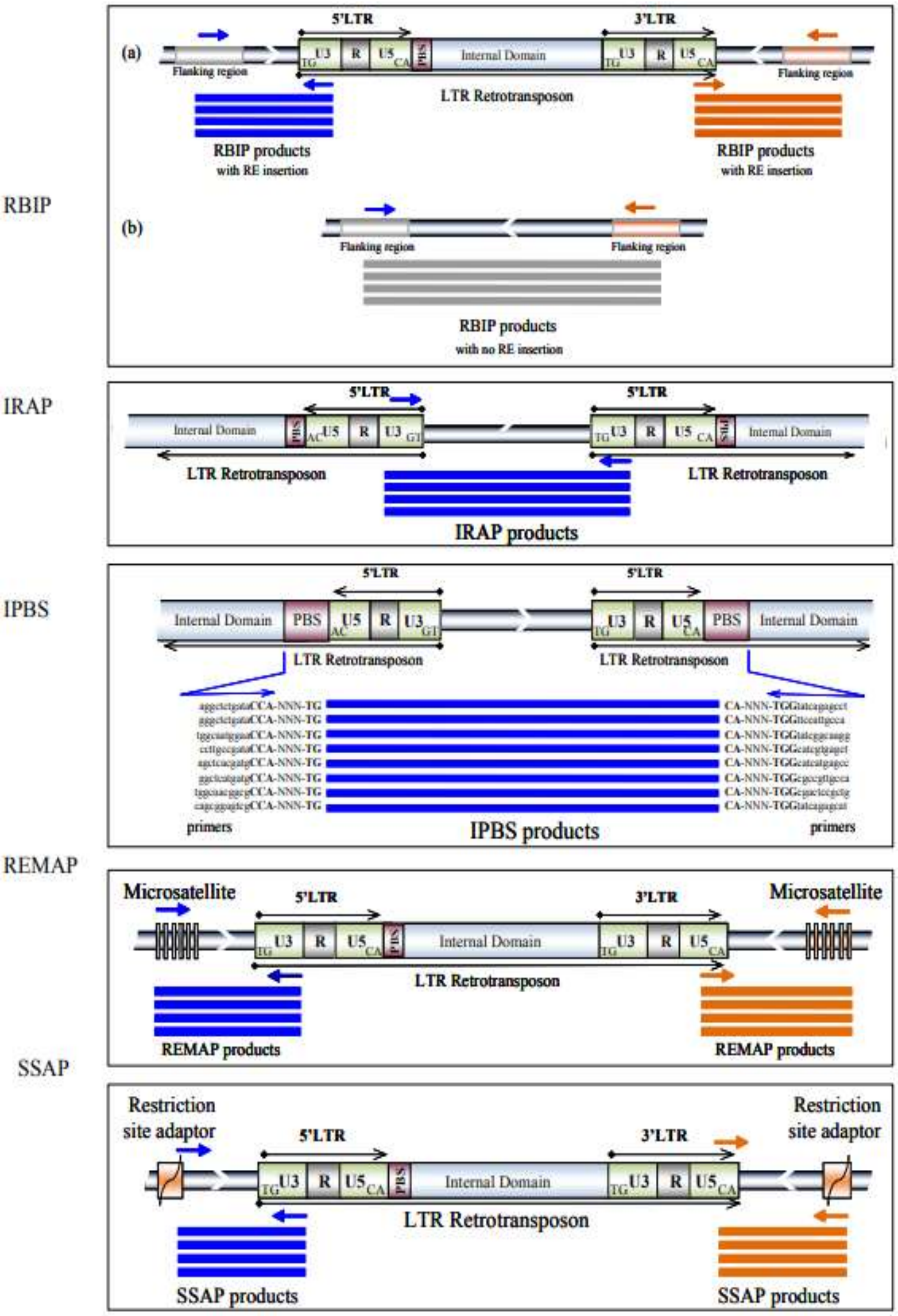

Figure 1. Retrotransposon-based molecular markers (Primers indicated by arrows) (Alzohairy, 2016).

IRAP (Inter Retrotransposon Amplified Polymorphism)

The number and size of the amplified regions of the inter-RT fragments were increased by various retrotransposon insertions. Therefore, we can use them as markers to detect polymorphism of the studied genotype. Primers should be selected as face outward from the LTRs of the LTR-RTs to amplify the region between the two RTs. The same or different RT families can be used for primer design. IRAP can also be performed with a single primer that matches the 5' or 3' end of the LTRs but points outward from the LTR itself. One disadvantages of this marker as follows; IRAP may produce too many fragments and good solubility gels were needed or may not produce products because the target amplification sites are too far to form amplicons. IRAP primers do not require radioactive or fluorescently labeled primers, whereas some markers like SSAP requires. This method has been widely used for genotyping of *Hordeum vulgare* and *Oryza sativa,* as well as *Musa*, *Brassica*, *Spartina*, *Triticum*, and *Solanum* species (Alzohairy, 2016).

Searching the Web of Science (WOS) by "IRAP" criteria revealed that approximately 120 articles in various plant species during 2010-2023, some examples of these studies were given in Table 1. Dilmen et al., (2023) used IRAP and REMAP markers to reveal the effects of salt stress and salicylic acid on retrotransposon polymorphism in wheat. **In** Ghaffarian and Mohammadi (2023), IRAP and REMAP markers were used for estimate the genetic diversity of *Melissa officinalis* populations from Iran. Both IRAP and REMAP markers had a high level of polymorphism and the average percent polymorphism was 96.35% and 90.78%, respectively. Marzang et al., (2020) used two RT-based marker systems, IRAP and REMAP to assess genetic variability and structure in a collection of 94 durum wheat genotypes from Iranian, Turkish and International cultivars. As a result, it was concluded that the genetic diversity of Iranian durum wheat is low. Shingote et al., (2019) used IRAP and ISSR markers to test the genetic fidelity of micropropagated clones of 47 sugarcane accessions, 20 polymorphic markers representing 98 loci were reported.

iPBS (Inter-primer Binding Sequence)

iPBS is one of the frequently used marker to demonstrate retrotransposon polymorphisms. The iPBS method is based on the amplification of the primary binding site by reverse transcriptase in the LTR retrotransposon (Çayır and Sevindik, 2022). The need for sequence information is a prerequisite for designing LTR-RT-based molecular barcodes. In this marker analysis, PCR amplification occurs between two nested primer binding sites of LTR-RTs. Because plants LTR-RTs are frequently mixed, intertwined, truncated, or inverted in the genome, LTR-RTs can be easily amplified using conservative iPBS primers. Retrotransposon motions and recombinations can also be observed because new inserts or recombinations will be polymorphic and will only appear in plant lines where insertions/recombinations have occurred (Alzohairy, 2016).

Searching the Web of Science (WOS) by "iPBS" criteria revealed that approximately 100 articles in various plant species during 2010-2023, some examples were given in Table 1. Kocak et al., (2023) studied 29 genotypes of the flax from the different sources including agricultural institutes and research centers in Türkiye by 12 iPBS and 33 ISSR markers. According to iPBS and ISSR markers data, there is consistency between agro-morphological results and the genetic diversity and population structure results. Carracedo et al., (2022) conducted study with LTR retrotransposon-based iPBS and IRAP techniques avocado accessions and genetic variations between cultivars have been characterized. In Demirel et al., (2018), the genetic identification and fingerprinting of 151 potato genotypes were determined by 45 iPBS markers. Also, it is reported that iPBS markers are efficient for the fingerprinting of potato germplasm. Baloch et al., (2015) studied 50 accessions from wild and cultivated *Lens* species with iPBS and ISSR markers, and they reported high level of polymorphism, at 82.92% and 51.92% with ISSR and iPBS markers, respectively.

Table 1. Some studies with retrotransposon based molecular markers on agriculture during 2010-2023.

RETROTRANSPOSON MARKER	SPECIES	REFERENCE
RBIP	**Pea (*Pisum* spp.)**	**Jing et al., (2010)**
	Pea (*Pisum sativum* L.)	**Martin-Sanz et al., (2011)**
	Jatropha (*Jatropha curcas* L.)	**Alipour et al., (2013)**
	Pear (*Pyrus* L.)	**Jiang et al., (2015)**
	Mango (*Mangifera indica* L.).	**Nashima et al., (2017)**
	Pea (*Pisum sativum* L.)	**Gixhari et al., (2014)**
	Sweet potato (*Ipomoea batatas* (L.) Lam.)	**Meng et al., (2021)**
RBIP, REMAP, SSAP	*Coffea* genus (31 species)	**Hamon et al., (2011)**
IRAP, REMAP	Durum wheat (*Triticum turgidum* L.)	**Marzang et al., (2020)**
	Maize (*Zea mays*)	**Yigider et al., (2020)**
	Artemisia anuua L.	**Valizadeh et al., (2021)**
	Rice (*Orzya sativa* L.)	**Arvas et al., (2022)**
	Lemon balm (*Melissa officinalis* L.)	Ghaffarian and Mohammadi (2023)
	Wheat (*Triticum aestivum* L.)	Dilmen et al., (2023)
IRAP	**Sugarcane (*Saccharum* spp.)**	**Shingote et al., (2019)**
	***Citrus* genus**	**Horibata and Kato (2020)**
	***Aegilops tauschii* Coss.**	**Sha et al., (2021)**
	***Pistacia* spp.**	**Chatti et al., (2022)**
IRAP, iPBS	**Avocado (*Persea americana*)**	**Carracedo et al., (2022)**
iPBS	***Lens* genus (7 species)**	**Baloch et al., (2015)**
	Potato (*Solanum tuberosum*)	**Demirel et al., (2018)**
	Wheat (*Triticum aestivum* L.)	**Pour et al., (2019)**
	Bermudagrass (*Cynodon* spp.)	**Bülbül et al., (2021)**
	Wheat (*Triticum aestivum* L.)	Nadeem (2021)
	Myrtle (*Myrtus communis*)	**Tüzün-Kis and İkten**

		(2022)
	***Gossypium* genus**	**Çayır and Sevindik (2022)**
	Flax (*Linum usitatissimum* L.)	**Kocak et al., (2023)**
REMAP	**Grape (*Vitis vinifera* L.)**	**Razi et al., (2020)**
	***Lallemantia* genus**	**Koohdar et al., (2021)**
REMAP, SSAP	**Pigeon pea (*Cajanus cajan*)**	**Maneesha and Upadhyaya (2017)**
SSAP	**Peach (*Prunus persica* (L.) Batsch)**	**Jiao et al., (2014)**
	***Malus* spp.**	Savelyeva **et al., (2017)**
	Wild barley (*Hordeum brevisubulatum*)	**Guo et al., (2018)**
	Maize (*Zea mays*)	**Roy et al., (2021)**

REMAP (Retrotransposon-Microsatellite Amplified Polymorphism)

REMAP combines one primer that binds to the LTRs of LTR-RTs and another primer that binds to adjacent locus-specific simple sequence repeats (SSRs) of the genome. This technique is only applicable when the SSR is located near retrotransposons. Amplification between the retrotransposon and a nearby SSR do not requires restriction enzyme digestion and adapter ligation to generate bands belonging to marker (Alzohairy, 2016).

Searching the Web of Science (WOS) by "REMAP" criteria revealed that approximately 90 articles in various plant species during 2010-2023, some examples of these studies were given in Table 1. Safiyar et al., (2022) used IRAP and REMAP markers to evaluate genetic diversity of *Aegilops tauschii* accessions and its association with *Triticum durum* and *Triticum aestivum*. The study revealed that retrotransposon-based molecular markers are a suitable and reliable method to evaluate genetic diversity and phylogenetic analysis in *Triticum* and *Aegilops* accessions. In Razi et al., (2020), 42 primers belonging to REMAP were used to determine insertional polymorphisms of retrotransposons in 75 cultivated and wild grape genotypes, and polymorphism ratio was calculated as 95.43%. According to their results, there is important level of diversity at molecular and pomological level for future grape breding studies. Koohdar et al., (2021) studied medicinally important plants belonging to *Lallemantia* genus with REMAP markers to reveal the species delimitation and species relationship in genus and investigate discriminating power of the studied markers.

SSAP (Sequence Specific Amplified Polymorphism)

The method is based on technical steps of AFLP (Amplified Fragment Length Polymorphism) technique. SSAP is the first retrotransposon-based barcoding technology. SSAP uses a primer that matches the 3' end of one LTR and the other primer pairs with a restriction site adapter usually for MseI or Pst, like AFLP. Two or three labeled selective nucleotides are necessary for primer pairs. The non-selective primer pair may also be useful when restriction enzymes have a long sequence of recognition sites or when the copy number of LTR-RTs is low. Primers that are highly polymorphic, yielding clear and reproducible SSAP banding patterns are candidate primers for further studies (Alzohairy, 2016).

Searching the Web of Science (WOS) by "SSAP" criteria revealed that approximately 50 articles in various plant species during 2010-2023, some examples of these studies were given in Table 1. In a study by Kour et al., (2014), various regeneration protocol trials were conducted in MS (Murashige and Skoog) media prepared for *Artemisia absinthium* L. plants grown *in vitro*. In order to understand the genetic effects as well as the phenotypic effects, the genetic stability of the plants was examined by using ISSR and SSAP molecular markers. As a result, both markers could detect somaclonal variations in plants regenerated with callus, while no variation was detected in plants regenerated from nodal explants. SSAP was found to be more useful in detecting variability compared to the ISSR molecular marker and concluded that the direct regeneration protocol would be beneficial for the production of plants loyal to the species of this medicinally important plant. Roy et al., (2021) developed SCAR and SSAP markers for maize, and reported that developed markers might be useful especially for the crops with no genetic sequence information.

RESULT AND CONCLUSION

Genetic diversity studies of plant gene resources constitute the guarantee of sustainability in agricultural production. Therefore, very valuable genetic resources should be used as breeding material for the development of new varieties (Erat and Balık, 2022). With the information obtained from genetic marker studies, it has been concluded that which samples should be preserved in the gene banks and the acquisition of genetic information is very effective in the selection of different species and cultivars rather than genetically similar to each other (Demir, 2015).

Transposon elements have extraordinary potential for altering genome structure and gene function, due to their ability to move from one place to another within the genome or to produce new copies of themselves at any genomic location (Vershinin et al., 2003). Retrotransposons are useful for many studies from assessment of biodiversity and genome evolution, gene mapping and estimation of genetic distance/similarity, assessment of basic derivation of varieties, detection of somaclonal variation, traceability and purity of food. These methods are quite extensive, as LTR retrotransposons are ubiquitous (Kalendar et al., 2011).

In plant biotechnology researches, retrotransposons play a significant role in plant genome evolution. Molecular markers based on retrotransposons provide wide applications in genetic diversity determination, genetic mapping, phylogenetic analysis, and variety genetic identification of genotypes. In addition, DNA markers are more effective, accurate and reliable for distinguishing varieties and closely related species when compared with traditional phenotypic markers (Ouyang et al., 2021). It has been determined that retrotransposon-based molecular markers are very effective and easily applicable in determining genetic variation of various crop plants (Aydın, 2016; Coşkun, 2019).

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ECONOMIC ANALYSIS OF 340 W SOLAR BASED DRIP IRRIGATION SYSTEM AND COMPARISON WITH ITS GASOLINE-BASED EQUIVALENT IN TURKIYE

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ABSTRACT
Nowadays, with a growing environmental pollution and an increasing energy demand, it demonstrates that energy is one of the most fundamental factors in defining the level of development of countries. Particularly, over the course of the pandemic period, energy needs in every aspect of daily life and the escalating in the usage of technological tools have once again shown the significance of energy resources and the process of energy generation. Accordingly, the use of alternative energy resources in the generation of electricity is swiftly increasing in the world. Generally, the most known and preferred renewable energy resources are hydropower, wind and solar but also biomass, geothermal, hydrogen and wave are other renewable energy resources. Solar energy, one of the recognized renewable energy resources, has a significant position in the generation of electricity worldwide. Türkiye is situated at the sunny belt of the world and is located between the 36th and 42nd northern parallels and the 26th and 45th east meridians. Hence, Türkiye has a high solar energy potential. Solar energy systems are consistently finding various application areas and a solar based agricultural irrigation is one of them. Water pumping systems are ordinarily affiliated to conventional electricity, gasoline generated electricity or gasoline-based engines. Solar water pumping systems are to reduce the use of fossil fuel-based electrical energy and consumption of fossil fuel. The usage of a gasoline-based water pumping systems requires not only costly fuels, but also create environmental negative impact such as rumble and atmospheric pollution. The gasoline-based water pumping system is more expensive than the solar water pumping system since has additional costs such as operating and maintenance costs apart from the cost of installation. Eco-friendly solar water pumping systems need less maintenance cost without fuel cost. This sustainable photovoltaic (PV) technology is similar to different conventional water pumping systems except that the source of power is solar energy. An incident solar radiation and the size of PV array determine the flow rate of pumped water. Solar water pumping systems gains significance lately on the score of unavailableness of electricity and increase in gasoline prices. An appropriately intended solar water pumping system concludes notable long-term cost savings as compared to conventional water pumping systems. In this study, the installation of 340 W off-grid solar system required to meet the energy requirement of 12 Volt DC submersible water pump used in the drip irrigating almost 3 acres of agricultural estate where irrigation was previously done with the gasoline-based pump located at Ezine, Çanakkale is performed. Solar irrigation system that was installed in April 2022, was used until November 2022. During this period, the operating time of the solar irrigation system was daily recorded. Also, the fuel cost was calculated with considering the daily gasoline prices as if the gasoline-based irrigation system was used. As a result of this study, the solar irrigation system amortized the installation cost within the mentioned period. In addition, the data obtained from this study and considering the lifetime of solar systems are observed that the specified off-grid solar drip irrigation system is an efficient, eco-friendly, and economical irrigation method rather than the gasoline-based irrigation system. Thus, the

proposed solar irrigation systems will constantly support the development of the agricultural sector.

Keywords: Agricultural irrigation, Economic analysis, Off-grid solar system, Renewable energy, Photovoltaic system

INTRODUCTION

Energy is needed for improved the quality of life and economic and social development all over the world. Today, since most of the electrical energy is generated from fossil-based resources, the requirements of energy cannot be met fully. The reduction of fossil fuels and environmental pollution such as global warming and greenhouse gas (GHG) emissions are two of the main reasons why energy demand cannot be met by fossil fuels. Therefore, recurrent crises occur in the electricity sector. This energy issue is a global concern, but the current trend has been switching from fossil-based energy to renewable energy. Increasing and expanding the use of renewable energy resources will contribute to the solution of these energy crisis and environmental problems (Reges et al., 2016). Generally, the most known and preferred renewable energy resources are hydropower, wind and solar but also biomass, geothermal, hydrogen and wave are other renewable energy resources (Yıldırım et al., 2018).

Solar energy is one of the most significant renewable energy sources due to its almost zero operational cost, long lifespan, and unlimited supply of energy. Solar energy is a clean resource of energy and has less negative impact on the environment than fossil-based resources. Although the initial investment costs of solar systems are relatively high, these systems amortize their costs in reasonable period of time. Solar energy technology constantly progresses with technological advancements. Solar energy will probably become the primary renewable energy resource in the future (Deveci et al., 2015).

Türkiye has a high solar energy potential and is situated at the sunny belt of the world and is located between the 36th and 42nd northern parallels and the 26th and 45th east meridians (Kotcioğlu, 2011). Sunlight durations in Türkiye vary seasonally. Daily sunlight duration is almost 5 hours in winter, 7 hours in spring, and 11 hours in summer months (Yüksel and Türkboyları, 2018). Furthermore, solar energy potential atlas of Türkiye is given in Figure 1. Colors of atlas illustrates total annually average values. Potential of solar energy is directly proportionate to sunlight duration of areas and significant amount of power could be obtained from solar energy in Türkiye, as can be seen from Figure 1 (Kabalci et al., 2016).

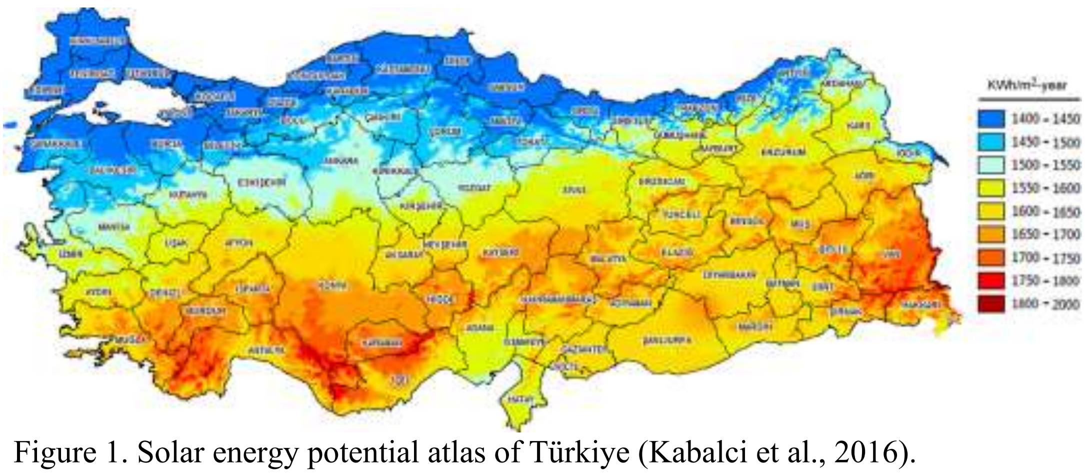

Figure 1. Solar energy potential atlas of Türkiye (Kabalci et al., 2016).

Solar energy systems are consistently finding various application areas and a solar based agricultural irrigation is one of them. Irrigation especially is one of the most significant steps for the most suitable production in the agricultural industry. Irrigation is identified as applying enough water to plants, crops, trees at regular interval for the healthy growth. Also, irrigation and cultivation are interrelated and can be used for crops and trees during periods of insufficient rainfall (Das et al., 2020). Drip irrigation which is a micro-irrigation technology delivers a controlled amount of water and nutrients to the root area of crop or tree through a network of pipes and drip drippers. It has been demonstrated that altering inadequate irrigation methods with drip irrigation could decrease water wasted during irrigation by 20% to 76% and raise crop water productivity by 15%. Also, smallholders can increase productivity of crop while saving water by adopting drip irrigation. In spite of mentioned benefits, drip irrigation is not extensively adopted among smallholders. High capital cost and increased labor requirements of drip irrigation compared to traditional irrigation methods such as flood and furrow irrigation are the main reasons for this situation (Grant et al., 2022).

In this study, the installation of 340 W off-grid on-off controlled solar system required to meet the energy requirement of 12 Volt DC submersible water pump used in the drip irrigating almost 3 acres of agricultural estate where irrigation was previously done with the gasoline-based pump located at Ezine, Çanakkale, Türkiye is performed. Solar based irrigation system that was installed in April 2022, was used until November 2022. A mentioned system basically consists of 2 PV panels (12 Volt, 340 Watt), 12 Volt DC submersible water pump and a circuit breaker. Solar irrigation system is planned without battery group since irrigation is manually realized between 8.00 and 19.00 hours during the day. Owing to the established on-off controlled solar based drip irrigation system, the water needs of agricultural crops are met. Also, it is detected that the solar irrigation system amortized the installation cost within the mentioned period by result of an economic analysis.

MATERIAL AND METHOD

Study Area

Çanakkale was established on both sides of the strait bearing its name and separating the European and Asian continents in the northwest of Türkiye. Çanakkale has 12 districts and it is surrounded by the provincial borders of Edirne, Tekirdağ and Balıkesir. The study is carried out in Ezine district of Çanakkale. The location of Ezine, Çanakkale in Türkiye is shown in Figure 2.

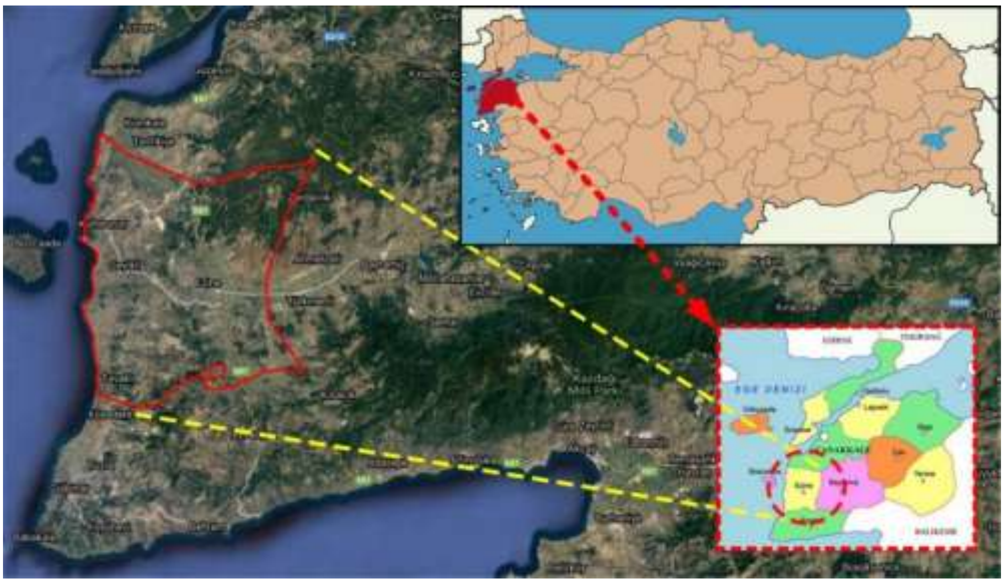

Figure 2. The location of Ezine, Çanakkale in Türkiye

Ezine is in the transition area of Marmara and Aegean Regions. In this respect, the climatic features seen in the two regions are reflected in Ezine. Summers are hot and dry and winters are mild and rainy. Precipitation is usually in the form of rain. In terms of annual precipitation, the rainiest months are November, December, March, and April. Also, the warmest months are July and August, with the average temperature between 25°C and 35°C and the amount of precipitation in these months is negligible. Therefore, agricultural irrigation is an important requirement, especially in summer.

The mentioned study is applied on approximately 3 acres of agricultural estate in Ezine shown in Figure 3. There are olive trees and various fruit trees in this agricultural estate. In addition, various vegetables such as pepper, tomato are grown on this agricultural estate. Before this study, agricultural irrigation needs were met by gasoline-based pump.

Figure 3. The agricultural estate in Ezine where the study is carried out

Comparison Between Solar and Gasoline-Based Water Pumping Systems in Use

Where the study is carried out, there is an artesian well with a depth of 33 meters in the agricultural estate. Before the solar-based water pumping system is installed, the system which consists of a hand water pump and a gasoline-based pump has been used for drip irrigation. Schematic diagram of the gasoline-based water pumping system for drip irrigation and photograph of the gasoline-based water pumping system in use is shown in Figure 4 and Figure 5, respectively.

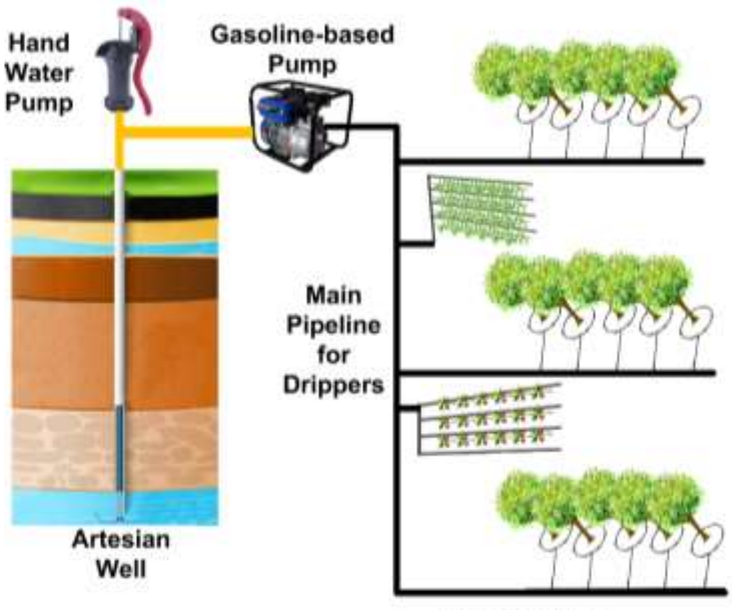

Figure 4. Schematic diagram of the gasoline-based water pumping system for drip irrigation

Figure 5. Photograph of the gasoline-based water pumping system in use

By adjusting the speed of the gasoline-based pump, the amount of freshwater for irrigation per unit time can be regulated. Thus, the need for irrigation of this agricultural estate has been met over the long years.

In April 2022, the solar-based irrigation system consisting of two solar panels, a circuit breaker for on-off control and a submersible pump was installed to meet the irrigation needs of mentioned agricultural estate. Scheme and photograph of the solar based drip irrigation system in use is shown in Figure 6 and Figure 7, respectively.

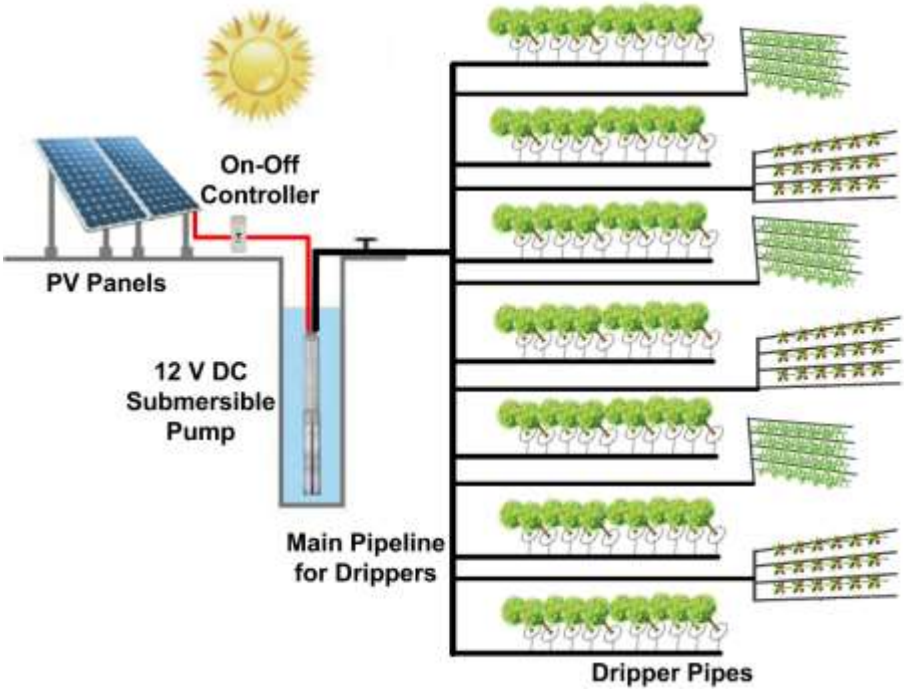

Figure 6. Scheme of on-off controlled solar-based water pumping system for drip irrigation

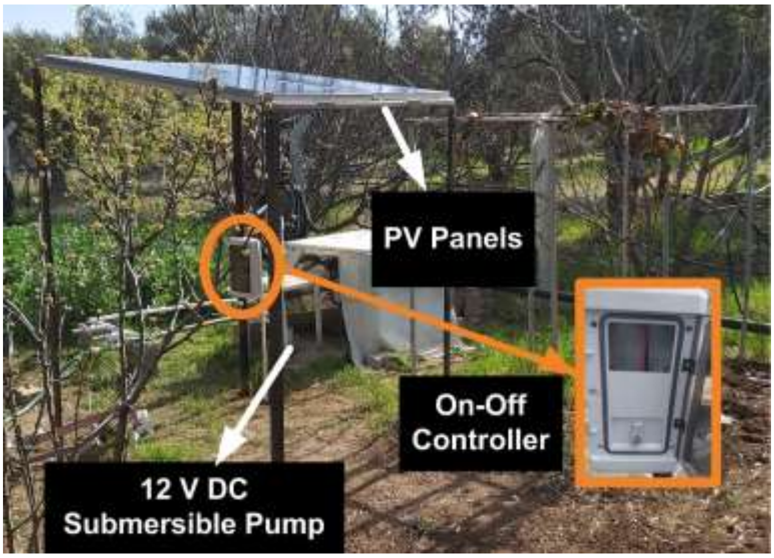

Figure 7. Photograph of the solar water pumping system in use

As seen in Figure 6 and Figure 7, the electrical part of the proposed system includes three main parts. These are PV panels, DC submersible pump and control unit, respectively. Battery-free solar based irrigation system is planned since irrigation is happened in the sunny hours of the day. So, installation cost of irrigation system is diminished. In the established system, there are two PV panels, each PV panels value is 12 Volt 170 Watt. 12 Volt, 340 Watt DC source is acquired by connecting these two PV panels in parallel. Specifications of PV panel are expressed in Table 1.

Table 1. Specifications of PV panel

Name	Rating
Panel Maximum Power	205 W
Peak Voltage	21.45V
Peak Current	9.6 A
Open Circuit Voltage	25.92 V
Short Circuit Current	9.9 A

Another part of the established system is DC submersible pump. The level of water of the artesian well is almost 6 meters under the ground and DC submersible pump is located at 13 meters under the ground. Specifications of DC submersible pump in use are shown in Table 2.

Table 2. Specifications of DC submersible pump

Name	Rating
Voltage	12 V
Power Consumption	130 W
Speed	2850 r/min
Maximum Flow Rate (Qmax)	2 m^3/h
Maximum Head (Hmax)	35 m

The last main unit of the proposed system is the control unit which consists only the circuit breaker. The circuit breaker in the control unit has two main functions. The first of these is to perform on-off control. The other is to protect PVs and the submersible pump by cutting the current flow in case of a possible fault.

As a result, it is determined that olive trees, various fruit trees and various vegetables within mentioned agricultural estate are successfully irrigated with on-off controller in the sunny hours of the day by the proposed system.

COST ANALYSIS

For solar systems, cost analysis, also known as cost-benefit analysis allows deciding whether the investment is economically worthwhile and compares the cost of implementation to its estimated total financial benefit.

In this study, the following steps are followed, respectively, while performing the cost analysis:

- First, the speed adjustment of the gasoline-based pump was made for arranging the amount of water flowing per unit time. Thus, it is ensured that this system transfers the same amount of water with a certain amount of gasoline as the solar based irrigation system. As a result of the adjustment, it was determined that if the irrigation is made by one liter of gasoline is placed in the tank of the gasoline-based pump, it is equivalent to three hours of solar-based irrigation. In other words, after the adjustment, approximately three hours of solar-based irrigation is equivalent to the irrigation of gasoline-based pump with one liter of gasoline. This means that approximately 0.00555556 liters of gasoline are used per minute for gasoline-based irrigation.
- Second, both the gasoline prices in the area where the agricultural estate is located, and solar-based irrigation times were recorded daily. Irrigation with solar energy started in April 2022 and continued until November 2022. The daily solar-based

irrigation times in this period are shown in Figure 8 and the daily change of gasoline price in Ezine during the mentioned period is shown in Figure 9.

- In the final step, cost calculation was made after the solar-based irrigation process was completed. Primarily, it was calculated how much fuel savings were daily achieved by using a solar-based irrigation system instead of a gasoline-based irrigation system. Monthly amount of saved fuel cost thanks to the solar based irrigation system is shown in Tablo 3. Then, the total saved fuel cost was calculated. Last, process of depreciation was evaluated by comparing the installation cost of the solar-based irrigation system with the possible fuel cost of gasoline-based pump.

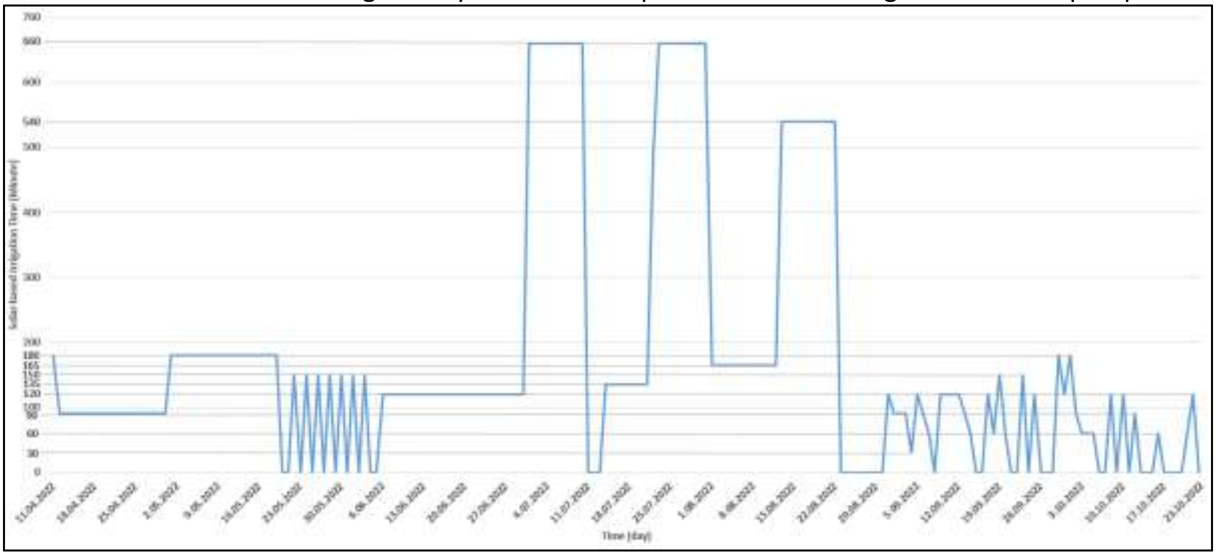

Figure 8. Daily change of irrigation time with solar-based irrigation system

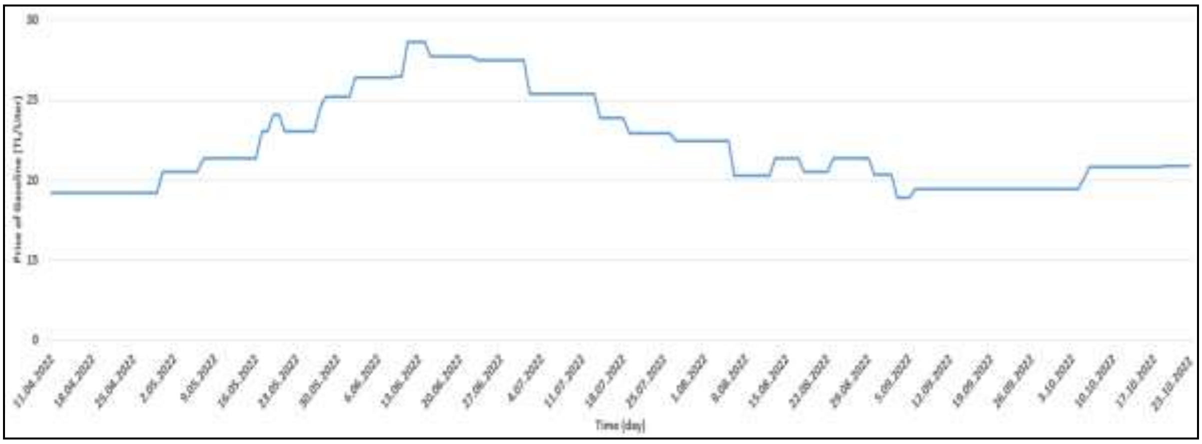

Figure 9. Daily change of gasoline price per liter in Ezine from April 2022 to November 2022 (https://www.petrolofisi.com.tr/arsiv-fiyatlari, accessed in November 2022)

Table 3. Monthly amount of saved fuel cost thanks to the solar based irrigation system

Month	**Cost of Saved Gasoline (TL)**
April 2022	202,27
May 2022	506,68
June 2022	501,88
July 2022	1889,35
August 2022	875,38
September 2022	233,63
October 2022	114,95

At the end of the specified irrigation process, amount of saved fuel cost is calculated as 4324.14 TL. The institutional cost of the solar-based irrigation system is 4174 TL. As it can be seen, the solar-based drip irrigation system amortized the installation cost. As a result, it can be said that no irrigation costs will arise during the lifetime of solar panels and the submersible pump.

CONCLUSIONS

The utilization of solar based agricultural drip irrigation systems has various benefits, including less dependence on fossil fuels, lower operating costs and decreased GHG emissions. However, the initial investment cost of the solar based drip irrigation systems can be an impediment, especially for farmers in developing countries.

In this study, the installation of 340 W off-grid solar based drip irrigation system for 3 acres of agricultural estate where irrigation was previously done with the gasoline-based pump located at Ezine District of Çanakkale Province in Türkiye is performed. Solar irrigation system that was installed in April 2022, was used until November 2022. The solar irrigation system amortized the initial investment cost within the mentioned period.

As a result, it is confirmed that the energy need in the irrigation duration for agricultural estates can be met from solar energy. The energy need can be met by adjusting the system capacity according to the size of the agricultural estates. The techno-economic analysis proffered in this study makes available to beneficial knowledge for farmers and policy makers in assessing the possibility, feasibility, and cost-effectiveness of a solar based drip irrigation system. Generally, this study emphasizes the potential of solar power to promote sustainable agriculture and decrease GHG emissions in the agricultural sector.

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THE IMPACT OF GEOTHERMAL ON LAND PRICES IN THE REGION

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ABSTRACT
Geothermal energy resources have positive effects on land purchase and sale criteria and agricultural land prices. The aim of this research is to investigate the effects of geothermal energy on land prices in Germencik county of Aydin province. The data of the research consist of face-to-face survey data obtained from producers who continue their agricultural activities in the region subject to the study. Descriptive statistical analyses were used to analyses the data obtained in accordance with the purpose of the study. According to the findings obtained as a result of the analysis of the data, more than 50% of the farmers stated that they have agricultural lands close to geothermal resources and that geothermal resources positively affect the economy in the region. 85% of the farmers stated that the economic value of the lands close to geothermal energy sources is high and that these agricultural lands are very valuable. The majority of the producers in the region subject to the research stated that geothermal energy plants affect the prices of fruit, vegetable, field and vineyard lands, facilitate irrigation in agriculture and increase productivity in agriculture. As a result; it was determined that geothermal energy resources have positive effects on agricultural land prices. While the decrease in energy costs increases the profitability of agricultural activities, it increases the potential of agriculture with special applications.

Keywords: Geothermals, Land Prices, Agriculture

INTRODUTION
Geothermal energy, which is one of the renewable energy sources, means the energy provided by hot water and its vapour coming out of the underground. The positive or negative effects of renewable energy sources on the environment have been the subject of many discussions (Külekçi 2009). Geothermal energy, which is one of the renewable energy sources, is a clean energy source. Compared to conventional energy sources, it is cheaper and ready to use. However, the minerals in the geothermal fluid cause water and soil pollution (Ataman 2007).

Geothermal Power Plants can cause serious environmental problems such as air and noise pollution, safety and land use. Recently, the effects of renewable energy sources on the environment continue to be a subject of debate. One of these debates is the impact of geothermal renewable energy sources on land pricing (Akova 2008). Land value is calculated not only by evaluating the land itself, but also by adding yield, assets, buildings if available, whether there are resources such as water, electricity, natural gas, topographical condition of the land and transaction costs (Töremen 2018). Researchers conducted before the establishment of geothermal power plants are very important in terms of the land where the power plant will be established, the structure of the land, its importance in terms of agriculture, which products are produced around the power plant, and how the power plant will benefit or harm them. In this study, the effects of geothermal power plants, one of the renewable energy sources, on land prices have been investigated by taking these situations into consideration. Determining the effects of GPP's on land prices may be important for policy makers both in expropriation processes and in determining the real estate declaration value. Geothermal energy is important in agriculture and many other factors on land. The aim of this study is to investigate the effects of geothermal power plants on land prices in the region.

MATERIAL AND METHOD

The main material of this study is the data obtained from the enterprises that carry out farming activities in the Germencik county of Aydin province. The main reason why the farmers in Germencik county are the main material is the intense geothermal energy resources in the region. These data were obtained by using a questionnaire prepared in accordance with the objectives. In this study, the sample size was determined randomly due to the Covid-19 pandemic process in Turkey and in the world in determining the number of farmers that could reflect the region. During the period when the pandemic measures were reduced by the state (April 2021), 20 farmers were interviewed randomly. Based on the data obtained from the producers, simple statistical analyzes were made in accordance with the purpose of the research.

FINDINGS

According to the findings obtained from the survey study of this research, it was determined that 40% of the farmers were primary school graduates, 10% secondary school graduates, 20% high school graduates and 30% university graduates. The average age of the producers participating in the research is 52 years.

In Table 1, while 30% of the farmers stated that they did not have sufficient knowledge about geothermal resources, 40% of the farmers stated that they had sufficient knowledge about geothermal resources.

Table 1. Knowledge Level of Farmers About Geothermal Resources

I have sufficient knowledge about geothermal resources		Frequency	%	Cumulative %
	I don not agree	6	30,0	30,0
	I am undecided	6	30,0	60,0
	I agree	7	35,0	95,0
	Absolutely I agree	1	5,0	100,0
	Total	20	100,0	

While 30% of the farmers participating in this study stated that Geothermal Power Plants did not affect the economic vitality, 50% of the farmers stated that they did (Table 2).

Table 2. The Impact of GPPs on Economic Vitality

GPPs have an impact on economic viability		Frequency	%	Cumulative %
	I strongly disagree	2	10,0	10,0
	I don not agree	4	20,0	30,0
	I am undecided	4	20,0	50,0
	I agree	8	40,0	90,0
	Absolutely I agree	2	10,0	100,0
	Total	20	100,0	

In Table 3, when these living participating households were asked whether they exceeded the house prices around the GPPs, 40% of the farmers did not agree with this statement, while 30% stated that they agreed with this statement. In Table 4, 70% of the farmers stated that the leakage of GPP pipes from their lands did not increase the land prices in the region, while 30% stated that they increased the prices.

Table 3. The Effect of Geothermal Power Plants in the Research Area on Housing Prices

GPPs increase house prices in the area		Frequency	%	Cumulative %
	I don not agree	2	10,0	10,0
	I am undecided	6	30,0	40,0
	I agree	6	30,0	70,0
	Absolutely I agree	6	30,0	100,0
	Total	20	100,0	

Table 4. Effect of GPP Pipes on Land Pricing

The passing of the GPP pipe in the land affects the land price		Frequency	%	Cumulative %
	I never agree	1	5,0	5,0
	I agree	13	65,0	70,0
	Absolutely I agree	6	30,0	100,0
	Total	20	100,0	

85% of the farmers participating in this research stated that the proximity of Geothermal Power Plants to agricultural lands increases the land prices (Table 5).

Table 5. Price Levels of Lands Near GPPs

Proximity to GPPs affects land price		Frequency	%	Cumulative %
	I never agree	1	5,0	5,0
	I don not agree	1	5,0	10,0
	I am undecided	1	5,0	15,0
	I agree	12	60,0	75,0
	Absolutely I agree	5	25,0	100,0
	Total	20	100,0	

In Table 6, 35% of the farmers participating in the research stated that the presence of Geothermal Power Plant wells on the land would not affect the land pricing positively, while 50% of the stated that it would positively affect them.

Table 6. Effect of Geothermal Power Plant Well on Land Prices

The presence of the GPP well on the land positively affects the price of the land		Frequency	%	Cumulative %
	I never agree	3	15,0	15,0
	I don not agree	4	20,0	35,0
	I am undecided	3	15,0	50,0
	I agree	6	30,0	80,0
	Absolutely I agree	4	20,0	100,0
	Total	20	100,0	

In this research, 20% of the farmers stated that Geothermal Power Plants do not affect the prices of agricultural lands in the region and 60% of the producers stated that Geothermal Power Plants have an impact on the prices of agricultural lands (Table 7).

Table 7. Impact of GPPs on the Prices of Orchards, Field Lands and Vineyards in the Region

GPPs have an impact on the prices of orchards, farmland and vineyard lands in the region		Frequency	%	Cumulative %
	I never agree	1	5,0	5,0
	I don not agree	3	15,0	20,0
	I am undecided	4	20,0	40,0
	I agree	9	45,0	85,0
	Absolutely I agree	3	15,0	100,0
	Total	20	100,0	

In Table 8, 65% of the farmers stated that Geothermal Power Plants did not increase the productivity of agricultural lands, while 20% stated that Geothermal Power Plants increased the yield of agricultural lands.

Table 8. Effect of Geothermal Power Plants on Efficiency

Yield increases in the land where GPPs are located		Frequency	%	Cumulative %
	I never agree	4	20,0	20,0
	I don not agree	9	45,0	65,0
	I am undecided	3	15,0	80,0
	I agree	2	10,0	90,0
	Absolutely I agree	2	10,0	100,0
	Total	20	100,0	

65% of the farmers participating in this research stated that Geothermal Power Plants reduced their olive and olive exports (Table 9). Most of the farmers who produce in the region stated that geothermals disrupt the ecological balance and negatively affect health.

Table 9. Effect of Geothermal Power Plants on Olive and Fig Exports

GPPs reduce olive and fig exports		Frequency	%	Cumulative %
	I never agree	2	10,0	10,0
	I don not agree	5	25,0	35,0
	I am undecided	10	50,0	85,0
	I agree	3	15,0	100,0
	Absolutely I agree	20	100,0	

SONUÇ

In the region subject to this research, figs, olives, cotton and many other agricultural products are cultivated along with the intensive geothermal power plants. It is important for sustainable life and sustainable development to objectively investigate the positive and negative effects on the regions where geothermal power plants are established. The scope of this study is limited to agricultural lands and producers in Germencik county of Aydın province. The degree of impact of geothermals on land prices may differ in other regions of Turkey and the world. Therefore, developing similar studies, sharing the results with the stakeholders of agricultural valuation activities and ensuring that it functions as a public system may provide solutions to the problems experienced in valuation activities. In this study, the effects of geothermal geothermal on land prices in Germencik county were investigated. According to the data and information obtained from farmers carrying out agricultural activities, it is stated that the economic value of the lands close to geothermal power plants is high. However, it can be said that geothermal power plants located in agricultural lands and the pipes passing through these lands limit the use of agricultural lands. In addition, it was stated that the yield of crops such as olives and figs decreased due to the pollution caused by geothermal power plants. As a result, farmers stated that even if the economic value of the lands close to geothermal power plants increases, geothermals harm people, plants, animals and the environment. For a sustainable life and sustainable development, healthy renewable energy sources that do not harm the ecology can be used.

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BREEDING STUDIES IN VITICULTURE

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ABSTRACT

Grapevine is one of the oldest species cultivated by humans, and throughout history, it has been an important source of nutrition with various evaluations. Globally, grapes are produced on 6.93 million hectares, yielding 73.5 million tons of grapes. Despite the decreasing agricultural areas, the world's population is increasing year by year. Therefore, the aim is to achieve higher yields and better quality products per unit area in plant production. Market demands for grapes vary according to different consumption purposes, and researchers effort to develop new grape varieties continue in this direction. To meet these demands, vine breeding programs are being carried out in various fields worldwide. The first breeding studies in viticulture began with seeing phylloxera and later fungal diseases in vineyards. The initial work on grapevine breeding started in the early 19th century with wine grape cultivation and continued in the late 19th century with table grape cultivation and rootstock breeding programs. The oldest breeding varieties emerged from cross-breedings different species between 1819 and 1849. Today, efforts to develop new varieties that is fungal resistant or tolerant to abiotic and biotic stresses are ongoing. Additionally, there is increasing interest in seedless, large, crunchy, and colorful berries to meet consumer expectations. Developing fungal resistant varieties, especially against powdery and downy mildew, is crucial for reducing the use of fungicides and preserving the environment and human health.

Keywords: Breeding, Viticulture, Seedless Grape, Fungal Diseases, Rootstock Breeding

INTRODUCTION

Grapes are one of the most extensively cultivated crops globally, and occupied approximately 6.93 million hectares of agricultural land. In today; 73.5 million tons of grapes are produced from these vineyards (FAO, 2023). Grapes are cultivated across the world for various purposes including table consumption, winemaking, raisin, jam and jelly production, cosmetics, and various other applications. Grapes are important agricultural products that enriches humankind's taste, culture, and economy with their varieties obtained for different purposes. Due to the increasing world population and decreasing agricultural areas, it is aimed to obtain quality and high-yield products per unit area. For these purposes, the expectations of producers and consumers are changing and the demand for new grape varieties is increasing. (Atak, 2022). In line with these demands, many researchers have obtained new grape varieties by cross-breeding, and these studies continue today. (Ergönül et al., 2018; Atak et al., 2019; Ebadi et al., 2009; Ilnitskaya et al., 2021).

The dates of cross-breeding extend to human history. The first hybridization studies in viticulture emerged with the spread of the phylloxera pest in vineyard areas. Breeding studies carried out in the early 19th century, especially for wine grape cultivation, later continued together with table grape cultivation and rootstock breeding programs. In this process, cross-breeding studies carried out between 1819 and 1849 led to the emergence of the first breeding varieties (Kambiranda et al., 2020). These cross-breedings have led to the emergence of varieties with colorful fruit skins and intense pigments in these berry skins. With modern grape cultivation, diseases and insects from North America were carried to Europe. This transport brought with it waves of root aphids, phylloxera (*Daktulosphaira vitifoliae*), powdery mildew (*Uncinula necator*), downy mildew (*Plasmopara viticola*), and black rot (*Guignardia bidwellii*) to vineyards in Europe, causing significant losses in the highly sensitive *Vitis vinifera* cultivars grown there. (Reisch et al., 2012).

As a result of the spread of these diseases and pests, important developments have occurred in viticulture. The most important of these is the need to use rootstocks to protect against phylloxera pest. In line with these needs, hybridization studies have been started, especially among American vines. (Campbell, 2005).

In viticulture, obtaining more productive, high-quality, and big beries table grape varieties with high market value and wine varieties with high must yield and good quality will only be possible through breeding studies, especially by hybridization studies (Ergül, 1994). Hybridization is the process of transferring pollen from a desired male parent flower to a female parent flower under controlled conditions to facilitate fertilization (Sağlam and Sağlam, 2023).

Vine breeding studies are among the priority issues of viticulture in Turkiye, as in the world. In recent years, the primary focuses in hybridization studies have included improving berry quality criteria, seedlessness, enhancing resistance to abiotic and biotic conditions, and obtaining early/late ripening varieties, among others. On the other hand, in these breeding studies that are time-consuming and labor-intensive; It is important to develop the right variety suitable for hybridization combinations (Karauz, 2013).

Hybrid breeding studies in viticulture are divided into two groups: intraspecific and interspecific hybridizations (Sağlam and Sağlam, 2023).

Intraspecific Hybridizations

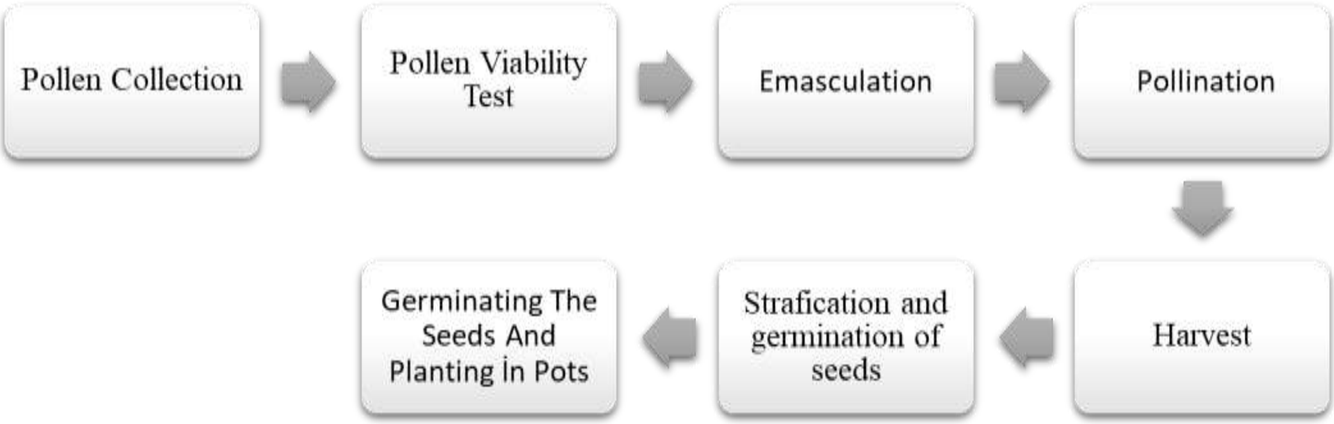
In grapevine breeding, intraspecific hybridizations are mostly made between varieties belonging to the *Vitis vinifera* species.

Intraspecific hybridizations are made for the following purposes:

- To collect the highly demanded features of two different grape varieties in one variety,
- To improve one or more characteristics of a popular but undesirable variety,
- To provide the desired aroma and increase yield and quality in varieties used for different consumption purposes
- Obtaining early and late varieties to increase market time,
- In addition to the existing varieties, to obtain varieties with larger and harder berry, larger clusters, and more skin colors,
- The aim is to obtain new seedless varieties due to the increase in demand for seedless grapes. (Fidan, 1985).

Interspecific Hybridizations

There are 60 species belonging to the *Vitis* genus in breeding studies carried out between these species for different purposes are called interspecific hybridizations. In interspecies hybridizations; The aim is to obtain a grapevine rootstock that is resistant to lime, drought and phylloxera, as well as tolerant to salt, has high adaptability and roots easily (Fidan, 1985).



The Stages of Hybridization

Pollen Collection

When a grape variety from which pollen will be collected enters the full bloom period, the bunches of this variety are shaken into a glass jar or bag that does not contain moisture and the pollen is dropped into them. If the pollen is not to be used immediately but stored, the pollen obtained is poured onto paper in a dry environment in the laboratory and dried there for 1-2 days. The dried bunches are sifted and stored in a container that does not contain moisture. Pollen is stored for long periods at -20˚C or below (Eibach et al., 2015).

Figure 1. The grape flowers is put on paper in the laboratory

Pollen Viability Test

Before hybridization; Viability tests are performed on pollens stored at -20˚C to determine their viability levels and are used for pollination. The viability test is; A few hours after the pollens are distributed into the prepared TTC solution with a brush, those stained red are identified as alive (Şahin and Dardeniz, 2023).

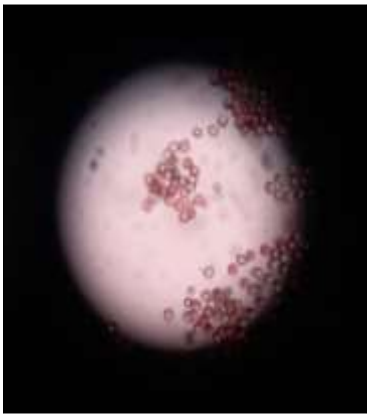


Figure 2. Microscope view of viable pollen

Emasculation

If the variety used as the main parent in hybridization is hermaphrodite, the emasculation process is carried out before the flowers open**.** Emasculation is the removal of the corolla (skullcap, hat) on the flower bud along with the male organs without damaging the female organ. A bag with a date written on it is placed over the cluster whose emasculation process has been completed. Varieties with female flowers do not require emasculation because they are self-sterile (Ergönül et al., 2018).

Figure 3. (a) The cluster that emasculated, (b) Female organ is ready for pollination

Pollination

After the emasculation process is completed, the bagged bunches are checked at regular intervals and are pollinated by the pollinator variety when sugary liquid is seen on the stigma. Since the sugary liquid on the female stigma disappears during very sunny and hot hours of the day, the pollination process should be carried out in the early morning hours. In pollination, the pollen from the male parent variety is applied to the stigma of the female parent variety using a brush, and this process is repeated several times during the flowering period. When pollinating different varieties, hands and brushes should be disinfected with 70% ethanol (Atak and Şen, 2021).

Figure 4. The photo of caps leaving and sugary liquid moist appear

12 to 24 hours after the pollen of the pollinator variety lands on the stigma, the sugary liquid disappears and the pistil begins to hold berry after its top turns to brownish (Sağlam and Sağlam, 2023).

Figure 5. The stage of berry setting

Harvest

The bunches obtained as a result of hybridization are harvested as soon as the fruits reach physiological maturity (Eibach et al., 2015). At the end of harvest, the seeds are separated from the fruit flesh, washed and dried, then stored in at +4 ^{o}C away from moisture until planting time (Sağlam and Sağlam, 2023).

Figure 6. The stage of collecting clusters and take the seeds from berry

Stratification of Seeds and Sowing

The seeds are tested for buoyancy before stratification. Seeds that float on top of the water are considered to be lifeless and those that sink to the bottom are considered to be alive (Conner, 2008). Dormant grape seeds were kept under controlled temperatures (+4 ^{o}C) for 3-4 months. Dormancy release changes with some plant growth regulator, Spiegel-Roy et al. recommended cyanamide, while Manivel and Weaver recommended gibberellic acid (GA_3) Ellis et al. recommended the following procedure for grape seed germination: 24 h soaking in H_2O_2 (5 M), followed by another 24 h soaking in 1000 ppm GA_3 and then planting. After the seeds are sprayed with fungicide, the seeds are taken into moist perlite to stratification. (Atak and Şen 2021).

Figure 7. Vitability test and stratification of seeds

Germinating The Seeds And Planting In Pots

After the seeds are planted in viols, when they have 3-4 leaves, they are transplanted into large containers to be developed or planted directly into the field (Ergönül et al., 2018).

Figure 8. Germinating seeds and growing F1 plants in the greenhouse

Targeted in hybridization breeding in viticulture; In addition to developing varieties that are resistant to earliness, lateness, seedlessness, yield and quality, diseases and pests, cold and drought, the development of new vine rootstocks that are resistant to lime, phylloxera, and nematodes (Özalp and Ergönül, 2015). Some varieties obtained in line with these goals are shown in the table below.

Table 1. Some Varieties Obtained Through Hybridization Breeding Worldwide and Their Prominent Characteristics (Vıvc, 2023)

Variety	Parentage	Utilization	Place and Year Registered	Important Features
Red Globe	Hunisa X Emperor	Table	1958 University Of California	Long Term Storage
Crimson Seedless	Emperor X C 33 - 199	Table	1983 USDA	Seedless – Late Ripening
Flame Seedless	Thompson Seedless X Cardinal	Table	1961 USDA	Seedless
Cardinal	Flame Tokay X Alphonse Lavallee	Table	1939 University Of California	Long Term Storage
Mıchale Palieri	Molınera X Alphonse Lavallee	Table	1958 Italy	Late Ripening

Perlette	*Koenıgın Der Weıngaerten X Sultanına*	Table – Raisin	1936 University Of California	Early Ripening
Summer Muscat	Fresno A4-162 X Fresno P100-111	Raisin	1984 USDA	Seedless – Early Ripening
Regent	Diana X Chamboucin	Wine	1995 JKI Geilweilerhof	Disease Resistant
Phoenix	Bacchus X Villard Blanc	Wine	1992 JKI Geilweilerhof	Disease Resistant
Syrah	Mondeuse Blanche X Düreza	Wine	1998 France	Late Ripening
Aravelle	Riesling X Cayuga White	Wine	2023 Cornell University	Disease And Cold Resistant
Arandell	Ny 84.0101.03 X Ny 88.0514.01	Wine	2013 Cornell University	Disease And Cold Resistant
Corot Noir	Seyve Vıllard 18-307 X Steuben	Wine	2006 Cornell University	Disease And Cold Resistant
St. Croix	Elmer Swenson 283 X Elmer Swenson 193	Wine	1974 Elmer Swenson	Cold Hardiness
Aromella	Tramınette X Ravat 34	Wine	2013 Cornell University	Cold Hardiness
La Rochelle	*Barlınka X Alphonse Lavallee*	Wine	1979 ARC Infruitec-Nietvoorbij	Late Ripening
Kober 5BB	*Vitis Berlandieri X Vitis Riparia*	Rootstock	1896 Sigmund Teleki And Franz Kober	Moist And Clay Soil Conditions
41B	Chasselas X *Vitis Berlandieri*	Rootstock	1882 Alexis Millardet And Charles De Grasset	Resistance To Lime
Demko 10-17A	Edna (America X Malaga) X *Vitis Simpsonii*	Rootstock	2012 USDA	Nematode Resistant
UCD GRN-1	Rupestrıs Alphonse De Serres X Cowart	Rootstock	2008 University Of California	Nematode Resistant
UCD GRN-2	Davıs L 514-30 X Rıparıa Gloıre	Rootstock	2008 University Of California	Nematode Resistant
UCD GRN-3	Davıs L 514-10 X *Vıtıs Champını* C 9038	Rootstock	2008 University Of California	Nematode Resistant
Matador	*101-14 Mgt X 3-1 A*	Rootstock	2011 USDA	Nematode Resistant
Freedom	Fresno 1613-59 X Dog Ridge 5	Rootstock	1974 University Of California	Nematode Resistant
Harmony	Fresno Seedling 39 X Fresno Seedling 5	Rootstock	1956 USDA	High Rooting Ability

Table 2. Varieties Obtained Through Hybridization Breeding in Turkey and Their Prominent Characteristics (Vıvc, 2023)

Variety	Parentage	Utilization	Place and Year Registered	Important Features
Barış	Cardinal × Beauty Seedless	Table	1991 Tekirdağ BAE	Seedless
Trakya İlkeren	Alphonse Lavallee × Perlette	Table	1991 Tekirdağ BAE	Early Ripening
Tekirdağ Çekirdeksizi	Alphonse Lavallee × Sultani	Table – Raisin	1991 Tekirdağ BAE	Seedless
Reçel Üzümü	Elhamra × Perlette	Table	1993 Tekirdağ BAE	Seedless - Late Ripening
Güz Üzümü	Emperor × Sultani	Table	1993 Tekirdağ BAE	Seedless - Late Ripening
Özer Karası	İtalia x Favli	Wine	2011 Tekirdağ BAE	Powdery Mildew Resistance – Late Ripening
Tekirdağ Sultanı	İtalia × Superior Seedless	Table	2011 Tekirdağ BAE	Early Ripening
Tekirdağ Misketi	İskenderiye Misketi × Sultani	Table	2011 Tekirdağ BAE	Seedless – Early Ripening
Bozbey	Queen × Beauty Seedless	Table	2011 Tekirdağ BAE	Early Ripening
Güz Gülü	Kırmızı Şam × Barış	Table	2011 Tekirdağ BAE	Seedless – Late Ripening
Özer Beyazı	Ribol × Güz Üzümü	Table	2016 Tekirdağ BAE	Seedless – Late Ripening
Emirali	Çınarlı Karası × Tekirdağ Çekirdeksizi	Table	2016 Tekirdağ BAE	Late Ripening
Süleymanpaşa Beyazı	A. Beyazı × (Uşuvi × S. Çekirdeksiz)	Table	2016 Tekirdağ BAE	Seedless – Late Ripening
Gönülçelen	Italia × Reçel Üzümü	Table	2016 Tekirdağ BAE	Late Ripening
Cengizbey	Ribol × Güz Üzümü	Table – Raisin	2016 Tekirdağ BAE	Mid-Season Ripening
Gürnil	Italia × Reçel Üzümü	Table	2016 Tekirdağ BAE	Female Flowers – Late Ripening
Kebeli	Ribol × Güz Üzümü	Table	2016 Tekirdağ BAE	Seedless – Late Ripening
Saklıkara	Ribol × Güz Üzümü	Table	2023 Tekirdağ BAE	Seedless
Uslu	Hönüsü × Siyah Gemre	Table	1990 Yalova ABKMAE	Early Ripening
Yalova Çekirdeksizi	Beyrut Hurması × Perlette	Table	1990 Yalova ABKMAE	Seedless
Ata Sarısı	Beyrut Hurması × Perlette	Table	1990 Yalova ABKMAE	Mid-Season Ripening
Yalova Misketi	Royal × Perle de Csaba	Table	1990 Yalova ABKMAE	Early Ripening
Yalova İncisi	Hönüsü × Siyah Gemre	Table	1990 Yalova ABKMAE	Early Ripening
Ergin Çekirdeksizi	Beyrut Hurması × Perlette	Table	1991 Yalova ABKMAE	Seedless

Atak 77	Beyaz Çavuş × H. Misketi	Table	2012 Yalova ABKMAE	Late Ripening
İsmetbey	Siyah Gemre × Royal	Table	2012 Yalova ABKMAE	Mid-Season Ripening
Pembe 77	A. Lavallee × M. Reine des Vignes	Table	2012 Yalova ABKMAE	Late Ripening
Yalova Beyazı	Beyaz Çavuş × Cardinal	Table	2013 Yalova ABKMAE	Early Ripening
Arifbey	Beyaz Şam × Müşküle	Table	2013 Yalova ABKMAE	Disease Resistant – Mid-Season
Samancı Çekirdeksizi	Beyaz Şam × Perlette	Table – Raisin	2013 Yalova ABKMAE	Seedless – Early Ripening
Ece	Mahrabaşı × Cardinal	Table	2016 Manisa BAE	Early Ripening
Lidya	Tahannebi × Cardinal	Table	2016 Manisa BAE	Late Ripening
Spil Karası	Mahrabaşı × Trakya İlkeren	Table – Raisin	2016 Manisa BAE	Early Ripening
Manisa Pembesi	Mahrabaşı × Cardinal	Table	2016 Manisa BAE	Mid-Season
Mesir	Mahrabaşı × Hamburg Misketi	Table	2016 Manisa BAE	Late Ripening

Conclusion

Today, in many countries, hybridization studies and the development of varieties resistant to diseases and pests, cold and drought are among the priority targets. In addition, studies continue to develop new vine rootstocks that are resistant to drought, lime, phylloxera, and nematodes.

Studies on obtaining new grape varieties that are superior in terms of earliness, seedlessness, yield, and quality have accelerated. Nowadays, as the consumer trend increases towards seedless, coarse and hard-grained, flavored and colored varieties, hybridization studies also change in line with consumer demands.

Developing disease-resistant varieties will provide benefits such as less use of pesticides and obtaining healthier grapes, protecting consumer and environmental health, reducing the producer's pesticide costs, and reducing residue problems in exports.

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THE EFFECT OF SOIL AND FOLIAR APPLICATION OF ALGAE EXTRACT ON SOME GROWTH PARAMETERS OF ALFALFA PLANTS

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ABSTRACT

Algae extract is used in many regions to increase yield and quality in sustainable agriculture, particularly in organic farming, by regulating plant growth, increasing resistance to disease and pests, and improving the reclamation of soil. For this purpose, the study was carried out to determine the effects of increasing doses (0, 0.1, 0.2, 0.3, 0.4 and 0.5%) of soil and foliar applications of algae extracts on some yield components of alfalfa plants under controlled greenhouse conditions according to a randomized experimental design with four replications. For this reason, some yield components (upper part of the plant and root length, fresh and dry weight of the upper part of the plant, fresh and dry weight of the root) were determined in alfalfa plants. According to the results of the study, the effects of increasing doses of soil and foliar applications of algae extracts on some yield components of alfalfa plants were found to be statistically significant ($p<0.01$). The highest effect on the length of the upper part of the alfalfa plant was observed with the application of 0.1% algae extract from the leaf. However, the best effect on root length was found in plants treated with 0.5% algae extract from the soil. In general, applications of algae extract from the soil resulted in higher fresh and dry weights of the upper parts of alfalfa plants. It was found that the fresh and dry weight of the root of the plant was more effective when 0.1% algae extract was applied from the soil. Generally, the lowest alfalfa yield component values were obtained in the control treatments.

Keywords: Alfalfa, Algae extract, Leaf, Soil, Yield components,

INTRODUCTION

Lucerne (alfalfa) is a protein-rich forage crop that is grown in the arid and semi-arid regions of the world and in Turkey (Arslan et al., 2020). Alfalfa, which has been called the queen of forage crops, is a highly productive forage plant with a wide range of adaptations and can be used in many ways. It is grown in almost every region of the country because it can be adapted to very different climatic and soil conditions. By encouraging strong root development, they enable plants to absorb more nutrients and water from the soil and increase the green parts by enhancing the formation of chlorophyll in plants, thus synthesizing more carbohydrates, proteins, etc., making plants more resistant to diseases and pests, and enabling plants to resist environmental stresses (abiotic stress) such as frost, drought, insufficient sunlight, excessive water, excessive heat, and excessive cold. They increase the resistance of products to storability.

They inhibit the multiplication of viruses. They reduce nematode damage. They increase the effectiveness of pesticides by 25%. They increase marketing and export value (Blunden et al., 1992). Spraying spinach plants with Goemar GA 14, an extract of

Ascophyllum nodosum, was found to increase the wet weight of the spinach (Gassan et al., 1992). Root and leaf application of Ascophyllum nodosum extract to tomato plants was found to significantly increase leaf green color. There have also been reports that seaweed extracts increase the amount of chlorophyll in cucumbers (Whapham et al., 1993). As a result of the use of seaweed extracts in world agriculture; it provides better root development and increases the germination and shelf life of fruit and vegetables. Many different effects have been recorded, such as darker colors, larger flowers and leaves, increased resistance to diseases and pests, stress conditions such as frost, drought, and unfavorable soil conditions, increased uptake of nutrients in the soil, and keeping plants young for longer (Hong et al., 1995).

The effects of increasing doses of seaweed fertilizer on the growth and some yield components of spinach and lettuce plants were investigated under greenhouse conditions. The application of seaweed fertilizer to the soil at increasing dosages supported the growth of both crops and showed differences according to the increasing doses. The effect of seaweed fertilizer applied in increasing doses on the length of the root and the wet and dry weight of the upper part of spinach plants was found to be statistically significant. For lettuce, the effect of seaweed fertilizer on the length of the upper part and root and on the wet and dry weight of the plant was found to be significant (Karaca and Yarımoğlu, 2020). In recent years, the use of commercial organic liquid fertilizers in greenhouses has become widespread to achieve earliness and variability. Nowadays, seaweeds are used in many countries either as liquid extracts or mixed straight into the soil. When mixed directly into the soil, the aim is to improve soil structure and maintain long-term soil productivity. Some marine algae that have been naturally washed ashore from the seas for many years have been used as fertilizer on fields (Güner and Aysel, 1996).

This study aimed to determine the effects of increasing doses of algal extract on some yield components of alfalfa plants from soil and foliar applications.

MATERIAL AND METHOD

This research was carried out under controlled greenhouse conditions (temperature 25±3oC, solar radiation 1750±50 kcal.m-2, and relative humidity 60±10% during the experiment) at the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Selçuk University. Some physical and chemical properties of the soil sample used in the study are given in Table 1.

Table 1. Some physical and chemical properties of the experimental soil

Parameters		**Results**	**Method**
Class of texture		Clay loam	Bouyoucos 1951
pH (1:2.5 s:water**)**		7.51	Richards 1954
EC (1:5 s:water**, µS/cm)**		172	U.S. Salinity Lab. Staff 1954
Lime	**%**	37.3	Hızalan and Ünal 1966
O.M.		1.22	Smith and Weldon 1941
N (NH_4-N + NO_3-N)	**$mgkg^{-1}$**	12.0	Bremner 1965
P (favorable)		10.0	Olsen et al. 1954
K (extractable		162	Soltanpour and Workman 1981
Ca (extractable)		5.84	
Mg (extractable)		242	
Na (extractable)		80	
Fe (available)		3.12	Lindsay and Norvell 1978
Zn (available)		0.68	
Mn (available)		5.82	
Cu (available)		0.67	
B		0.80	Richards 1954

O.M.: Organic matter

The seaweed fertilizer Proton Algine-S, which is a commercial product, was used as an algae extract. Proton is obtained from the wet processing of Ascophyllum nodosum, the most biologically active and nutrient-rich seaweed used in agriculture. Some of the chemical properties of the algae extract (seaweed fertilizer) used in the study are given in Table 2. The algae extract used in the experiment was rich in organic matter, alkaline in pH, and hypersaline in salinity, as shown in Table 2. This fertilizer contains 1.5% alginic acid.

Table 2. Some chemical properties of the algae extract used in the study

Content	**% w/w**	**Macro E.**	**% w/w**	**Micro E.**	**mgkg^{-1}**
O. M.	43	N	1.9	Fe	200
pH	9-10	P	2.8	Cu	5.8
EC (dSm^{-1})	43.5	K	9.5	Mn	11.8
Protein	6-8	Ca	0.2	Zn	98
Carbohydrates	38-49	S	1.3	B	98
Alginic acid	1.5	Mg	0.6	Mo	3.8
Mannitol	3.5-6.5	Na	1.6		

O. M.: organic matter

The study was conducted in 48 pots (3 kg oven-dried soil) with 2 treatments (soil and foliar) x 6 doses (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) x 4 replications. After surface sterilization of Bilensoy-80 alfalfa seeds with 0.5% sodium hypochlorite, they were planted in pots containing 3 kg of soil and doses of seaweed fertilizer. Basic fertilization was applied according to the nutrient requirements of the soil. The foliar application was done twice: once 1 month after the planting of alfalfa seeds and once 2 months after the planting. The alfalfa plants were harvested after flowering and some growth parameters were taken (length of plant upper part and roots, wet and dry weight of plant upper part, wet and dry weight of roots).

The data obtained from the greenhouse experiment, which was designed with 4 replicates according to the randomized design, were subjected to analysis of variance by using the Minitab 19 statistical program.

RESULTS AND DISCUSSION

The study investigated some growth parameters of Bilensoy-80 alfalfa by soil and foliar application of increasing doses of seaweed extract (0, 0.1, 0.2, 0.3, 0.4, and 0.5%). According to the results of this experiment, the effect of algae extract applied to the soil and leaves with increasing doses on the length of the upper parts of the plant was found to be statistically significant ($p<0.01$). It was found that the length of the upper parts of the alfalfa plants generally decreased with increasing doses when applied in the soil. The highest lengths of the upper stem of the plants were observed in plants treated with 0.1% and 0.3% algae extract (Figure 1). The lowest upper stem length was found in plants treated with 0.5% algae extract (Table 3). In the case of foliar applications, it was found that there was generally a reduction in plant length with an increase in the dose of algae extract applied. Foliar applications of 0.1-

0.3% of seaweed extract have been found to be more effective (Figure 1). Spraying spinach plants with Goemar GA 14, an extract of Ascophyllum nodosum, was found to increase the wet weight of the spinach (Gassan et al., 1992). It was found that the application of Ascophyllum nodosum extract to the roots and leaves of tomato plants resulted in a significant increase in the green color of the leaves. It has also been reported that extracts of seaweed can increase the amount of chlorophyll in cucumbers (Whapham et al., 1993).

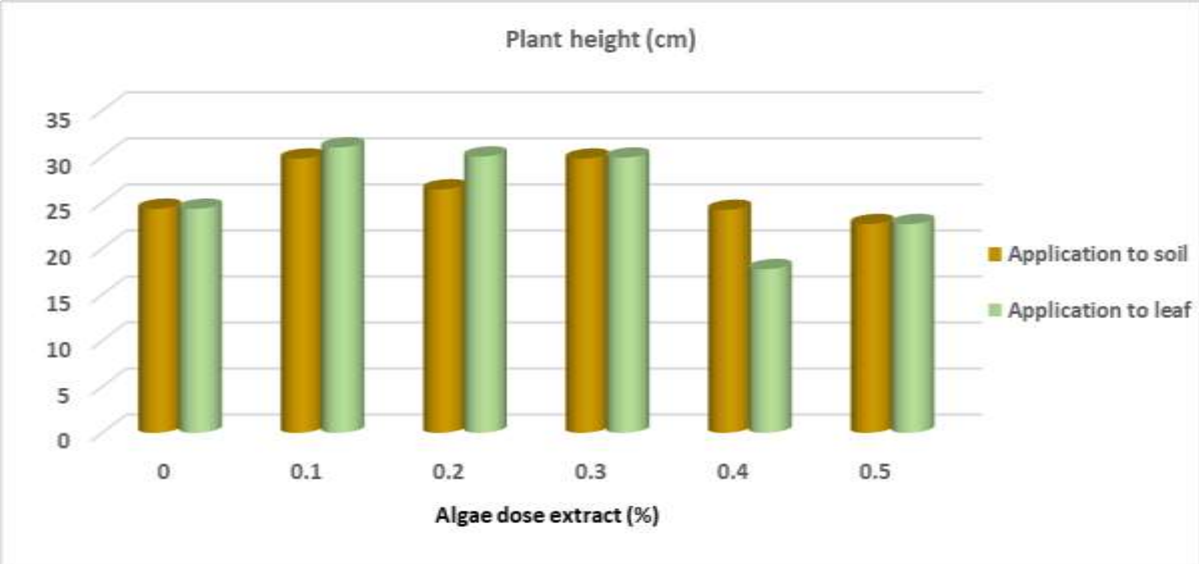

Figure 1. Effects of algal extract applied to soil and leaf on plant stem length with increasing dosage

Root lengths of alfalfa plants varied between 12.33-19.22 cm when soil and leaf treatments were evaluated (Table 3). The highest effect on root length was observed in plants treated with 0.5% algae extract (Figure 2). The effect of algae extract applied to soil and leaves at increasing doses on plant root length was found to be statistically significant ($p<0.01$). According to the root lengths of alfalfa plants among different applications, the highest effect was determined in the plants grown in the algae extract applied at a rate of 0.3% in foliar applications. It was observed that higher values were obtained for root lengths in soil applications.

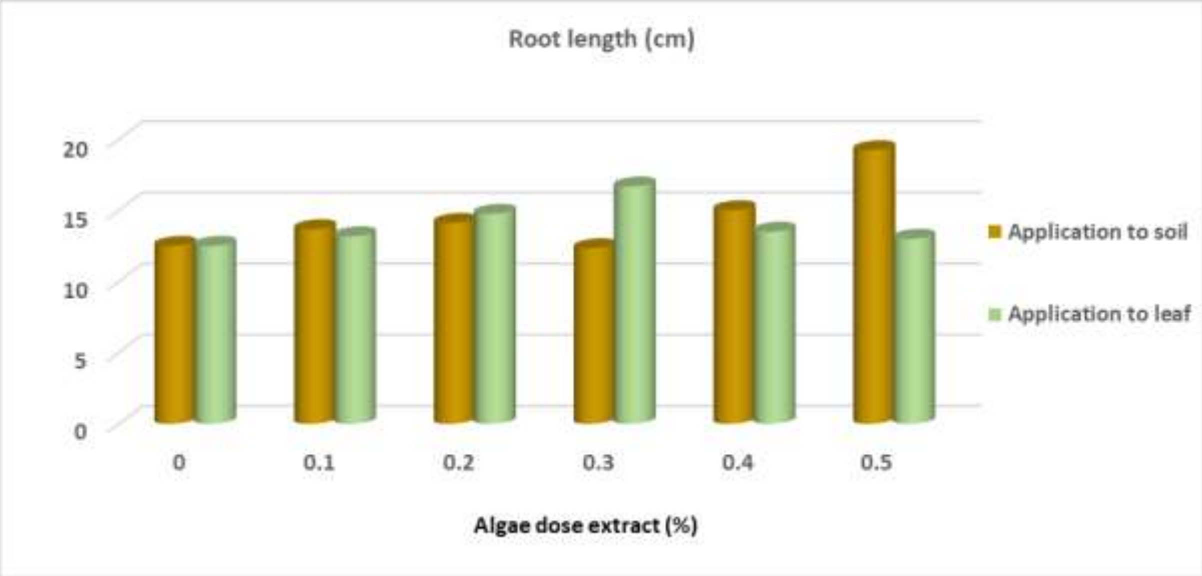

Figure 2. Effects of soil and leaf application of algae extract on root length with increasing dosage

When soil and foliar applications were analyzed, it was determined that the wet weight of the upper parts of alfalfa plants varied between 4.47 and 11.50 g. The highest plant upper part wet weight of 11.50 g was determined in the soil with a 0.3% application dose. This value was followed by the plants grown in the algae extract at a dose of 0.1% applied to the soil (Table 3). The lowest wet weight of the upper part of the plant was observed in the foliar application at a dosage of 0.4%. It was found that the algae extract applied from the soil had better results than the algae extract applied from the leaves (Figure 3).

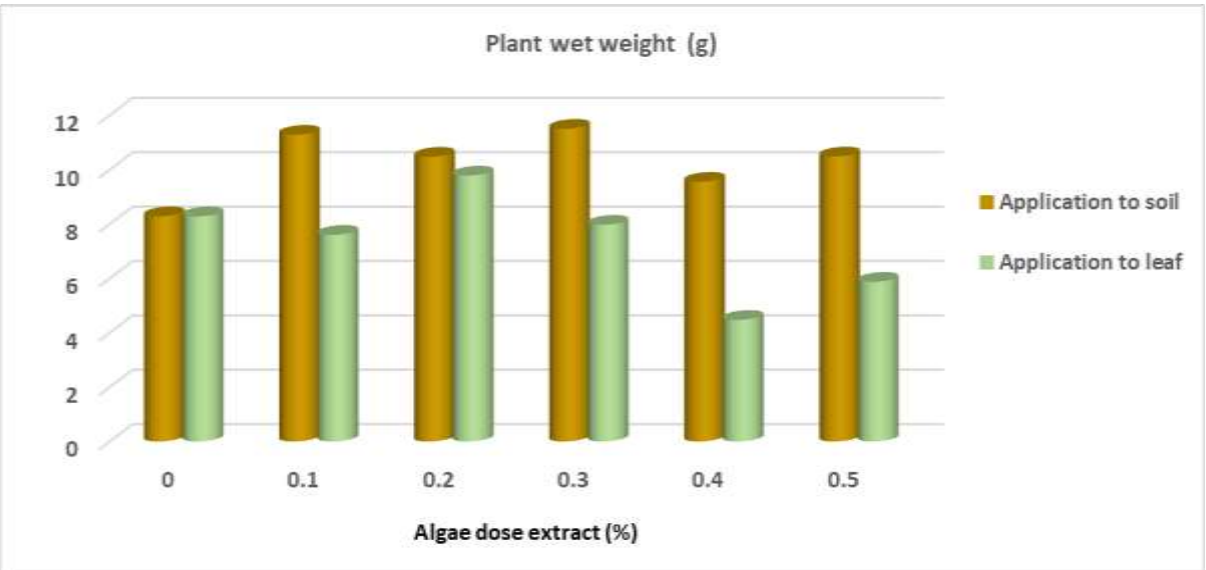

Figure 3. Effects of algae extract applied to soil and leaves on the wet weight of the upper parts of the plant at increasing doses

The effect of algae extract applied to soil and leaves at increasing doses on root wet weights of alfalfa plants was found to be statistically significant ($p<0.01$) (Table 3). It was determined that the root wet weights of alfalfa plants varied between 3.90 and 11.10 g. The highest wet weight of the plant roots was observed in the plants that were treated with 0.1%

algae extract in the soil (Figure 4). The lowest wet weight of the roots was found in the plants treated with 0.4 % of algae extract (Table 3). In the foliar applications, it was observed that the root wet weights of the plants decreased as the dose of algae extracts increased. In foliar applications, it was observed that 0.1-0.3% doses of algae extract were significant in root wet weight (Figure 4). Soil application of seaweed fertilizer at increasing doses supported plant growth in both cultivars and showed differences with increasing doses. The effect of the seaweed fertilizer applied in increasing doses on the length of the root and the wet and dry weight of the upper part of the spinach plant was found to be statistically significant. In lettuce, the effect of seaweed fertilizer on the length of the upper part of the plant and the root and on the wet and dry weight of the plant was found to be significantly significant (Karaca and Yarımoğlu, 2020).

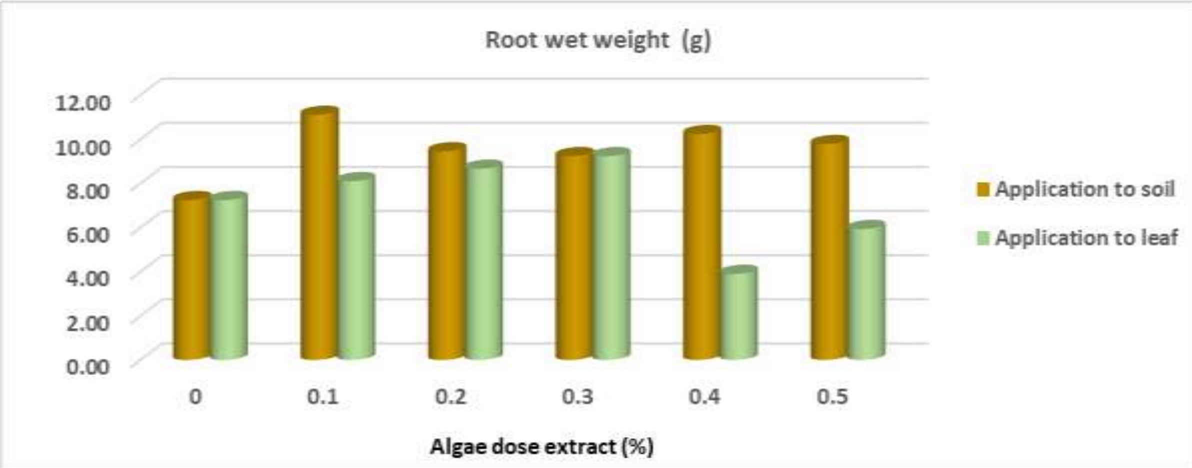

Figure 4. The effects of algae extract applied to soil and leaves on root wet weight with increasing doses

Soil and foliar applications of increasing doses of algae extract on the dry weight of the upper parts of alfalfa plants were found to be statistically significant ($p<0.01$). It was found that both soil and foliar applications of algae extract decreased with increasing doses when plant dry weights were evaluated. The highest dry weight of the upper part of the plant was 4.10 g for plants treated with 0.3% soil application of algae extract. This value was followed by the plants grown in the algae extract at a dose of 0.1% that was applied to the soil (Table 3). The lowest dry weight of the upper part of the plant was observed with foliar application at 0.4%. It was found that the soil-applied algae extract gave better results on the dry weight of the plant than the foliar-applied algae extract (Figure 5). In the dry weight of the plant, the most effective dose of the algal extract applied by foliar application was determined to be 0.2%.

Figure 5. The effects of algae extract applied to soil and leaves on the dry weight of the upper part of the plant with increasing doses

The effect of the algae extract applied to the soil and to the leaves at increasing doses on the root dry weights of the alfalfa plants was found to be statistically significant ($p<0.01$) (Table 3). It was found that the root dry weights of alfalfa plants varied between 2.01 and 3.87 g.

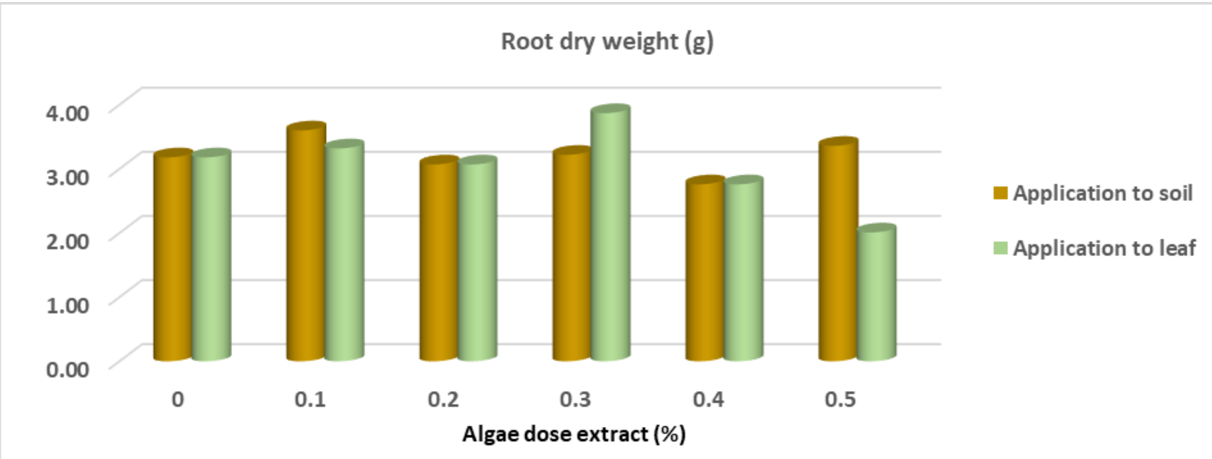

Figure 6. Effects of soil and foliar algae extract on root dry weight with increasing doses

The highest dry weight of the roots was observed in the plants that were treated with 0.3% algae extract (Figure 6). The lowest root dry weight was found in plants treated with 0.5% algae extract (Table 3). In foliar applications, it was determined that the root dry weights of the plants decreased as the dose of algae extract applied increased. In foliar applications, it was observed that 0.1-0.3% doses of the algae extract were more effective in root dry weight (Figure 6). The highest effect of algae extract applied through the soil on plant root dry weight was observed at a 0.1% application rate.

Table 3. Effects of soil and foliar application of increasing doses of algae extract on some yield components of alfalfa plants

Treatment	Dose	PL	RL	PWW	KYA	BDW	RDW
		cm		g			
Soil	0	24.33 bc	12.5 e	8.28 c	7.24 e	2.90 de	3.18 abc
	0.1	29.78 a	13.67 cde	11.28 a	11.10 a	3.83 ab	3.60 ab
	0.2	26.44 b	14.11 cde	10.47 ab	9.45 bc	3.70 abc	3.07 bc
	0.3	29.78 a	12.33 e	11.50 a	9.23 bcd	4.10 a	3.22 abc
	0.4	24.22 bc	15.00 bc	9.55 b	10.23 ab	2.96 cde	2.76 cd
	0.5	22.67 c	19.22 a	10.48 ab	9.78 bc	3.10 bcde	3.36 abc
Leaf	0.0	24.33 bc	12.5 e	8.28 c	7.24 e	2.90 de	3.18 abc
	0.1	31.00 a	13.17 de	7.60 c	8.10 de	3.20 bcd	3.32 abc
	0.2	30.00 a	14.75 cd	9.78 b	8.66 cd	3.70 abc	3.07 bc
	0.3	29.89 a	16.67 b	7.97 c	9.23 bcd	3.21 bcd	3.87 a
	0.4	17.78 d	13.50 cde	4.47 e	3.90 g	1.80 f	2.76 cd
	0.5	22.67 c	13.00 de	5.87 d	5.93 f	2.29 ef	2.01 e
LSD		**2.78 ****	**1.81 ****	**1.16 ****	**1.28 ****	**0.78 ****	**0.74 ****
CV %		**7.32**	**8.74**	**9.13**	**10.54**	**17.43**	**16.82**

****: p<0.01, *:p<0.05** (PL: Plant upper stem length, RL: Root length, PWW: Wet weight of the upper part of the plant, BDW: Dry weight of plant upper parts, KYA: Kök yaş ağırlık, RDW: Root dry weight)

CONCLUSIONS

In the study, the effects of increasing doses (0%, 0.1, 0.2, 0.3, 0.4, and 0.5) of algae extract (seaweed fertilizer) on some yield components of Bilensoy-80 clover cultivar plants were found to be statistically significant ($p<0.01$). It was found that some yield components of alfalfa plants generally decreased with increasing doses of algae extract applied through the soil and foliar applications. It was found that soil-applied algae extract provided better results than foliar-applied algae extract in both wet and dry plant weights.

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THE EFFECT OF SOIL AND FOLIAR APPLICATION OF MACRO-ALGAE AT INCREASING DOSES ON THE NUTRIENT CONTENT OF THE ALFALFA PLANTS

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ABSTRACT

Algae are considered fertilizer in many countries because they aerate the soil, maintain moisture, are as rich in nitrogen as farmyard manure, and contain trace elements. Today, seaweed fertilizers are used in many countries, either as a liquid extract or mixed directly into the soil. Therefore, the study was conducted to determine the effects of increasing doses (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) of seaweed extract (seaweed fertilizer) on the nutrient content of alfalfa plants under controlled greenhouse conditions according to a randomized experimental design with 4 replicates. For this reason, some nutrients (P, K, Ca, Mg, Fe, Cu, Mn, Zn, and B) were determined in Alfalfa plants. According to the results of the study, the effects of increasing doses of soil and foliar application of seaweed extract on the nutrient content of alfalfa plants were found to be statistically significant ($p<0.01$). The highest phosphorus and potassium contents of alfalfa plants were found in plants treated with 0.4% seaweed fertilizer. The highest calcium and magnesium contents were determined in the plants treated with 0.4% algal extract from the leaves compared to the control treatments. It was observed that the foliar application of seaweed fertilizer generally affected plant microelement content.

Keywords: Alfalfa, Algae extract, Nutrients, Leaf, Soil,

INTRODUCTION

Alfalfa is a highly nutritious forage crop for animals due to its high levels of protein, minerals, and vitamins (Engin and Mut, 2018). They are the source of macro- and micro-nutrients for plants. Chelating the micro-elements that the plant cannot absorb in the soil provides the plant with the highest levels of these nutrients and balances them in the plant. They increase side branching and fruit set in fruit trees. They also reduce flower and fruit droppings. They help increase crop yield by up to 30%. They increase yield and improve quality by providing a balanced and long-term uptake of macro and micro-nutrients from the soil (Blunden et al., 1992).

Seaweed extracts belong to a broader class of agricultural biostimulants that have the potential to improve yield and quality in crops and cropping systems, increase plant tolerance to abiotic stresses, enhance biotic stress and nutrient use efficiency, and improve plant tolerance to different soil types (Shukla et al., 2019; Rouphael and Colla 2020). For example, in greenhouse/field studies, foliar or soil applications of seaweed extracts can increase yields in sugarcane, strawberry, vegetables, and tomatoes (Mattner et al., 2018; Shukla et al. 2019; Arioli et al., 2020; Hussain et al., 2021).

Liquid fertilizers from natural sources such as seaweed are rich in organic matter, micro- and macro-elements, vitamins, fatty acids, as well as growth regulators (Crouch and Van Standen, 1993). When seaweed products remain in the soil for a long time, they decompose easily under natural conditions and produce high levels of nitrogen (N) and calcium (Ca). They also contain trace elements such as Manganese (Mg), Boron (B), Iron (Fe), Zinc (Zn), Cu, and Co. All these effects of algae are due to compounds such as macro- and micro-elements (N, Ca, Mg, Mn, B, B, Fe, Zn, Cu, Co), plant growth regulators (auxins, cytokinins, gibberellins, abscisic acid) and betaines (Hong et al., 1995). The effect of liquid seaweed extract (Kelpak) on the growth and nutrient content of lettuce was investigated, and it was reported that Kelpak increased the yield of the crop and the content of Ca, K, and Mg in the leaves (Grouch et al., 1990). Sayed et al., (2015), In a study, evaluated the effect of a seaweed extract on the growth, yield, and chemical composition of moringa and alfalfa plants. Seaweed extract was applied twice, 30 and 45 days after planting, and 15 and 30 days after each harvest, at 0 and 1.5 g/L. The results showed that foliar application had the highest wet and dry plant weights at a 1.5 g/L application dose. The data also showed that the macro- and micro-nutrient and protein content of moringa and alfalfa had the highest significant levels, especially in the first cutting. Di Mola et al., (2020b) reported that foliar application of biostimulants, particularly seaweed extract and protein hydrolysates, improved the quality of romaine lettuce grown at different nitrogen levels, including unfertilized lines.

The aim of this study was to determine the effects of increasing doses of soil and foliar application of algae extracts on some macro- and micronutrient contents of alfalfa plants.

MATERIAL AND METHOD

This experiment was conducted in the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Selçuk University, under controlled greenhouse conditions (temperature 25±3^{o}C, solar radiation 1750±50 kcal.m^{-2}, and relative humidity 60±10% during the experiment). Some physical and chemical properties of the soil sample used in the study are given in Table 1.

Table 1. Some physical and chemical properties of the research soi

Parameters		Results	Method
Class of texture		Clay loam	Bouyoucos 1951
pH (1:2.5 s:water**)**		7.51	Richards 1954
EC (1:5 s:water**, µS/cm)**		172	U.S. Salinity Lab. Staff 1954
Lime	**%**	37.3	Hızalan and Ünal 1966
O.M.		1.22	Smith and Weldon 1941
N (NH_4-N + NO_3-N)	**mgkg^{-1}**	12.0	Bremner 1965
P (favorable)		10.0	Olsen et al. 1954
K (extractable		162	Soltanpour and Workman 1981
Ca (extractable)		5.84	
Mg (extractable)		242	
Na (extractable)		80	
Fe (available)		3.12	Lindsay and Norvell 1978
Zn (available)		0.68	
Mn (available)		5.82	
Cu (available)		0.67	
B		0.80	Richards 1954

O.M.: Organic matter

The seaweed extract used was Proton Algine's seaweed fertilizer, a commercial product. Proton is obtained by processing fresh Ascophyllum nodosum, the most biologically active and nutritious seaweed used in agriculture. Some chemical properties of the seaweed extract (seaweed fertilizer) used in the study are given in Table 2. As can be seen from Table 2 below, the seaweed extract used in the experiment is rich in organic matter, has an alkaline pH, and is classified as extremely saline. This fertilizer contains 1.5% alginic acid.

Table 2. Some chemical specifications of the algal extract used in the experiment

Content	% w/w	Macro E.	% w/w	Micro E.	$mgkg^{-1}$
O.M.	**43**	**N**	**1.9**	**Fe**	**200**
pH	**9-10**	**P**	**2.8**	**Cu**	**5.8**
EC (dSm^{-1})	**43.5**	**K**	**9.5**	**Mn**	**11.8**
Protein	**6-8**	**Ca**	**0.2**	**Zn**	**98**
Carbohydrates	**38-49**	**S**	**1.3**	**B**	**98**
Alginic acid	**1.5**	**Mg**	**0.6**	**Mo**	**3.8**
Mannitol	**3.5-6.5**	**Na**	**1.6**		

O.M.: Organic matter

The study was conducted in 48 pots (3 kg oven-dry weight) with 2 treatments (soil and foliar) x 6 doses (0, 0.1, 0.2, 0.3, 0.4 and 0.5 %) x 4 replications. Bilensoy-80 seeds were surface sterilized with 0.5% sodium hypochlorite and planted in pots containing 3 kg doses of soil and seaweed fertilizer. Basic fertilization was applied according to the nutrient needs of the soil. Foliar fertilization was applied twice, once 1 month after the sowing of alfalfa seeds and once 2 months after the sowing of alfalfa seeds. Alfalfa plants were harvested after flowering and some element contents (P, K, Ca, Mg, Fe, Cu, Mn, Zn, and B) were determined in the upper part of the plant.

The data obtained from the greenhouse experiment, designed as a randomized four-replication design, were analyzed by ANOVA analysis of variance using the Minitab 19 statistical program.

RESULTS AND DISCUSSION

In the experiment, some nutrient contents of soil and foliar applications of algae extract at increasing doses (0, 0.1, 0.2, 0.3, 0.4, and 0.5 %) were determined in the alfalfa cultivar Bilensoy-80. According to the data obtained, the effect of algae extract applied to soil and leaves in increasing doses on the macro- and micro-element contents in the upper part of the plant was found to be statistically significant ($p<0.01$). The phosphorus content of alfalfa plants varied between 0.28-0.34%. The highest phosphorus content was observed in plants that had applied 0.4% algae extract to the soil (Figure 1). The lowest plant phosphorus content was found in control and 0.5% algal extract-treated plants (Table 3). In foliar applications, the 0.4% dose of algae extract was observed to be the most important (Figure 1).

The potassium content of alfalfa plants varied between 1.49 and 3.40% when soil and foliar applications were evaluated (Table 3). It was found that the highest effect on plant potassium content was observed when 0.5 % algae extract was applied to the soil (Figure 2). Among the different treatments, it was observed that higher values were obtained for the potassium content of alfalfa plants in soil applications. The effect of algal extract applied to soil and foliar at increasing doses on the calcium content of the alfalfa plant was found to be statistically significant ($p<0.01$) (Table 3). The highest calcium content was observed in plants treated with 0.4% algae extract through the leaves (Figure 3). The lowest calcium content was found in the plants treated with 0.4% algae extract from the soil (Table 3). It was observed that 0.3 and 0.5% doses of algae extract were more important for calcium content when applied to soil (Figure 3).

When soil and foliar applications were evaluated, the magnesium content of alfalfa plants varied between 0.19 and 0.30 % (Table 3). It was found that the highest effect on the magnesium content of the plant was observed in the plants treated with algae extract through the leaves (0.1, 0.2, and 0.4%) (Figure 4). It was observed that higher values were obtained in foliar applications for the magnesium content of the alfalfa plant among different treatments. The effect of liquid seaweed extract (Kelpak) on the growth and nutrient content of lettuce was investigated, and it was reported that Kelpak increased the crop yield and Ca, K, and Mg content in leaves (Grouch et al., 1990). The effect of liquid seaweed extract (Kelpak) on the growth and nutrient content of lettuce was evaluated, and it was reported that Kelpak increased crop yield and Ca, K, and Mg content in leaves (Grouch et al., 1990).

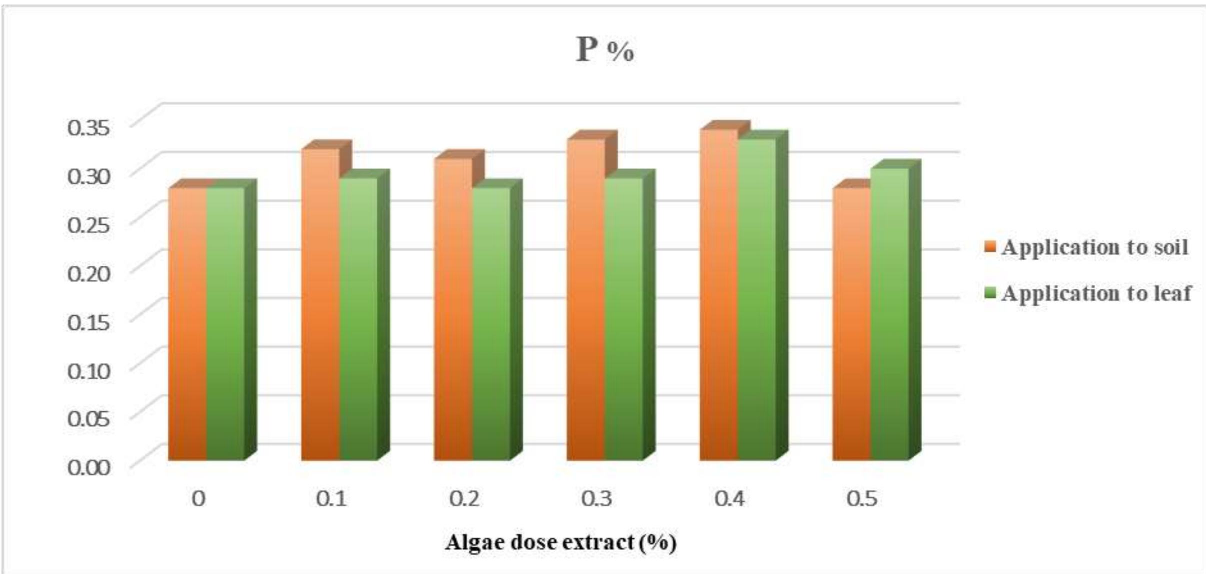

Figure 1. Effects of increasing doses of algae extract applied to soil and leaves on the phosphorus (P) content of alfalfa plants

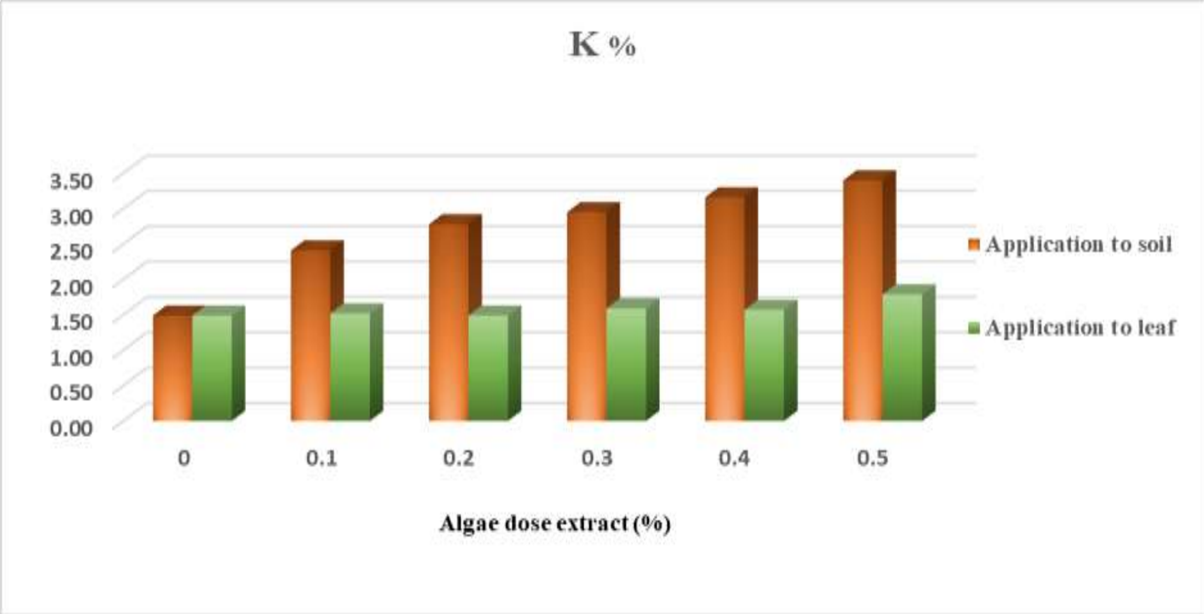

Figure 2. Effects of increasing doses of algae extract applied to soil and leaves on the potassium (K) content of alfalfa plants

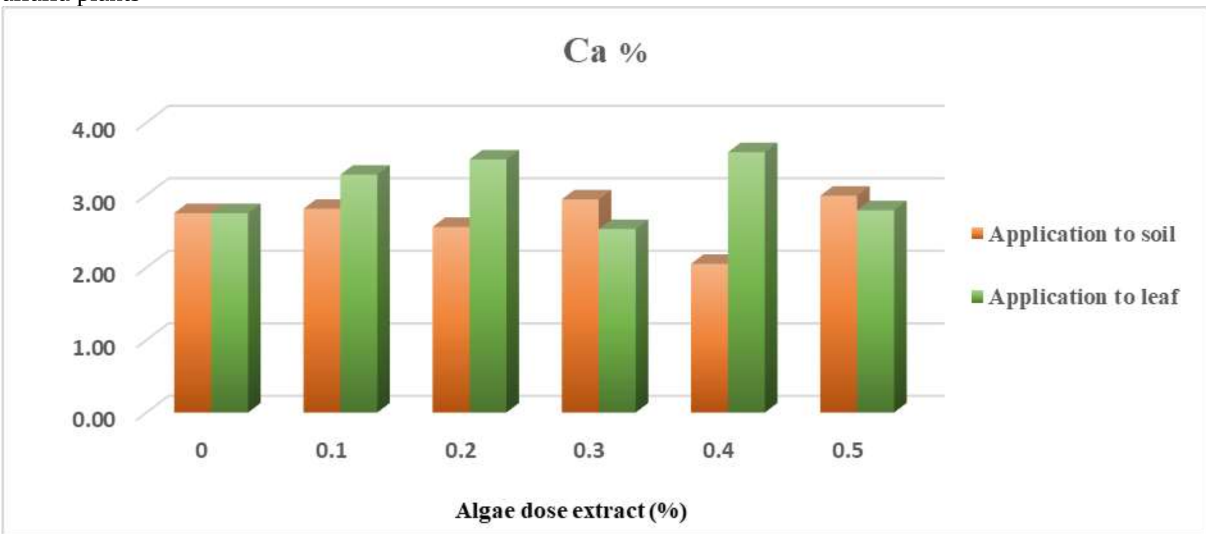

Figure 3. Effects of increasing doses of algae extract applied to soil and leaves on the calcium (Ca) content of alfalfa plants

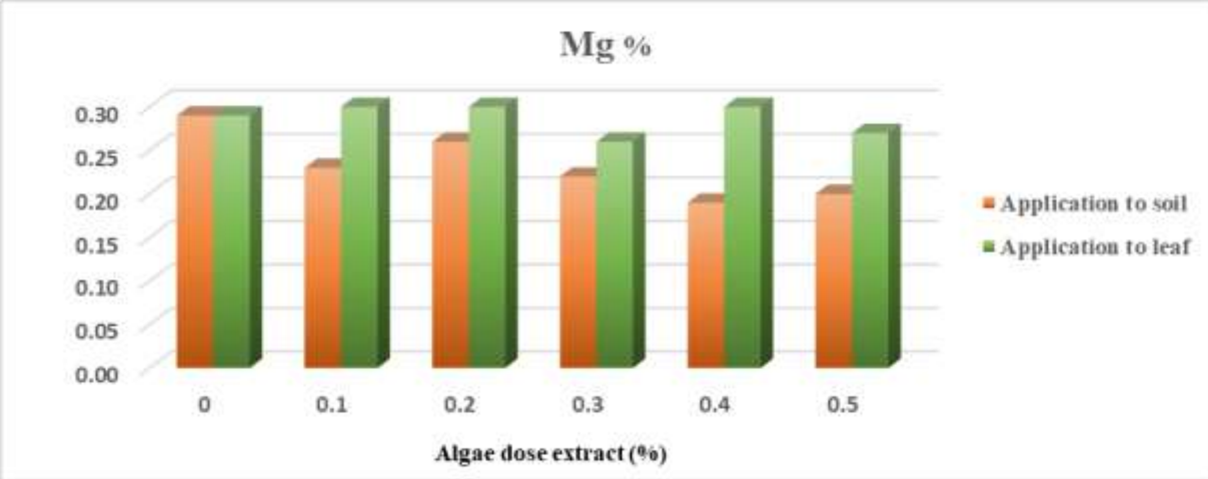

Figure 4. Effects of increasing doses of algae extract applied to soil and leaves on the magnesium (Mg) content of alfalfa plants

The effect of the algae extract applied to the soil and to the leaf in increasing doses on the iron content of the alfalfa plant was found to be statistically significant ($p<0.01$) (Table 3). The highest iron content was observed in the alfalfa plants treated with 0.1 % of the algae extract (Fig. 5). The lowest iron levels were found in the control plants (Table 3). For foliar applications, it was observed that the iron content of the algae extracts decreased with increasing doses (Figure 5). When the copper content of alfalfa plants in soil and foliar applications was evaluated, it was found to vary between 3.92-8.08 mgkg-1 (Table 3). It was found that the highest effect on plant copper content was observed in plants with a 0.2% foliar application of algae extract (Figure 6). In the case of soil application of algae extract, the 0.1% dose was found to be the most significant.

The highest effect of algae extract applied in increasing doses on the manganese content of alfalfa plants was observed at the 0.5% dose applied from the soil. This value was followed by the 0.3% dose. The lowest manganese content was found in plants treated with 0.3% algae extract applied through the leaves (Figure 7).

The effect of increasing doses of algae extract applied to soil and leaves on the zinc content of alfalfa plants was found to be statistically significant ($p<0.01$) (Table 3). The highest zinc content was observed in alfalfa plants treated with 0.3% algal extract applied through the leaf (Figure 8). The lowest zinc levels were found in plants grown at a 0.5% dose of algae extract (Table 3). In soil applications, it was observed that zinc content decreased with increasing doses of algae extract (Figure 8). The highest effect on the boron content of alfalfa plants was found in plants that were applied with 0.2% dose of algae extract through the leaves. The lowest boron content in alfalfa plants was found when the 0.4% dose was applied through the soil. Sayed et al., (2015) investigated the effect of algal extract on the growth, yield, and chemical composition of moringa and alfalfa plants. The results showed that the highest values of the wet and dry weights of the plants were obtained at an application dose of 1.5 g/L in the foliar application. The data also showed that the content of macro- and micro-nutrients and protein in moringa and alfalfa was significantly higher, especially in the first cut. Soil or foliar application of algae has been reported to improve the physiological status of plants and increase their drought tolerance (Dela et al., 1988).

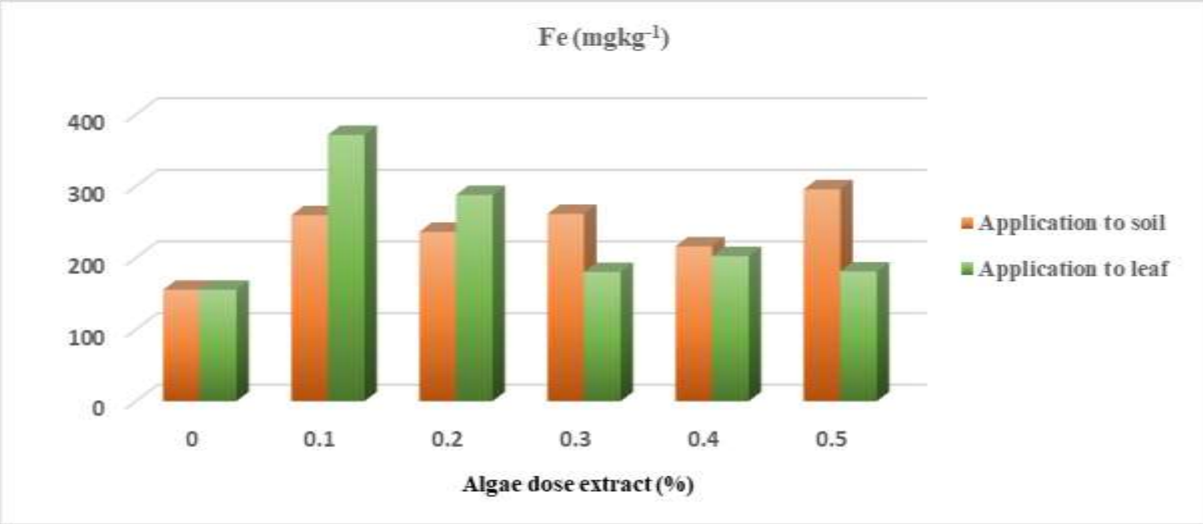

Figure 5. Effects of increasing doses of algae extract applied to soil and leaves on the iron (Fe) content of alfalfa plants

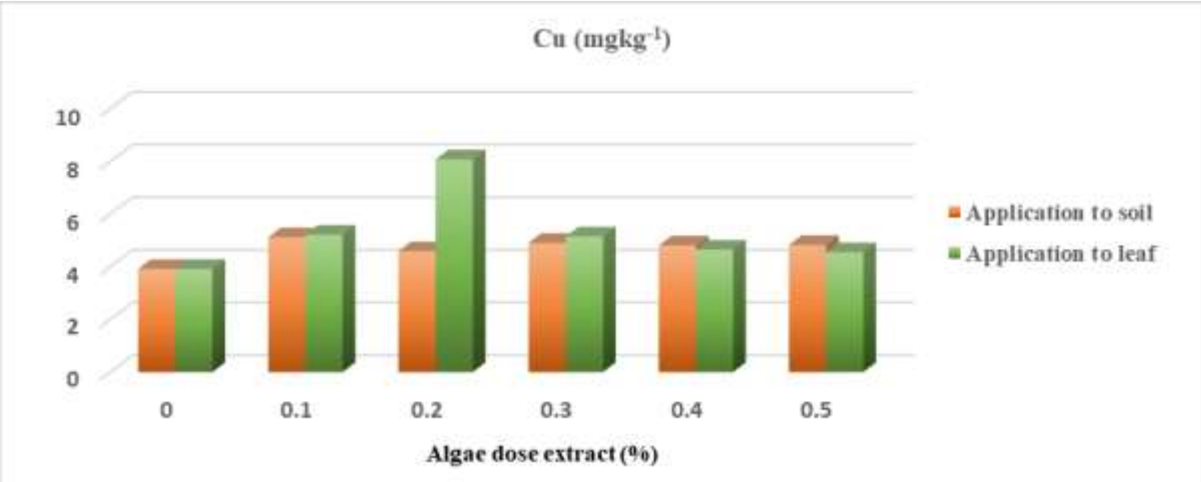

Figure 6. Effects of increasing doses of algae extract applied to soil and leaves on the copper (Cu) content of alfalfa plants

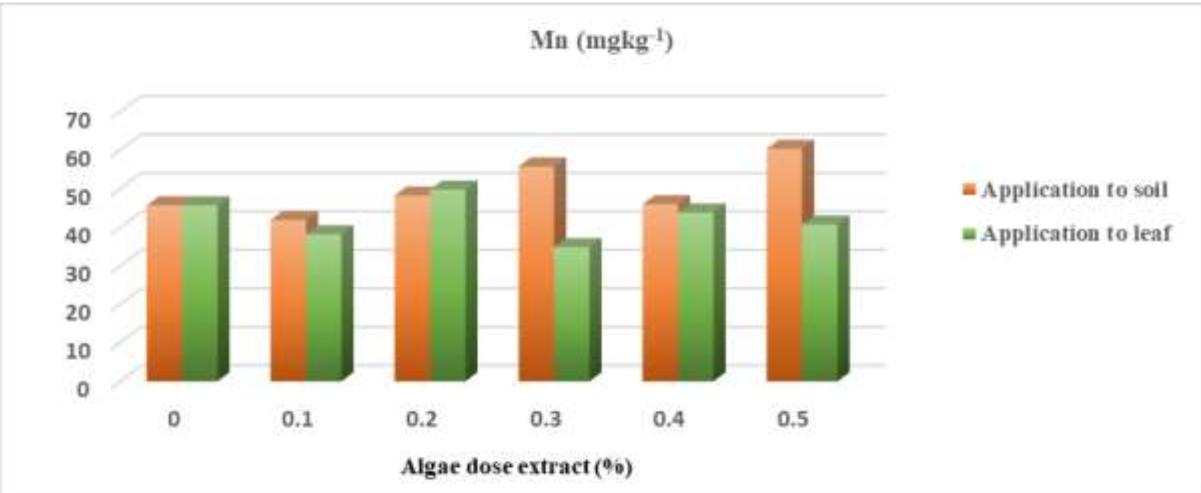

Figure 7. Effects of increasing doses of algae extract applied to soil and leaves on the manganese (Mn) content of alfalfa plants

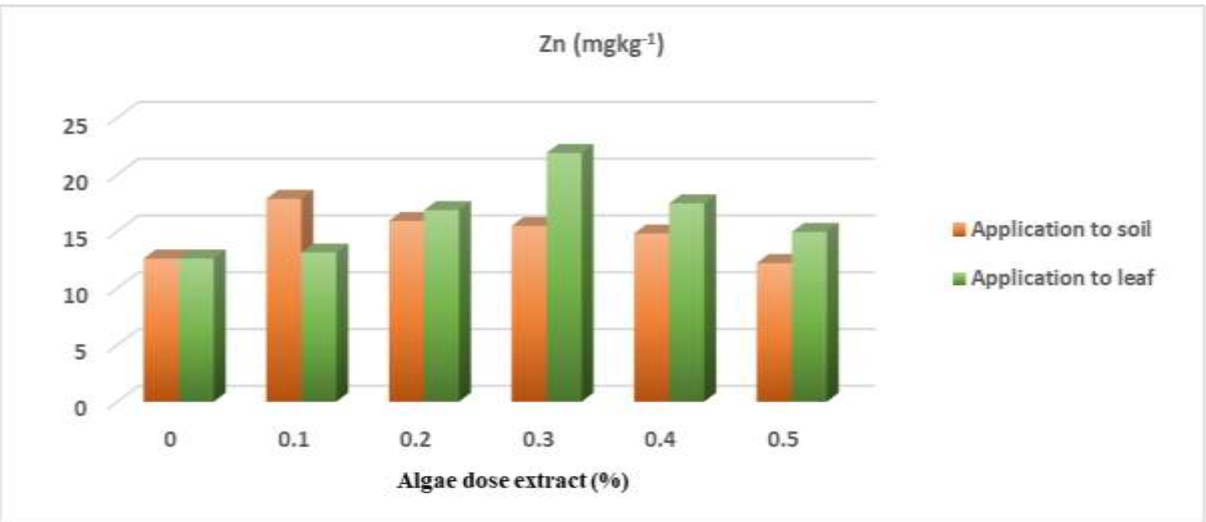

Figure 8. Effects of increasing doses of algae extract applied to soil and leaves on the zinc (Zn) content of alfalfa plants

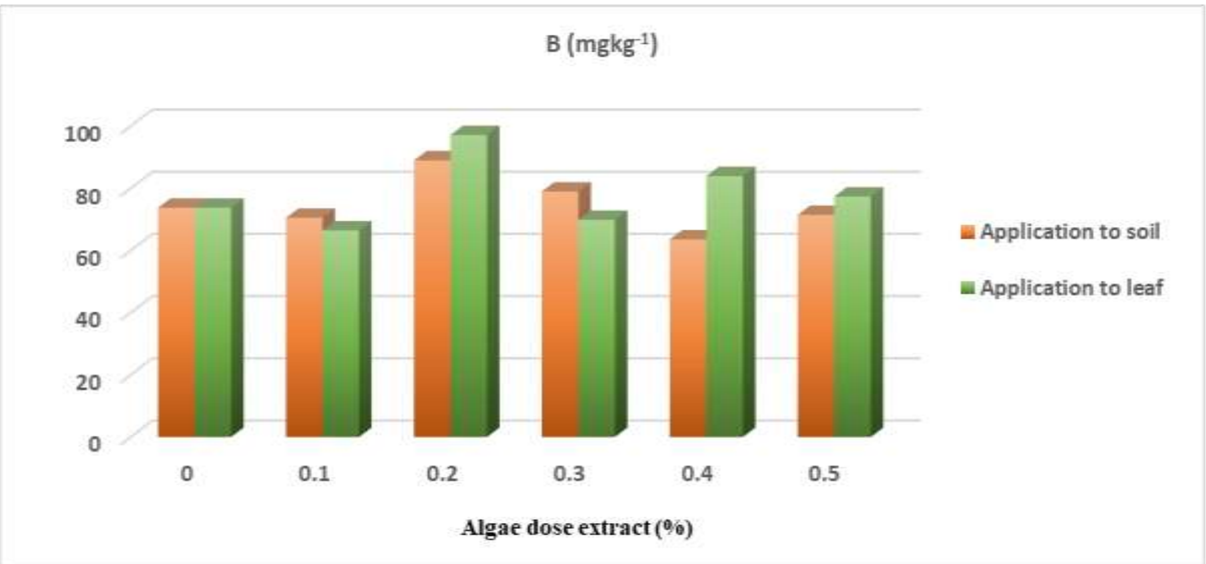

Figure 8. Effects of increasing doses of algae extract applied to soil and leaves on the boron (B) content of alfalfa plants

Treatment (%)	Dose (%)	P	K	Ca	Mg	Fe	Cu	Mn	Zn	B
		%				$mgkg^{-1}$				
Control		0.28±0.01 e	1.49±0.04 f	2.75±0.25 c	0.29±0.009 ab	155.55±18.98 g	3.92±0.01 c	45.72±3.12 de	12.60±1.44 f	73.77±5.40 def
Toprak	**0.1**	0.32±0.005 bc	2.41±0.24 d	2.81±0.48 c	0.23±0.02 d	259.68±1.24 c	5.12±0.42 b	41.93±1.38 fg	17.86±1.04 b	70.51±5.80 fg
	0.2	0.31±0.007 cd	2.78±0.07 c	2.56±0.06 c	0.26±0.01 c	236.37±10.09 d	4.59±0.44 bc	48.28±1.40 cd	15.91±0.95 cde	88.98±4.53 b
	0.3	0.33±0.01 ab	2.95±0.21 c	2.94±0.29 bc	0.22±0.01 de	261.56±3.87 c	4.91±0.06 b	55.82±1.38 b	15.47±1.47 de	78.99±3.09 cd
	0.4	0.34±0.009 a	3.16±0.01 b	2.05±0.17 d	0.19±0.02 e	216.20±12.34 e	4.81±0.77 b	46.01±2.66 de	14.81±0.80 e	63.57±5.37 h
	0.5	0.28±0.01 ef	3.40±0.01 a	2.99±0.006 bc	0.20±0.009 de	295.78±7.72 b	4.83±0.88 b	60.41±0.72 a	12.21±0.73 f	71.45±3.24 efg
Leaf	**0.1**	0.29±0.004 e	1.52±0.20 f	3.28±0.55 ab	0.30±0.02 a	371.75±16.26 a	5.22±0.50 b	38.20±0.65 h	13.13±1.24 f	66.46±3.51 gh
	0.2	0.28±0.01 ef	1.49±0.14 f	3.49±0.39 a	0.30±0.02 a	287.88±24.6 b	8.08±0.48 a	49.80±0.62 c	16.86±1.02 bcd	97.16±3.02 a
	0.3	0.29±0.003 e	1.59±0.04 f	2.53±0.29 c	0.26±0.007 c	180.30±13.49 f	5.15±0.47 b	34.86±2.84 i	21.90±1.24 a	69.85±6.68 fg
	0.4	0.33±0.008 abc	1.57±0.06 f	3.59±0.40 a	0.30±0.008 a	202.58±3.04 e	4.65±0.23 b	43.87±2.73 ef	17.46±0.35 bc	83.93±1.80 b
	0.5	0.30±0.30 de	1.79±0.12 e	2.79±0.09 c	0.27±0.02 bc	181.11±9.30 f	4.55±0.47 bc	40.74±1.10 gh	14.94±1.23 e	77.37±2.31 de
LSD		**0.016 ****	**0.19 ****	**0.46 ****	**0.026 ****	**18.60****	**0.72 ****	**2.77 ****	**1.58 ****	**6.24 ****
CV %		**3.67**	**6.14**	**11.12**	**7.03**	**5.37**	**9.87**	**4.19**	**6.97**	**5.66**

Table 3. Effects of soil and foliar applications of algae extract with increasing doses on some macro- and micro-elements of the alfalfa palnts

CONCLUSIONS

In the study, the effects of increasing doses (0%, 0.1, 0.2, 0.3, 0.4, and 0.5) of soil and foliar application of algae extract (seaweed fertilizer) on some macro and micro elements of Bilensoy-80 clover cultivar plants were found to be statistically significant ($p<0.01$). It was found that both soil and foliar applications of algae extract on some macro and micro elements of the alfalfa plant generally increased the nutrient elements in comparison with the control application. It was found that both soil and foliar applications of algae extracts were effective on the macro- and micro-elements of the alfalfa plant. However, further research with different environmental conditions, different growth substrates, and different plants is needed to confirm these results.

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EFFECTS OF DIFFERENT ORGANIC MATERIALS ON SOME YIELD COMPONENTS AND NUTRIENT CONTENT OF BEAN CROPS

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ABSTRACT

ABSTRACT

The use of organic materials in agriculture plays an important role in recycling plant nutrients and protecting soil health. Organic materials have a positive effect on the physical, chemical, and biological properties of the soil, as well as promoting plant growth and crop yields. For this purpose, the study was carried out under greenhouse conditions according to a randomized experimental design with three replications to determine the effects of different organic materials (sewage sludge, mushroom compost, barnyard and chicken manure) and doses (0, 1.5 and 3 t/de) on the growth and nutrient content of bean plants. To achieve this objective, some growth parameters (fresh and dry weight of the upper part of the plant, fresh and dry weight of the root) and nutrient concentrations in the upper part of the plant (P, K, Ca, Mg, Fe, Zn, Mn, Cu and B) were determined in bean plants. According to the results of the study, the effect of different organic materials and doses on some yield components and nutrient contents of the bean plant was found to be statistically significant ($p<0.01$). In comparison with the control crop, farmyard manure applied at 1.5 t/de was effective on the fresh weight of the upper part of the plant, while farmyard manure applied at 3 t/de was effective on the dry weight of the upper part of the plant. In addition, sewage sludge applied at 3 t/de was found to be effective on the fresh and dry root weights of bean plants. Furthermore, it was found that sewage sludge applied at 3 t/de was effective on the fresh and dry root weights of bean plants. On the other hand, the highest phosphorus, calcium, and magnesium contents of bean plants were found in plants treated with 1.5 t/de sewage sludge. It was observed that farmyard manure application was generally effective on plant microelement content. In general, the highest values of yield components and nutrients were obtained from farmyard manure and sewage sludge applications.

Keywords: Bean, Nutrients, Organic material, Yield components,

INTRODUCTION

The amount of organic matter in the soil is an important factor in maintaining and improving the fertility of the soil for sustainable agricultural production (Önal et al., 2003). Beans, one of the most important edible legumes produced and consumed in developing countries, are a food product rich in carbohydrates and protein content that is produced and consumed in our country in a significant way in terms of human nutrition. This plant, which belongs to the family of legumes, contains high levels of protein, about 22–30% in its grains, and is considered to be a very important nutrient in human nutrition. It is rich in calcium, potassium, magnesium, phosphorus, carbohydrates, and vitamins. It is also one of the most important vegetable sources of protein (Akçin, 1988). As a result of the study conducted to determine the effects of five different animal manures (chicken, sheep, goat, horse, and cattle) on yield and quality in tomato production, it was found that yield, fruit diameter, fruit length, fruit hardness, fruit weight, pH, and vitamin C content were significantly affected by animal manures (Ceylan et al., 2000).

They inhibit the multiplication of viruses. They reduce nematode damage. They increase the effectiveness of pesticides by 25%. They increase marketing and export value (Blunden et al., 1992). Spraying spinach plants with Goemar GA 14, an extract of Ascophyllum nodosum, was found to increase the wet weight of the spinach (Gassan et al., 1992). Root and leaf application of Ascophyllum nodosum extract to tomato plants was found to significantly increase leaf green color. There have also been reports that seaweed extracts increase the amount of chlorophyll in cucumbers (Whapham et al., 1993). As a result of the use of seaweed extracts in world agriculture; it provides better root development and increases the germination and shelf life of fruit and vegetables. Many different effects have been recorded, such as darker colors, larger flowers and leaves, increased resistance to diseases and pests, stress conditions such as frost, drought, and unfavorable soil conditions, increased uptake of nutrients in the soil, and keeping plants young for longer (Hong et al., 1995).

Erdal and Tarakçıoğlu (2000), investigated the effects of organic materials such as tea waste, tobacco powder, hazelnut pulp, and livestock manure on maize plant growth and some nutrient contents. 2 t/de organic materials were mixed into the soil and incubated at field capacity for 15 days. Maize plants were grown for 3 months at the end of the incubation period. At the end of the study, it was found that the dry weight of the plant and the content of N, P, K, Fe, Cu, and Zn in the plant increased to different levels depending on the organic material. Inal et al. (2015) evaluated the effects of processed poultry manure (0, 5, 10 and 20 g/kg) and biochar (0, 2.5, 5, 10 and 20 g/kg) on soil chemical properties and growth of beans (Phaseolus vulgaris) and maize (Zea mays) plants in a calcareous soil. In the incubation study, both processed poultry manure (PPM) and biochar decreased the pH and the concentration of plant-available Fe in the soil, but increased the available P, Cu, Mn and Zn. PPM and biochar increased the concentrations of the exchangeable cations (K, Ca and Mg) in the soil. The application of PPM and biochar increased the growth of maize and beans. PPM and biochar increased the concentrations of N, P, K, Ca, Fe, Zn, Cu and Mn in bean plants. PPM and biochar applications increased N, P, K, Zn, Cu and Mn in maize plants. Çakır and Çimrin (2018) investigated the effects of increasing rates of sewage sludge (0%, 2.5%, 5%, 7.5%, and 10%) on root and seedling development and some plant nutrient contents on the yield of maize plants. They reported statistically significant increases in N, P, K, and Ca and insignificant increases in Mg of experimental soil. The total fresh and dry yield of maize plants increased significantly (P 0.05) in all treatments compared to the control. Üçok et al., 2019, determined the effects of solid vermicompost and chicken manure on the yield, some quality characteristics, and plant nutrient contents of lettuce. The experiment included solid vermicompost, solid poultry manure, chemical fertilizer, and control (K) treatments. According to the results obtained from the study, the highest total yield, the highest marketable yield, and the highest average head weight were obtained from the TG + CG treatment. In terms of the macro-elements analyzed the highest levels of nitrogen were found in the TG + CG, SG + CG, and CG treatments, the highest levels of phosphorus were found in the control treatment, and the highest levels of potassium, calcium, and magnesium were found in the TG treatments. Erdem and Karaaslan, 2023; determined the yield and yield components of some rapeseed varieties using different organic fertilizer applications and chemical fertilizers in their study. In the study, 2 rapeseed varieties (Exstorm, Es Hydromel) were placed in the main plots and 6 fertilizers (solid cattle manure, liquid cattle manure, sheep manure, vermicompost, chicken manure) were placed in the sub-plots as organic fertilizer and chemical fertilizer. The highest values for grain yield and oil yield were obtained with the Exstorm variety and the highest values for grain yield and oil yield were obtained with the application of chemical fertilizers. Chemical fertilizer application was followed by chicken manure application.

This research was carried out to determine the effects of different organic material applications on some yield components and nutrient contents of bean plants.

MATERIAL AND METHOD

This research was carried out under controlled greenhouse conditions (temperature 25±3°C, solar radiation 1750±50 kcal.m^{-2}, and relative humidity 60±10%) at the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Selçuk University. Some of the physical and chemical properties of the soil sample used in the study are given in Table 1.

Table 1. Some physical and chemical properties of the experimental soil

Parameters		**Results**	**Method**
Class of texture		Clay loam	Bouyoucos 1951
pH (1:2.5 s:water)		7.51	Richards 1954
EC (1:5 s:water, µS/cm)		172	U.S. Salinity Lab. Staff 1954
Lime	**%**	37.3	Hızalan and Ünal 1966
O.M.		1.22	Smith and Weldon 1941
N (NH_4-N + NO_3-N)	**mgkg^{-1}**	12.0	Bremner 1965
P (favorable)		10.0	Olsen et al. 1954
K (extractable		162	Soltanpour and Workman 1981
Ca (extractable)		5.84	
Mg (extractable)		242	
Na (extractable)		80	
Fe (available)		3.12	Lindsay and Norvell 1978
Zn (available)		0.68	
Mn (available)		5.82	
Cu (available)		0.67	
B		0.80	Richards 1954

O.M.: Organic matter

Table 2. Some physical and chemical properties of organic materials

Organic Materials		**Barnyard manure**	**Chicken manure**	**Sewage Sludge**	**Mushroom compost**	**Method**
pH		**7.89**	**8.01**	**6.45**	**7.02**	**Anonymous, 1978**
EC (dSm^{-1})		**5.70**	**7.16**	**3.81**	**5.97**	
O.C.	**%**	**26.34**	**29.74**	**25.94**	**35.55**	**Kacar, 1995**
C/N		**17.72**	**14.51**	**31.90**	**16.90**	
N		**1.49**	**2.05**	**0.82**	**2.11**	
P		**0.71**	**2.44**	**0.81**	**0.53**	**Olsen et al. 1954**
K		**2.56**	**2.87**	**1.29**	**2.12**	**Kacar, 1995**
Ca		**3.03**	**9.22**	**4.24**	**2.46**	
Mg		**0.91**	**0.88**	**0.96**	**0.49**	
Fe		**5614**	**1507**	**5219**	**2776**	**Lindsay and Norvell, 1978**
Cu		**25.68**	**63.36**	**87.79**	**19.05**	

Mn	**(mg kg^{-1})**	**411.45**	**404.59**	**375.40**	**191.61**
Zn		**52.62**	**197.34**	**236.56**	**18.48**
B		**20.91**	**18.14**	**39.88**	**19.48**

O.C.: Organic carbon

The study was conducted in 48 pots (3 kg oven-dried soil) with 4 organic materials (barnyard manure, chicken manure, sewage sludge, mushroom compost) x 3 doses (0-1.5-3 t/ha) x 4 replications. After surface sterilization of Sarıkız bean seeds with 0.5% sodium hypochlorite, they were planted in pots containing 3 kg of soil and doses of organic material. Primary fertilizer was applied according to soil nutrient requirements. The beans were harvested after the flowering period, and the required parameters were determined (fresh and dry weight of the plant upper parts, fresh and dry weight of the roots, and nutrient concentrations of the plant upper parts P, K, Ca, Mg, Fe, Zn, Mn, Cu, and B).

The data obtained from the greenhouse experiment, which was designed with 4 replications according to the randomized design, were analyzed by ANOVA using the Minitab 19 statistical program.

RESULTS AND DISCUSSION

Crop yield components

In this study, some yield components and nutrient concentrations of Sarıkız bean plants were determined with increasing doses of different organic materials. According to the data obtained from the study, there were differences in the fresh weight of the upper parts of the plant between the treatments. These differences were found to be statistically significant ($p<0.01$). It was determined that the fresh weight of the upper part of the bean plant varied between 14.59-28.89 g. The highest fresh plant upper weight of 28.89 g was observed with 1.5 t/de farmyard manure application rate. This value was followed by the plants grown at 3 t/de application dose of barnyard manure (Table 3). The lowest fresh weight of the upper part of the plant was obtained in the control treatment. The effects of increasing doses of different organic materials on the dry weight of the upper part of the plant were found to be statistically significant ($p<0.05$) when compared with the control plants. The dry weight of the upper part of the bean plants varied between 3.07 and 4.47 g. The highest effect on the dry weight of the upper part of the bean plant was observed at a dose of 3 t/de barnyard manure applied. This value was followed by an application rate of 3 t/de of chicken manure. According to the data obtained from the study, the effects of the different organic materials and of the application of increasing doses on the fresh weight of the roots of the bean plants were found to be statistically significant ($p<0.01$). The highest root wet weight of 15.14 g was obtained from the plants grown in the 3 t/de application of sewage sludge and the lowest fresh weight of 4.5 g was obtained in the control treatment (Table 3). Higher root wet weights were observed in plants from barnyard manure and sewage sludge applications. The effects of increasing doses of applications on the root dry weight of bean plants were found to be statistically significant ($p<0.01$). It was found that the root dry weight of Sarıkız bean plants varied between 0.49-1.12 g within the treatments. The highest root dry weight of bean plants was obtained from plants treated with 3 t/ha sewage sludge, and the lowest dry weight was found in the control treatment (Table 3). Inal et al. (2015) evaluated the effects of processed poultry manure (0, 5, 10, and 20 g/kg) and biochar (0, 2.5, 5, 10, and 20 g/kg) on soil chemical properties and the growth of beans (Phaseolus vulgaris) and maize (Zea mays) plants in a calcareous soil. PPM and biochar applications increased the growth of maize and bean plants. Çakır and Çimrin (2018) investigated the effects of increasing rates of sewage sludge (0%, 2.5%, 5%, 7.5%, 10%) on the root and seedling development and some plant nutrients of maize plants. The total fresh and dry yield of maize plants increased significantly ($p<0.05$) in all treatments compared to the control ($p<0.05$). Üçok et al. 2019; determined the effects of solid vermicompost and poultry manure on the yield, some quality parameters, and plant nutrient content of lettuce. According to the results of the study, the highest total and marketable yields and average head weights were obtained from the TG + CG treatment. Erdem and Karaaslan (2023) determined the yield and yield components of some rapeseed varieties using different organic and chemical fertilizer applications in their study. The highest values of grain yield and oil yield were obtained from the variety Exstorm, and the highest values of grain yield and oil yield were obtained from the application of chemical fertilizer.

Nutrient content of upper plant parts

The effect of the different organic materials and of the increasing doses applied on the phosphorus content of the upper parts of the bean plants was found to be statistically significant, and the values were found to vary between 0.09 and 0.15% (Table 4). The highest content of phosphorus in the upper part of the bean plants was found in the plants treated with 1.5 t/de of sewage sludge, and the lowest content was found in the control treatment. The highest effect on the potassium content of the bean plant was shown by a 3 t/de application dose of chicken manure. This value was followed by mushroom compost (3 t/de) (Table 2). The effects of increasing organic material doses on the calcium content of bean plants were found to be statistically significant ($p<0.01$). Calcium contents in the upper parts of the plants varied between 3.68 and 5.37%, with the highest value found in plants treated with 1.5 t/dr sewage sludge (Table 4). Magnesium contents were found to be between 0.47-0.58%. The effect of the different organic materials on the content of micro-elements in the bean plants with increasing doses was found to be statistically significant ($p<0.01$). It was found that the iron content of the plant varied between 76.83-179.86 mgkg^{-1} and that the highest effect on iron content was given by barnyard manure applied at a dose of 3 t/de. This effect was followed by applications of 1.5 t/de of sewage sludge, 3 t/de of chicken manure, 1.5 t/de of barnyard manure, and 3 t/de of mushroom compost. The highest copper content of the bean plant was found to be 12.74 mg kg^{-1} in a 1.5 t/ha application of barnyard manure (Table 4). The manganese content varied between 122.49 and 194.08 mg kg^{-1}, and the highest manganese content was found when applying 1.5 t/de of barnyard manure. The highest value was followed by the application of 1.5 t/de poultry manure. The lowest zinc content in the upper part of the bean plant was found in the control treatment. The highest zinc content of 23.07 mg kg^{-1} was observed in the plants that were grown with an application rate of 3 t/de of sewage sludge. It was observed that this value of zinc content in cultivated plants was followed by a dose of 1.5 t/de of sewage sludge (Table 4). The effect of the organic materials applied in increasing doses on the boron content of the plants was found to be statistically significant ($p<0.01$), with the lowest boron content determined in the control application and the highest boron content determined in the 3 t/de doses of chicken manure that were applied. Ceylan et al. (2000), It was reported that N, Ca, Mg, Fe, Zn, Mn, and Cu contents in leaves increased with the application of animal manure as a result of the study conducted to determine the effects of five different animal manures (chicken, sheep, goat, horse, and cattle) on yield and quality in tomato cultivation. In an experiment conducted by Erdal and Tarakçıoğlu (2000) to investigate the effects of organic materials such as tea waste, tobacco powder, hazelnut pulp, and barnyard manure on maize plant growth and some nutrient contents, 2 t/de of organic materials were mixed into the soil and incubated at field capacity for 15 days. At the end of the research, it was found that the dry weight of the plant and the N, P, K, Fe, Cu, and Zn contents of the plant increased to varying levels in relation to the organic material. Inal et al. (2015) reported that both processed poultry manure (PPM) and biochar reduced soil pH and plant-available Fe concentrations, but increased available P, Cu, Mn and Zn.

Table 3. Effect of different organic materials on some yield components of bean plant

Treatment	Dose	PUFW	RFW	PUDW	RDW
	t/de	g/plant			
C	**0**	14.59±0.95 d	4.50±0.77 c	3.07±0.40 b	0.49±0.01 d
BM	**1.5**	28.89±2.41 a	11.77±2.12 ab	4.10±0.97 ab	0.70±0.05 bcd
CHM		25.44±0.68 ab	6.78±1.55 bc	4.03±0.26 ab	0.74±0.12 bcd
SSL		21.46±1.66 bc	5.87±0.44 c	3.99±0.45 ab	0.66±0.03 cd
MC		17.73±1.05 cd	9.29±1.44 bc	3.62±0.06 ab	0.68±0.05 bcd
BM	**3**	27.61±1.66 a	11.81±2.13 ab	4.47±0.22 a	0.95±0.07 ab
CHM		25.33±0.09 ab	8.80±0.98 bc	4.19±0.11 ab	0.82±0.03 bc
SSL		26.96±1.27 a	15.14±1.70 a	4.03±0.05 ab	1.12±0.16 a
MC		19.30±1.07 cd	9.72±2.02 abc	3.79±0.14 ab	0.82±0.03 bc
LSD		2.33**	2.69**	0.69*	0.143**
CV %		5.89	16.87	10.23	10.76

****: p<0.01, *:p<0.05** (O.M.: organic materials, C: Control, BM: Barnyard manure, CHM: Chicken manure, SSL: Sewage sludge, MC: Mushroom compost, PUFW: Plant upper part fresh weight, PUDW: Plant upper part dry weight, RFW: Root fresh weight, RDW: Root dry weight)

Table 4. Effect of different organic materials on some nutrient components of bean plants

O.M.	Dose	P	K	Ca	Mg	Fe	Cu	Mn	Zn	B
	t/de	%				mgkg^{-1}				
C	**0**	0.09±0.009d	1.17±0.046f	4.16±0.20cd	0.55±0.021ab	76.83±10.52e	8.05±0.40c	147.36±10.53bc	12.43±0.39d	15.54±0.26c
BM	**1.5**	0.12±0.01a-d	1.85±0.07c	5.09±0.19ab	0.48±0.031b	130.56±12.55bc	12.74±0.62a	194.08±16.31a	14.10±1.91cd	19.12±0.80a
CHM		0.14±0.02abc	1.86±0.14bc	4.55±0.11abc	0.49±0.02b	88.44±8.96cde	9.91±0.94b	186.85±10.18ab	17.37±0.69b	17.88±0.59abc
SSL		0.15±0.025a	1.42±0.01de	5.37±0.15a	0.58±0.04a	159.32±4.21ab	10.44±0.54b	177.89±7.75ab	20.87±1.05a	16.46±0.75bc
MC		0.11±0.013bcd	1.57±0.051d	4.82±0.68abc	0.54±0.034ab	82.20±5.05de	9.96±0.39b	169.33±37.30ab	15.26±0.91bc	17.44±1.31abc
BM	**3**	0.12±0.009a-d	1.17±0.076c	3.68±0.12d	0.47±0.017b	179.86±34.7a	10.53±0.62b	122.49±9.85c	15.40±0.60bc	18.74±1.29ab
CHM		0.14±0.014ab	2.30±0.043a	4.31±0.36bcd	0.48±0.04b	136.15±19.34b	9.81±0.81b	160.38±6.91abc	16.67±0.16bc	19.63±0.19a
SSL		0.13±0.006abc	1.25±0.085ef	4.77±0.29abc	0.50±0.013ab	86.71±3.95de	9.51±0.45bc	158.42±7.75abc	23.07±0.97a	16.15±1.21c
MC		0.09±0.006cd	2.05±0.02b	5.08±0.17ab	0.47±0.018b	120.76±0.16bcd	9.00±0.51bc	178.67±11.61ab	16.60±0.21bc	16.31±0.64bc
LSD		**0.02****	**0.104****	**0.44****	**0.04****	**21.57****	**0.89****	**23.25****	**13.80****	**1.27****
CV %		**11.35**	**4.22**	**6.55**	**5.5**	**12.61**	**6.15**	**9.64**	**15.22**	**5.02**

****: p<0.01, *:p<0.05** (O.M.: organic materials, C: Control, BM: Barnyard manure, CHM: Chicken manure, SSL: Sewage sludge, MC: Mushroom compos

CONCLUSIONS

In the study, the effects of different organic materials (sewage sludge, mushroom compost, cattle manure, and chicken manure) applied in increasing doses (0, 1.5, and 3 t/ha) to soil with a clay loam texture on some growth parameters of bean plants and nutrient contents in the upper parts were found to be statistically significant ($p<0.01$; $p<0.05$). According to the data obtained, the highest effect on the growth parameters of the bean plant was observed in the farmyard manure and sewage sludge treatments, while the lowest effect was generally observed in the control treatments. On the other hand, it was observed that the sewage sludge and poultry manure treatments were generally effective on the macroelement contents in the upper parts of the plant. In this study, especially the supplementation of organic materials to soils can be used in terms of its positive effects on both plant growth and nutrient contents. However, the properties of the organic materials used should be taken into consideration, especially the pathogenic microorganisms and heavy metal contents of sewage sludge.

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DIFFERENT PHOSPHORUS SOURCES IN BROILERS DIET – UTILIZATION AND ENVIRONMENTAL POLLUTION

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ABSTRACT

The current study was conducted to evaluate the digestibility of phosphorus from monocalcium phosphate (MCP), as an inorganic P source in broilers diet, and to determine the faecal P output. A total of 200 1-day-old (Cobb 500) broilers, both sexes, obtained from the local hatchery, were included in the trial and divided in two groups, by one hundred birds each. During the 42 experimental days, broilers were fed a diets which differed only in MCP origin. Cr_2O_3 was added to the diets at a 0.5% level as an indigestible marker. By 10 birds from each group were placed into individual balance cages, at day 11 and day 25, in order to provide the collection of faeces during 5 consecutive days. Daily phosphorus intake, fecal P output and percentage of apparent tract digestibility of P were measured, in two ages of broilers. Determined differences have shown the influence of source on P utilization ($P<0.05$). It was concluded that the P utilization was not significantly affected by the age of broilers. Tested parameter may be used in estimation of level of P that in this way merged into the environment. Additionally, these are valuable information for optimization of complete feed mixture for broilers regarding P needs, which is one of the most important ways of dealing with phosphorus pollution.

Keywords: Phosphorus, Broilers, Feed, Pollution

INTRODUCTION

Phosphorus (P) play role in great number of metabolic functions, as activator or cofactor of many enzyme systems, it participates in maintaining osmotic and acid-base balance, it is involved in the metabolism of fats, proteins, carbohydrates, but also energy metabolism as an integral element of energy-rich compounds (ATP, ADP, GTP) (Wu et al., 2008; Proszkowiec-Weglarz and Angel, 2013).

P in diet originate from plant sources and mineral sources. Plant P is bound in phytin form, poorly available for absorption (50-60%) (NRC, 1994), which results in a high percentage of its excretion into the environment. Inorganic P, as highly digestible, contribute to adequate P supply, but the amount of P that that exceeds the body's needs is excreted in feces (Dilger et al., 2006; Pavlović et al., 2018). Therefore, it is important to determine the percentage of phosphorus digestibility that indicates the rate of absorbed P from the meal, as well as the rate of its excretion into the environment. These data can be used for optimal diet supplementation, in order to reduce the negative impact of P as a pollutant, by reducing P excretion, but also to reduce feed costs and preserve non-renewable natural P sources. The aim of this trial was to evaluate the P digestibility and rate of P excretion from two different MCP used as inorganic P sources in broilers diet.

MATERIAL AND METHOD

A total of 200 1-day-old (Cobb 500) broilers, both sexes, obtained from the local hatchery, were included in the trial and divided in two groups, by one hundred birds each. During the 42 experimental days, broilers were fed a diets which differed only in MCP origin. Birds were fed a starter diet days from 1 to 21, grover diet days 22 – 35 and finisher diet days 36 – 42 (Table 1). Cr_2O_3 was added to the diets at a 0.5% level as an indigestible marker.

Table 1. Composition of experimental diets

Ingredient [g/kg]	**1 – 21 day**	**22 – 35 day**	**36 – 42 day**
Corn	541.20	604.5	625.5
Soybean meal	230.0	160.00	100.0
Soy grits	180.0	190.00	230.00
MCP	14.30	13.00	13.00
Salt	3.50	3.50	3.50
Limestone	16.00	14.00	13.00
Mineral-vitamin premix	15.00	15.00	15.00
Total	1000	1000	1000

By 10 birds from each group were placed into individual balance cages, at day 11 and day 25, in order to provide the collection of excreta during 5 consecutive days. Individual balance cages (45cm × 50cm × 45cm), were equipped with feeder and drinker on the front side of the cage, and tray under the cage. Measurement of feed consumption and collection of excreta were enabled per bird. Samples were stored at –20 °C, until further analyses.

Daily phosphorus intake, fecal P output and percentage of apparent total tract digestibility of P were measured, in two ages of broilers. Samples of feed and feces were analysed for dry matter (DM), Ca, P and Cr content. Measured concentration of P and DM will be used for determination of P digestibility according to following equations:

Pd = Pi – Pf

Pi = Fi × P diet

Pf = Ex × P excreta

where Pd is daily digested P of each bird (mg/day), Pi is daily intake of P of each bird (mg/day), Pf is daily faecal P output (mg/day), Fi is daily feed intake of each bird (mg/day), P diet is P concentration in feed, Ex is daily faeces weight of each bird and P excreta is P concentration in faeces.

Apparent total tract digestibility and were calculated as follows:

% ATTD = 100 – 100 × (Cr_2O_3feed × P excreta)/(Cr_2O_3feed × P diet)

Data were analysed by using Graph Pad Prism 6.0. software (Graph Pad Software Inc., San Diego, CA, USA). All values are expressed as means and standard error of means. T test was performed to assess the significance of differences among experimental groups. Levels of $P<0.05$ were considered as significant.

RESULTS AND DISCUSSION

No statistically significant difference between the experimental groups were observed in feed consumption and the rate of DM digestibility. Daily P intake was higher and daily faecal P output was lower in O-I experimental group (Table 2). Daily digested P and apparent total tract digestibility were higher in the same group of broilers.

Table 2. Digestibility and fecal phosphorus output at two ages of broilers

	O-I	**O-II**
I period		
Daily feed intake (g)	75,7 ± 1,89	74,3 ± 2,22
ATTD DM (%)	72,95 ± 4,67	70,46 ± 4,52
P intake (mg/day)	598±45,86	595±91,64
Fecal P output (mg/day)	186,3 ± 50,72	205,2 ± 30,23
Digested P (mg/day)	411,7 ± 50,72	389,8 ± 30,23
ATTD of P (%)	68,85 ± 8,48	65,51 ± 5,08
II period		
Daily feed intake (g)	140 ± 3,64	137 ± 3,31
ATTD DM (%)	81,34 ± 3,79	77,03 ± 4,47
P intake (mg/day)	939 ± 27,04	906 ± 31,75
Fecal P output (mg/day)	283 ± 90,79	298 ± 79,87
Digested P (mg/day)	656 ± 90,79	608 ± 79,87
ATTD of P (%)	69,86 ± 9,67	67,11 ± 8,82

Animal manure is used to fertilize agricultural areas in traditional conditions of agricultural production, so it represents a significant source of P emissions into the environment. Lack of detailed calculations on the amount of animal waste and the contained P lead to use a rough estimate of the available data, as an indicator of the potential harmful effect of P excreted in this way. If it is assumed that the biomass of animals remains relatively constant, P amount in animal manure is compatible with the amount of P originating from the consumed feed. Scholz (2013) showed that about 50% of consumed P is lost to the environment. In livestock breeding, the annual P input originating from plant feed is estimated at 3.9 million tons per year. Another P source in diet are mineral additives, for which input is estimated at one million tons per year (Liu et al., 2008). It is considered that about 5 million tons of P is intake by farm animals through feed, and thus subjected to the feed - animal - environment cycle. Rough estimate of the P utilization from the complete feed mixture is about 50%, thus farm animal manure is responsible for 2.5 million tons of phosphorus reaching agricultural land (Liu et al., 2008). However, degree of P utilization varies depending on the animal species, the concentration of total P in feed, the chemical form of P in feed, the concentration of calcium and phosphorus:calcium ratio in diet, the use of the enzyme phytase etc. Therefore, a more accurate assessment of the P amount of released into the environment is necessary, in order to predict its potential harmful effects.

The determined amount of fecal P output in our trial was 186,3 mg/kg and 205,2 mg/kg in I period, and 283 mg/kg and 298 mg/kg in I period, for O- I and O-II group of broiler respectively. Other authors reported different values, from 115 to 315 mg/day excreted P (Rodehutscord et al., 2005), 651 to 1808 mg/kg DM of feces (Dilger et al., 2006), 475 - 591 mg/day (Liu et al., 2012), 205 - 1025 mg/day (Liu et al., 2013), from 0,31 to 2,08g/kg DM (Perryman et al., 2016). Reported results varied along with the experimental conditions, age of broilers, the length of the faeces collection period, concentration and chemical form of P in diet, etc. In addition, obtained results are expressed in a different way, as excreted P per day, per bird, or per kg or DM of feces, which makes the literature data incomparable.

Percent of P digestibility in our trial ranged from 65,5% to 69,9% in two age periods of broilers, which is in line with findings of other authors (Leske and Coon, 2002; Perryman et al., 2016). Liu et al. (2013) determined higher value for P digestibility (from 73,6% to 85,5%), while Shastak et al. (2012) proved significantly lower values (from 54,9% to 56,3%). These differences may be due to different sources of inorganic phosphorus, different concentrations of total P in diet, different meal formulations. The ratio of calcium to phosphorus in feed can be a limiting factor for dietary P absorption. In our trial the Ca:P ratio was not the limiting factor, due to uniform ratio in experimental mixtures (P= 0.584) (Figure 1).

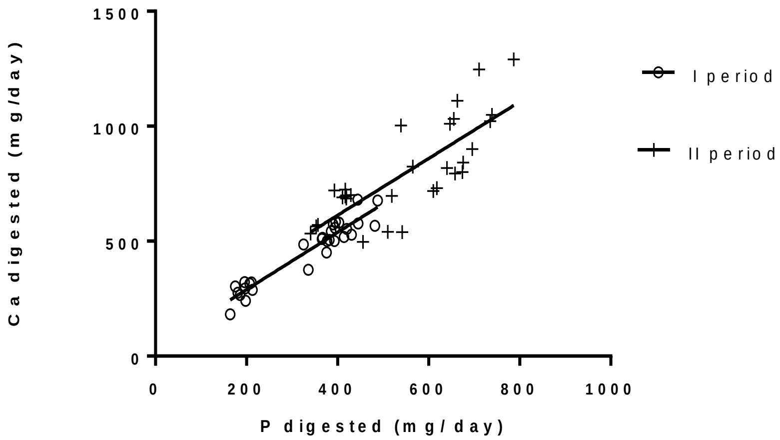

Figure 1. Ratio of absorbed calcium and absorbed phosphorus from broilers diet

Total number of poultry in Serbia in 2017 was 16,338,000, of which 4,981,000 are broilers, while other categories of chickens make up 10,964,000, and the remaining number is made up of all other types of poultry (turkeys, geese, ducks, etc.) (Statistical Office of the Republic of Serbia, 2023). Thus, 27,81% of total poultry number make broilers. In commercial farming, the excreted P is expected to be at the level of here presented results. Adding P in excess, apart from the ecological one, has a financial aspect. P is relatively expensive mineral, sources of which are globally non-renewable.

Unlike some other methods of testing mineral P sources (the degree of mineralization of bone tissue, the concentration of P and alkaline phosphatase in the blood), which provide qualitative data, by determining the degree of digestibility quantitative data are obtained, important for the formulation of diet. Negative effect of P reaching the environment in this way is reflected on the mineral status of the soil, but also by leaching from agricultural land on water. An increased amount of phosphates in natural waters can cause an excessive growth of plankton, algae and aquatic plants, and with it an increased consumption of oxygen and the development of partially anaerobic conditions, which negatively affects other aquatic life (Liu et al., 2008). Numerous researches aimed at develop a strategy to reduce the negative environmental impact of P originating from poultry production. There are two bases for the approach in solving this problem: 1. improving the utilization of P from diet and 2. reducing the P concentration in meal according to needs of animal (Saylor, 2006; Manangi and Coon, 2006). Increasing the P utilization is achieved by supplementing the meal with phytase. Adding exogenous phytase to the diet, will increase the level of phytic P breakdown, and thus reduce the amount of P added from inorganic sources (Naves et al., 2015; Humer et al., 2015). Additionaly, by using nutrients with a lower content of phytic P, the degree of its utilization increases. In addition, by reducing the level of total and digestible P in diet, is avoided its addition in excess. It has been proven that the combination of these measures can reduce the concentration of total phosphorus in poultry manure by 36% (Saylor, 2006).

Livestock production has become a globally significant environmental problem, both in terms of biological and chemical hazards, and in terms of the emission of P as a pollutant. World production has changed, in a way that most of the production take part in a certain number of countries, which are the biggest producers (Amanna et al., 2018). Therefore, today in certain regions poultry production is constantly increasing, such as USA and China. This leads to changes in the structure of producers and production technology, and large capacity farms become dominant. Their production is characterized by high farming technology, high levels of productivity, and large production capacities. Consequently, a large number of poultry become concentrated, in certain regions, along with the amount of manure and P (Arata et al., 2022). In China, 36% of total P emission into the environment originate from farms (Liu et al., 2008). Adequate assessment of the P amount thus reaching the environment is the start point for the implementation of measures to reduce these concentrations.

CONCLUSIONS

In order to preserve natural P resources, as well as to reduce its harmful effects on the environment, the addition of P in excess must be prevented, by an adequate formulation of broilers diet, and by adding the enzyme phytase, which will increase the level of breakdown and absorption of phytic P. In that purpose, it is necessary to know the digestibility percentages of P from commercially available feeds, in order to be able to correct the ration according to the existing problem. In addition to the ecological aspect, a financial aspect of this approach, will allow reducing the addition of inorganic P to the complete feed mixture, which is important to producers.

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DETERMINATION OF IRRIGATION SCHEDULING AND CROP WATER CONSUMPTION OF PUMPKIN BY USING CROPWAT PROGRAMME IN NEVŞEHIR PROVINCE

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ABSRACT

Approximately 35% of the cultivation of pumpkin is met from Nevşehir in Turkey. The widespread cultivation of pumpkin also reveals the necessity of irrigation scheduling in Nevşehir. On the other hand, the increase in population, the decrease in water resources day by day, and the large volume of water used in agricultural production, which is necessary for food supply, make it necessary to carry out irrigation scheduling in the cultivation of all plants. The aim of this study is to determine the plant water consumption and irrigation scheduling of the pumpkin cultivation by using Cropwat scheduling in Nevşehir. In this study, the irrigation amount and crop water consumption were determined for 5, 7, 14 and 21 days irrigation intervals of the pumpkin cultivation according to the climatic characteristics of Nevşehir with Cropwat scheduling. The irrigation amounts were 396 mm, 379.6 mm, 263.4 mm and 218.2 mm for 5, 7, 14 and 21 days, respectively. Crop water consumption were 464.9 mm, 446.2 mm, 355.4 mm and 287.6 mm when 5, 7, 14 and 21 days were selected, respectively. When the results obtained were compared with the other results in the literature, it was understood that the highest yield was obtained from the cultivation of pumpkin with 7 days irrigation interval. An average yield of 100 kg da^{-1} is obtained in irrigated pumpkin cultivation in Nevşehir. According to the data obtained from the results, it was suggested that irrigation every 21 days would be appropriate for producers with limited irrigation opportunities, and in other cases, irrigation at 7-day irrigation intervals was recommended.

Keywords: Pumpkin, Irrigation, Cropwat, Nevşehir

INTRODUCTION

Determining the irrigation water consumption in crop production is a very important issue in terms of making the irrigation schedule. It is essential to know the crop water consumption in order to determine the irrigation water requirement and to plan the irrigation systems. Crop water consumption is used synonymously with evapotranspiration. Evapotranspiration is the total amount of water given to the atmosphere by evaporation from the soil surface and transpiration from plant leaves. It is usually expressed as depth (mm). In practice, it is very difficult to measure and evaluate transpiration and evaporation separately. In reality, there is no need for this in terms of irrigation (Güngör and Yıldırım, 1989; Kaya and Özdengiz, 2013). Irrigation scheduling is a concept that is defined as the determination of the irrigation time and the amount of water to be applied and that ensures the optimization of water use (Baştuğ, 1994; Kaya and Özdengiz, 2013).

Irrigation scheduling and estimation of crop water consumption are of great importance in the assessment of water stress and effective management of water resources. In the calculations of crop water consumption, it can be calculated by using soil properties, climate parameters and crop characteristics. In the world and in our country, fast and accurate calculations can be made using computer models (Kartal et al., 2019).

Various models have been developed for the determination of crop water consumption. One of them is the CROPWAT software developed by FAO, which uses climate parameters, soil and plant characteristics. CROPWAT, developed by Smith (1992), is frequently used by researchers in order to estimate actual and comparison crop water consumption using climatic data (Surendran et al., 2015; Yahaya et al., 2015; Karaca t al. 2017). It may not be possible to obtain climatic data such as temperature, precipitation, humidity, sunshine duration, wind speed and the ETo value calculated using these data for every point in the geographical area. However, with CROPWAT, inferences can be made about the entire geographical region by taking measurements from certain places in the study area (Karaca t al. 2017).

Cucurbitaceae (Cucurbitaceae) family includes a large number of cultivated crops with high economic value, and the cultivation of plants in this family is widely carried out in many regions of our country due to the favorable ecological conditions in our country. The most cultivated species in the genus Cucurbita L. (Cucurbitaceae) are Cucurbita pepo L. (Squash), C. moschata Duch. (Pumpkin) and C. maxima Duch. (Chestnut squash). The seeds of these pumpkin species are used as snacks. C. pepo is the type of zucchini for snacks most cultivated by farmers. It is thought that the agricultural production of C. pepo, whose homeland is Mexico and Central America, entered our country from the Thrace region and became widespread (Yanmaz and Düzeltir, 2003; Ülkücü et al., 2022).

It is thought that the planting areas have increased due to the short vegetation period of the pumpkin, its ability to be grown with rain water without irrigation, higher yields when irrigated, and its high economic value. Pumpkin seeds are grown mostly in the Central Anatolia Region. In the Central Anatolia Region, Nevşehir takes the first place in production, which accounts for 35% of Turkey's confectionery pumpkin production. This is followed by Kayseri with 29% and Konya with 11% (TUIK, 2020; Ülkücü et al., 2022).

The aim of this study is to predict the comparative plant water consumption (ETo), which is the main data for determining the crop water consumption of the pumpkin with high agricultural production potential in Nevşehir province, by using the FAO Penman-Monteith method by using long-term climate data and to create the irrigation program of the pumpkin.

MATERIAL AND METHOD

Nevşehir is located in the Central Anatolia Region between 38° 12' and 39° 20' north latitudes and 34° 11' and 35° 06' east longitudes. Except for the Derinkuyu district, which is in the closed basin of Konya, the province, which completely falls into the Middle Kızılırmak

Basin, is in the middle of Turkey in terms of its location, and its surface area is 5,392 km². It occupies 7 per thousand of the country's territory. In Nevşehir, continental climate is dominant. Summers are hot and dry, winters are harsh and cold. Precipitation falls mostly in spring and autumn. The climatic features of the Kızılırmak Valley become more severe. Since Nevşehir province is located in the central Anatolian Region, which is closed to sea effects due to the Northern and Southern Anatolian mountain systems, precipitation is low. However, precipitation is more abundant in the Kızılırmak Valley and the slopes of this valley. Since the winters in the province are very harsh, precipitation usually falls in the form of snow. The average annual precipitation in the Merkez District is 388.7 mm. This average decreases to 353.2 mm in Derinkuyu District, which enters the Konya Closed Basin of Nevşehir. The rainiest months in the Merkez District are December and March, and the least rainy months are July and August (Anonymous, 2023).

In the study, CROPWAT software developed by FAO was used to calculate ETo values. The aforementioned software is a computer program used to determine the amount of water to be given to the plant by using local climate data (such as precipitation, evaporation, wind, temperature, sun exposure) and plant data. The FAO Penman-Monteith method given in Equation 1 and Equation 2 is based on the calculation of crop water consumption in the program.

$$ET_O = (0,480\Delta(Rn-G)+\gamma\ (900/T+273)U2(ea-ed))/(\Delta+\gamma(1+0.34U2)) \qquad \text{Equation 1}$$

Eto: Crop Water Consumption, Mm/D; Rn: Net Radiation, MJ/(M^2D); G: Soil Heat Flux, MJ/(M^2D); T: Average Air Temperature, ^oC, U2; Wind Speed at 2 m Elevation, M/S; (Ea−Ed): Vapor Pressure Gap, Kpa; Δ: Slope of Vapor Pressure Curve, Kpa/^oC; Γ: Psychometric Coefficient, Kpa/^oC

$$ETc = KcXET_0 \qquad \text{Equation 2}$$

Etc: Crop water consumption, Kc: Crop coefficient; ET_0: Reference crop water consumption.

Crop water consumption and irrigation intervals were tested at intervals of 5, 7, 14 and 21 days in CROPWAT software. CROPWAT is started by selecting the location of the region to be studied first, after the Nevşehir province is selected in the CROPWAT software, the climate data of the region (maximum and minimum temperature, humidity, wind speed and solar radiation) are entered. Figure 1 shows the climate data entered into the CROPWAT software.

Monthly ETo Penman-Monteith - C:\Users\Nevu\Desktop\nevşehir_cropwat\NEVSEHIR_iklim....

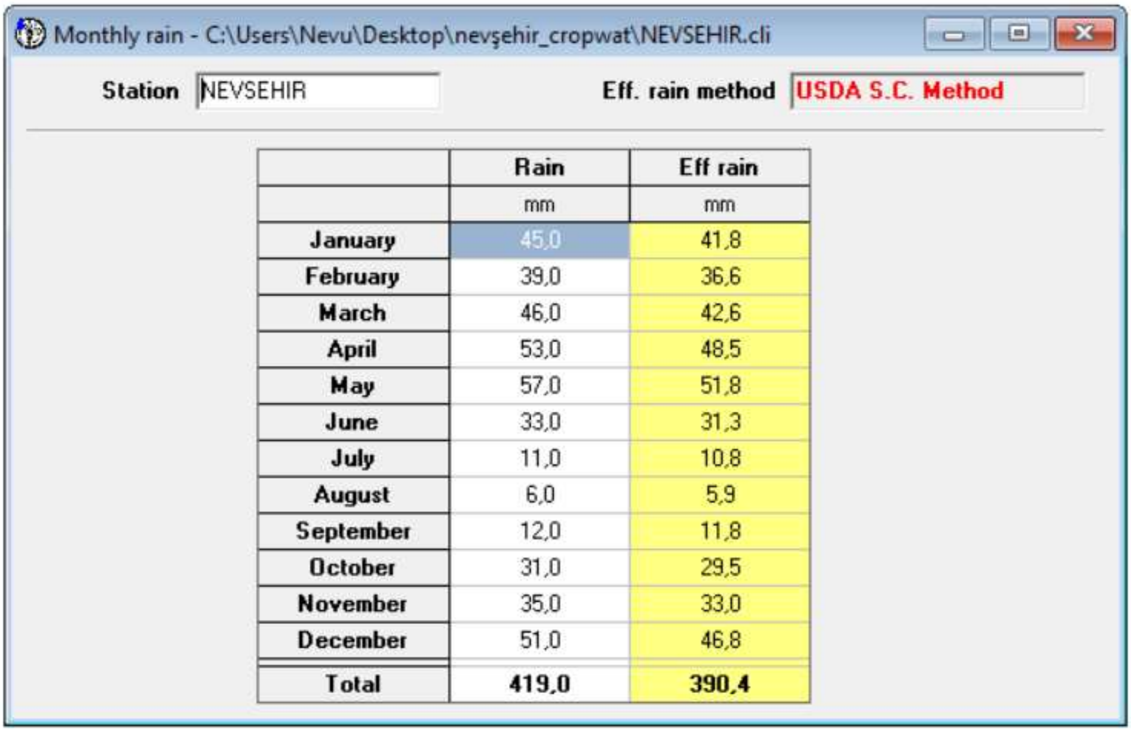
Country: Nevşehir   Station: NEVSEHIR

Altitude: 1260 m.   Latitude: 38,61 °N   Longitude: 34,71 °E

Month	Min Temp	Max Temp	Humidity	Wind	Sun	Rad	ETo
	°C	°C	%	km/day	hours	MJ/m²/day	mm/day
January	-4,3	3,2	78	285	4,4	7,7	0,86
February	-3,1	5,0	70	233	4,3	9,6	1,22
March	-1,0	9,6	61	233	5,1	13,1	2,01
April	3,3	15,3	52	225	6,5	17,5	3,22
May	6,9	19,8	54	173	7,8	21,0	3,96
June	9,8	24,0	49	156	9,9	24,5	4,91
July	11,4	27,6	43	164	12,5	27,8	5,90
August	11,1	27,7	40	156	12,1	25,6	5,51
September	8,3	23,9	43	130	10,1	20,0	3,98
October	5,2	17,7	53	156	7,6	13,8	2,59
November	1,9	11,6	66	164	5,6	9,1	1,47
December	-1,7	5,7	77	233	4,1	6,9	0,93
Average	**4,0**	**15,9**	**57**	**192**	**7,5**	**16,4**	**3,05**

Figure 1. Climate data for Nevşehir province in CROPWAT

Rain data of Nevşehir province is added. The CROPWAT image of Nevşehir rain data is given in Figure 2.



	Rain	Eff rain
	mm	mm
January	45,0	41,8
February	39,0	36,6
March	46,0	42,6
April	53,0	48,5
May	57,0	51,8
June	33,0	31,3
July	11,0	10,8
August	6,0	5,9
September	12,0	11,8
October	31,0	29,5
November	35,0	33,0
December	51,0	46,8
Total	419,0	390,4

Figure 2. Rain data for Nevşehir province in CROPWAT

Soil properties of Nevşehir province were selected as "Medium (loam)" in CROPWAT software. In Figure 3, a visual of the soil properties is given.

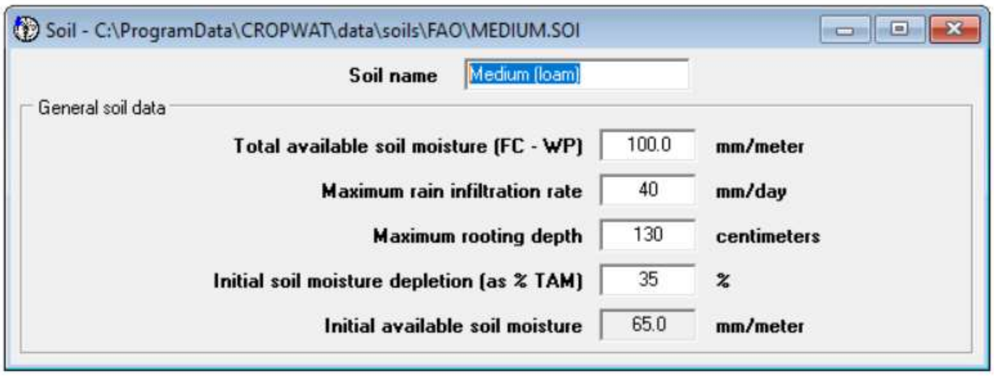


Figure 3. Soil properties for Nevşehir province in CROPWAT

The pumpkin plant coefficient values for Nevşehir province were entered into the program as 0.50, 1 and 0.65 for the beginning, middle and last periods, respectively (FAO, 2023). The initial, development, middle and last period lengths of the pumpkin were determined as 20, 35, 35 and 20 days, respectively (Yavuz et al., 2015). Vegetative data of the pumpkin are given in Figure 4.

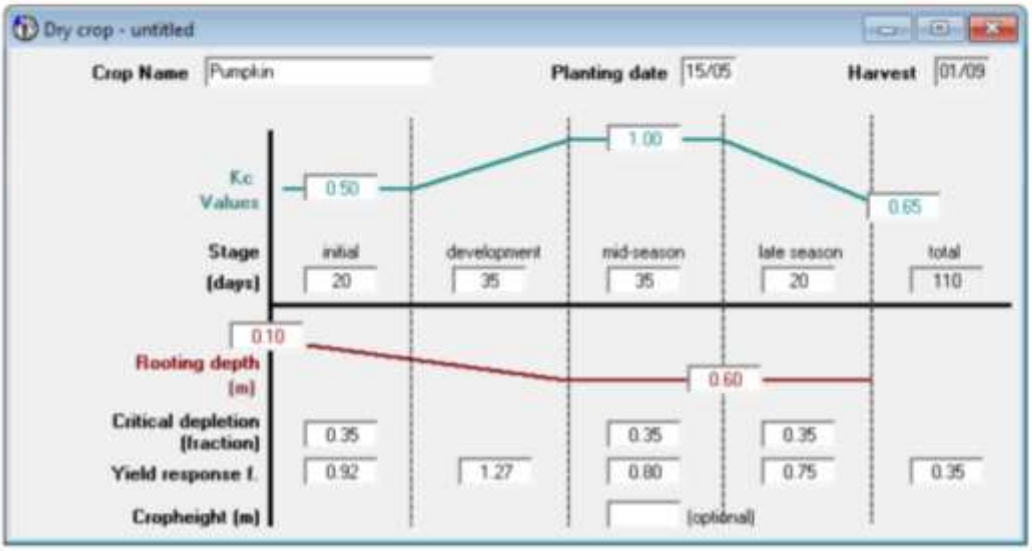


Figure 4. Vegetative data for pumpkin in CROPWAT

RESULTS AND DISCUSSION

In the CROPWAT software, the irrigation amount and crop water consumption of pumpkin were determined at the irrigation intervals of 5 days, 7 days, 14 days and 21 days. As a result, the amount of irrigation water that should be given to the pumpkin was 396 mm, 379.6 mm, 263.4 mm and 218.2 mm at intervals of 5 days, 7 days, 14 days and 21 days, respectively. According to the amount of irrigation obtained, the crop water consumption values were 464.9 mm, 446.2 mm, 355.4 mm and 287.6 mm at intervals of 5 days, 7 days, 14 days and 21 days, respectively. Irrigation data obtained at 5 days, 7 days, 14 days and 21 days irrigation intervals by using the Nevşehir province climate data of the pump are given in Figures 5, 6, 7 and 8, respectively.

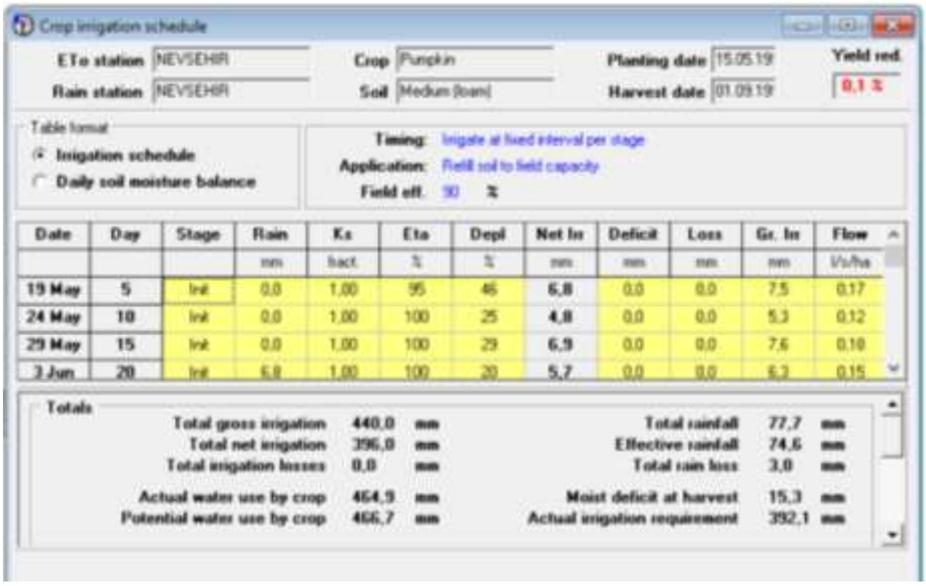


Figure 5. CROPWAT results based on 5 days irrigation interval in the pumpkin

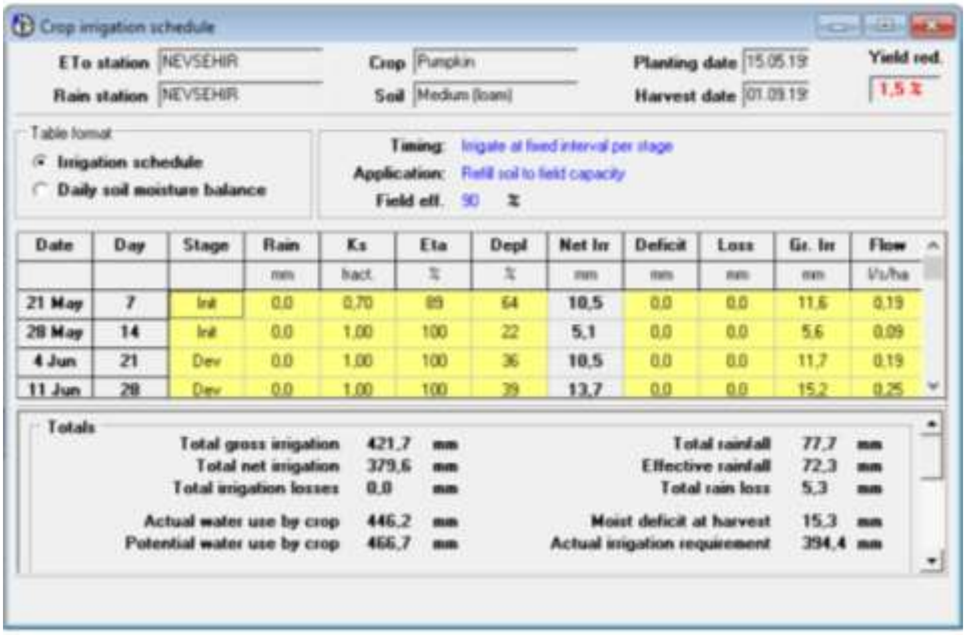


Figure 6. CROPWAT results based on 7 days irrigation interval in the pumpkin

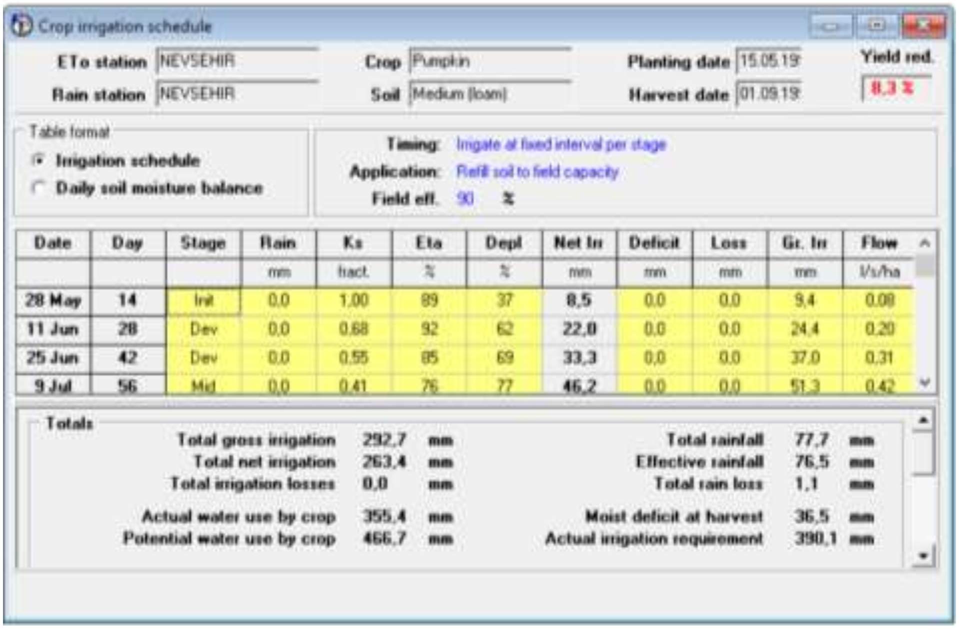


Figure 7. CROPWAT results based on 14 days irrigation interval in the pumpkin

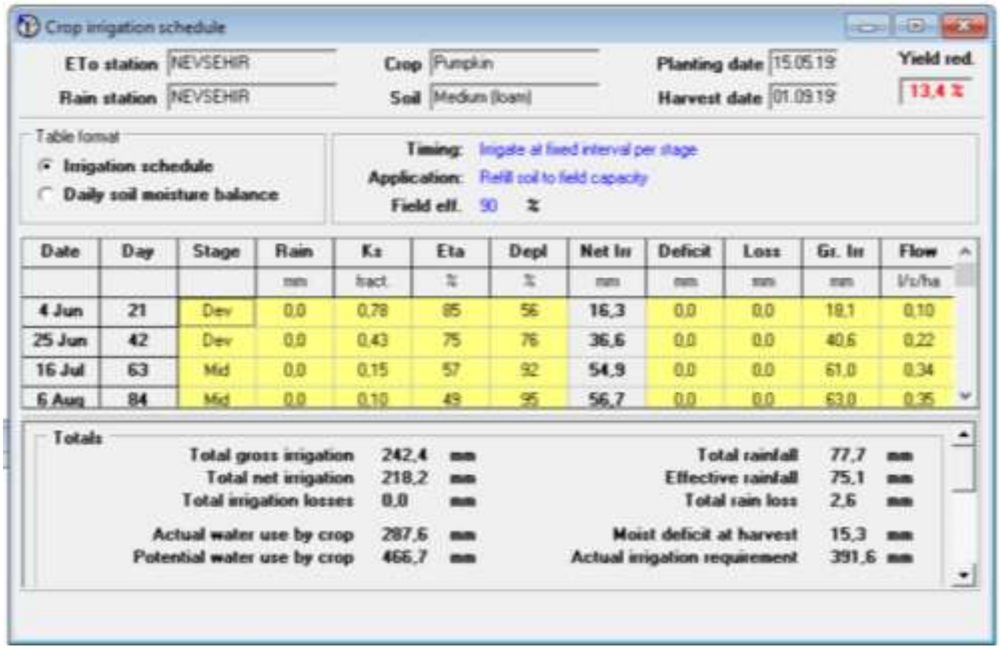


Figure 8. CROPWAT results based on 21days irrigation interval in the pumpkin

Average yield value in dry and wet pumpkin cultivation in Nevşehir is 100 kg da^{-1}. However, Ghanbari et al. (2007) obtained 170.1, 83 and 48.1 kg da^{-1} seed yield from pumpkinfor snacks during 7, 14 and 21 days of irrigation. When the results were evaluated, it was seen that the yield increased as the irrigation interval was shortened. It is thought that the yield will increase in the 5 day irrigation interval. Based on the results mentioned above, it has been understood that crop water consumption and irrigation program can be made with the CROPWAT software. In addition, many researchers emphasized that it is easy to use CROPWAT software to determine irrigation interval and crop water consumption (Dong, 2018; Khaydar et al. 2021; Gabr, 2022). The use of programs such as CROPWAT, which can easily calculate irrigation schedules and crop water consumption, in conditions where the crop water status cannot be physically monitored, can provide convenience to many researchers and producers. The CROPWAT program is recommended in order to make the necessary irrigation requirements in crop production in a healthy way.

CONCLUSIONS

As a result of this study, it was estimated that the pumpkin irrigated with a 5 day irrigation interval would give the highest yield. With the CROPWAT program, it has been understood that the irrigation interval, irrigation schedule and crop water consumption of other crops can be determined as in the pumpkin. In this study, it was emphasized that the data on soil, plant and climate information used in the CROPWAT program should be taken in a way that best represents the region to be studied.

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POMOLOGICAL PROPERTIES OF SOME CORNELIAN CHERRY (*CORNUS MAS* L.) GENOTYPES

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ABSTRACT

Cornelian cherry (*Cornus mas* L.), a fruit-bearing shrub or small tree, has been traditionally appreciated for its culinary uses and medicinal properties. In the present study, phenotypic variation of 10 seedlings originated accessions of cornelian cherry was investigated to select the superior trees. The highest values in terms of pomological characteristics; fruit width 17,22 mm, fruit length 23,28 mm, seed width 6.84 mm , seed length 14.92 mm , fruit weight 5,28 g, fruit flesh weight 4.81 g, seed weight 0.59 g genotypes were determined to have. SSC is between 8.75% and 18.66%, titratable acidity is between 1.74% and 3.82%, pH 2.96 and 3.48.

Keywords: Breeding, Fruit quality, Cornelian cherry, Physical properties

INTRODUCTION

Cranberry (*Cornus mas* L.) belongs to the order Cornales, family Cornaceae, genus Cornus. There are over 65 species in the genus Cornus, the majority of which are shrubs or tiny trees that can be found in temperate, tropical, and subtropical climates. Cranberry is common in the mountains of southern and central Europe, southwestern Asia, eastern and western of North America and Central America, South America and eastern Africa (Dinda et al., 2016).

Cornelian fruits are used to make food, syrup, and jam, and have recently been fermented to make a low alcoholic functional beverage with excellent nutritional content (Mantzourani et al., 2019; Nouska et al., 2016). It has mostly been used historically to treat gastrointestinal health issues (Deng et al., 2013). Chinese herbal medicine has used cornelian cherry fruits for their tonic, analgesic, and diuretic properties. In Europe, fruit extract of cornelian cherry is also used for cosmetic purposes in place of synthetic astringents, and it is claimed that this has a positive effect on skin tone (Sara et al., 2008).

Red and black fruit consumption is crucial for maintaining good health and preventing diseases including cancer, heart disease, cancer, and illnesses associated with aging (Terry et al., 2001). Red and black fruits are high in natural antioxidants, which defend against dangerous free radicals and are thus connected with lower cancer and heart disease incidence and mortality rates, among other health benefits (Shui and Leong, 2006).

The goal of this research was to identify the pomological features of ten Cornelian cherry (*Cornus mas* L.) genotypes from Turkey and nominate the most beneficial for preservation, future research, and breeding.

MATERIAL AND METHOD

Study area

Aksu Village (Kestel/Bursa) is included in the Marmara Region. The altitude is 470 meters. The region has a mild continental climate. Due to its distance from the sea, it has drier air compared to other districts.

Fruit material

Fruits of ten different native Cornelian cherry (*Cornus mas* L.) genotypes were harvested in around Aksu village of Bursa city, Turkey's Marmara Region. The trees were naturally developed and were close to being thirty years old. The codes AKS01 to AKS10 were assigned to each genotype discovered. The harvesting took place in 2021 and 2022, when the fruits of the genotypes under investigation had achieved commercial maturity. Three replicates were employed, with each repeat including 30 uniform cornelian cherry genotype fruits. The fruits collected from the genotypes were then taken to the lab for testing.

Pomological features

The weight of fruit, fruit flesh and seed of per genotypes were determined using a 0.01g-sensitive weighing. The measurements of both the length and width (diameter) of fruit and seed were made using a 0.01 mm-sensitive digital compass.

Soluble solids content, pH, and titratable acidity

At room temperature, the total soluble solids content (TSS) was evaluated using a digital refractometer (Model HI-96801 Hanna, Germany). A Hanna-HI 98103 pH meter was used to measure the pH value, which was calibrated using pH 4.0 and 7.0 buffer solutions. Titratable acidity was determined by titrating the sample with 0.1 NaOH until the pH reached 8.01, and the result was stated as percent citric acid equivalent.

RESULTS AND DISCUSSION

Table 1 shows the results of pomological features. The highest values in terms of pomological characteristics; fruit width 17,22 mm, fruit length 23,28 mm, seed width 6.84 mm , seed length 14.92 mm , fruit weight 5,28 g, fruit flesh weight 4.81 g, seed weight 0.59 g genotypes were determined to have.

Table 1. Pomological features of genotypes

Genotypes	Fruit Width	Fruit Length	Fruit Weight	Seed Width	Seed Length	Seed Weight	Fruit Flesh Weight
AKS01	16,7 ± 0,15	22,58 ± 0,22	5,12 ± 0,14	6,25 ± 0,16	14,47 ± 0,22	0,57 ± 0,04	4,81 ± 0,12
AKS02	15,87 ± 0,17	21,45 ± 0,24	4,87 ± 0,24	6,3 ± 0,24	13,75 ± 0,42	0,54 ± 0,02	4,43 ± 0,15
AKS03	17,22 ± 0,18	23,28 ± 0,16	5,18 ± 0,2	6,12 ± 0,22	12,41 ± 0,28	0,59 ± 0,06	4,61 ± 0,13
AKS04	8,15 ± 0,14	11,17 ± 0,18	2,53 ± 0,18	3,28 ± 0,42	7,94 ± 0,18	0,28 ± 0,01	2,31 ± 0,18
AKS05	4,85 ± 0,12	13,04 ± 0,34	5,28 ± 0,12	3,42 ± 0,28	8,48 ± 0,29	0,33 ± 0,08	2,69 ± 0,12
AKS06	5,63 ± 0,82	12,4 ± 0,58	2,81 ± 0,23	6,84 ± 0,23	14,92 ± 0,27	0,31 ± 0,04	2,56 ± 0,14
AKS07	5,04 ± 0,52	22,28 ± 0,62	5,05 ± 0,32	6,55 ± 0,14	14,28 ± 0,12	0,56 ± 0,06	4,6 ± 0,02
AKS08	14,98 ± 0,34	20,25 ± 0,31	4,59 ± 0,64	5,95 ± 0,18	12,98 ± 0,25	0,51 ± 0,01	4,18 ± 0,08
AKS09	7,18 ± 0,13	19,09 ± 0,17	4,33 ± 0,16	5,61 ± 0,29	12,23 ± 0,14	0,48 ± 0,03	3,94 ± 0,24
AKS10	11,54 ± 0,15	15,6 ± 0,33	3,54 ± 0,27	4,58 ± 0,27	10,12 ± 0,53	0,40 ± 0,04	3,22 ± 0,22

Table 2. shows the results of SSC, TA and Ph values of Cornelian cherry genotypes. SSC is between 8.75% and 18.66%, TA is between 1.74% and 3.82%, pH 2.96 and 3.48.

Table 2. Soluble solids content, pH, and titratable acidity features of genotypes

Genotypes	SSC	TA	pH
AKS01	12,85 ± 0,15	3,71 ± 0,05	3,38 ± 0,17
AKS02	11,73 ± 0,24	3,52 ± 0,08	3,21 ± 0,07
AKS03	18.66 ± 0,23	3,82 ± 0,13	3,26 ± 0,13
AKS04	9,15 ± 0,3	2,92 ± 0,12	3,48 ± 0,12
AKS05	8,89 ± 0,13	1,74 ± 0,37	3,12 ± 0,06
AKS06	13,27 ± 0,28	2,02 ± 0,26	3,43 ± 0,09
AKS07	15,34 ± 0,16	1,81 ± 0,09	3,08 ± 0,04
AKS08	8,75 ± 0,16	3,32 ± 0,08	3,03 ± 0,13
AKS09	16,77 ± 0,18	2,58 ± 0,29	3,38 ± 0,08
AKS10	14,28 ± 0,28	2,56 ± 0,14	2,96 ± 0,12

Previous studies have shown varying results with regards to the length and width of cornelian cherry fruit. The pomological characteristics of the fruit are predominantly affected by environmental factors and genotype, as demonstrated by Güleryüz et al. (1998) and Demir and Kalyoncu (2003). Our findings are consistent with prior research. According to Erzincan's study, fruit weight ranged between 1.44 and 4.24 grams, with most fruit sizes falling between 9.6-15.8 mm and 14.1-22.8 mm (Selçuk and Özrenk, 2011). Additionally, Oblak (1980) reported an average fruit weight of 1.78 grams for a population of wild fruit in Slovenia. Studies were conducted in Bolu, Zonguldak, Karabük and Bartin between 1996-1998 to determine the best cornelian cherry in both natural and cultivated populations. These studies revealed that average fruit weight ranged from 1.02 to 4.07 grams (Yalcinkaya and Eti, 1999). In a separate study, ten different types of naturally grown cornelian cherry in the Derebucak district of Konya were found to weigh between 3.65 – 4.57 g (Turkoglu et al., 1999). In a separate study carried out in Konya, it was discovered that the weight range of the selected fruit was between 1.496 g and 4.116 g (Demir and Kalyoncu, 2003). Another study conducted by Tural and Koca (2008) in Samsun disclosed that the weight of the naturally grown fruit was between 0.39 g and 1.03 g, with the length of the fruit measuring between 14.24 mm and 22.20 mm. Furthermore, a majority of the fruit measured between 9.59 mm and 13.21 mm in length.

Selcuk and Özrenk's (2011) study on cornelian cherry found that the water soluble dry matter content ranged from 9.0% to 17.7% at a pH of 2.9-5.7. Additionally, a population study in Slovenia showed that naturally grown cornelian cherries had an average TSS of 20.6%, total sugar of 7.42%, and a pH of 3.38 (Oblak, 1980). Another study conducted in Trabzon reported a range of 8% to 13.5% for the total dry matter (Karadeniz, 2001). In a separate study carried out in Zonguldak, the concentration of soluble solids was recorded to be in the range of 12.1 to 16.9%. Tural and Koca (2008) determined the concentration of soluble solids in cornelian cherry using chemical analysis and found it to be in the range of 28.19% to 15.88%. The same researchers also reported the total acidity to be between 1.10 and 2.53%. Similar studies (Demir and Kalyoncu 2003; Yılmaz et al., 2009) found total acidity between 4.69% and 1.24%. The studies show that the chemical compounds present in the samples are consistent, despite ecological differences influenced by environmental and maintenance requirements (Gerçekçioğlu, 1998).

CONCLUSION

Our research findings are consistent with those of other genotypes grown both domestically and internationally. However, the use of alternative cultivation technologies is expected to yield superior results. Additional information regarding the genotype's value can be derived from characteristics such as phenolic compounds, antioxidant capacity, and pollen biology. Conducting further research of this nature will provide valuable insight and identify genetic materials with promising attributes.

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PEPPER SEEDLING DEVELOPMENT AFTER APPLICATION OF LEAF FERTILIZER PROTIFERT LN 6.5

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ABSTRACT

The main goal of the present study was to establish the effect of different ways for the application of the leaf fertilizer Protifert LN 6.5 on the development of pepper seedlings. The experiments were carried out in the Experimental field of the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria, with pricked-out seedlings of two pepper varieties Kurtovska kapia 1619 and Bulgarski rotund. In the stage of four leaves the leaf fertilizer Protifert LN 6.5 was used in two ways- as foliar and as a growing media application in three concentrations-1.0%, 2.0%, and 3.0%. Twenty days after application the morphological development was established. Following parameters: weight and volume of roots, weight, high, and diameter of the stem, as well as weight, number, and area of leaves, were established. Dry vegetative weight, number of flower buds, and content of total chlorophyll were determined. The ratio between individual organs to the formation of total vegetative weight and Index of development were calculated. A strong positive correlation between high and weight of the stem as well as between the number and area and weight of leaves were established. Polynomial regression between concentration and total vegetative weight with high determination coefficients R^2=0.64 and R^2=0.9 was determined. The best development of the pepper seedlings was observed in the foliar treatment of 2.0% Protifert LN 6.5, which can recommend for practice application.

Keywords: morphology, vegetation, fertilization, leaf area, vegetative ratio, amino acids

INTRODUCTION

In vegetable production, seedling cultivation is a major agrotechnological practice with high economic value and has been set up as a special independent industry (Dufault, 1998). The development of seedlings is an extremely important point for the successful and sustainable cultivation of vegetable crops (Demir et al., 2010; Shopova et al., 2014). The adaptation and survival of vegetable crops under field conditions, as well as the economic efficiency of production, largely depend on their quality. In this sense, the status and initial growth and development of pepper seedlings are essential for future plant productivity (Trejo-Téllez et al., 2020). De Grazia et al. (2004) also emphasize that, from an agronomic point of view, the quality of pepper seedlings is particularly determined by their initial and uniform development. At the same time, Resh (2012) and García-Jiménez et al. (2017) add that pepper plants from well-developed seedlings have much stronger and faster growth in the field, they develop much better, and this determines the lower investment in plant cultivation.

Improving the quality of pepper seedlings can be successfully implemented by applying various stimulants and especially using the foliar application of fertilizers (Souri and Sooraki, 2019). By providing a suitable nutritional regime, better development of pepper seedlings is achieved, and their growth can be controlled by precising the concentrations and ratios of applied nutrients (De Grazia et al, 2008; Marschner, 2012). Improving the development of pepper plants under the influence of foliar fertilizers was also reported by Massimi et al. (2023) as well as Haytova (2013). In this regard, very good results are obtained by using foliar fertilizers with amino acid content. Al-Said and Kamal (2008) emphasize that amino acids are the fundamental ingredients in the process of plant synthesis and directly or indirectly affect the physiological activity and growth of plants. Yaghoub et al. (2019) add that the exogenous application of preparations based on amino acids supports and stimulates plant development. As a result of amino acids being major initiators in the biological synthesis of proteins, they induce cell growth stimulation (Rai et al., 2002; AL-Nassrallah and AL-Asadi, 2023). Significantly better growth and productivity of pepper were found after the use of some amino acids (Al-Zayadi, 2021).

The foliar fertilizer Profitert LN 6., which has a high content of amino acids, over 40%, has a strong effect on plant development. Several authors have found a stimulating effect on some agricultural crops when applying preparations from the Protifert group (Amara et al, 2016; Laita et al, 2022). A particularly strong impact, both on vegetative growth and productivity, was observed by the action of Protifert on pepper grown under field conditions (Kos et al., 2011; Barrantes, 2018). However, there is a lack of studies on the influence of this preparation on the development of pepper seedlings, which determined the direction of our research.

The main goal of the present study was to establish the effect of different methods and concentrations of the application of the leaf fertilizer Protifert LN 6.5 on the development of pepper seedlings.

MATERIALS AND METHODS

The experiments were conducted in the Experimental Field of the Agricultural University-Plovdiv, Bulgaria and the scientific laboratories of the Departments "Horticulture" and "Microbiology and Environmental Biotechnology" with pepper varieties Kurtovska kapia 1619 and Bulgarski rotund. The seeds for seedling production were sown in styrofoam trays with 176 holes during the first ten days of March. In the phase of two true leaves, the seedlings are pricked into number 8 pots. Plants were grown in a plastic unheated greenhouse. The applied substrate was in a ratio of 3:1 peat: perlite. The peat Domoflor Mix 4 of the company UAB Domoflor, Vilnius, Lithuania, with the following characteristics: 100% white peat a particle size 0-10 mm, pH 5.5-6.5, EC 0.6-0.7 mCm/cm, fertilizers N:P: K – 14:16:18 kg.m^3 - 1 was used. All necessary agrotechnological practices were applied to ensure the normal growth and development of seedlings.

At the development of four leaves, the foliar fertilizer Protifert LN 6.5 of the company SICIT 2000, Italy, was tested in the following concentrations: 1.0%, 2.0%, and 3.0%. The application was carried out in two ways: foliar – 6-8 ml/plant until the plants are well wetted and soil - by watering with 30 ml of solution per plant, of each concentration. The control plants, depending on the application method, were sprayed or watered with the same amount of water. Twenty-five days after the treatment, an analysis of their morphological development was performed on 15 plants. Fresh and dry weights, as well as volume (according to the displaced amount of water in a measuring cylinder with an accuracy of 1 cm3) of the root system, were determined. Stem height (length of whole plant , diameter, and fresh and dry weight were measured. The number, fresh and dry weight of the leaves, as well as the leaf area, were determined. The leaf area was established using a leaf area meter, the WinDias Image Analysis System of the company Delta-T Devices, Cambridge, UK. Measurements were taken for all formed leaves, regardless of their size. Dry matter was analyzed according to the method described by Georgiev et al. (1988). The number of flower buds per plant was determined. The content of total chlorophyll was analyzed by measuring on 5 well and fully developed leaves from the middle layers of 10 plants, with a chlorophyll meter SPAD -509 of company Konica Minolta. The total vegetative weight is represented as the sum of the weights of the separate plant organs. The plant development index was calculated using the equation:

Ig = $\frac{\Sigma + (P - 100)}{n}$, where P is a relative value compared to the control of the investigated indicator, n – number of the investigated indicators (Panayotov, 2004).

The resulting data were subjected to static processing. Analyzes of variance (ANOVA), correlation and regression were performed according to the methods described by Fowel and Cohen (1992). Due to the similarity in the trend of the obtained results, the representative data are averaged values from three years of studies.

RESULTS AND DISCUSSION

García-Jiménez et al. (2017) and Vidigal et al.2(011) point out that the basic elements to evaluate the growth and development, as well as the quality of pepper seedlings, are above all its morphological characteristics. Under the influence of the foliar fertilizer Protifert LN 6.5, a stimulating effect on the development of the root system was observed regardless of the method and concentration of its application (Table 1). The weight of the roots for both varieties tested was the highest at a concentration of 2.0%, applied foliar, with 63.55% for Kurtovska kapia 1619 and with 40.83% for Bulgarski rotund over the control. These values are lower for the soil treatment, but they are also the highest compared to the other variants in this method of application. At 3.0%, a decrease was observed compared to the previous concentration, but the weight was greater than that of the control plants. The effect of this foliar fertilizer is the weakest at 1.0%, but higher than untreated seedlings. The volume of the root system follows the same trend, reaching up to 18.66 cm3 and 14.83 cm3 in foliar treatment and up to 14.5 cm3 and 13.48 cm3 in soil treatment, for Kurtovska kapia 1619 and Bulgarski rotund respectively. Again at 2.0% the root dry weight was higher except for the foliar application in the kapia type variety. An increase in root dry mass as a result of both foliar and soil application of amino acid fertilizers to pepper was also found by Padem et al. (1999). Souri et al (2019), and Serralta-Interian et al (2020) observed a stimulating effect on the development of the pepper root system from the application of foliar fertilizers with an amino acid composition. The results are statistically significant except for those for a concentration of 1.0%. The stated stimulatory effect of Protifert LN 6.5 on the root system of pepper seedlings gives reason to assume that these plants will take root and adapt much better and faster when planted under field conditions.

Some differences, depending on the method of application, are observed in the morphological features of the stem, with the foliar treatment having the highest values for the studied characters at a concentration of 2.0%, while for the soil it was at 3.0% (Table 2). When applying 2.0% Protifert LN 6.5 through the leaves, the height of the stem of Kurtovska kapia 1619 seedlings exceeded the control by 29.28%, and that of Bulgarski rotund by 18.8%. A similar effect of amino acid action in pepper was reported by Padem et al (1999) and AL-Nassrallah and AL-Asadi (2023). By 46.34% and by 13.04%, the diameter of the stems is thicker for the two varieties, respectively. Significantly stronger growth was also observed for its fresh weight, with the increase compared to the control being approximately 3 g to 4 g, and the dry weight increased by 13.76% in the kapia type variety. The data for the results obtained at this concentration are mathematically proven. These results coincide with those obtained by Trejo-Téllez et al. (2020) that reported an increase in the stem weight of pepper plants when treated with amino acid preparations. A strong positive correlation was established for the formation of stem weight from its height with coefficients r=0.81 and r=0.75, respectively for Kurtovska kapia 1619 and Bulgarski rotund. For both the previous and the following concentration, the values were smaller, but significantly greater than those of the control, and at 3.0% they were higher than those at 1.0%.

With soil fertilization, a gradual increase of up to 3.0% is established. Stem height was 19.15% and 30.81% higher than untreated plants for Bulgarski rotund and Kurtovska kapia 1619, respectively. A larger stem diameter was developed by plants of the var. rotundum, reaching 0.59 cm, compared to 0.48 cm for the control. There is also an increase of 3.52 g and 5.37 g in weight, more pronounced for the Bulgarski rotund. The results are statistically proven. With this method of treatment, the dry weight for the variety with a conic (kapia) shape of the fruits is the highest at 1.0%, but also for the other concentrations it exceeds the control, while for Bulgarski rotund this is the case for 3.0%.

Table 1. Morphological characteristics of the root system

Variants	Foliar application			Soil application		
	Kurtovska kaipa 1619					
	Weight (g)	Volume (cm^3)	Dry Weight (%)	Weight (g)	Volume (cm^3)	Dry Weight (%)
Control	10.7	9.80	7.80	8.76	10.76	9.83
1.0%	10.85	10.30	8.93	10.3	10.98	9.98
2.0%	17.50	18.66	8.72	12.0	14.5	13.97
3.0%	13.48	15.98	8.83	10.81	12.16	11.99
LSD p=0.05%	2.6	3.1	1.5	2.2	1.3	2.1
	Bulgarski rotund					
Control	9.82	11.15	9.41	8.23	8.52	9.69
1.0%	10.45	11.48	9.16	9.33	9.66	9.81
2.0%	13.83	14.83	9.61	13.35	13.48	9.82
3.0%	11.00	11.82	9.48	11.33	11.83	8.76
LSD p=0.05%	2.6	3.3	1.6	3.8	2.9	2.0

The development of the leaves (Table 3) follows the trend indicated for the root system, a stronger development among all the variants tested was observed with 2.0% Protifert LN 6.5. The number of leaves at this concentration for Kurtovska kapia 1619 was 4.47 and 4.0 above the control and for Bulgarski rotund – 2.67 and 2.32, respectively for foliar and soil application. Such an effect of foliar fertilizers with amino acids on pepper was established by García-Jiménez et al. (2017) and Souri and Sooraki (2019). Significant stimulation is also established on leaf area, and in both varieties, it is higher in the soil application and reaches maximum dimensions in Bulgarski rotund - 337.65 cm2. Relative increase, however, compared to the control, is the highest with foliar application of Kurtovska kapia 1619, with 62.13%. Amara et al (2016) and Al-Hassani and Majed (2019) also reported a similar effect on pepper leaf area when applying foliar fertilizers containing amino acids. There is a strong positive correlation between the number of leaves and the leaf area with correlation coefficients between r= 0.82 to r= 0.87, respectively, in leaf treatment of Bulgarski rotund and Kurtovska kapia 1619, as an exception to this dependence in soil treatment of Bulgarski rotund, where the correlation is also positive, but medium r=0.50. The fresh weight of the leaves also increased by approximately 36%, being the highest for Kurtovska kapia 1619 with leaf treatment - 13.82 g. Between the number of leaves and their weight, there is also a strong correlation dependence with coefficients from r=0.69 to r=0.85, while in the case of soil-applied Protifert LN 6.5, and in the case of Bulgarian ratund, it is positive and medium with r=0.55. The dry weight increased within narrower limits between 2.83% to 17.61% for Bulgarski rotund, soil and foliar application, respectively, and the values for the other variety occupied an intermediate position. The indicated differences are statistically significant. At the remaining two tested concentrations, the data for the above-mentioned signs are also higher than those of the control, and in many cases at 1.0% they exceed those at 3.0%.

Table 2. Morphological behaviors of the stem

Variants	Foliar application				Soil application			
	Kurtovska kaipa 1619							
	Heigh t (cm)	Diameter (cm)	Weigh t (g)	Dry Weight(%)	Height (cm)	Diameter (cm)	Weight (g)	Dry Weight(%)
Control	18.1	0.41	4.50	18.0	18.5	0.42	5.48	10.97
1.0%	20.3	0.43	5.33	19.0	19.6	0.45	6.83	17.45
2.0%	23.4	0.60	9.15	20.5	22.0	0.49	7.40	14.63
3.0%	21.5	0.45	6.00	18.7	24.2	0.48	9.00	14.77
LSD p=0.05%	3.0	1.2	3.5	1.9	2.1	0.8	2.8	3.2
r*			0.81				0.84	
	Bulgarski rotund							
Control	19.2	0.46	7.82	14.70	17.33	0.48	6.29	13.03
1.0%	19.33	0.54	9.48	14.74	20.11	0.51	8.50	14.40
2.0%	22.81	0.52	10.2	14.91	20.50	0.55	9.48	13.05
3.0%	20.42	0.50	9.2	14.70	20.65	0.59	11.66	18.88
LSD p=0.05%	2.8	0.8	2.6	1.4	2.2	0.7	2.2	2.4
r*			0.75				0.87	

*with stem height

The total root and leaf-stem weight (Table 4) increased at all tested concentrations of Protifert LN 6.5. It is higher in the foliar treatment, except for Bulgarski rotund, where this is at a concentration of 3.0%, soil application. Seedlings developed the highest total vegetative weight after exposure to 2.0%. The increase for the foliar treatment was by 82.29% and 36.44%, and for the soil treatment - by 34.06% and 48.90%, respectively for Kurtovska kapia 1619 and Bulgarski rotund. The results are mathematically proven. Figure 1 shows the participation of individual plant organs in the formation of the total vegetative weight. It is noteworthy that there are no significant differences between individual concentrations in a given variety and method of application. In all varieties, the weight of the root system takes the largest share, followed by that of the leaf and lastly the weight of the stem. According to Simidchiev and Kanazirska (1984), quality pepper seedlings are characterized by the ratio among the weight of individual organs, which is correspond with our results. The absence of large differences with the control plants shows that those treated with Protifert LN 6.5 have also developed harmoniously, following a similar trend to that of the untreated ones, no excessive growth of a given organ was observed, which is a prerequisite for their better behavior at field conditions, for their faster rooting and successful development. A polynomial regression relationship was established (Figures 3 and 4) between the concentrations and the total vegetative weight with high coefficients of determination R^2=0.64 and R^2=0.90 for foliar application and R^2=0.9 and R^2=0.8 for soil application, respectively for Kurtovska kapia 1619 and Bulgarski rotund. By means of these coefficients can evaluate what percentage of the variance of the outcome variable is due to the action of the factor variable. In this sense, between 64% and 90% of the cases, the application of Protifert LN 6.5 will cause the indicated total vegetative weight development of pepper seedlings.

Table 3. Morphological features of leaves development

Variants	Foliar application				Soil application			
	Kurtovska kaipa 1619							
	Number	Leaf area(cm^2)	Weight (g)	Dry Weight (%)	Number	Leaf area(cm^2)	Weight (g)	Dry Weight (%)
Control	13.83	188.80	7.00	14.55	12.33	227.20	7.66	16.40
1.0%	15.68	242.64	10.20	19.80	13.33	264.94	8.83	17.37
2.0%	18.30	306.11	13.82	14.57	15.00	309.79	9.96	18.94
3.0%	13.96	218.40	8.83	16.90	13.83	232.60	9.62	19.25
LSD p=0.05%	2.4	28.2	2.6	2.2	1.1	32.3	2.1	1.6

r*		0.87	0.80			0.82	0.85	
	Bulgarski rotund							
Control	12.82	227.40	9.50	15.50	13.16	246.82	9.65	14.44
1.0%	13.3	243.14	12.25	16.98	15.16	248.14	10.00	14.23
2.0%	16.83	314.73	13.00	16.80	15.48	337.65	13.16	14.85
3.0%	16.5	286.96	11.16	15.05	13.33	280.32	10.50	16.23
LSD p=0.05%	3.0	34.4	2.8	1.3	2.0	29.8	1.6	1.3
r*		0.82	0.55			0.50	0.69	

* with number of leaves

Table 4. Total vegetative weight (g)

Variants	Kurtovska kaipa 1619		Bulgarski rotund	
	Foliar application	Soil application	Foliar application	Soil application
Control	22.20	21.90	27.14	24.17
1.0%	26.38	25.96	32.18	27.83
2.0%	40.47	29.36	37.03	35.99
3.0%	28.13	29.43	31.80	33.29
LSD p=0.05%	3.5	3.8	4.1	3.5

In addition to vegetative organs, Protifert LN 6.5 also exerts a strong stimulating effect on generative development, tracked down through the number of flower buds formed (Table 5). According to Panayotv (2014), the presence of flower buds is an indicator of quality and well-developed pepper seedlings. At all concentrations, the number of flower buds was higher than that of untreated plants. It is highest at 2.0% foliar application, except for the soil application of the leaf fertilizer at Kurtovska kapia 1619, where this is for the higher concentration. Seedlings from variant 2.0% developed approximately 4.5 flower buds, compared to 1.4 for the controls, and the highest values were recorded for Bulgarski rotund.

García-Jiménez (2017) also reported stronger flower bud development in pepper seedlings as a result of foliar stimulation of the plants.

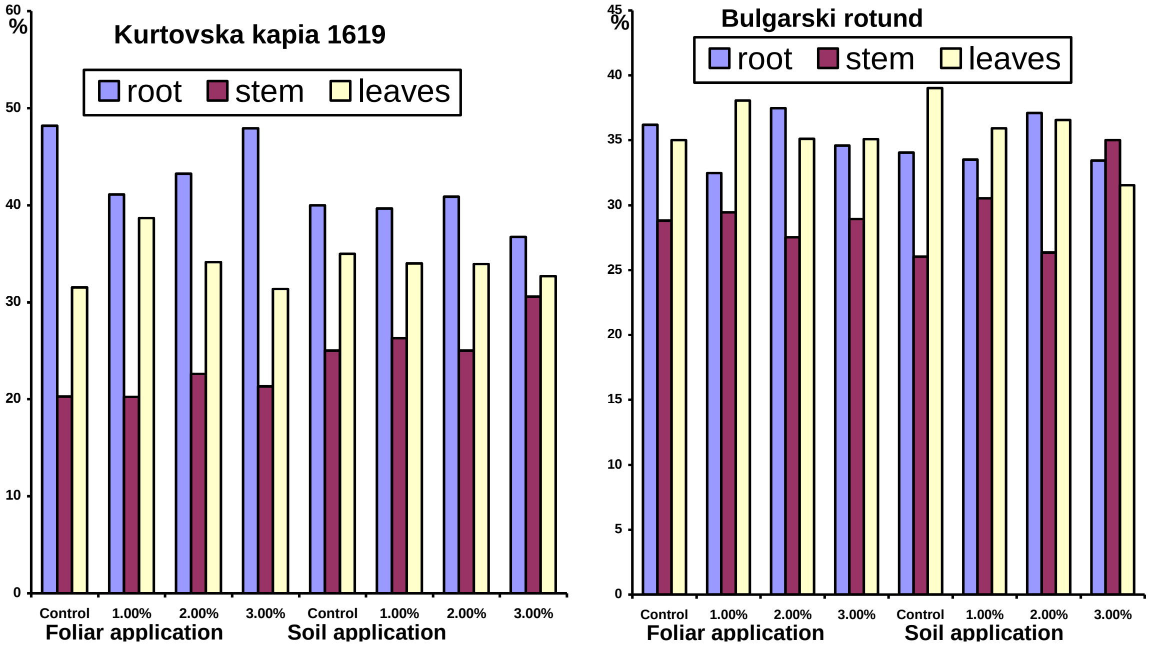

Figure 1. Distribution by organs of the total vegetative mass of seedling plants (%)

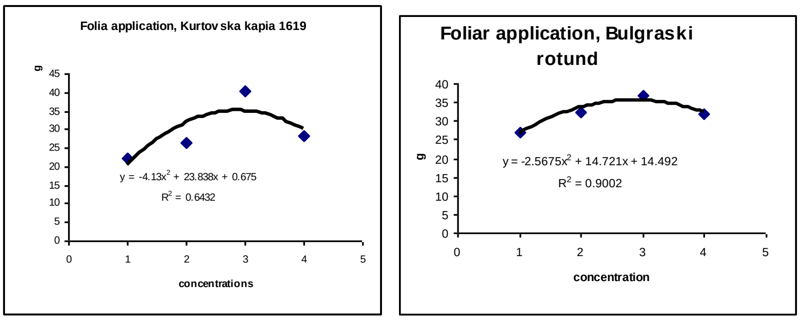

Figure 2. Regression dependences between concentration and total vegetative weight in leaf application of Protifert LN 6.5

Concentrations: 1-0%; 2-1.0%; 3-2.0%; 4-3.0%

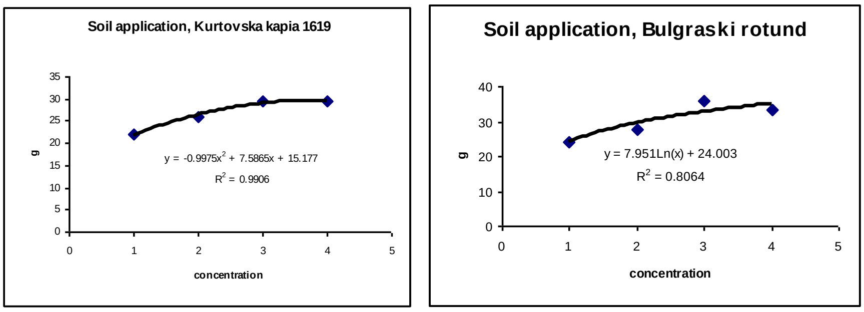

Figure 3. Regression dependences between concentration and total vegetative weight in soil application of Protifert LN 6.5

Concentrations 1-0%; 2-1.0%; 3-2.0%; 4-3.0%

The content of total chlorophyll (Table 6) varies within narrow limits. In most variants, as a result of the action of the foliar fertilizer, its content is higher, except for 1.0% foliar application and 3.0% soil application for Kurtovska kapia 1619 and 2.0% foliar treatment for Bulgarski rotund. Some differences were found between the two varieties. In the case of Kurtovska kapia 1619, the highest values for both methods of application were recorded at the concentration of 2.0%, while in Bulgarski rotund, regardless of the method of use, the chlorophyll is the most at 1.0%. The data are statistically significant. A strong effect of foliar fertilizers with amino acids on the chlorophyll content in pepper leaves was also noted by Karakurt et al. (2009) and Souri and Sooraki (2019).

Table 5. Number of flower buds per the seedling plant

Variants	Kurtovska kaipa 1619		Bulgarski rotund	
	Foliar application	Soil application	Foliar application	Soil application
Control	1.16	1.17	1.33	1.82
1.0%	2.48	1.18	3.83	2.33
2.0%	4.66	1.90	4.83	4.42
3.0%	2.67	2.5	2.66	2.98
LSD p=0.05%	1.1	1.2	2.0	1.6

A Summarizing expression for the effectiveness of the foliar fertilizer Protifert LN 6.5 is the plant development index (Table 7). In all variants, it is positive, but with a specific meaning and a strong variation between individual concentrations, which is a result of their different effect on the studied indicators. A higher index is reported with the foliar application of this fertilizer. In both varieties and in the tested ways of use, it is the highest at a concentration of 2.0%. The above-mentioned stronger influence of this concentration on most of the observed behaviors of the seedling plants was also reflected in its higher values, 66.59 and 41.51 for foliar application and 36.32 and 33.85 for soil application, respectively for Kurtovska kapia 1619 and at Bulgarski rotund. The lowest is the development index at 1.0%, except for leaf treatment in Bulgarian ratoon, where it is at 3.0%.

Table 6. Content of total chlorophyll (SPAD)

Variants	Kurtovska kaipa 1619		Bulgarski rotund	
	Foliar application	Soil application	Foliar application	Soil application
Control	52.16	55.00	55.13	53.10
1.0%	50.83	56.50	58.40	54.76
2.0%	54.96	59.06	51.60	53.36
3.0%	54.70	52.70	57.46	54.20
LSD p=0.05%	2.2	2.4	2.0	2.1

Table 7. Index of the development of seedling plants

Variants	Kurtovska kaipa 1619		Bulgarski rotund	
	Foliar application	Soil application	Foliar application	Soil application
Control	0	0	0	0
1.0%	18.69	15.24	22.66	11.14
2.0%	66.59	36.32	41.51	33.85
3.0%	27.46	27.94	16.68	26.23

CONCLUSION

The foliar fertilizer Protifert LN 6.5, with amino acid content, significantly stimulates the vegetative development of pepper seedlings. The influence of the concentration is very clearly expressed, and for most signs the effect of the foliar application is more considerable than that of the soil application.

The morphological behaviors, both of the root system and of the leaves, have the highest values when applying 2.0%. Varieties response was observed in terms of stem growth, which was strongest for Kurtovska kapia 1619 at 2.0% and for Bulgarski rotund at 3.0%.

Treated pepper seedlings maintained harmonious development, no excessive growth or significant change in the ratio between the weights of individual organs was observed, which is a prerequisite for its better adaptation and growth upon planting. These plants also developed a greater number of flower buds, the most at the 2.0% concentration.

This gives reason to recommend in practice for seedlings growing to use of the foliar fertilizer Protifert LN 6.5 in a concentration of 2.0% by foliar application.

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STUDY ON THE STORABILITY OF CAPE GOOSEBERRY (*PHYSALIS PERUVIANA* L.)

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ABASTRACT

The goal of this study was to establish the duration of the storage in which the seed of cape gooseberry keeps high sowing and viability qualities. The objects of this investigation were the cape gooseberry seed stored in the laboratory, non-controlled conditions in paper pouches. The seed was produced in 2016 year in the Experimental field of the Agricultural University-Plovdiv, Bulgaria, and sowing qualities were tested annually to the seventh year. The seeds from Plovdiv and Obrazec 1 varieties were harvested in botanical maturity. Several parameters of the germination status and vigor, as well as morphological behaviors of the seedlings and some germination index, were established. Preservation of high sown qualities was reported until the 6th year with germination higher than 80%, but in the 7th year the vitality dropped sharply and this indicator had zero values. The seedling fresh weight depends strongly on the length of hypocotyls and embryo root which is also proven by the established high correlation dependencies with coefficients r=0.7 and r=0.90. Already in the second year, the deviation of seedling development was observed. The relationship between storage period and germination rates and vigor index is described very well by the established linear regression with determination coefficients of R^2=0.89 and R^2=0.96, respectively. The variety response of different sowing and quality parameters towards storage was established.

Keywords: germination, vigour index, ageing, deterioration, viability

INTRODUCTION

Most vegetable crops are mainly propagated by seeds (Souza, 2014; Shopova, 2014). The genus *Physalis* belongs to the family *Solanaceae*, according to Legge (1974), Fisher et al. (2007) and Yildirim et al. (2011) it includes more than 100 species. Popova et al. (2020) pointed out that the most economically important of these is cape gooseberry (*Physalis peruviana* L.). The plants are grown for their attractive fruits, with a bright orange or yellow color. They are enveloped by the expanded sepals, which have dried and completely cover the fruit. This is a specific feature of this species, which distinguishes it from other species of the genus *Physalis*. On the other hand, the fruits have a very pleasant and refreshing taste (Panayotov and Popova, 2014).

This species, as indicated by Rufato et al. (2008) and Souza et al. (2016), can also be propagated by vegetative method, using stem cuttings or in vitro. However, the most widely used is its cultivation through seeds, which must be with high vitality, and this is the most efficient way of its production (Slageren, 2003; Muniz, 2011).

The quality of the seeds mainly determines the possibility of successful reproduction and development of the plants (Panchev, 2015; Panchev, 2019). Their vital status, apart from the applied technology for their production, depends to a very large extent on their correct storage. It must be carried out under such conditions as to reduce the natural deterioration of their vitality. This depends a lot on the environment in which they are stored, with the greatest importance in this direction there are the air temperature and humidity, as well as the preservation of their health status (Barua et al., 2009; Panayotov, 2015 and Souza et al., 2016). Seed moisture is also essential, which according to ISTA (2013) prescriptions and as Labbé (2003) points out, must be below 13%.

There are different ways and technologies of storage, as well as types of storage houses for seeds, the main aim of which is to minimize the metabolic processes, thereby reducing the deterioration and loss of vitality and preserving their initial germination for a longer time. The storage under controlled microclimate and uncontrolled conditions, for long-term storage in gene banks and, although less often, seed preservation in liquid or vapour nitrogen are used (Panayotov, 2014; Souza et al., 2016). The most widely applied method of seed storage for farmers' needs, for a shorter or relatively longer period, is under common, uncontrolled conditions. This is a much cheaper method and is associated with significantly lower investments (Panayotov, 2015).

The scientific research on the storage of cape gooseberry seeds is quite limited. Similar assertions are made by Pichardo-González et al. (2010). Nunes et al. (2018) obtained seeds from green-yellow and straw-yellow calyx fruits, which they stored in paper envelopes under common, uncontrolled conditions, and found that after storage, the highest viability between 96% - 98% was reported when germination was carried out at a temperature of 27^0C.

In the natural aging of cape gooseberry seeds, Pichardo-González (2014) found that, as a result of storage, the average annual rate at which germination capacity decreased was 11.2% and a strong loss of vitality was reported at 7 years. Pérez-Camacho et al. (2008), however, reported that these seeds stored under common conditions lost approximately 9% germination per year. Souza et al. (2016) recommended that to maintain high seeding qualities, the seeds from this crop should be stored at temperatures between 5^0C and 25^0C.

The main aim of the present study was to monitor the changes occurring in the vital status of cape gooseberry (*Physalis peruviana* L.) seeds as a result of storage under ambient conditions, as well as the period until which they retain high sowing qualities.

MATERIAL AND METHODS

The experiments were carried out in the experimental field and scientific laboratory of the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria with cape gooseberry (*Physalis peruviana* L.), variety Plovdiv and accession Obrazec 1. The plants were grown according to the adopted technology for Bulgaria, described by Panayotov & Popova

(2013) with seedlings produced by sowing on 03.2015 and planting on 05.2015 by the scheme 70 x 50 cm, and all the necessary for normal plant development agrotechnological practices during the growing season were applied. Seeds were obtained from fruits in the full botanical maturity phase, washed, dried and placed in paper bags under laboratory conditions. In the same year, the vital state of the seeds was analyzed, and these data served as a control.

Each year for seven years, until 2022, these analyzes were conducted. The germination energy and germination in four replicates of 100 seeds, according to the recommendations of ISTA (2013) and the approach described by Sbrussi (2014) and Nunes (2018) were determined. The seeds for germination were placed in Petri dishes, diameter 10 cm with two folds of Whatman 1 filter paper, moistened with 4 ml of water and placed in the germination chamber.

According to methods described in detail by Panayotov (2015), the uniformity of germination (Strona, 1966) and also the Mean germination time calculated according to the given below equation were determined.

$$M.G.T. = \frac{\Sigma(G \times T)}{F}, \text{ where}$$

T – day in which the seed is germinated, G – numbers of seeds that germinated in this,

F – final number of germinated seeds (Battle and Whittington, 1969).

The time to obtain 50% germination (T50) by Coolbear et al. (1984), modified by Farooq et al. (2004), and described by Farooq (2005) was calculated.

When calculating germination, fresh weight was measured for all seedlings from four replicates and recalculated for one seedling. The dry mass of seedlings was determined according to the method described by Georgiev et al. (1980).

Embryo root and hypocotyl lengths were measured on the day of germination determination on 15 seedlings from each replicate. For this purpose, 25 seeds in four replicates, in moistened filter paper Watman 1 in the form of a roll, were placed in upright glass cylinders so that the seedlings developed in a vertical position, maintaining the necessary humidity, and germination took place under the same conditions as for germination.

Deviations from the normal development of the seedlings were observed, according to Wellington (1970) and ISTA (2013) and including in this study: the presence of a short embryo root; lack of: branches in the embryo root, hairs on the embryo root and on the hypocotyl and also unopened cotyledons.

Vigor was calculated using the method "Initial vegetative production of seeds - method for determination of the vigor" (Panayotov, 2013). The following parameters were also determined: coefficient of the velocity of germination and germination rate index, both described in detail by Kader (2005).

The data were subjected to analysis of variance ANOVA, correlation and regression analyses. These analyses are described by Fowel and Cohen (1992).

RESULTS AND DISCUSSION

Seed storability is one of the main aspects of agronomic science and practice and an essential unit in the seed production system, but the mechanisms underlying seed aging and deterioration are largely unclear (Raikar et al., 2011; Hang et al., 2015; Yan et al. et al., 2018). Alahakoon et al. (2021) pointed out that rapid loss of germination and vigor is a common problem in seed storage. Therefore, the most complete assessment of storage capacity is by examining the changes that occur in the sowing qualities. Determination of germination energy is in a shorter time when those seeds that have greater vitality and higher potential have germinated (Copeland and McDonald, 2001; Traynov, 2021). In the two cape gooseberry studied accessions, its values, as a result of storage, evenly and slightly decrease until the fifth year, after which in the sixth year, than the first one, they sharply reduced by 68.32% (Plovdiv) and by 85.24% (Obrazec 1) to reach zero after another year (Table 1). A similar trend is observed for germination, which is a generalized expression and accepted standard for seed classification (Black et al., 2006). For this trait, the obtained data, both between individual years and between the two tested genotypes, have small differences. The initial germination is over 98% and it gradually decreases. Slightly lower values are recorded for the seeds of Obrazec 1. The decrease is the strongest in the third year, approximately 4%, but the seeds still retain high germination. It can be noted that the average annual decrease is approximately 2.5%. This is appropriate to consider in the mass production and storage of seeds (Pérez-Camacho, 2008). An extremely sharp decrease and reaching zero values are reported at year 7^{th}. Pichardo-González et al (2014) obtained similar results for physalis. Differences are statistically significant except between the first and second year. From the obtained results, it can be pointed out that within the *Solanaceae* family, cape gooseberry is characterized by a good to moderate shelf life.

A linear regression relationship (Figure 1) was established between the storage period and germination with high coefficients of determination R^2=0.892 and R^2=0.896 for Plovdiv and Obrazec 1, respectively. These coefficients show what percentage of the dispersion of the resulting variable is attributed to the action of the factor variable and in 89% of cases the storage period will produce the indicated germination trend. High determination coefficients of regression relationships with the germination of cape gooseberry seeds were also reported by Nunes et al. (2018).

Deterioration of seed quality during storage has a much more significant effect on the germination time of 50% of the seeds. The initial seeds are with T_{50} of 3.8 days for the Plovdiv variety and 3.5 days for Obrazec 1. Throughout the storage period, this time increased to reach 9.9 days and 9.3 days in the 6th year, respectively, for the mentioned two genotypes.

The greatest increase in the time for germination of 50% of the seeds was found between the 5th and 6th years, with an increase of 3.4 days (Plovdiv) and 4.3 days (Obrazec 1). The average annual decrease is 1.22 days and 1.04 days, respectively, for the two cape gooseberry accessions.

Table 1. Viability properties of cape gooseberry seeds after storage

Year	Plovdiv			Obrazec 1		
	Germination energy (%)	Germination (%)	T_{50} (day)	Germination energy (%)	Germination (%)	T_{50} (day)
1	89.64	98.96	3.8	89.20	98.60	3.5
2	89.92	96.92	5.5	87.96	95.96	4.3
3	85.90	92.90	6.0	86.80	91.00	4.9
4	83.66	90.66	6.3	81.84	90.84	5.2
5	81.90	90.00	6.5	80.96	90.00	5.0
6	21.32	89.9	9.9	3.96	87.92	9.3
7	0	0		0	0	
LSD p=0.05	3.1	2.2	4.4	2.9	2.4	2.7

To follow in more detail the effect of storage on the vital status of the seeds, the mean germination time, indicating the days required for the germination of one seed, was also examined (Table 2). Changes up to year 4th are within narrow limits. More significant differences were observed in year 5th, the increase in seed germination time was one day. The influence of aging is very clear in the sixth year, as this time increases by 4.63 days for the Plovdiv variety and by 6.2 days for Obrazec 1, compared to the initial data, and only for one year - between the fifth and sixth, it has increased respectively with 3.64 days and with 5.09 days. Deterioration of the seeds also affects the seed uniformity very strongly. A well-expressed genotype response was also observed for this indicator. Better results were reported for Obrazec 1, wherein the first year simultaneously germinated averages 38.0% of the seeds, against 30.53% for the other variety. Already from the third year, a significant decrease of 5% is observed, reaching in the 6th year with 13.39% and 20.69% for Plovdiv and Obrazec 1, respectively. As a result of the storage average every year the losses of uniformity of germination are 2.68% for Plovdiv and 4.17% - for Obrazec 1. Data on uniformity of germination are mathematically proven except for the second year in Obrazec 1.

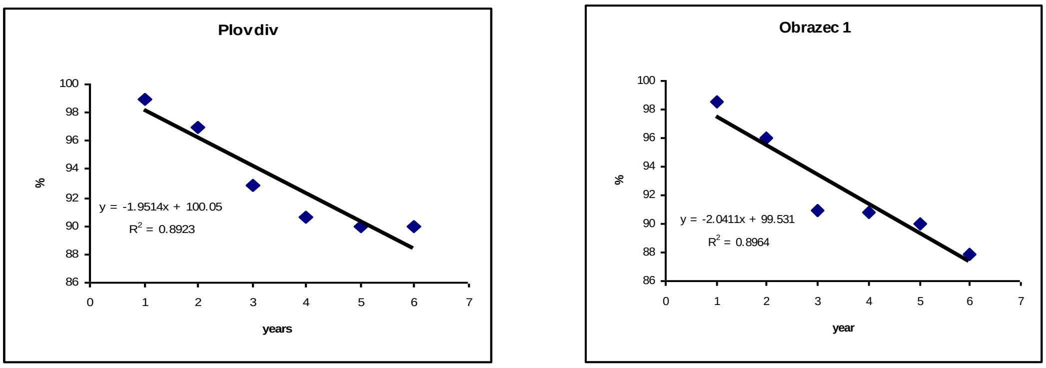

Figure 1. Regressions of germination and period of storage

Seedling morphology was also affected by storage time (Table 3). The length of the embryo root varies within narrow limits, and the differences between the two genotypes are small. The strongest decrease, compared to the initial measurements, was found at year 6th with 58.68% and 53.07%, for Plovdiv and Obrazec 1, respectively. The trend for hypocotyls length is also close. The reduction between the first and the sixth year for the Plovdiv variety is 1.22 cm and significantly more for Obrazec 1 - by 2.35 cm, with it, from the third year onwards, the seedlings with substantially shorter lengths are observed. Changes in the weight of a seedling are more visible after the fifth year and very pronounced after the sixth one. It is characteristic that in the second and third years of storage, the fresh weight of seedlings remains very close to those of the initial year, with the differences being minimal, for Plovdiv as well as Obrazec 1. Strong positive correlations were found between the fresh weight of a seedling and the embryo root and hypocotyls lengths with high correlation coefficients of r=0.83 to r= 0.84 and from r=0.78 to r= 0.91, respectively. The dry weight of the seedling changes slightly until the third year, after which it is lower, compared to the initial values by 57.89% for Plovdiv and by 61.22% for Obrazec 1.

In addition to the dimensions of the seedlings, storage also strongly affects the deviations from their normal development (Table 4). Despite the slight variation indicated for the lengths of the embryo root and the hypocotyls, not all seedlings are developing normally, as already in the second year the deviations increased by 45.37% for Obrazec 1 and with 74.37% for Plovdiv. On an average year, the percentage of seedlings with deviations increases by approximately more than 5%, which is important and should be taken into account when storing cape gooseberry seeds for commercial purposes.

The most common deviation is unopened cotyledons, but the percentage of seedlings with such deviation, on average for all varieties, is not high -37.58%. This percentage is the highest for lack of hairs on the embryo root and reaches 49.3%, but this deviation is registered the most seldom, followed by short embryo roots. Bewley et al. (2013) point out that one of the common signs of seed deterioration is the presence of abnormally developed seedlings.

Table 2. Germination features of cape gooseberry seeds after storage

Year	Plovdiv		Obrazec 1	
	Mean germination time (day)	Uniformity of germination (%)	Mean germination time (day)	Uniformity of germination (%)
1	4.02	30.5	4.12	38.0
2	4.15	27.4	4.37	36.1
3	4.28	25.0	4.88	33.3
4	4.92	24.7	4.94	28.9
5	5.01	20.3	5.23	25.0
6	8.65	17.1	10.32	17.3
LSD p= 0.05	2.2	3.0	3.2	2.4

The successful storage of the seeds is very directly related to their vigor (Mbofung et al., 2013; Souza et al., 2016). One of the most common problems in seed storage according to Alahakoon et al. (2021) is the rapid loss of vigor. Vigor indicates the ability of seeds not only to germinate but also to develop normal seedlings under a wide range of environmental conditions (AOSA, 1991; Panayotov, 2015). In this sense, its determination is extremely important for the production of cape gooseberry in different areas. As the storage period increases, vigor decreases uniformly (Table 5). From the initial value of vigor of 17.23 for the Plovdiv variety, in the sixth year, it reaches 11.17, and in the case of Obrazec 1, this reduction is by 5.63 or approximately more than 30%. On average, every year of storage, the seeds of cape gooseberry lose 1.2 and 1.6 points of their initial vigor. Pichardo-González et. al. (2010) also found that the deterioration of the vital indicators of cape gooseberry seeds during aging and storage reflected very strongly also on a decrease in vigor.

Table 3. Morphological characteristics of cape gooseberry seedling after storage of the seeds

Year	Plovdiv				Obrazec 1			
	LER (cm)	LH (cm)	FW (mg)	DW (%)	LER (cm)	LH (cm)	FW (mg)	DW (%)
1	3.63	4.42	16.90	1.90	2.45	4.70	13.06	1.96
2	2.92	4.34	16.88	1.75	2.16	4.46	13.05	1.95
3	2.62	4.30	16.81	1.70	2.35	3.61	13.05	1.55
4	2.30	4.11	15.40	1.10	2.33	3.55	13.00	1.20
5	1.94	4.02	15.21	1.10	2.23	3.77	11.17	1.20
6	1.50	3.20	12.03	1.10	1.15	2.35	10.13	1.10
	r=0.83	r= 0.91			r= 0.84	r=0.78		
LSD p= 0.05	1.3	2.2	2.2	0.7	1.9	1.1	2.4	0.8

LER-Length of embryo root, LH-Length of hypocotyls, FW-Fresh weight of one seedling,

DW- Dry weight of seedlings; r-correlation coefficient with fresh weight

Table 4. Deviation of cape gooseberry seedlings after storage (%)

Year	Plovdiv		Obrazec 1	
	%	Type and % to the total number of deviation	%	Type and % to the total number of deviation
1	9.5	HR-52.2; H-47.84	10.8	BR-38.8; H-51.2
2	16.6	SR-28.8; BR-35.2; C-36.0	15.7	H-55.3; C-44.7
3	19.5	BR-48.8; C-41.2	20.9	SR-42.8; BR-27.2; C-30.0
4	22.4	HR-45.5; BR-30.0; C-24.5	24.6	SR-41.5; H-26.5; C-32.0
5	28.3	H-42.3; C-57.7	31.3	HR-48.8; C-41.2
6	36.8	SR-35.5; H-36.6; C-27.9	38.6	SR-25.0;BR-15.5;H-20.5; C-39.0

SR-Short embryo root, BR- Lack of branches in embryo root, HR-Lack of hairs on the embryo root, H-Lack of hairs of hypocotyls, C-Unopened cotyledons

The influence of storage of cape gooseberry seeds on their vigor is very well seen from the established linear regression relationships (figure 2) with high coefficients of determination for Plovdiv R^2=0.967 and for Obrazec 1 R^2=0.962, which shows that in 96% of the storage, such seeds will receive the stated reduction in vigor. The importance of studying vigor is even more apparent when comparing the change in germination and vigor, expressed as a percentage, relative to the initial values of the first year, accepted to be 100%. It can be seen from Figure 3 that the decrease in vigor occurs much earlier and is much faster than that of germination. By the third year, these two indicators have a very close course of development. Then, in the 4th year, while the percentage reduction of germination was by 8.4% and by 8.9%, the values of vigor had already decreased by 20% and by 24% for Plovdiv and Obrazec 1, respectively. In the sixth year of storage, the germination has changed little and still maintains relatively very high values, having lost approximately only about 10%, but the vigor has decreased very sharply and the losses reach 32% and 35% of the initial results for the indicated more above genotypes. That is why, in addition to determining germination, the establishment of vigor is much more important for seed quality.

Table 5. Behaviors of vitality status of cape gooseberry seeds after storage

Year	Plovdiv			Obrazec 1		
	V	CVG	GRI	V	CVG	GRI
1	17.23	25.63	21.11	16.08	23.30	27.05
2	16.71	25.53	26.98	15.27	23.28	24.37
3	15.85	21.69	24.83	14.47	20.19	22.34
4	13.78	20.33	23.38	12.22	19.74	20.86
5	12.40	19.90	21.20	13.35	19.64	20.48
6	11.71	10.55	11.49	10.45	9.98	10.21
LSD p= 0.05	4.4	3.0	2.4	2.9	5.2	3.6

V-vigor index, CVG-coefficient of velocity of germination,

GRI–germination rate index (%/day)

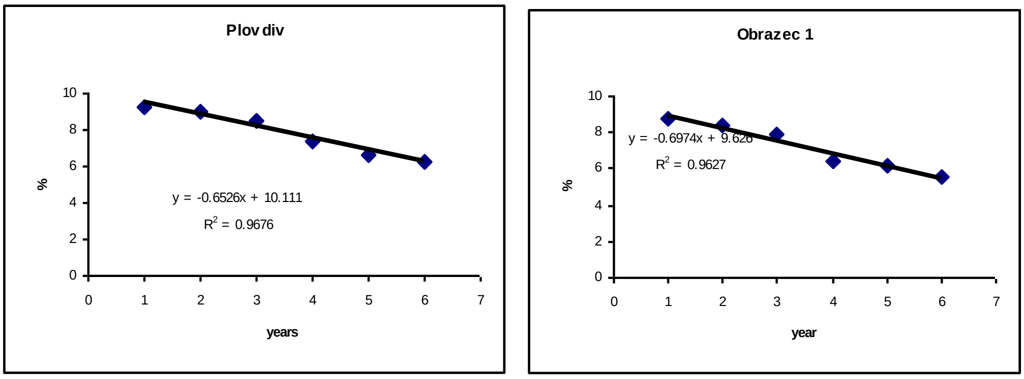

Figure 2. Regressions of vigor and period of storage

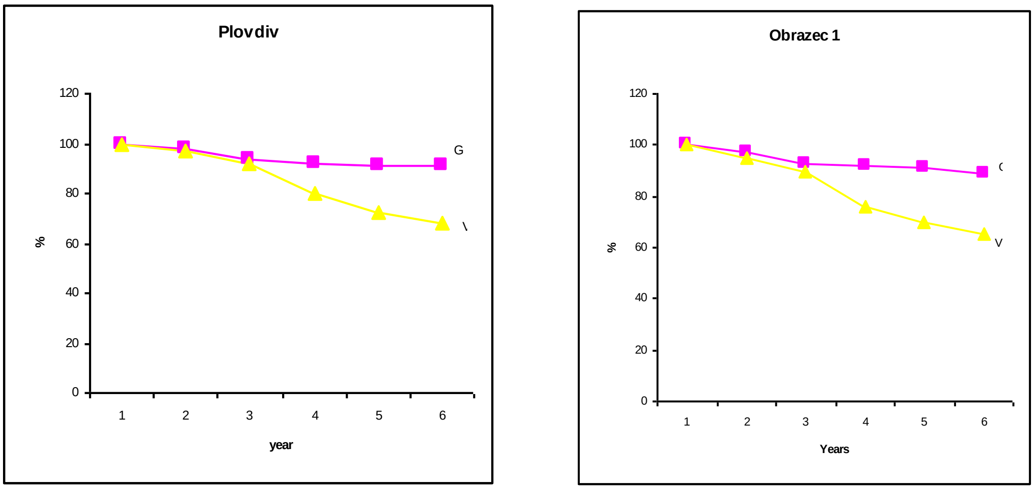

Figure 3. Changes of the germination (G) and vigor (V)

The assessment of the status of cape gooseberry seeds is completed by establishing the coefficient of the velocity of germination, as it gives information on the rapidity of germination, which means the germination of a larger number of seeds in a shorter time (Kader, 2005). Results show very clearly the influence of storage on seed quality deterioration. As the storage period increases, this coefficient decreases strongly, and in the sixth year, it is approximately more than 50% lower relative to those of the first one. In the second year of storage for the seeds of both genotypes the values are very close to the initials. In the sense of the seeds storage is also important the evenly distribution over time of the percentage of germinated seeds during the whole period of the germination. This is established using the germination rate index, which however, does not correlate with the daily peaks of the germination (Kader, 2005). High values of this index are reported for almost the entire period of storage, which is evidence of the faster course of germination in these variants and it’s even spread throughout the reporting period. An exception is observed in the sixth year, when its values decrease sharply, by more than half compared to the other variants, and this means a delay of the rate of the germination.

CONCLUSIONS

In store, the seeds of cape gooseberry (*Physalis peruviana* L.) retain high sowing qualities, mainly in terms of germination, until the sixth year. Weak changes begin to be observed in the fourth year and increase in the sixth one, but still germination is high and it reaches zero values in the seventh year. A linear regression relationship was established between germination and the storage period with high coefficients of determination R^2=0.89.

A stronger deterioration in mean time and uniformity of germination is reported from the fifth year of storage. In this period, the fresh weight of the seedling decreases more significantly and there is a high positive correlation between it and the lengths of the embryo root and the hypocotyls. The storage of seeds from the second year causes a significant increase in seedling deviations from their normal development.

The seed vigor index decreases significantly from the fourth year, and its changes occur much earlier and are stronger compared to those of germination. A linear regression relationship was established between vigor and storage period with high coefficients of determination R^2=0.96.

For the seed production and trade of high-quality cape gooseberry (*Physalis peruviana* L.) seeds, it is recommended that they be stored until the fifth or, at most, the sixth year after their seed extraction.

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POSSIBILITY OF USING FACTORY BLACK TEA WASTE IN ANIMAL FEEDING

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ABSTRACT
Tea (*Camellia sinensis*) is the most consumed beverage in the world after water. Tea leaves are usually harvested from late March and early April to July each year. Tea can be classified as black, green and oolong tea according to processing procedures. The percentage of each type of tea produced and consumed in the world is: 78% black, 20% green and 2% oolong tea. The chemical composition of tea is quite complex as it has different classes of chemical compounds including polyphenols, alkaloids, amino acids, proteins, minerals, vitamins and carbohydrates. Catechins in tea leaves polyphenols are components with high antioxidant activity. They are classified as (±)-catechin C, (-)-epicatechin EC, (+)-gallocatechin GC, (-)-epigallocatechin EGC, (-)-epicatechin gallate ECG and (-)-epigallocatechin gallate EGCG, respectively. Epigallocatechin gallate is the most abundant catechin in green tea, accounting for 50% of total catechins. Catechins act as antioxidants by sequestering metal ion, scavenging free radicals that can damage DNA and contribute to cancer, metabolic dysfunctions, clotting, and atherosclerosis. The reason why tea is the 2nd most consumed beverage in the world is that it has such high antioxidant activity. However, with so much production, tea production wastes occur as fabrication waste. Black tea waste, which occurs as a by-product during the production of black tea from fresh tea, is not utilized in any way other than being used as fuel and compost. Since it is thrown into nature in an uncontrolled manner, it causes great environmental pollution and causes ecological and economic damage. In this review, the results of the research on the possibilities of using factory black tea waste as an alternative source in animal feeding were evaluated.

Keywords: Tea production, Factory black tea waste, Animal feeding, Roughage

INTRODUCTION

Ruminant animals need roughage due to the anatomical structure and physiological functions of their digestive systems. The quality of the roughage taken by the animals and how much of it is converted into meat, milk, etc. by the animal is also an important factor. In daily rations, at least 40-50% of the total dry matter calculated for milking cows and at least 10-15% of the dry matter in fattening cattle should be met from roughage sources. In amounts below these ratios, some metabolic disorders, feed intake, milk fat and milk yield and live weight gain decrease (Aksoy et al., 2000).

One of the biggest problems of the country's animal husbandry is the insufficient production of quality roughage and the high cost of the existing production. Enterprises with insufficient or poor quality roughage resources utilise more concentrate feed resources for milk yield, thus ration cost increases and profit decreases. For this reason, it is necessary to have quality roughage resources (clover, corn silage, etc.) or feed resources that can be substituted for these (industrial by-products, wastes, residues, etc.), which are indispensable for dairy and beef cattle enterprises. The number of bovine animals in Turkey is 17.30 million and the number of small ruminants is 56.23 million (TURKSTAT 2023a). When roughage production in Turkey is examined, approximately 33 million tons of roughage is produced, 18 million tons from field agriculture and 15 million tons from meadows and pastures (TURKSTAT, 2023b). According to the bovine animal unit (BAU), there is a need for about 86 million tonnes of roughage, which means that Turkey has a roughage deficit of 53 million tonnes. The insufficient quality roughage deficit is tried to be met with resources that can be alternative to these feeds.

Tea

Tea is a product dating back to 3000 BC. It is the most consumed beverage after water in the world and tea is cultivated in about 30 countries (Tang et al., 2002). Black tea constitutes 78% of the world tea production and is mostly grown for this purpose in western countries. The 20% of tea production is grown and consumed as green tea, and the demanders of this product are mostly Asian countries. Finally, 2% of tea production is produced as oolong tea and it is consumed intensively in South China (FAO, 2021).

Table 1. World Dry Tea Production (FAO, 2021)

Countries	**Quantity (Thousand Tonnes)**
China	2.473
India	1.325
Kenya	440
Srilanka	350
Vietnam	260
Turkey	234
Indonesia	139
Argentina	81
Iran	101
Japan	81
Other Countries	615
Total	6.099

Tea Production Stages

Withering

After the tea leaves are collected, they come to the factory. In tea factories, withering of tea takes place as the first stage. The withering process is used to reduce the moisture content of 70-80% in the tea leaves to 50-55%. In this way, the tea leaves become suitable for the next stage, the Rolling process. The withering process of tea leaves is done in the form of natural, artificial and freezing withering.

Natural Withering

Fresh tea leaves are withered under normal weather conditions. In the natural withering process, tea leaves are laid on shelves with a spacing of 10-15 cm on the shelves with a capacity of 0.5 kg per m^2. Natural withering has a duration of 16-20 hours, but it has the quality of being better quality tea than artificial withering. In natural withering, plenty of air is also provided. The disadvantage of natural withering is that there may be a difference in tea quality with the change of weather conditions.

Artificial Withering

The purpose of artificial withering in tea leaves is to realize the process in a short time by setting the appropriate temperature for withering. 32 ^{0}C is the appropriate temperature for artificial withering, if high temperature is applied, the withering time can be shortened, but the quality of the teas with fast withering is negatively affected.

Freeze Withering

The purpose of freeze withering is to reduce the withering time in black tea production. The amounts of polyphenols and catechins in freeze withered tea leaves are similar to those of naturally withered tea leaves for 16 hours. The freezing temperature of the tea leaves is -20^0 C and lasts approximately 2 hours. At the end of this time, the tea leaves placed in the withering chamber are laid so that 9 kg per 1 m^2. For the thawing process, it is sufficient to expose 35^0 C hot air flow for 30 minutes.

Figure 1. Withering Process of Tea Leaves

Rolling

The Rolling process of tea leaves is the process of crushing, bending and disintegration of the withered tea leaves with machines, the cell sap in it comes out and the oxidation process starts. In tea factories Rolling process is carried out twice.

First Rolling (Straight Rolling)

The first Rolling of tea leaves is done with the help of flat (leaf) Rolling machines. These Rolling machines can take at least 300 kg of withered tea leaves into their chambers. A Rolling machine can fill and empty tea leaves 8 times in one shift. Each Rolling time takes approximately 45 minutes.

Figure 2. Rolling Process of Tea Leaves (Flat Rolling)

After the first Rolling process, the tea leaves are slowly discharged. Tea leaves are first passed through the rotervan in factories with rotervan, and directly through the Fresh tea sieves in those without rotervan. After the teas are aerated in the sieves, they are sent to the pressed or belly Rollingers for the second Rolling process.

Second Rolling

The second crimping process is performed by press crimping or core crimping. Press Rolling is the more recommended method. The coarse tea leaves, which are not sufficiently broken down in the first crimping, are provided to be broken down more in the core crimping or press crimping under pressure, the cell membrane of the leaf is cracked and the cell sap inside is taken out and better oxidation conditions are prepared. The teas removed from the second Rolling are sent to the oxidation unit after being ventilated in the Fresh tea sieves (in those with rotervan, they are passed through the rotervan again).

Press Rolling

The duration of press Rolling is approximately 40 minutes. During this time, the leaves are pressed at least 3 times. A pressure of 90-135 kg is applied on the tea for 5-6 minutes. After 5-6 minutes, the press is lifted into the air and the machine is operated without press for 5-6 minutes. In this way, the temperature of the tea leaves, which are heated due to excessive friction during pressurisation, is reduced.

Figure 3. Rolling Process of Tea Leaves (Press Rolling)

Belly Rolling

The Rolling time is 15 minutes in the Rolling with belly. Exceeding this time causes the tea to heat up and causes loss of quality. It is not recommended to take an excessive amount of Rollinged tea to belly Rolling. If the Rolling is not sufficient, the Rolling process is completed by extending the Rolling times.

Figure 4. Rolling Process of Tea Leaves (Bellied Rolling**)**

Oxidation

The oxidation process of tea leaves is the stage of formation of the desired brightness, colour, smell, astringency and aroma in black tea as a result of the biological change of the chemical substances in the cell sap of the rollinged fresh tea leaf with the help of oxidase enzyme. As a result of the oxidation process, green coloured tea leaves turn into copper red colour and an apple scented aroma is formed. The importance of the oxidation process is a stage that determines the quality of black tea.

Factors Affecting Oxidation

a-) Time

Oxidation time refers to the time from the start of rolling to the completion of the oxidation process, rather than the time the teas spend in the oxidation section. This period should not exceed 3.5 hours. The oxidation time can be increased or decreased in order to eliminate the mistakes made in the rolling and withering phases and to bring the colour and odour of the tea to the desired level. Increasing the oxidation time increases the astringency and decreases the brightness. The longer the oxidation time, the more colour and less quality in the tea, and the shorter the oxidation time, the less colour and more quality in the tea.

b-) Heat

In oxidation, the temperature varies between 21-32^0 C depending on weather conditions. An ideal oxidation in tea varies between 24-26^0 C. Below 21^0 C and above 32^0 C, oxidation slows down, whereas it stops when it rises to 66^0 C. The brightness and vibrancy of tea liquor increases at low temperature oxidation. At high temperature, a dull and dull colour is formed. At low temperatures, more Tiflavin (TF) is formed. Brightness, vibrancy and hardness are determined by the amount of TF. Thearubigin formation accelerates if the temperature rises. This has a negative effect on black tea

c-) Humidity

The relative humidity of the environment during oxidation is desired to be around 90-95%. In order to avoid darkening, the humidity should be kept high.

d-) Laying Thickness and Density

Depending on the laying thickness, the temperature of the tea leaves varies. If the leaves are observed to heat up during oxidation, the laying thickness is reduced. If the leaves are laid too thin, the temperature decreases, but oxidation does not continue properly. The thickness should be such that the air can reach it. Thick paving reduces the surface area exposed to the drying effect of air. Thick laying shows better results than thin laying, which is considered favourable.

e-) Oxygen Concentration

Oxidation occurs only in the air environment and thanks to the oxygen of the air. Oxygen is the basis of oxidation. Plenty of oxygen is needed for TF formation. If fresh air is not supplied, there may be sections where the temperature has risen towards the lower parts of the places where the tea leaves are laid and not enough oxygen has not reached. TF formation will accelerate here and as a result, various properties of the black tea produced will be adversely affected. During oxidation, withered tea leaf particles should be in good contact with air. Thus, catechins can be converted into theaflavin and tirubigin. Excessive air causes lower temperatures and accelerates cooling by evaporation. This creates an undesirable situation in oxidation.

f-) Other Factors Affecting Oxidation

Bright and light coloured tea leaves oxidise better than dark coloured ones. At the same time, young leaves oxidise in a shorter time. Coarse leaves need more Rolling process than fine leaves. In this respect, fine leaves, which are more easily shredded, oxidise better.

Figure 5. Oxidation Process of Tea Leaves

Drying

Drying is done to reduce the moisture content of tea leaves to 2-4%. The most important purpose of drying is to stop enzyme oxidation. In this way, the loss of the properties and substances obtained is prevented. There are mainly two oven settings for drying tea leaves. The first is the thickness (pallet) setting and the second is the speed (belt-pulley or variator) setting. The thickness setting allows the tea to be laid in thin and thick layers. The speed setting determines the time the tea stays in the oven.

The oxidised teas given to the oven come out of the oven in 32 minutes on the 1st belt, 27 minutes on the 2nd belt, 21 minutes on the 3rd belt, 17 minutes on the 4th belt and 12 minutes on the 5th belt. Ovens are generally operated on the 1st and 2nd belt.

Factors Affecting Drying

The temperature of the air entering the oven, the temperature flow rate, the time the tea stays in the oven and the thickness of the leaves laid on the pallet are among the factors affecting the drying process. While the inlet temperature in drying is 90-100^0 C, the outlet temperature is kept between 45-65^0 C according to the hood shape of the drying ovens.

Figure 6. Drying Process of Tea Leaves

Classification

In the sorting stage of tea leaves, the baked teas are passed through standard sieves and separated according to their thickness, fineness and quality. After the teas are baked, they can be well sorted with 2-4% moisture content. Since the humidity of the teas that are kept waiting and cannot be kept well will increase and gain elasticity, their classification cannot be done well and they become harmful to health by moulding in a short time. In the sieve frames of the grading sieves, sieve wires of 8-10-12-20-30 numbers are used. Sieve frames are placed in the form of 8-10-12-20-30 numbers from top to bottom on decked plates.

Figure 7. Sorting Process of Tea Leaves

Dry Tea Yield

Dry tea yield depends to a large extent on the moisture content of the Fresh leaf. This may vary not only according to weather conditions, but also due to the pruning period.

Factors affecting tea yield

a-) Collection standard

When the coarsely picked teas are excessively shredded in the rolling machines, the yield decreases.

b-) Fading degree

When over or under wilted leaves are shredded excessively in the crimping machines, the ratio of good grade and yield decreases.

c-) Fading Time

As the wilting time is prolonged, leaf transpiration consumes some of the available starch to provide energy, resulting in a reduction in dry matter content of up to 1 %.

d-) Fading temperature

Withering at high temperatures may result in a dry matter reduction of more than 1%.

e-) Dry matter of dried tea

An excess of 1% moisture in dry tea increases the yield value by 0.25% on average.

The moisture content of the green leaf varies from 69 to 83 %. The leaf with moisture lost on the surface can have a value between 69% and 76%. With this situation, the yield of tea quickly processed into product from dry day leaves (without surface moisture) collected from the plant varies between 31% and 24% (anonymous, 2020).

Black Tea Waste

It is thought that black tea waste can be used as an alternative to quality roughage in ruminant feeding. Turkey among the top 5 in world tea (Camellia Sinensis) production, and although the production area remains almost the same, the amount of production is gradually increasing. As of 2017, 1 million 300 thousand tonnes of fresh tea, 325.000 tonnes of dry tea (on DM basis), 334.750 tonnes of dry tea containing 2-3% moisture, 253.500 tonnes of black tea and 81.250 tonnes of black tea waste are produced in our country (Kacar, 1987; TURKSTAT, 2021).

Black tea waste, which occurs as a by-product during the production of black tea from fresh tea, causes a great environmental pollution, that is, ecological and economic damage, as it is not evaluated in any way other than being used as fuel and compost and it is thrown into nature in an uncontrolled manner. This waste, which is a food industry by-product but not used in any way other than fuel or compost, was found to be rich in total tannin (5.7%), energy (2442 kcal ME /kg DM), crude fiber (19.8%) and crude protein (18.2%) on dry matter basis (Çelebi et al., 2014).

In black tea processed in the factory and ready for consumption, theophylline constitutes 0.02%, theobromine 0.2-0.4% and caffeine 1-5% of its dry weight (Lin et al., 1998; Perva-Uzunalić et al., 2006; Yang et al., 2007). Caffeine in the tea plant is formed as a result of the breakdown of nucleic acids. Since this breakdown continues in the withering stage, the caffeine content of black tea increases. Volatile acids constitute 10-30% of total black tea volatiles (Çalıkoğlu and Bayrak, 2006). At the same time, tannins in tea have antioxidant, antiparasitic and antimicrobial effects (Şen and Erten, 2022).

Table 2. Chemical composition (% DM) of factory black tea waste (Çelebi et al., 2014).

Component	(%)
Dry matter	92.10
Crude protein	18.20
Crude fiber	19.80
Ether extract	1.60
Ash	5.80
ADF	35.40
NDF	46.40
ME (kcal/kg)	2442

ADF: acid detergent fibre, NDF: neutral detergent fibre, ME: Metabolic energy, kcal: calories, kg: kilogram

Tannin

Tannins are water-soluble polyphenolic compounds (condensed and hydrolyzable) that exhibit antinutritional effects on ruminants by forming complexes with ration proteins (Ağma Okur et al.) In addition to inhibiting the growth and activity of ruminal microflora, they limit nitrogen supply to animals (Goel, 2005). Hydrolyzable tannins are molecules containing polyols (usually D-glucose) as the core centre. The hydroxy groups of these polyols are partially or completely esterified with phenolic groups such as gallic acid (gallotanes) or elagic acid (elagitanes). Hydrolyzable tannins esterified with gallic acid are called gallotanenes and those esterified with elagic acid are called elagitanenes (Kanberoğlu, 2006). Tannic acid is an important gallotanene belonging to the hydrolyzable class (Hagerman, 1992). The most important difference of condensed tannins from hydrolyzable tannins is that they do not have a sugar-containing part (Albertse, 2002). There is another intermediate tannin group that combines the properties of hydrolyzable tannins and condensed tannins, this tannin class is called "catechin tannins" (Graham, 1992). The antioxidant activities of tea catechins are ranked as epigallocatechin gallate>epigallocatechin>epicatechin gallate>epicatechin. Phenols in tea are responsible for its antioxidant activity. It is reported that tea catechins, especially epigallocatechingallate, inactivate urease enzyme (Önenç et al., 2006). Hydrolysed tannins can be explained by the inhibition of microbial deaminase activity (Leinmüller and Menke, 1990). The role of tannins in animal nutrition is limited. While the tolerance threshold of tannins in poultry is <1%, this figure is reported to be 3-5% in cattle and 8-10% in sheep and goats (Şen and Erten, 2022).

Possibility of Using Tea Waste in Animal Feeding

Jayasuriya et al. (1978) reported that the waste generated after the use of black tea can be used up to 18% in ruminant rations and will significantly reduce the cost of the ration and increase net profit.

Factory black tea wastes containing tannin were added to the ration and used in the feeding of angora goats, but it was observed that they were not consumed willingly. It was reported that goats could consume a maximum of 150 g of tea waste during the acclimatisation period (İmik and Şeker, 1999).

Tea waste was used in various ratios in Akkaraman lamb rations and it was stated that tea waste used at the rate of 10% instead of oat straw did not have any negative effect on fattening performance, total ration dry matter digestibility and animal health (İmik et al., 2002).

It was reported that 0.5% green tea supplementation to the ration showed low plasma cholesterol levels in dairy cow (Crespy and Williamson 2004).

It was determined that green tea waste can be added to the ration of lactating cows as an alternative feed source to roughages up to 5.0% on a dry matter basis, and can even replace 25% of alfalfa hay to be added to the ration on a dry matter basis (Kondo et al., 2004).

It was found that the addition of tea seed saponins to rations containing different ratios of concentrate feed linearly decreased protozoal number, ammonia-N production and methane production, increased total gas production, short chain fatty acid production, metabolisable energy value measured at 24th hour of incubation, and significantly decreased *in vitro* true dry matter and organic matter digestibility (OMD) (Hu et al., 2005).

Nishida et al. (2008) reported that 20% green tea pulp supplementation to dairy cow rations did not have a negative effect on rumen fermentation and increased antioxidant activity and vitamin E levels in the ration.

In the silages made with black and green tea pulp additives, high amounts of lactic acid, propionic acid and butyric acid were observed at the end of 45 days of fermentation, while the amount of tannin decreased. In addition, it was reported that green tea pulp additive decreased the pH level of silage (Kondo et al., 2014).

Kaya et al. (2014) examined the effect of factory black tea waste on laying hens. It was reported that 2% and 4% tea waste supplementation to the ration showed high antioxidation effect.

It was reported that the addition of tea waste extract on 10% barley showed high by-pass effect (Nasehi et al., 2017).

When the factory wastes of the teas collected in different seasons (1st, 2nd and 3rd season) were evaluated, crude protein, *in vitro* ME and OMD values increased as the season period passed: NDF and ADF values decreased (Özyılmaz, 2019).

Conclusion

Feed costs constitute 70-80% of the costs in livestock enterprises. It is necessary to balance the ration for an economic animal husbandry. Roughages in the ration constitute an important part in terms of both efficiency and health for the animal. The deficit of roughage in Turkey has led researchers to search for alternatives. In particular, it is discussed that the products that are characterised as wastes, which occur during production in the factory, can be an alternative to roughage. Black tea waste is also important among these products. Depending on the production and factory processes, approximately 80 thousand tonnes of black tea waste is generated. When this waste is left to the nature, it causes environmental pollution. In addition, it is not economically beneficial because it is not utilised. In the studies carried out, the limited usability of black tea waste in animal feeding has been revealed by researchers. The factor that determines this limit is determined by the tannins in the tea. Livestock can tolerate tannin consumption up to a certain level. When the tolerance threshold is exceeded, negative effects on animal health can be seen. As a result, more studies should be carried out to reduce the tannin effect of factory black tea wastes in terms of animal nutrition. In this way, a waste product will be utilised to reduce the roughage deficit.

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RECENT ADVANCES IN THE POTENTIAL APPLICATIONS OF WATER TREATMENT FOR THE REMOVAL OF EMERGING CONTAMINANTS

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ABSTRACT

The rapid increase in industrialization has caused the generation of more compounds that have potential risks, resulting in serious water pollution issues for living beings and the environment. In this context, the presence of emerging contaminants (ECs) in water supplies, mainly from pharmaceutical products, cosmetics, pesticides, and dyes, is currently increasing global concern. The removal of these effluents, which have high toxicity, persistence, and bioaccumulation, using conventional methods cannot be fully successful. Biological treatment technologies are insufficient for non-biodegradable organic micropollutants. Hence, advanced oxidation processes (AOPs) including ozonation, Fenton, sonochemical, and heterogeneous photocatalysis methods, have been applied to the removal of ECs as an alternative method. Reactive radical species, in particular hydroxyl radicals, are formed, and these radicals are responsible for organic degradation. Among AOPs, heterogeneous photocatalysis has attracted great attention due to its effectiveness and versatility. Since these treatment methods have certain disadvantages, a combination of hybrid technologies can also be integrated to ensure efficient ECs removal from water.

Keywords: Advanced oxidation processes, emerging contaminants, heterogeneous photocatalysis, water treatment.

INTRODUCTION

Emerging contaminants (ECs) can be described as either a chemical or a material that causes a potential threat to human health or the environment by failing to meet the regulated health standards. A long daily list of products, including pharmaceuticals, cosmetics, plasticizers, pesticides, flame retardants, etc., are the sources of ECs. Besides, the presence of antibiotic-resistant genes and antibiotic-resistant bacteria in the environment may have a high potential for generating ECs (Berendonk et al., 2015; Pereira et al., 2015; Rout et al., 2021; Shahid et al., 2021).

ECs can be categorized as endocrine-disrupting chemicals, industrial and agricultural compounds, pharmaceuticals, and personal care products. Endocrine-disrupting chemicals is a term that describes compounds that interfere with the physical hormone systems and can cause damage to future generations. This group includes highly heterogeneous chemical molecules such as phthalates, bisphenol A, polybrominated diphenyl ethers, diethylstilbestrol, ethinyl estradiol, dichlorodiphenyltrichloroethane, etc. (Rout et al., 2021; Shahid et al., 2021). Insufficient treatment for recalcitrant organic pollutants in industrial and domestic wastewater treatment plants often leads to the presence of ECs in the discharged effluents (Morin-Crini et al., 2022). Pharmaceuticals and personal care products are detected as EC around 70%, in environment samples. For instance, antibiotics, antidepressants, lipid regulators, analgesic compounds, 4-benzophenone, and sunscreen agents are ECs usually observed in wastewater (Rout et al., 2021). Improper discharge of pharmaceuticals and personal care products into water has become as the use of drugs, veterinary medicines, and cosmetics is increasing , leading to adverse effects on humans and wildlife (Ebele et al., 2017).

The long-term exposure to the constant release of ECs has been reported to have common adverse environmental effects and can thus result in chronic effects on aquatic life and human and animal health due to their tendency to form complex, persistent, and bioaccumulation potentiality in nature. Consequently, existing water treatment methods are crucial to remove ECs from water to deal with this pollution issue (Morin-Crini et al., 2022; Rout et al., 2021).

WASTEWATER TREATMENT PROCESSES

Conventional wastewater treatment processes are standard methods to eliminate suspended and colloidal particulates, dissolved organics, nutrients, and pathogens. Coagulation, flocculation, adsorption, and sedimentation are the main processes used for suspended solids in conventional water treatment systems. Activated carbon, biochar, carbon nanotubes, and clay minerals are widely used as adsorbent materials. These processes are not sufficiently designed for EC removal since the application can only occur by adsorption of the prepared flocs (Rodriguez-Narvaez et al., 2017; Rout et al., 2021; Shahid et al., 2021). The biological processes follow a biodegradation mechanism using algae, fungi, and bacteria. However, no single biological treatment method is effective for most ECs (Saidulu et al., 2021).

Advanced oxidation processes (AOPs) are believed to be a promising and effective wastewater treatment containing a wide range of effluents that lead to a better quality unharmful species. The main principle is based on the generation of short-life reactive species, such as highly oxidized hydroxyl radicals (Ahmed et al., 2017; Quiñones et al., 2015a).

Ozone technology occurs through the direct attack of ozone reaction on ECs, and then a radical species generation process that follows highly rapid reactions with pollutants. The high operating cost is the major drawback of the ozonation process. Moreover, this process is not usually capable of mineralizing the organic pollutants in domestic wastewater since it has a selectivity nature, and this can lead to the presence of ozonation by-products in the effluents. For this reason, the combined use of ozone and agents such as UV light and/or catalysts can increase the mineralization efficiency of ozonation (Almomani et al., 2016; Quiñones et al., 2015a). A pilot-scale application using solar photocatalytic ozonation reported a high mineralization efficiency of ECs (Quiñones et al., 2015b).

The Fenton process is one of the most studied AOPs in that the hydroxyl radical plays a dominant role, and electron transfer occurs between hydrogen peroxide as the oxidant and the dissolved Fe^{2+} ion as the catalyst at pH = 2-3 in acidic conditions (Salimi et al., 2017). However, a large volume of $Fe(OH)_3$ sludge forming in this process is required to apply an additional treatment. Photo-Fenton (Fe^{3+}/H_2O_2/UV-light) was developed as an alternative method to overcome this issue. There are several studies reported on the degradation of ECs using photo-Fenton application (Cavalheri et al., 2023; Clarizia et al., 2017; De la Cruz et al., 2012; Machado et al., 2023).

Among the AOPs, heterogeneous photocatalysis is recognized as a preferred green technology with safety and cost effectiveness characteristics. This method has attracted attention, especially TiO_2 based, for the removal of ECs in wastewater (Arfanis et al., 2017; Chen et al., 2021; Chen et al., 2023; Gurkan et al., 2012; Miranda-García et al., 2011; Salimi et al., 2017). Although photocatalysis is efficient for EC removal, its widespread application and commercialization has faced notable challenges such as catalyst reusability, operating conditions, and reactor designs (Fu et al., 2023; Zhang et al., 2023).

CONCLUSIONS

Despite substantial studies on the removal of ECs from wastewater, most wastewater treatment research is laboratory-based and has been applied using synthetic water. Further investigations are recommended to expand the existing laboratory knowledge and upscale into pilot plants by designing a prototype reactor. The contribution of hybrid water treatment methods such as a combination of AOP and biological processes can be used to achieve efficient and economical treatment goals. Overall, additional research is essential to develop more profitable and environmentally friendly nanomaterials/catalysts to cope with industrial wastewater treatment and achieve a sustainable future and cleaner environment.

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THE PHOTOCATALYTIC DEGRADATION OF A THIAZINE DYE VIA CuO NANOPARTICLES

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ABSTRACT

Water pollution containing dyes is a serious concern for human health and the environment. Photocatalysis is a wastewater treatment process that can be carried out to remove these dyes from water. Photocatalysts play a key role in this environmentally friendly and cost-effective technique. CuO is a p-type metal oxide that has attracted great interest during the past years since it is non-toxic, easy-handle, and has high optical absorption.

This study focused on evaluating the efficiency of methylene blue (MB) upon using CuO photocatalyst under UV light irradiation. MB was selected as the model of a thiazine cationic dye. Besides, CuO nanoparticles were characterized by FT-IR, XRD, SEM, and Raman spectroscopy. The morphology of the CuO photocatalyst consisted of various-shaped polyhedral particles. The XRD diffractogram of CuO revealed the characteristic monoclinic structure of the CuO phase. This work may provide a promising transition metal-based catalyst for the degradation of wastewater containing thiazine dyes released from different industries.

Keywords: CuO, decolorization, heterogeneous photocatalysis, methylene blue, thiazine dye.

INTRODUCTION

Advanced oxidation processes (AOPs) with their strong oxidation capabilities can be a potential innovative solution for wastewater treatment. The mechanism of action of AOPs, particularly photocatalysis, is commonly carried out by reactive oxygen species such as hydroxyl radicals, which degrade the recalcitrant pollutants to CO_2, H_2O, and inorganic acids (Mahbub and Duke, 2023; Saviano et al., 2023). Photocatalysis is an environmentally friendly, energy-saving, and sustainable process that can be applied to the breakdown of dyes and effluents in wastewater from the textile industry (Okpara et al., 2023).

Methylene blue (MB) is a popular and highly consumed cationic dye widely used in the textile industry for coloring silk, wool, and cotton. It is a toxic, carcinogenic, and non-biodegradable thiazine dye that can leach into wastewater and cause environmentally hazardous impacts on human and aquatic life. (Doan, 2023; Khan et al., 2022). Therefore, the efficient removal of MB is essential before discharging into water bodies. Recently, photocatalysis has been used as a beneficial water treatment method for the photocatalytic degradation of this harmful dye (Din et al., 2021). In this regard, metal oxide-based photocatalysts, particularly CuO, have attracted much consideration due to their excellent physical and chemical properties as a p-type semiconductor (Raizada et al., 2020; Sibhatu et al., 2022). CuO is an environmental photocatalyst applied on the photocatalytic degradation of dyes such as basic violet 3 (Sorbiun et al., 2018), congo red (Arunadevi et al., 2018), MB

(Akter et al., 2021; Bruno et al., 2021; George et al., 2022; Rafique et al., 2023; Vimala Devi et al., 2017), methyl red (Tamuly et al., 2014), MB and methylene violet (Sonia et al., 2015), direct red 81 and victoria blue (Singh and Bansal, 2020), methyl green and methyl orange (Aroob et al., 2023).

In this study, CuO nanoparticles were characterized using Fourier transform infrared spectrometer (FTIR) with attenuated total reflection (ATR), Raman spectroscopy, X-ray diffraction (XRD), and Scanning electron microscopy (SEM) spectroscopic techniques. The photocatalytic degradation efficiency of CuO nanoparticles was also investigated in the presence of MB under UV light irradiation.

MATERIAL AND METHOD

Copper(II) oxide (CuO, ACS) was obtained from Thermo Scientific and MB ($C_{16}H_{18}ClN_3S{\cdot}2H_2O$) was purchased from Merck. The chemical structure of MB dye (λ_{max} = 664 nm, MW = 319.85 g/mol) was given in Figure 1. The chemicals were used without further treatment. All aqueous solutions were prepared with distilled water.

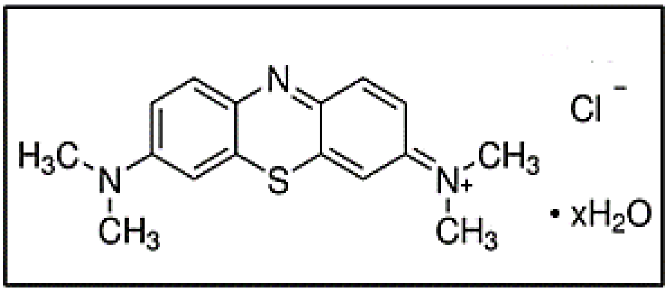

Figure 1. The chemical structure of MB.

FTIR spectra were recorded in the range of 4000–400 cm^{-1} using a Thermo Scientific Nicolet 6700 spectrometer equipped with an attenuated total reflection accessory. Dispersive Raman spectroscopic measurement was performed on a Thermo Scientific DXR Raman Microscope with an applied Ar^+ laser power of 10 mW at λ = 532 nm. The XRD diffractogram was carried out on a Rigaku-D/MAX-Ultima diffractometer with Cu Kα radiation at λ = 1.54 Å. The diffraction intensity was obtained in the range of 5-80° with a scan rate of 2° min^{-1}. SEM analysis was performed on a FEI-Philips XL30 Scanning Electron Microscope with an accelerating voltage of 10 kV.

The photocatalytic activity tests were carried out in a cylindrical Pyrex reaction vessel. A black-light fluorescent lamp (125 W, λ_{max} = 365 nm) irradiated from the top of the reactor as the light source. The light intensity was $I_o = 1.65 \times 10^{16}$ quanta/sec (Parker, 1997). The photocatalytic experiments were performed without pH adjustment. CuO nanoparticles dose amount used in experiments was 0.25 g/L and the initial MB concentration was 10 mg/L. The irradiated solution (50 mL) was immediately filtered through 0.22 µm cellulose acetate filters. The absorbances of the specimens were monitored by a Thermo Scientific Genesys 10S double beam spectrophotometer.

RESULTS AND DISCUSSION

FTIR spectroscopy was utilized to examine the presence of the functional groups in CuO nanoparticles and the spectrum was presented in Figure 2. The observed bands at 420 cm^{-1}, 471 cm^{-1}, 523 cm^{-1}, and 594 cm^{-1} could be related to the characteristic Cu-O stretching vibration modes (Islam et al., 2021).

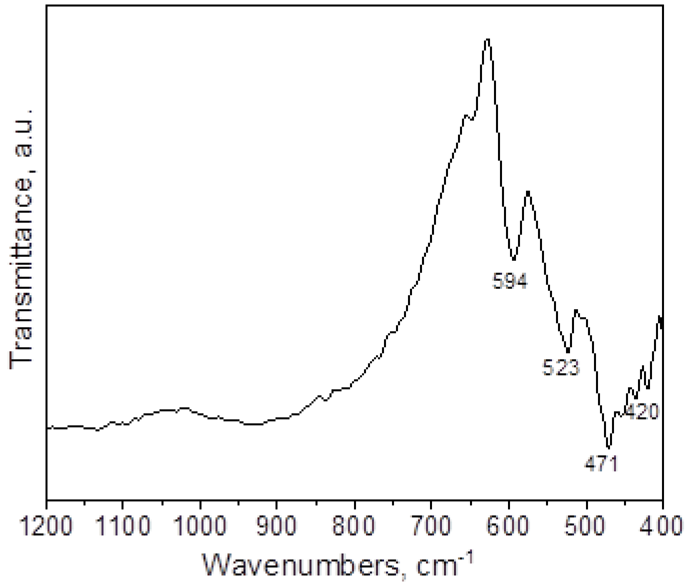

Figure 2. FTIR spectrum of CuO nanoparticles.

The Raman spectrum of CuO nanoparticles was displayed in Figure 3. The three bands located at 293 cm^{-1}, 343 cm^{-1}, and 633 cm^{-1} were attributed to the A_g, B_{g1}, and B_{g2} modes of CuO, respectively (Islam et al., 2021).

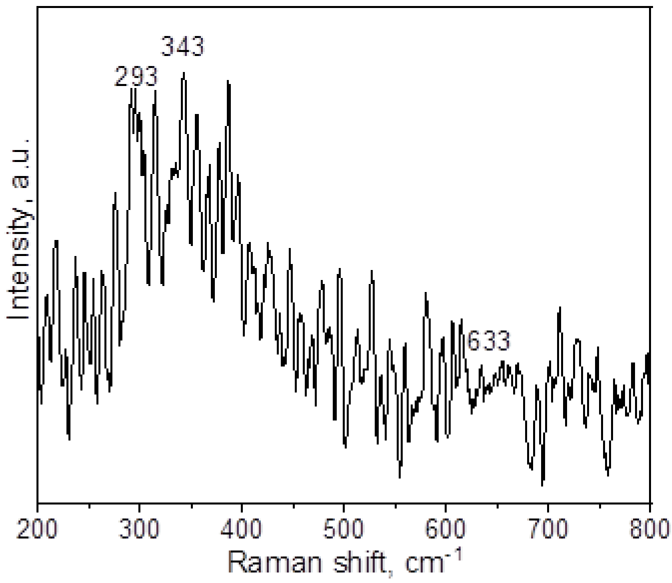

Figure 3. Raman spectrum of CuO nanoparticles.

The XRD diffractogram of CuO revealed two intense peaks at 2θ = 35.56° and 38.72°, which were related to the characteristic planes of (−1 1 1) and (1 1 1) monoclinic structure of CuO, respectively. The other diffraction peaks observed at 2θ = 32.54°, 48.80°, 53.36°, 58.32°, 61.56°, 65.84°, 66.30°, 68.12°, 72.44°, and 75.02° corresponded to the (1 1 0), (−2 0 2), (0 2 0), (2 0 2), (−1 1 3), (0 2 2), (−3 1 1), (2 2 0), (3 1 1), and (0 0 4) planes of monoclinic CuO crystals (space group C2/c). The data was in accordance with the standard (JCPDS card no. 89-5895). The crystallite size of CuO nanoparticles was calculated using the Scherrer equation (Equation 1) with reference to the planes (−1 1 1) and (1 1 1) of CuO.

$$D = K \lambda / (\beta \cos\theta) \tag{1}$$

where K = 0.9, λ is the X-ray wavelength (1.5418 Å), θ is the Bragg angle, and β is the full width at half maximum intensity (FWHM, radians) (Scherrer, 1918). The calculated crystallite sizes of CuO were 31 nm and 26 nm with reference to planes (−1 1 1) and (1 1 1), respectively.

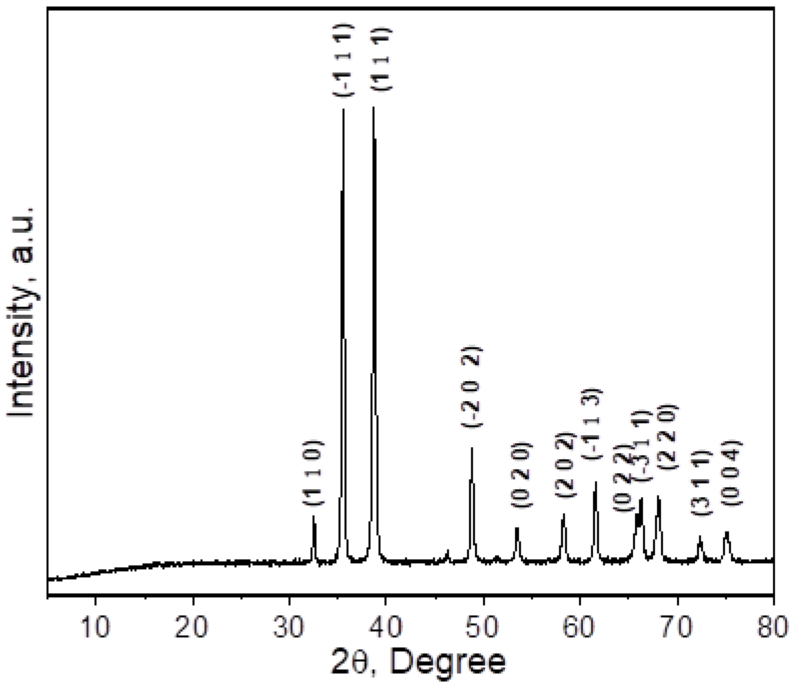

Figure 4. XRD spectrum of CuO nanoparticles.

SEM images of CuO nanoparticles were presented in Figure 5. The morphology of CuO nanoparticles consisted of a variety of polyhedral shaped particles.

Figure 5. SEM images (left) x100000, (middle) x50000, (right) x20000 of CuO nanoparticles.

The degree of MB decolorization by using CuO nanoparticles (Figure 6) was calculated by the following equation (1).

Decolorization, % = $((A_{,o} - A)/A_o))$ x100 (1)

where,

$A_{,o}$ = initial absorbance of MB and A = absorbance of MB at irradiation time t.
The photocatalytic degradation of MB in the presence of CuO upon 300 min irradiation was 36%.

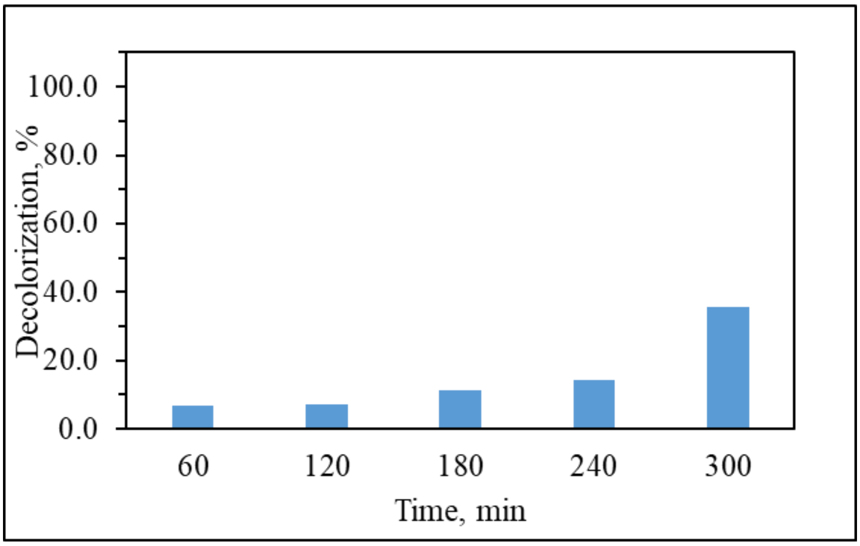

Figure 6. Removal efficiencies of MB upon using CuO nanoparticles.

CONCLUSIONS

In summary, the structural and morphological properties of CuO nanoparticles were identified. FTIR spectrum indicated the presence of functional groups belonging to CuO. XRD analysis confirmed the structure of monoclinic CuO nanoparticles. The surface morphology CuO consisted of various polyhedral shaped particles. The photocatalytic degradation of MB in the presence of CuO nanoparticles under UV light irradiation was found to be 36%.

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ALGAL COENOSES OF SHALLOW ROCKY COASTS OF THE ADRIATIC SEA IN ALBANIA

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Studies on algae of shallow rocky coasts in Albania are limited and so are the existing data. The aim of this study is to know species composition and algal cover in the rocky coasts of the Adriatic Sea in Albania, as well as their seasonal differences between the study areas.
The study was carried out in the all rocky areas of the Albanian Adriatic coastline, namely Cape Rodoni, Kallm, Spille, and Triport in the spring, summer, and autumn seasons.
The study presents the list of species composition of macroalgae for each studied area and the percentage of algal cover. Comparisons of these data were made between the four studied areas and between the three seasons. The dominant classes of algae wer: Cyanophyceae; Florideophyceae with families Rodomelaceae, Corallinaceae, Rhodymeniaceae, Callithamniaceae; Phaeophyceae with families Sargassaceae, Dictyotaceae, Stypocaulaceae; Ulvophyceae with families Ulvaceae, Caulerpaceae, Cladophoraceae. Kallmi and Triport areas had the highest algal cover in the spring and autumn seasons, while Kallmi and Spille had the highest algal cover in the summer season. In the Spille area it was recorded a large number of algal species, which were not found in the other three areas.
In this paper are also given considerations on possible natural and anthropogenic factors that affect the algal coenoses of the studied areas.

Key words: marine macroalgae, algal cover, species composition, natural and anthropogenic impacts.

Introduction

The macroalgae of the rocky shores of the Albanian coast of the Adriatic Sea have been poorly studied. The existing data on macroalgae coenoses are very few and sporadic.
Some important studies on the macroalgae of the Albanian Adriatic coast have been published in Anonymus, 2002; Ercegovic A.,1952; Ercegovic A., 1960; Kashta L., 1987, 1992-93; Kashta L. & Pizzuto, F., 1995; Kashta L., 1995-1996; Kashta L., 1995-1996, 1999, 2006; Xhulaj M. & Kashta L., 2007. Most of the existing data are from deep infralittoral and circalittoral, while the shallower parts, including midlittoral and upper infralittoral have been poorly investigated. Most recent data belong to assessments mainly related to proclamation of marine protected areas in the Adriatic coast of Albania, and they are mainly presented in technical projects reports and rare in scientific papers, such as Beqiraj et al. (2011), Beqiraj & Kashta (2014), Beqiraj et al. (2014), Blanfuné et al. (2016), Gogo & Kashta (2013), Kashta et al. (2005), Kashta & Beqiraj (2009), Kashta et al. (2010), Fraschetti et al. (2011), Maiorano et al. (2011). Some data have also been presented in students' master theses and PhD theses, but not presented in scientific publications, like journals, conferences, or other scientific events.
Rocky areas in the Albanian part of Adriatic Sea are very short segments and very sensitive in ecological and environmental point of view. During the three last decades, the environmental

impact is considerably influenced by the urban and touristic development of the country (Fraschetti et al. 2011).

Materials and methods

Benthic samples have been taken during three seasons: spring, summer and autumn, in four rocky coastal areas along the Adriatic coast of Albania: Rodoni Cape, Kallm, Spille and Triport (Figure 1). Samples of macroalgae were taken in shallow water, including the supralittoral, midlittoral and upper limit of infralittoral. The samples were taken through standard methods for benthic sampling in hard bottoms, after the methods of Bianchi et al. 2004, Salomidi 2003, and Zenetos et al. 2000. In each site the sampling was done along three transects, distanced 50 m from each other.

Total algal cover in percentage has been evaluated in all sampling sites. It has been evaluated the species composition in each site, cover in percentage of each species in each sample, and the average cover of each species in each site. A comparison between sampling sites has been conducted regarding the differences in macroalgal cover and species number. Identification of macroalgae was based on atlases, identification keys, monographs and other relevant publications, referring to Cerrano et al. (2004), Mojetta & Ghisotti (1994), Riedl (2010), Trainito (2011). Taxonomic classification of macroalgae has followed the system of WoRMS (World Register of Marine Species).

Figure 1. Map of Albania with the sampling sites: 1. Rodoni Cape; 2. Kallm; 3. Spille, 4. Triport


a) Rodoni Cape b) Kallm


c) Spille d) Triport

Figure 2. Photos of the sampling sites, a) Rodoni Cape, b) Kallm, c) Spille, d) Triport.

Results and discussions

The total number of macroalgae taxa recorded in the three seasons in the four studied areas was 50 (Appendix 1), of which 43 taxa were found in spring, 41 in summer and 25 taxa in autumn. The highest number of taxa was recorded in Kallm (41) and the lowest number of taxa was recorded in Rodoni Cape (21). The low number of taxa in Rodoni Cape maybe related to the exposure of the coast, under direct impact of the waves. The impact of pollution from beach tourism can be considerable at this site, taking into consideration the fact that the sea currents in this area have a south-north direction (according to Pano 2015). About 1 km in south of this site lies the beach of Saint Peter (Shën Pjetër), which is quite populated during the summer.

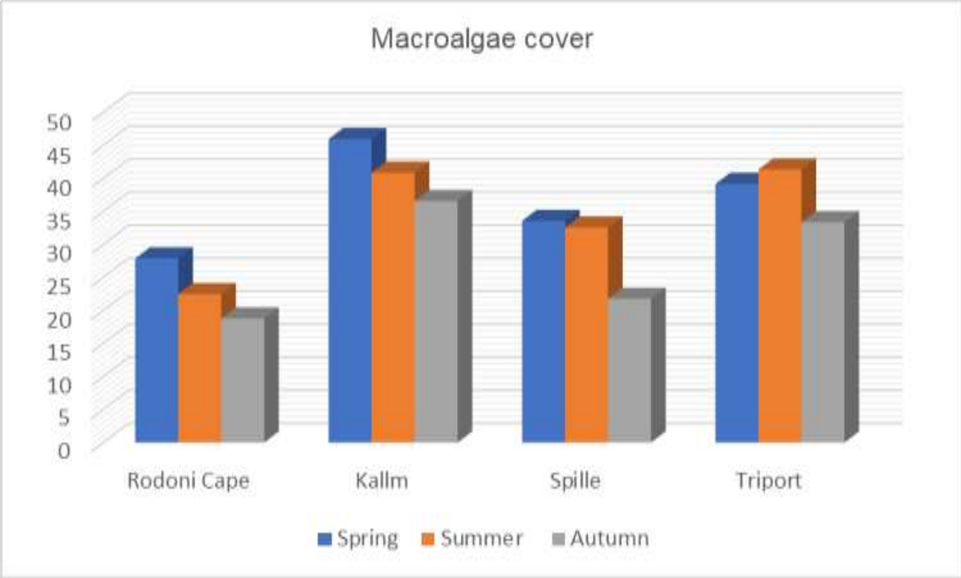

Figure 3. Macroalgae cover in percentage for each sampling site in each season.

As it is shown in figure 3, Kallm was the site with the highest macroalgae cover in the three seasons followed by Triport, which in summer had the highest cover from all sites. The lowest macroalgae cover was found in Rodoni Cape. The lowest algal cover in Rodoni Cape, also corresponds to the lowest number of species found in this site compare to the other sites.

A difference in algal cover and also in species composition has been evident between seasons, too. The highest number of species and the highest algal cover has been recorded in spring season in all sampling sites (Fig. 4). Kallm represents the site with the highest macroalgae cover and number of species in three seasons, with the exception of Triport, which in summer presents the highest algal cover compared to the other sites.

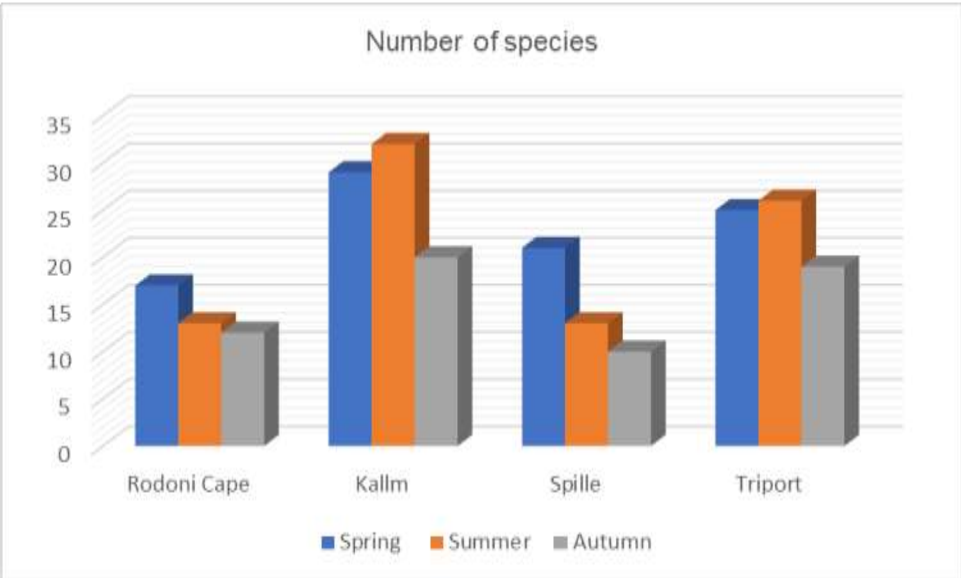

Figure 4. Number of macroalgae species found in each sampling site in each season.

From the **Chromista** kingdom there were recorded 16 taxa belonging to: Phylum Ochrophyta , part of Class Phaeophyceae represented by Fam. Sargassaceae (7 species), Fam.

Dictyotaceae with 6 species, Fam. Stypocaulaceae with 2 species, and Fam. Fucaceae with only one species. From the **Plantae** kingdom there were recorded 34 taxa belonging to Phylum Rhodophyta and Phylum Chlorophyta. Rhodophyta was recorded with 21 species, distributed in 2 classes and 11 families as below: Class Florideophyceae represented by Fam. Rhodomelaceae with 6 species, Fam. Corallinaceae (4 species), Fam. Callithamniaceae with 2 species, Fam. Delesseriaceae with 2 species and families Ceramiaceae, Phyllophoraceae, Cystocloniaceae, Sphaerococcaceae, Rhodymeniaceae, Peyssonneliaceae with 1 species recorded; Class Bangiophyceae with Fam. Bangiaceae with 1 species recorded. Rhodophyta represented the largest number of species during the whole period of this study. Phylum Chlorophyta was recorded with 13 species distributed in 7 families, part of Ulvophyceae. Ulvophyceae was represented by Fam. Ulvaceae (4 species), Fam. Cladophoraceae (3 species), Fam. Halimedaceae (2 species), and families Valoniaceae, Caulerpaceae, Dasycladaceae, and Polyphysaceae with 1 species recorded. The most represented families in species number were Sargassaceae (7 species), followed by Dictyotaceae and Rhodomelaceae with 6 species and afterwards Corallinaceae and Ulvaceae with 4 species each.

Number of species of each class was as following: Floridophyceae with 20 species, Phaeophyceae with 16 species, Ulvophyceae with 13 species, Bangiophyceae and Cyanophyceae with 1 species.

Class Floridophyceae was the class with the highest species diversity, while the class with the highest cover was Phaeophyceae.

In this study an invasive alien macroalgae species was found, Caulerpa racemosa (Forsskål) J.Agardh, 1873 of the family Caulerpaceae, known as native to Australia. This species was found in Kallm and Triport sites. Also, another important species found in this study was *Fucus virsoides* J.Agardh, 1868 of the family Fucaceae that is an endemic species in the Adriatic Sea. This species was found in Rodoni Cape, Kallm and Spille sites. In most of existing databases, Albania has not been mentioned as a distribution site of *Fucus virsoides*, although its presence in the Albanian coast has been published since many years already, in Kashta, 1995-1996. Albania should be considered as the most southern distribution of this species in the Adriatic Sea.

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Appendix 1. The list of macroalgae species and their systematic position

Taxa	Rodoni Cape	Kallm	Spille	Triport
Chromista				
Ochrophyta				
Phaeophyceae				
Fucales				

Sargassaceae				
1. *Treptacantha barbata* (Stackhouse) Orellana & Sansón, 2019	+	+	+	+
2. *Cystoseira crinita* Duby, 1830		+	+	+
3. *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, 1975	+	+	+	+
4. *Ericaria amentacea* (C.Agardh) Molinari & Guiry, 2020	+	+	+	+
5. *Cystoseira foeniculacea* (Linnaeus) Greville, 1830		+	+	+
6. *Cystoseira* C.Agardh, 1820			+	+
7. *Sargassum vulgare* C.Agardh, 1820		+	+	+
Fucaceae				
8. *Fucus virsoides* J.Agardh, 1868	+	+	+	
Dictyotales				
Dictyotaceae				
9. *Dictyopteris polypodioides* (A.P.De Candolle) J.V.Lamouroux, 1809	+	+	+	+
10. *Dictyota dichotoma* (Hudson) J.V.Lamouroux, 1809	+	+		+
11. *Dictyota fasciola* (Roth) J.V.Lamouroux, 1809		+		
12. *Dictyota* J.V.Lamouroux, 1809	+	+		+
13. *Padina pavonica* (Linnaeus) Thivy, 1960	+	+	+	+
14. *Taonia atomaria* (Woodward) J.Agardh, 1848			+	
Sphacelariales				
Stypocaulaceae				
15. *Halopteris scoparia* (Linnaeus) Sauvageau, 1904	+	+	+	+
16. *Halopteris filicina* (Grateloup) Kützing, 1843		+		+
Plantae				
Rhodophyta				
Florideophyceae				
Ceramiales				
Ceramiaceae				
17. *Ceramium virgatum* Roth, 1797		+	+	+
Rhodomelaceae				
18. *Laurencia obtusa* (Hudson) J.V.Lamouroux, 1813	+	+		+
19. *Palisada perforata* (Bory) K.W.Nam, 2007		+		

20. Laurencia J.V.Lamouroux, 1813	+	+	+	
21. Rytiphlaea tinctoria (Clemente) C.Agardh, 1824		+	+	+
22. Alsidium corallinum C.Agardh, 1827				+
23. Halopithys incurve (Hudson) Batters, 1902	+	+		+
Callithamniaceae				
24. *Callithamnion granulatum* (Ducluzeau) C.Agardh, 1828			+	
25. Spyridia filamentosa (Wulfen) Harvey, 1833		+		
Delesseriaceae				
26. Dasya baillouviana (S.G.Gmelin) Montagne, 1841			+	
27. Dasya C.Agardh, 1824		+		
Corallinales				
Corallinaceae				
28. *Jania virgata* (Zanardini) Montagne, 1846	+	+	+	+
29. Ellisolandia elongata (J.Ellis & Solander) K.R.Hind & G.W.Saunders, 2013		+	+	+
30. Corallina Linnaeus, 1758	+	+	+	+
31. Jania rubens (Linnaeus) J.V.Lamouroux, 1816		+		
Gigartinales				
Phylloporaceae				
32. *Phyllophora crispa* (Hudson) P.S.Dixon, 1964			+	
Cystocloniaceae				
33. Hypnea musciformis (Wulfen) J.V.Lamouroux, 1813	+	+	+	+
Sphaerococcaceae				
34. *Sphaerococcus coronopifolius* Stackhouse, 1797		+		
Rhodymeniales				
Rhodymeniaceae				
35. Botryocladia botryoides (Wulfen) Feldmann, 1941	+	+		
Peyssonneliales				
Peyssonneliaceae				
36. Peyssonnelia heteromorpha (Zanardini) Athanasiadis, 2016				+
Bangiophyceae				
Bangiales				

Bangiaceae				
37. *Bangia fuscopurpurea* (Dillwyn) Lyngbye, 1819		+		
Chlorophyta				
Ulvophyceae				
Ulvales				
Ulvaceae				
38. *Ulva linza* Linnaeus, 1753		+		
39. *Ulva lactuca f. rigida* (C.Agardh) Hylmö		+	+	+
40. *Ulva intestinalis* Linnaeus, 1753		+		
41. *Ulva* Linnaeus, 1753	+	+	+	+
Cladophorales				
Cladophoraceae				
42. *Cladophora fracta f. prolifera* (C.Agardh) Rabenhorst	+	+	+	+
43. *Cladophora* Kützing, 1843		+	+	
44. *Chaetomorpha aerea* (Dillwyn) Kützing, 1849				+
Valoniaceae				
45. *Valonia utricularis* (Roth) C.Agardh, 1823		+		
Bryopsidales				
Halimedaceae				
46. *Halimeda tuna* (J.Ellis & Solander) J.V.Lamouroux, 1816	+	+		+
47. *Flabellia petiolata* (Turra) Nizamuddin, 1987				+
Caulerpaceae				
48. *Caulerpa racemosa* (Forsskål) J.Agardh, 1873		+		+
Dasycladales				
Dasycladaceae				
49. *Dasycladus vermicularis* (Scopoli) Krasser, 1898	+	+		+
Polyphysaceae				
50. *Acetabularia acetabulum* (Linnaeus) P.C.Silva, 1952	+	+		+
Total	**21**	**41**	**26**	**32**

MOLLUSCS FROM HIMARA COAST, IONIAN SEA, ALBANIA

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Abstract

Himara is part of the Ionian coast in southern Albania. Data on molluscs of the shallow coast of Himara are limited, while there are more detailed data for the deeper waters of the infralittoral of this area. This study was carried out in four sites of this area: Guma, Llaman, Porto Palermo, and Qeparo.
Molluscs were collected on shallow rocky shores in supralittoral, midlittoral and the upper part of the infralittoral, in October 2022. This study provides data on species composition and abundance of the mollusc populations. The mollusc groups with the highest presence and abundance were gastropods of the families Patellidae, Trochidae, Cerithiidae, Littorinidae, Risoiidae, and bivalves of the Family Mytilidae.Among the species found in this study are threatened and alien species for the Mediterranean Sea. Data on the species composition and abundance of the molluscs populations were analyzed in comparison between the four sites of the study. Considerations on possible factors affecting the mollusc populations in the studied area are also presented.

Key words: marine malacofauna, threatened species, alien species, algal cover.

Introduction

Himara lies along the Ionian coast of Albania. It is situated in the southwestern part of the country, from the extreme south of Llogara Pass to Qeparo Mountain in southeast, with a straight length of 22 km. The total length of coastline, rocky coast and clefts is about 26 km (Pano, 2015). Southern Coast is crucial for marine biodiversity as flora and fauna include species of various origin.
Existing studies on malacofauna of the Himara coast are scarce and related to deep infralittoral and circalittoral , mainly focused on the evaluation of areas proposed as marine protected areas (Beqiraj et al., 2008; Beqiraj & Kashta, 2013; Beqiraj, 2014; Beqiraj & Ballesteros, 2018; Fraschetti et al., 2011; Kashta et al., 2005; Kashta et al., 2007; Kashta & Beqiraj, 2009; Pititto et al., 2009). Also, there are few studies on marine alien species of Albania, including the coast of Himara (Beqiraj & Zenetos, 2021; Katsanevakis et al., 2011; Zenetos et al., 2016). Meanwhile, there are no studies on the rocky shore communities of the Himara coast. This is the first study on malacofauna of the rocky shores of this area. The marine and coastal environment of this area has high-value economic, social and ecological recourses for the country, but, on the other hand, it represents one of the most vulnerable territory from tourism development.
The area from Porto Palermo bay, to Llamani Bay, has been proclaimed a Marine and Coastal Protected Area in July 2022.

Material and Method

Sampling was carried out in autumn season, October 2022, according to standard methods for benthic sampling in hard bottoms, after Bianchi et al. 2004, Salomidi 2003, and Zenetos et al. 2000 Sampling was carried out in shallow waters at a depth of up to 1m in four sites, from north to south of the coast of Himara: Guma, Llamani Bay, Porto Palermo Bay and Qeparo. The sampling aimed at collecting molluscs sheltered in algae as well as on the bare rocks. Algal cover samples were also taken, in order to get a more complete knowledge of the biocenoses.

For each site, it was sampled in 3 transects, at a linear distance of 50-100 m from each other. For each transect, 3 samples were taken. A total of 9 samples were taken for each site, and a total of 36 samples were taken for all (4) sites. Samples were taken quantitatively by collecting and evaluating the malacofauna within a 50 cm x 50 cm frame. This frame was divided into 16 small squares in order to facilitate quantitative assessment of the molluscs. Within these squares, the number of individuals or the cover in percentage for colonial animals, such as Melarhaphe, Mytilaster, Vermetus, etc., was evaluated. On bare rocks the collection of the molluscs was done by hand and forceps. On rocks covered by macroalgae, the whole algal cover with all included invertebrates has been collected within the sampling frame. After sampling, the collected material was stored in 4% formaldehyde and transported to the laboratory.

The taxonomic identifications of molluscs were mainly based on existing literature from the Mediterrenean, as well as larger databases: Cossignani 1992, Clemam checklist, D'Angello & Gargiullo 1991, Gianuzzi-Savelli 1994, 1997, 1999, 2001, 2003, Pope & Goto 1991, 1993, Riedl 1991, Millard 2001. The systematic position of molluscs was referred to WoRMS (World Register of Marine Species).

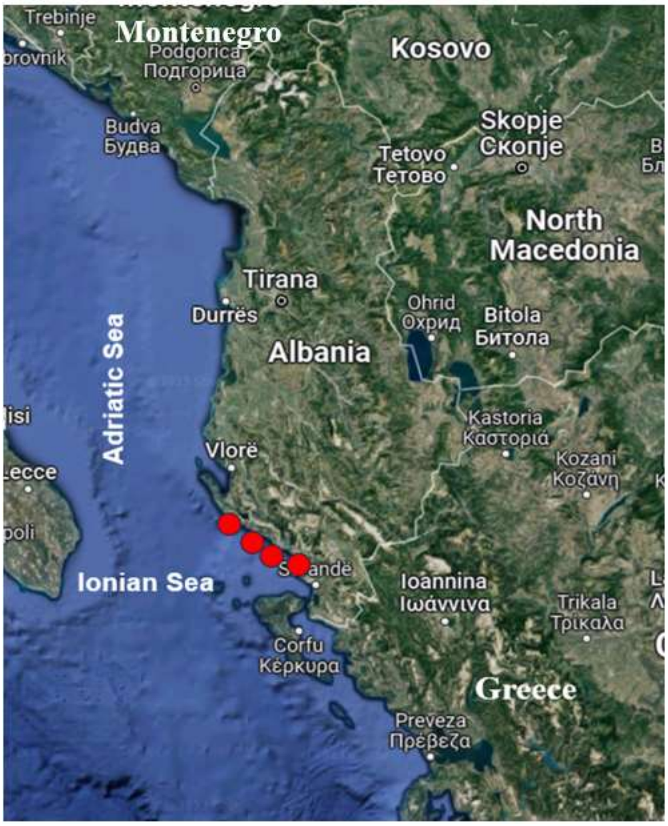

Figure 1. Map of the Himara coast, with the sampling sites indicated in red color.

a) Guma b) Llaman

c) Porto Palermo d) Qeparo

Figure 2. Photos from the four sampling sites, a) Guma, b) Llaman, c) Porto Palermo, d) Qeparo.

Total abundance and average abundance for each species and for each sampling site have been evaluated. The following indexes and coefficients have been evaluated:

1. Constancy: C = a / p * 100 where: C – Constancy

a – the number of samples where the assessed species is present

p – Total number of samples.

According to Blanc et al. 1976 , based on the value of the costancy, the following classification of species has been done:
- Constant species (K > 50%);
- Accompanying species (25% ≤ K ≤50%);
- Occasional species (K < 25%)

2. The species similarity coefficient Sokal & Sneath (after Blanc et al., 1976) has been evaluated, according to the formula in the following:

i = a / a+2*(b+c) where:
a – the number of common species for both sites;
b – the number of species present only in the first site;
c – number of species present only in the second site.

3. In order to evaluate the degree of diversity, the following indexes were calculated and evaluated according to Begon et al., 2006, for each season and sampling station:
   a) Shannon-Weaver $H'=\sum$ pi ln (pi)
   b) Pielou: $J= H'/ \ln S$
   c) Margalef: $M = (S-1) / \ln N$
   d) Simpson $(1/D) = 1/ \sum (pi^2)$,

where:
p = n/N
n = number of individuals of one particular species
N = total number of individuals
S = number of species.

Results and discussion

The total number of taxa recorded in the four studied areas was 60, and belong to three classes of molluscs: Polyplacophora, Gastropoda and Bivalvia, with the highest number of species recorded from gastropods, with 40 species (see Appendix 1).

The total number of taxa recorded for each site was: Guma 31, Llaman 20, Porto Palemo 24 and Qeparo 32. As shown in Appendix 1, the number of species that were found in only one site were 9 in Guma,4 in Llaman, 6 in Porto Palermo, and 13 in Qeparo. The largest number of gastropods was found in Guma and the lowest number in Llaman, while the largest number of bivalves was found in Qeparo, followed by Llaman, while the lowest number of bivalves was found in Guma (Appendix 1). The species with the highest average abundance for each site were respectively, in Guma: *Pisania striata* (4.4), *Phorcus (Monodonta) turbinatus* (3.28), *Columbella rustica* (6.28) and *Patella caerulea* (5.71); in Llaman : *Musculus costulatus* (9), *Modiolus adriaticus* (9.83), *Mytilus galloprovincialis* (4.16), *Patella caerulea* (3.16); in Porto Palermo: *Musculus costulatus* (40.33), *Pisania striata* (9.32), *Phorcus (Monodonta) turbinatus* (8.88), *Bittium reticulatum* (9.66), *Patella ulyssiponensis* (5.11), *Patella rustica* (4.88); in Qeparo: *Musculus costulatus* (55.8), *Modiuolus adriaticus* (22.6), *Bittium reticulatum* (21.4), *Musculus discors* (14.2), *Patella caerulea* (10.2).
Two alien mollusk species for the Mediterranean has been recorded: the gastropod *Cellana rota* and the bivalve *Brachidontes pharaonis*. The relatively high species number and the presence of alien species show the importance of the studied area at national and regional level. Polyplacophorans were found only in Llaman with two species *Acanthochitona fascicularis* and *Rhyssoplax olivacea*.

The families with the highest abundance were the gastropods Trochidae, Patellidae, Buccinidae, Pissaniidae, Columbellidae and Cerithiidae, and the bivalves of the Family Mytilidae (Table 1). The highest abundance among the whole collected mollsucs has been recorded for the mytilids, followed by the patellids, which show an evident difference compared to the other groups in all sampling sites. The site with the highest abundance of these two families was Qeparo. Comparing the four sampling sites, the lowest number for the most abundant families has been recorded in Guma. The family with the largest number of species in all sites was Mytilidae.

The largest number of mollusc species in Qeparo may be related to the diversity of microhabitats, caused by freshwater inputs, as surface water and ground water, pouring from karstic coastal rocks in this area. Consequently, brackish water conditions are also present there.
The large number of species in Guma maybe related with the algal cover. From the field observations during sampling, in this site it was noticed a high cover and a large number of macroalgae species, mainly of the class Phaeophyceae with predominance of *Ericaria amentacea* and *Cystoseira compressa*, which serve as shelter and food for most of the mollusc species found in this site.

Some of the recorded species have been considered as species with a high level of threat (VU and CR) at a national scale (table 2), referring to the Red List of Threatened Species of Albania, after the Ministry of Environment (2013), where most of them are gastropods 6 species and 3 bivalves. Some of them are threatened from direct collection for trading in markets and restaurants mainly local, while many others, although they are not the object of trade, are threatened from degradation of coastal habitats, and from water pollution, as a result of human impacts. *Lithophaga lithophaga* is a species threatened at international scale, and it belongs to Annex II of the Barcelona Convention (Convention for the Protection of the Mediterranean Sea from Pollution). During the last three decades, this species has been collected intensively throughout the rocky Albanian coast, mainly on the Ionian coast, and currently it became very rare already. Although it is a protected species, it is served in restaurants in Albania, and illegally exported abroad.

Table 1. The average abundance for the most abundant families for each sampling site.

Families	*Guma*	*Llaman*	*Porto Palermo*	*Qeparo*
Trochidae	4.57	1.83	8.88	3.33
Patellidae	11	6.33	11.11	14.33
Pissanidae	4.57	0.16	9.22	1.22
Cerithiidae	3.14	-	10.11	21.77
Columbellidae	8.28	0.16	1.44	0.11
Mytiliiidae	5.28	33.8	47.11	93.6

Table 2. List of species threatened at national scale.

Gastropoda		**Threat level at national scale**
1.	*Patella caerulea (Linnaeus, 1758)*	VUA1c
2.	*Patella rustica Linnaeus, 1758*	VUA1c
3.	*Patella ulyssiponensis Gmelin, 1791*	VUA1c
4.	*Osilinus (Monodonta) turbinatus (Born, 1778)*	VUA2b
5.	*Diodora graeca (Linnaeus, 1758)*	VUA2b
6.	*Bittium reticulatum (da Costa, 1778)*	VUD2
Bivalvia		
7.	*Lithophaga lithophaga (Linnaeus, 1758)*	VUA1a
8.	*Mytilus galloprovincialis Lamarck 1819*	VUA1c
9.	*Mytilaster minimus (Poli 1795)*	CRD1

Figure 3. The total average abundance of molluscs for each site: Guma, Llaman, Porto Palermo and Qeparo.

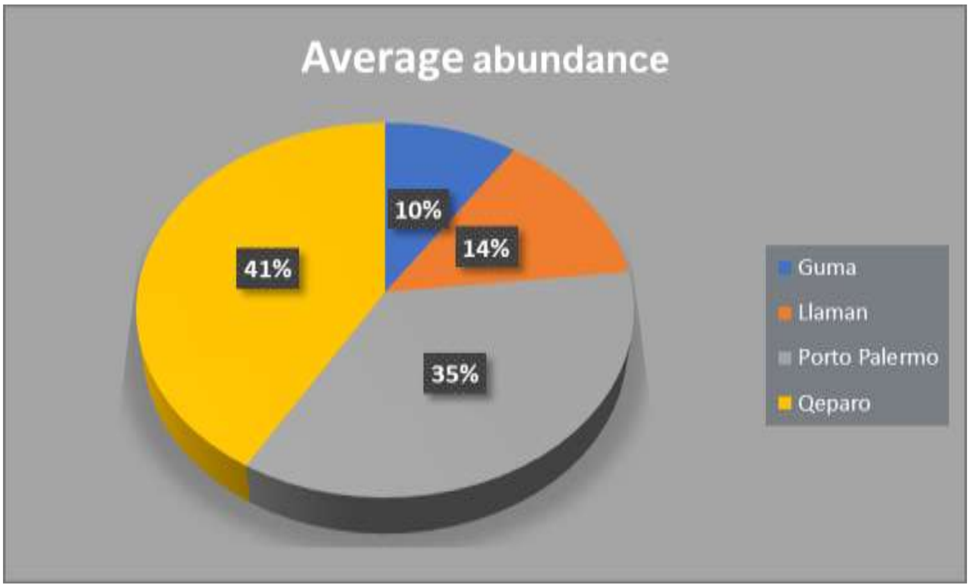

Table 3. Species similarity coefficient (Sokal & Sneath) between sampling sites.

Sites	*Guma*	*Llaman*	*Porto Palermo*	*Qeparo*
Guma		20.63%	28.81%	20.51%
Llaman	20.63%		23.07%	23.33%
Porto Palermo	28.81%	23.07%		20%
Qeparo	20.51%	23.33%	20%	

As can be seen from Table 3, in general, species similarity is of medium scale (according to Blanc et al., 1976) between sites.
Porto Palermo had the highest species similarity with the other sites. Qeparo had the lowest species similarity with the other sites, and this may be related to the special salinity conditions in this area, due to freshwater inputs, as already mentioned here above

Referring to the evaluated diversity indexes (Shannon & Weaver (H'), Pielou (J), Margalef (M) and Simpson (D) (table 4), it is noted that for Guma and Llaman, the diversity of the molluscs community is of a good degree, while for Porto Palermo and Qeparo, the diversity is of an average degree. In general, the indexes present the highest values for Guma and Llaman, and the lowest values for Porto Palermo and Qeparo, with the exception of the Margalef index, which presents the highest values for Guma and Qeparo, and the lowest values for Llaman and Porto Palermo.

Table 4. Diversity indexes in each sampling site.

Sites / ***Indexes***	***Guma***	***Llaman***	***Porto Palermo***	***Qeparo***
Shannon & Weaver (H')	2.679	2.446	1.922	1.905
Pielou (J)	0.795	0.791	0.613	0.555
Margalef (M)	7.771	5.520	4.901	6.072
Simpson (D)	0.094	0.120	0.246	0.225

Table 5. Percentage of species according to the values of constancy for each sampling site.

Sites	**Constant species**	**Accompanying species**	**Occasional species**
Guma	17%	24%	59%
Llaman	20%	40%	40%
Porto Palermo	30%	22%	48%
Qeparo	19%	7%	74%

In Qeparo it was recorded the lowest degree of stability of the molluscs community, referring to the values of the constancy (table 5), according to the assessment based on Blanc et al. 1976. While in Porto Palermo and Llaman, based on the constancy values the degree of stability of the molluscs community is considered as average.

Considering the results and findings in this study, the main factors that affect mollucs populations at the shallow rocky coast of Himara are related to macroalgal cover, diversity of microhabitats, and human impacts, mainly from tourism development.

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Appendix 1.

Table 6. Total list of species recorded in the four sampling sites: Guma, Llaman, Porto Palermo and Qeparo, in Himara Coast.

Taxa	**Guma**	**Llaman**	**Porto Palermo**	**Qeparo**
Mollusca				
Polyplacophora				
1. *Rhyssoplax olivacea* (Spengler, 1797)		+		
2. *Acanthochitona fascicularis* (Linnaeus, 1767)		+		
Gastropoda				
3. *Patella caerulea* Linnaeus, 1758	+	+	+	+
4. *Patella rustica* Linnaeus, 1758	+	+	+	+
5. *Patella ulyssiponensis* Gmelin, 1791	+	+	+	+
6. *Cymbula safiana* (Lamarck, 1819)	+			
7. *Cellana rota* (Gmelin, 1791)				+
8. *Iothia fulva* (Müller O.F., 1776)				+
9. *Diodora gibberula* (Lamarck, 1822)	+		+	
10. *Diodora dorsata* (Monterosato, 1878)	+			
11. *Clanculus corallinus* (Gmelin, 1791)	+			
12. *Jujubinus exasperatus* (Pennant, 1777)	+			
13. *Gibbula ardens* (Von Salis, 1793)	+	+		
14. *Gibbula umbilicaris* (Linnaeus, 1758)	+			
15. *Gibbula varia* (Linnaeus, 1758)	+			+
16. *Phorcus (Monodonta) articulatus* (Lamarck 1822)				+
17. *Phorcus (Monodonta) mutabilis* (Philippi, 1846)				+

18. *Phorcus (Monodonta) turbinatus* (Born, 1778)	+	+	+	+
19. *Cerithium vulgatum* Bruguière, 1792	+		+	+
20. *Bittium reticulatum* (da Costa, 1778)	+		+	+
21. *Cerithidium perparvulum* (Watson, 1886)				
22. *Melarhaphe (Littorina) neritoides* (Linnaeus, 1758)	+		+	
23. *Rissoa similis* Scacchi, 1836				+
24. *Rissoa variabilis* (Von Mühlfeldt, 1824)				
25. *Alvania lineata* Risso, 1826				+
26. *Alvania discors* (Allan, 1818)				+
27. *Alvania cimex* (Linnaeus, 1758)	+		+	
28. *Circulus striatus* (Philippi, 1836)			+	
29. *Vermetus triquetrus* Bivona Ant. 1832	+			
30. *Vermetus* sp. Daudin, 1800	+			+
31. *Hexaplex (Truncularıopsis) trunculus* (Linnaeus, 1758)				+
32. *Ocinebrina edwardsii* (Payraudeau, 1826)			+	
33. *Ocinebrina hispidula* (Pallary, 1904)			+	
34. *Ocenebra ingloria* (Crosse, 1865)			+	
35. *Pisania striata* (Gmelin, 1791)	+	+	+	+
36. *Aplus scacchianus* (R. A. Philippi, 1844)	+			
37. *Tritia incrassata* (Strøm, 1768)	+			+
38. *Columbella rustica* (Linnaeus, 1758)	+	+	+	+
39. *Enginella leucozona* (Philippi, 1844)				+
40. *Tarantinaea (Fasciolaria) lignaria* (Linnaeus, 1758)	+			
41. *Conus mediterraneus* Hwass in Bruguière, 1792			+	
42. *Aplysia fasciata* Poiret, 1789	+			
Bivalvia				
43. *Arca noae* Linnaeus, 1758				+
44. *Mytilus galloprovincialis* Lamarck, 1819	+	+	+	+

45. *Mytilus edulis* Linnaeus, 1758	+	+	+	+
46. *Mytilaster minimus* (Poli, 1795)	+	+	+	+
47. *Mytilaster lineatus* (Gmelin, 1791)		+		
48. *Mytilaster sp.* Monterosato, 1884				
49. *Musculus costulatus* (Risso, 1826)	+	+	+	+
50. *Musculus discors* (Linnaeus, 1767)		+	+	+
51. *Lithophaga lithophaga* (Linnaeus, 1758)			+	
52. *Modiolus adriaticus* Lamarck, 1819	+	+	+	+
53. *Modiolus barbatus* (Linnaeus, 1758)		+		+
54. *Brachidontes pharaonis* (P. Fischer, 1870)	+	+		
55. *Modiolula phaseolina* (Philippi, 1844)		+		
56. *Anomia ephippium* Linnaeus, 1758	+		+	
57. *Ostrea edulis* Linnaeus, 1758		+		+
58. *Ostrea stentina* Payraudeau, 1826				+
59. *Chama gryphoides* Linnaeus, 1758				+
60. *Pododesmus (Monia) patelliformis (Linnaeus, 1761)*				+
Total	**31**	**20**	**24**	**32**

RECENT ADVANCES IN ANTIMICROBIAL FOOD PACKAGING FOR CHEESE PRESERVATION

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ABSTRACT

Cheese is a very suitable growth medium for microorganisms, which can cause food spoilage and health problems. Packaging plays a significant role in the protection of the food products and improves the shelf life by preventing contamination. Particularly, antimicrobial packaging has come to the fore in recent years. Antimicrobial compounds are involved in a synthetic or natural polymer substrate, thus forming an antimicrobial film for the improvement of microbial safety and quality of the product. Polysaccharides, proteins, and lipids have been mostly used as biopolymers for the formation of antimicrobial films, together with antimicrobial compounds like titanium oxide nanoparticles, plant essential oils and extracts as antimicrobial compounds. This review focuses on the recent advances in antimicrobial food packaging for cheese preservation.

Key Words: Antimicrobial, packaging, biopolymer, film, cheese

INTRODUCTION

The nutritional and physicochemical characteristics of cheese create a favorable environment for microorganism growth, thus reducing the shelf-life of the product and causing health risks. Especially hard and semi-hard cheeses are prone to cross contamination throughout their long ripening process. Therefore, coating with packaging materials has long been used to prevent cheeses from microbial spoilage (Paidari et al., 2023). Proper packaging is one of the most suitable ways of ensuring the microbial quality, while maintaining the sensory, textural, and physicochemical properties specific to the type of cheese (Jafarzadeh et al., 2021). Although synthetic packaging materials meet these requirements, the serious environmental concerns coming together have given rise to sustainable and active packaging materials recently. Antimicrobial packaging is a form of active packaging which is obtained by embedding the antibacterial agents into a polymer matrix or using antimicrobial activity of the polymer itself, coating onto the packaging surface or immobilizing via non-covalent or covalent links (Appendini and Hotchkiss, 2002). The successfully applied antibacterial agents include inorganic ones such as oxidized nanoparticles (NPs), and natural ones like chitosan, nisin, natamisin, lysozyme, and essential oils. This review overviews the recent advances in antimicrobial food packaging for preservation of different kinds of cheeses.

Modified Atmosphere Packaging (MAP) Modifications

Packaging systems like vacuum packaging and MAP have been used up to the last decade to limit the microbiological changes during the storage period of cheese. Modification of the internal atmosphere in cheese packages by carbon dioxide, oxgen, and nitrogen gases is proved to be a very effective way of controlling microorganisms (Ščetar et al., 2019). The advances in this method have been made on joining the antimicrobial compounds and MAP. Silver NPs in sodium alginic acid solution were used in combination with MAP for coating Fior di Latte cheese, enhancing the shelf-life (Gammariello et al., 2011a). Also, chitosan addition during cheesemaking process, and MAP application prolonged the shelf-life of Stracciatella cheese (Gammariello et al., 2011b). Potassium sorbate in sodium alginate-based coating in combination with MAP was used for coating Mozzarella cheese (Mastromatteo et al., 2014), advancing the preservation and doubling the shelf-life. Silver NPs were also loaded in the same coating under MAP on Fior di Latte cheeses (Mastromatteo et al., 2015). The obtained conditions exhibited serious antibacterial activity against *Staphylococcus aureus* (*S. aureus)*, *Pseudomonas aeuroginosa (P. aeuroginosa)*, *Escherichia coli (E. coli)*, and *Candida albicans (C. albicans)*. Furthermore, the total bacteria, mold and yeast, and coliform counts decreased during storage.

Biopolymers

Consumers and producers are more interested in edible, biodegradable and environment friendly packaging materials lately (Iqbal et al., 2021). Biopolymers have found extensive use as food packagings due to their biodegradability, biocompatibility, chemical stability, and low carbon footprint. Generally, the types of antimicrobial biopolymers for food packaging are polysaccharide-, protein-, and lipid-based. Polysaccharide-based biopolymers such as chitosan (CS), starch, cellulose, alginate, gum Arabic, and agar are non-toxic and biodegradable (Mohamed et al., 2020). Whey protein, soy protein, zein, and gelatin are widely used in protein-based packaging materials, with regard to their availability, biodegradability, and release control of antimicrobial agents into the packaging system. Lipids are used as components of antimicrobial packagings, including resins, waxes (carnauba, beeswax, candelilla), oils and fats (essential oils (EOs), extracts). These biopolymers are applied as edible films and coatings. An edible film is a thin layer (less than 0.3 mm) formed on food surface, while an edible coating is placed on food after forming, and both can be eaten as an integral part of the food product (Jeevahan and Chandrasekaran, 2019). Edible films remain on the surface of the cheese to release the antimicrobials slowly, in a long period of the antimicrobial effect which is advantegous especially in ripened cheeses (Fajardo et al., 2010; Jafarzadeh et al., 2021).

The nanoparticulation of biopolymers and antimicrobial agents like metal oxides, bacteriosins, plant EOs and extracts have offered good solutions for specialized usage of food packaging and coating materials (Paidari et al., 2023). Metal oxide NPs like zinc oxide (ZnO-NPs), copper oxide (CuO-NPs), titanium dioxide (TiO_2-NPs), aluminium oxide (Al_2O_3-NPs), magnesium oxide (MgO), silica (SiO_2-NPs), gold (Au-NPs), and silver (Ag-NPs) have been used in edible films and coatings in cheese preservation. The EOs show strong antimicrobial activity against pathogens, attributed to their high content of phenolic compounds like eugenol, carvacrol, thymol, cinnamaldehyde, and etc. (Burt, 2004; Sharafi et al., 2023).

CS-based Packagings

CS is one of the most widely used biopolymers in the manufacture of food packaging and coating, with superior film forming ability, biodegradability, safety, and potent antimicrobial property (Youssef et al., 2019). Incorporation of CS with antimicrobial agents has been studied in packaging of different kinds of cheeses. Natamycin was used in CS-based coating of Saloio cheeses and as a result there was a decrease in yeast and mold counts (Fajardo et al., 2010). CS coating with lysozyme and natamycin increased the shelf-life of

Halloumi cheese by 5 days (Mehyar et al., 2018). Additionally, nanofibers containing nisin-loaded poly-γ-glutamic acid/CS were examined as potential anti-listerial materials on Cheddar cheese (Cui et al., 2017), indicating promising results for active packaging for cheese preservation. CS, cellulose, and nisin containing antimicrobial bilayer film was developed for the inactivation of *Listeria monocytogenes (L. monocytogenes)* on the surface of ultrafiltered White cheeses (Divsalar et al., 2018). Metal oxide added CS packagings have also been the subject of much research. CS-NPs, TiO_2-NPs, and polyvinyl alcohol (PVA) were mixed to prepare a novel bionanocomposite and used as a packaging material for soft White cheese, demonstrating excellent antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* (Youssef et al., 2015). Mold growth on the surface of Ras cheese could be inhibited by a film consisting of CS/PVA and TiO_2-NPs (Youssef et al., 2019). White cheese samples were wrapped with CS nanofiber and ZnO-NPs included films, resulting in a significant decrease in the growth of bacteria (Amjadi et al., 2019). The CS coating with ZnO-NPs had significant inhibitory effect against *E. coli* O157: H7 on the surface of White brined cheese (Al-Nabulsi et al., 2020). It was observed that CS coating alone had nearly similar effect of antimicrobial activity. The antimicrobial effect of CS-based edible coating containing ZnO-NPs was tested against *L. monocytogenes* on White brined cheeses, resulting with reduced counts of bacteria (Olaimat et al., 2022).

Plant extracts are the other studied materials with CS for antimicrobial packaging of cheeses. Boldo extract added gelatin-CS edible films were produced and applied on sliced Prato cheese, resulting in significant antimicrobial protection against psychrotrophic microorganisms and low development of coliforms (Bonilla and Sobral, 2019). Roselle calyx extract, and chitosan, guar gum and ZnO containing nanocomposite films were applied to Ras cheese, and inhibitory effect against *L. monoctygenes* and *E. coli* was achieved (El-Sayed et al., 2020). CS-based films, with fibrous chestnut and tannin-rich extracts were used as a package for Gouda cheese, which has been contaminated with *Pseudomonas fluorescens* (*P. fluorescens*), *E. coli*, and *Penicillium commune* (Kõrge et al., 2020). The packaging material protected the surface of Ras cheese nearly three months from yeasts, molds, and other bacteria growth. Chestnut and tannin-rich extracts added films showed protective properties against mycotoxins and had good wrapping properties. Also, a nanocomposite film of CS with nanoclay as a surface coating had significant antibacterial activity and successfully limited coliform, *E. coli*, *Salmonella* spp., *Staphylococcus* spp., and yeast and mold growth in Gouda cheese (Mohammadzadeh-Vazifeh et al., 2020).

Starch- and Cellulose-based Packagings

An antimicrobial sachet containing microcellular foam starch with embedded rosemary oil (RO) and thyme oil (TO) was developed by Han et al. (2014) for the inhibition of *L. monocytogenes* in shredded Mozzarella cheese, resulting in a delay in growth of this bacteria. The volatile oils also limited the growth of lactic acid bacteria and total aerobic bacteria. However, the sachet treatment produced a distinct odor which is not favored by the panelists. Starch/halloysite/nisin nanocomposite film was used on Minas Frescal cheese, which was previously inoculated with *L. monocytogenes* (Meira et al., 2016), resulting in complete inhibition of bacterial growth. Oliveira et al. (2007) found that natamycin included cellulose films were effective in *Penicillium roqueforti* inhibition in Gorgonzola cheese. The high hydrostatic pressure treatment of oregano EO-cellulose acetate films was used for packaging Coalho cheese, which was contaminated with *L. monocytogenes*, *E. coli*, and *S. aureus* (Goncalves et al., 2021). Combination of the antimicrobial film with 400 MPa high pressure treatment for 10 min resulted in greater inhibition of the target microorganisms during storage. Natamycin added carboxymethyl cellulose (CMC) coatings were used in preservation of Mozzarella cheeses, inhibiting *Aspergillus flavus (A. flavus)*, *Aspergillus niger* (*A. niger*), and *C. albicans* (Azhdari and Moradi, 2022).

Alginate-based Packagings

Potassium sorbate added sodium alginate (SA)-based coating was developed for Mozzarella cheese, for the inhibition of *Enterobacteriaceae* spp. and *Pseudomonas* spp. (Lucera et al., 2014). Nanoemulsion-based mandarin fiber-oregano EO-SA coatings were applied onto low fat cut cheese, and coatings, and decreased *S. aureus* count, inhibited psychrophilic bacteria, mold and yeast growth, and extended shelf-life (Artiga-Artigas et al., 2017). The authors stated that carvacrol in oregano EO caused the antimicrobial effect, which was earlier proved to present strong inhibitory activity against *Salmonella typhimurium (S. typhimurium)*, *E. coli*, *L. monocytogenes*, and *S. aureus* (Burt, 2004). Novel antimicrobial edible coatings based on antimicrobial microcrystalline cellulose, and probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium lactis*) were developed with CS, SA, and CMC to be used on ultrafiltered soft cheese (El-Sayed et al., 2021). SA and CS films had the highest antimicrobial effect against *S. aureus*, *S. typhimurium*, *L. monocytogenes*, *E. coli*, *Bacillus cereus*, *A. niger*, and *A. flavus*. In another study, SA-glycerol-thymol rich zeolite nanocomposite films were developed for packaging of Cottage cheese (Giannakas et al., 2022). A significant decrease in *S. aureus* ATCC1538 was seen during storage, indicating a good bacteriostatic activity.

Agar- and Gum Arabic-based Packagings

The silver montmorillonite NPs were embedded into an agar-based matrix and prolonged the shelf-life of Fior di Latte cheese inhibiting the growth of *Pseudomonas* spp. (Incoronato et al., 2011). *Enterococcus avium* enterocins were used successfully as antimicrobial agents on agar edible films on Tybo cheeses and goat cheeses, especially contaminated with *L. monocytogenes* (Guitián et al., 2019). Soft goat cheeses' humid matrix facilitated rapid diffusion of the antimicrobials, since the drier matrix of semi hard cheeses' allowed the release of the antimicrobials gradually, prologing the inhibition. Aloe vera oil added edible agar-gelatin films were developed for Kashar cheese packaging (Isık et al., 2023). The films had antimicrobial activity against *E. coli*, *S. aureus*, *A. niger*, and *C. albicans*. Also, total bacterial, and yeast and mold counts were lower than the control samples. Encapsulation of cinnamon EO with gum Arabic-based membrane were used for packaging and extended the shelf life of string cheese sticks from 3 to 8 weeks, with the inhibition of *E. coli* O157:H7, and reduction in total bacteria counts (Ali et al., 2021).

Gelatin-based Packagings

The antimicrobial activity against *L. monocytogenes* of chicken bone gelatin films used for wrapping Mozzarella cheeses was increased with the increase in cinnamon bark oil (CBO) added (Kim et al., 2018). CBO contains trans-cinnamaldehyde as the major component for its antimicrobial activity. Gelatin nanofibers were embedded into moringa oil/CS nanoparticles resulting in an antimicrobial packaging for cheese, with high activity against *S. aureus* and *L. monocytogenes* (Lin et al., 2019). Since bacteriophages provide a convenient alternative to antibiotics, edible gelatin films containing bacteriophages were used to pack fresh cheese contaminated with *S. aureus* (Weng et al., 2021). The cheeses packed with films exhibited higher microbial reduction than the control samples. Gelatin films were also prepared by the addition of extracts of Moringa oleifera leaves to wrap Ricotta cheese (Mezhoudi et al., 2022). Microbial spoilage was reduced with low levels of yeast and mold counts attributed to the phenolics coming from Moringa oleifera extract. Lemon peel EO rich in limonene was used in gelatin nanofibers for packaging of sliced Kashar cheeses (Dogan et al., 2022). The antimicrobial effect of packaging material was greater on *S. aureus* ATCC 29213 than *E. coli* ATCC 35218, and the growth of total aerobic mesphilic bacteria, and yeast and molds were suppressed. Eugenol and oreano EO containing gelatin-CS-based edible films were applied as fresh cheese packaging, resulting in antimicrobial activity against *S. aureus*, *E. coli*, and reduction in total coliform and mesophilic aerobic bacteria counts (Méndez et al., 2023).

Whey protein-based Packagings

Whey protein concentrate (WPC), and whey protein isolate (WPI) have been used as components of antimicrobial packagings lately. Natamycin added WPC coatings used for packaging of White cheeses strongly inhibited *S. aureus* growth compared to *Pseudomonas* spp. and *Enterobacteriaceae* (Henriques et al., 2013). Sorbitol, WPI, and mint EO containing edible films were prepared for coating Lor cheese (Kavas and Kavas, 2014). The antimicrobial films limited the growth of *E. coli* O157:H7, *L. monocytogenes*, *S. aureus*, yeasts and molds artificially contaminated to the cheese samples, extending the shelf-life. Thyme and clove EOs added sorbitol-WPI based films were used for coating Kashar cheese artificially contaminated with *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus* (Kavas et al., 2015). A significant relationship was found with films and antimicrobial activity due to the reduction in bacterial counts. *E. coli* O157: H7 and *S. aureus* counts decreased in Kashar cheese samples coated with ginger EO added WPI-alginate films during 30 days of storage, owing to oleoresin and sesquiterpene hydrocarbons of ginger EO (Kavas et al., 2016). Cheddar cheese was preserved with carvacrol containing WPI fibers, with higher antimicrobial activity against *Salmonella enteritidis* (Wang et al., 2019). Fabrication of thymol-loaded PVA/WP nanofiber mats were developed as novel antimould surface coating material with a remarkable limitation of *Aspergillus parasiticus* on Kashar cheese surface (Tatlisu et al., 2019). Oregano EO, garlic EO, nisin and natamisin added WPI films were also used for the storage of Kashar cheese and slices (Seydim et al., 2020). Oregano added WPI films showed higher antimicrobial activity against *E. coli* O157: H7, and nisin added WPI films had higher bactericidal effect against *L. monocytogenes* during storage. Moreover, natamisin added WPI films better inhibited *Penicillium* spp. Essential oil added WPI films were recommended as sliced cheese separator for increasing the safety and quality during storage of Kashar cheese. Furcellaran and WPI films containing green tea extracts were prepared to be used as a packaging of Quark cheese (Pluta-Kubica et al., 2021). The package showed bacteriostatic effect against *S. aureus*, and total bacteria count decreased in almost all samples during storage. Since most of the films had negative effect on sensory properties of cheese samples, they were not appropriate for acid-curd cheese packaging. WPC, rosemary, and sage extracts were used to produce active edible packaging materials in coating soft cheeses (Kontogianni et al., 2022). No spoilage or pathogenic bacteria were found until the end of 60 days of storage. In both rosemary and sage extracts, rosmarinic acid was the main phenolic compound. The use of these packaging materials was recommended so that they could be consumed with cheese avoiding the waste, and whey protein films could also increase the protein value of the product. Natamycin, citric acid, and melanin containing WPI films were developed and used successfully for antimicrobial coating of goat cheeses (Ferraz et al., 2023).

Soy protein- and Zein-based Packagings

CS, soy protein isolates (SPI) and SA included edible coating on Mozzarella cheese was effective in preserving (Zhong et al., 2014). SPI films embedded cardamom EO were synthesized for packaging Iranian white cheese (Hajirostamloo et al., 2022). The films inhibited *S. typhimurium* and *S. aureus* growth due to cardamom EO concentration, which consists of antimicrobial tannins and polyphenols. Pomegranate peel extract in zein films were developed for sustaining antimicrobial effect in Kalari cheese packaging (Mushtaq et al., 2018). The films have reached a significant reduction in total bacteria, yeast and molds counts. Natamycin added alginate and zein films were developed to limit the mold growth on surface of Kashar cheeses inoculated with *A. niger*, and *Penicillium camamberti* (Saritas Kucuk et al., 201920). Zein films exhibited higher antifungal activity at high natamycin concentrations than alginate films. Antimicrobial edible zein coatings were developed by encapsulation of cineole-rich rosemary EO and bay EO (Goksen et al., 2020). Bay EO showed higher antibacterial activity than rosemary EO against *L. monocytogenes* and *S. aureus*, with increasing activity during storage. The aerobic mesophilic bacteria were also inhibited in coated semi-hard cheese slices.

Resin- and Wax-based Packagings

Salicylate-layered double hydroxide-resin coated polyethylene terephthalate packages were used for extending the shelf-life of Mozzarella cheeses (2 et al., 2021). There were serious reductions in coliform, yeast and mold, *Pseudomonas* spp., and total mesophilic aerobic bacteria counts. Beeswax was used as an edible coating in Kashar cheese, resulting in a reduction of mold counts (Yilmaz and Dagdemir, 2012). Zein composite films with carnauba wax, candelilla wax, and beeswax incorporated with lysozyme were tested on Kashar cheeses inoculated recently with *L. monocytogenes* ATCC 7644 (Unalan et al., 2013). The zein-carnauba wax film caused a significant reduction bacterial count.

Pulsed Light Technology

Pulsed Light (PL) is a promising non-thermal technology using short bursts of intense light to decontaminate food and packagings (Moraes et al., 2020). Recent studies have proved the potential of this method in cheese preservation. The efficiency of PL technology on *P. fluorescens*, *E. coli* O157:H7, and *Listeria innocua (L. innocua)* inoculated Cheddar and processed cheese surfaces was evaluated (Proulx et al., 2015). The results of the study showed good reductions in bacterial counts. Sodium benzoate and citric acid added starch films, irradiated with PL technology were used for packing Cheddar cheese slices inoculated with *L. innocua* (Moraes et al., 2020). The PL treatment was effective in decontamination, however quality parameters of the products were affected negatively.

Photodynamic Inactivation

The photodynamic inactivation (photosensitization) is the application of light doses in a wavelength suitable with the chosen photosensitizer which is a non-toxic dye, resulting in the formation of singlet oxygen and free radicals to damage microorganism cells (Saraiva et al., 2021; Miazaki et al., 20222). Curcumin was successfully used as a photosensitizer in photodynamic inactivation of *S. aureus* in pasteurized Coalho cheeses (Santos et al., 2019). Also, the effect of curcumin in an edible coating was determined in Minas Frescal cheese packaging against *P. fluorescens* (Saraiva et al., 2021). The samples treated with photodynamic inactivation with curcumin exhibited lower bacterial counts. Erythrosine was also used as a photosensitizer with alginate to form an antimicrobial edible coating in the preservation of Ricotta cheeses (Miazaki et al., 2022). *Salmonella* and *S. aureus* were not detected in photodynamic inactivated samples, since reduction in coliform count was only obtained when high amounts of erythrosine was used.

CONCLUSION

This review focused on the recent advances in antimicrobial food packagings for cheese preservation. The safety of edible films and coatings composed of biopolymers is guaranteed, however the packagings containing NPs have still concerns to be eliminated such as the potential toxicological effects on human and environment. Also, there are limited studies on pulsed light technology and photodynamic inactivation. Therefore, the future studies should focus on the use of new biopolymers and NPs, the revealization of all the aspects of the mentioned novel technologies, and the effects of these developments on cheese quality.

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DETERMINING THE PHYSICOCHEMICAL AND BIOACTIVE PROPERTIES OF SPECIFIC MELON VARIETIES AND INVESTIGATING INTERRELATIONSHIPS

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ABSTRACT

Melon is an important commercially grown species from the cucurbit family. For this study, 22 different melon varieties were used. The experiment was conducted in the research and development field of Verim Ziraat Ltd. Co. in Adana province, Türkiye. The trial was designed using a randomized block design with two replications, and each replication consisted of 10 plants. In the study, various fruit characteristics were examined, including fruit weight (g), fruit length (cm), fruit diameter (cm), fruit flesh thickness in longitudinal section (cm), Hue* and C*, TSS (Total Soluble Solids), titration acidity, TSS/acidity (TS/TA ratio), ascorbic acid, pH, seed number, seed weight, seed width, seed length, as well as fructose, glucose, and sucrose contents. Statistical analysis was performed using the Tukey test in the JMP 7.0 software package to identify significant differences between the melon varieties in terms of each characteristic at a significance level of 0.05. Additionally, the relationships between these 18 characteristics were examined using correlation matrix and UPGMA methods with the NTSYS 2.1 software package

Keywords: Melon, variety, fruit characteristics, physicochemical properties, bioactive properties

INTRODUCTION

Melon, known scientifically as *Cucumis melo* L., is a tropical plant species with a rich botanical and cultural history. The genus Cucumis, to which *Cucumis melo* belongs, encompasses a wide variety of gourd-type plants that produce fruits of diverse shapes, sizes, colours, and flavours. Its genetic make-up consists of 2n=24 chromosomes, contributing to its diverse array of attributes. The fruit itself exhibits considerable morphological diversity, encompassing traits such as size, shape, colour, texture, taste, and content. Due to this remarkable variation, *Cucumis melo* stands out as the most diversified species within the *Cucumis* genus (Kirkbride, 1993; Whitaker and Davis, 1962; Jeffrey 1980; Bates and Robinson, 1995).

It is a widely popular vegetable enjoyed both in Türkiye and around the world. With a global production of 28.6 million tons, it holds a significant presence in various culinary cultures. Notably, China takes the lead by producing 14 million tons, followed by Türkiye with 1.6 million tons, India with 1.5 million tons, Kazakhstan with 1.3 million tons, and other countries contributing a total of 10 million tons (FAO, 2021).Turkey stands out not only as a major global contributor to melon production but also as a geographical region harbouring a significant secondary centre of genetic diversity for melons, stretching from Minor Asia to Japan (Pitrat et al., 1997). In Turkey, local melon varieties not only exhibit rich diversity varieties (Sensoy et al., 2007; Sari and Solmaz, 2005; Sari et al., 2008), but also the recorded melon are increasing year by year, and the diversity of these melon types within the species is observed to change and be registered in accordance with consumer preferences.

The attractiveness of melons can be attributed to their delightful aroma, aromatic qualities, and their nutritional richness, comprising antioxidants, vitamins, polyphenols, and minerals (Soltani, 2021). Melons are also rich in a significant amount of biologically active compounds, including ash, fibre, protein, phenolic compounds, carbohydrates, tocopherols, phospholipids, and sterols, all of which offer beneficial effects to human health (Aziz and Tahir, 2022). Melon fruits are utilized in various ways within the food, cosmetic, and pharmaceutical industries (Gómez-García et al., 2020).

Melon displays a significant degree of variability, encompassing a wide array of leaf, flower, and fruit characteristics. Recent research, conducted by Pitrat in 2017, has identified 19 distinct horticultural groups within the *Cucumis melo* species. These groups consist of various melon varieties such as agrestis, kachri, chito, tibish, acidulus, momordica, conomon, makuwa, chinensis, fexuosus, chate, dudaim, chandalak, indicus, ameri, cassaba, ibericus, cantalupensis, and inodorus. Many of these melon groups are actively cultivated in Turkiye. The genetic diversity of melons (*Cucumis melo* L.) has been extensively investigated in various studies (Kaçar et al., 2012; Dantas et al. 2015; Ermiş and Aras, 2017; Maleki et al. 2018; Saputro et al. 2020; Singh et al. 2020; Pandey et al. 2021, Aziz and Tahir, 2023). These studies have employed a wide range of analyses, including the assessment of factors such as the physical appearance, growth patterns, internal structures, sensory qualities, chemical composition, and molecular characterization of melon fruits. Among these analytical approaches, the initial and most crucial criterion for classifying and distinguishing different melon genotypes has proven to be the meticulous examination of their morphological characteristics (Aziz and Tahir 2022)

In this specific research, the primary objective was to investigate the physicochemical and bioactive attributes of 22 different melon cultivars. Through an extensive analysis, the study aimed to elucidate the intricate associations among these cultivars concerning their characteristics. By exploring aspects such as nutritional composition, structural features, and bioactive compounds, the research aimed to provide insights into the diverse characteristics of melon cultivars and their potential implications for various applications. These findings may contribute to the preservation of genetic variation within melon varieties and promote their utilization in breeding programs for further development and enhancement of other melon cultivars.

MATERIALS AND METHODS

A total of 22 hybrid and open-pollinated melon varieties, officially registered with the Ministry of Agriculture and Forestry, were selected for this research (see Table 1 for the list of these cultivars). The study was conducted in the research area of Verim Ziraat Ltd. Company, located in Adana, Türkiye, spanning from February to July 2016. The study was specifically carried out in geographic zones defined by coordinates 37.02710 latitude and 35.15311 longitude.

Table 1. Melon cultivars utilized in the research

Name	Variety	Company
Adahan	*Cucumis melo* var. *cantalupensis*	Verim Ziraat Ltd. Co.
Alabanda	*Cucumis melo* var. *cantalupensis*	Beta Agriculture A.Ş.
Alara	*Cucumis melo* var. *inodorus*	AD-Rossen Seeds
Albella	*Cucumis melo* var. *inodorus*	Beta Agriculture A.Ş.
Asude	*Cucumis melo* var. *inodorus*	Asgen Agriculture A.Ş.
Sarıkız	*Cucumis melo* var. *inodorus*	Vatan Seed Ltd. Co.
Chems	*Cucumis melo* var. *inodorus*	HM Clause Seeds A.Ş.
Dağhan	*Cucumis melo* var. *cantalupensis*	Verim Ziraat Ltd. Co.
Duru	*Cucumis melo* var. *cantalupensis*	Verim Ziraat Ltd. Co..
Hanzade	*Cucumis melo* var. *cantalupensis*	Verim Ziraat Ltd. Co.
Işıl	*Cucumis melo* var. *inodorus*	Yüksel Seeds A.Ş.
Man 15025	*Cucumis melo* var. *reticulatus*	Syngenta Seeds A.Ş.
Merlin 4300	*Cucumis melo* var. *reticulatus*	AMC-TR Agriculture
Mertcan	*Cucumis melo* var. *inodorus*	Yüksel Seeds A.Ş
Napolyon	*Cucumis melo* var. *cantalupensis*	Genetika Seeds Ltd.
Nasa	*Cucumis melo* var. *cantalupensis*	United Genetics Turkey Seeds A.Ş.
Natal	*Cucumis melo* var. *inodorus*	Rijk Zwaan Ltd. Co.
Nefise	*Cucumis melo* var. *cantalupensis*	Vatan Seed Ltd. Co.
Paşa	*Cucumis melo* var. *inodorus*	Yüksel Seeds A.Ş.
Sweety	*Cucumis melo* var. *cantalupensis*	Koltar Seeds Ltd. Co
VCR 601	*Cucumis melo* var. *cantalupensis*	Genetika Seeds Ltd.
Zeynep	*Cucumis melo* var. *cantalupensis*	Verim Ziraat Ltd. Co.

On February 10, 2016, seeds were sown in seedling trays. The seedling growing medium consisted of a mixture of peat and vermiculite in a 2:1 ratio. When the seedlings reached the 4-5 leaf stage, they were transplanted into the field on March 24, 2016. The planting arrangement involved a row spacing of 2.0 meters, with a plant spacing of 0.8 meters within rows. The experiment was set up using a randomized block design with two replications. Each replication comprised 10 plants, resulting in a total of 20 plants per melon variety studied according to the UPOV test guidelines (UPOV 2019). Table 2 displays the climate data (min, max and average temperature, total precipitation, sunbathing time, sunbathing intensity) for Adana, Turkey from February to July during the year 2016.

Table 2. Meteorological data of the year 2016.

	Temperature ^{0}C			Total Precipitation	Sunbathing time (h)	Sunbathing intensity
Month	Min	Max	Average	(mm=kg/m²)		(cal/cm²)
February	4.1	28.5	14.0	95.8	6.0	288.8
March	6.4	27.7	15.7	64.8	6.2	387.3
April	10.7	33.8	20.5	35.4	7.9	503.9
May	13.1	33.2	21.6	69.2	7.1	520.9
June	16.8	40.2	27.1	43.4	9.8	611.2
July	22.5	38.3	29.5	0.2	10.0	607.6

Physiological Properties Assessment of Fruit Characteristics

In this study, a comprehensive assessment of various fruit characteristics was conducted. The parameters under examination included fruit weight (FW, g), fruit length (FL, cm), fruit diameter (FD, cm), fruit flesh thickness in longitudinal section (FFT, cm), as well as the evaluation of L*, Hue and Chroma (CR‑300, Konica Minolta, Osaka, Japan) values (McGuire,1992). The chroma values (C*) represent the intensity of colour, while the hue angle (h*) characterizes the specific colour tone (0° corresponds to red-purple, 90° to yellow, 180° to bluish-green, and 270° to blue). Additionally, the study encompassed an analysis of seed number, seed weight, seed width, and seed length, which were assessed at the maturity stage using five randomly selected plants per plot (UPOV, 2019).

Physicochemical and Bioactive Properties Assessment of Fruit Characteristics

The total soluble solids content (TSSC) was measured using a digital refractometer (Atago, Model ATC-1E). pH value was measured using a pH meter (Hanna, H14222) according to the AOAC method. The titratable acidity (TA) of the melon fruits was measured using a pH meter (WTW InoLab, pH720, Germany). The samples were titrated with a 0.1 N sodium hydroxide solution until reaching a pH endpoint of 8.1, and the result was expressed as a percentage, following the method described by Cemeroglu (2010). The primary soluble carbohydrates found in melon fruits, including sucrose, fructose, and glucose, were assessed using HPLC (High-Performance Liquid Chromatography), following the methodology outlined by Bartolomé et al. (1995). The results were expressed as grams of soluble sugar per 100 grams of fresh weight (g $100g^{-1}$).

For the analysis of ascorbic acid content, using an HPLC method (Lee and Coates, 1999), where 10 µl portions of the sample or standard were injected into a Zorbax ODS column (4.6 mm×25 mm) along with a Zorbax Cl8 guard column. Ascorbic acid was consistently eluted using 2% KH_2PO_4 (adjusted to pH 2.4 with phosphoric acid) at a flow rate of 0.5 ml/min. The eluate was continuously monitored through UV detection at 245 nm.

Statistical analysis

Differences between the groups in terms of each feature were assessed for significance at the 0.05 level using the Tukey test in the JMP 7.0 software package. Furthermore, the relationships between these 18 features were explored through the correlation matrix and UPGMA methods using the NTSYS 2.1 software package. Data were subjected to Numerical Taxonomy Multivariate Analysis using the NTSYS-PC Version 2.1 software (Exeter Software, Setauket, N.Y.) (Rohlf, 1993). Initially, the data, which exhibited continuous variation, was standardized using the Standardization module within the program. Subsequently, correlation coefficients among varieties were calculated using the correlation matrix in the SIMINT module. Finally, a dendrogram depicting the genetic relatedness of genotypes was constructed using the Unweighted Pair Group Method Arithmetic Average (UPGMA) method based on the correlation matrix.

RESULTS and DISCUSSION

The melon varieties were categorized into three horticultural clusters of *Cucumis melo* L., following the botanical classification outlined by Pitrat (2017) and Raghami et al. (2014). These groups consisted of cantalupensis, inodorus, and reticulatus. The melon varieties used in our study belong to different subspecies that are commercially valuable. In total, 22 different melon varieties were utilized. Out of these varieties, 11 belong to the cantalupensis subspecies, 9 to the inodorus subspecies, and 2 to the reticulatus subspecies.

The analysis of variance data indicates significant discrepancies among the evaluated melon varieties in terms of fruit weight, fruit length, fruit diameter, and fruit flesh thickness in the longitudinal section. Among these varieties, the commercial hybrid "Hanzade" exhibited the highest fresh weight, while the "Nasa" variety from the cantalupensis group demonstrated the lowest. Moreover, genotypic distinctions were identified in fruit length, with "Paşa" displaying the greatest length, and "Sweety" being the smallest. Regarding to fruit diameter (FD), Mertcan and Hanzade exhibited the largest diameter, while Natal had the smallest. When examined in terms of fruit flesh thickness, it was generally observed that melons belonging to the cantalupensis groups had thicker flesh, whereas those belonging to the inodorus groups of melons had thinner flesh ($p<0.05$).

The study found that both the 1000-seed weight and the number of full seeds per fruit had a statistically significant relationship ($p<0.05$). Specifically, when considering the 1000-seed weight (in grams), the Alara variety produced the heaviest seeds, weighing in at 41.27 grams. On the other hand, the Mertcan variety yielded the highest seed count, totalling 1235 seeds. Substantial variation was observed across all seed characteristics, indicating significant diversity within the seed attributes. The study's results further indicated that the width of melon seeds ranged from 3.60 to 5.54 cm, while the length ranged from 8.21 to 13.11 cm. This variation in seed size and shape suggests that the physical properties of melon seeds can vary significantly, depending on factors such as the specific melon variety and the environmental growth conditions in which they are cultivated (Makanjuola, 1972).

Table 3. Mean performance for fruit weight, fruit length, fruit diameter, fruit flesh thickness in longitudinal section, thousands of seed weight, number of seeds, seed width, and seed length of 22 melon varieties

Varieties	FW (g)	FL (cm)	FD (cm)	FFT (cm)	1000 SW (g)	NS (no)	SW(cm)	SL (cm)
Adahan	1.775 DE	19.60 C-F	15.70 B-E	5.13 A-E	21.27 G	517 K	4.24 EFG	8.21 K
Alabanda	1.423 F-I	16.40 F-J	14.53 D-H	4.00 F-J	32.47 BC	340 M	4.58 D	12.15 B
Alara	1.694 DEF	18.30 E-I	13.30 HI	3.17 J	41.27 A	807 F	5.54 A	11.12 CD
Albella	2.235 C	22.00 BC	14.00 C-F	3.70 IJ	31.37 CD	1 100 B	3.60 J	10.38 E
Asude	1.689 D-G	18.73 D-I	13.30 E-I	3.27 J	29.80 D	119 O	5.24 AB	11.43 C
Sarıkız	2.237 C	24.53 AB	15.47 C-F	4.33 E-I	16.97 H	1 115 B	4.09 GH	9.80 FG
Chems	1.973 CD	17.20 F-J	14.00 E-I	4.73 C-H	17.20 H	1 057 C	3.66 IJ	10.85 D
Dağhan	2.134 C	15.60 IJ	15.47 C-F	5.10 A-E	15.47 HI	574 J	3.90 HIJ	9.30 HI
Duru	2.214 C	16.20 G-J	15.00 D-H	5.97 A	23.60 FG	981 D	4.06 GH	10.35 E
Hanzade	4.481 A	18.87 C-H	17.50 AB	5.37 C-H	26.03 EF	594 IJ	4.20 E-H	9.32 HI
Işıl	1.681 EFG	18.00 E-I	14.50 D-H	4.73 C-H	26.07 E	621 HI	3.96 GHI	9.34 GHI
Man 15025	3.382 B	24.13 AB	15.73 B-E	5.90 AB	13.73 I	724 G	4.49 DE	11.09 CD
Merlin 4300	1.400 GHI	24.63 AB	15.70 B-E	4.93 B-G	34.53 B	245 N	4.16 FGH	8.97 IJ
Mertcan	1.453 FGH	19.23 C-G	19.00 A	4.50 D-I	13.90 I	1 235 A	4.70 CD	12.55 B
Napolyon	2.161 C	19.10 C-G	16.87 BC	5.00 A-F	14.47 I	925 E	4.55 D	9.48 GH
Nasa	853 J	16.67 F-J	16.87 BC	4.90 B-G	29.13 D	580 J	4.16 FGH	10.26 EF
Natal	1.162 I	15.73 HIJ	12.30 I	3.97 G-J	26.53 E	362 M	5.29 A	13.11 A
Nefise	1.598 EFG	21.60 BCD	13.60 GHI	4.53 D-I	30.13 CD	106 O	4.47 DEF	9.52 GH
Paşa	2.215 C	27.13 A	16.00 BCD	3.83 HIJ	30.90 CD	634 H	4.97 BC	8.74 J
Sweety	1.183 HI	14.70 J	14.20 D-H	4.63 D-I	17.57 H	593 IJ	4.42 DEF	9.22 HI
VCR 601	1.461 FGH	18.03 E-I	13.70 F-I	5.97 A	15.77 HI	802 F	4.23 EFG	12.59 B
Zeynep	1.538 EFG	20.47CDE	15.20 C-G	5.73 ABC	23.57 G	481 L	4.51 DE	9.19 HIJ
CV (%5)	4.86	5.29	3.84	6.92	3.26	1.45	2.26	1.44
Prob > F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*Different letters in the same column indicate statistically significant differences between varieties (p< 0.05)
FW: Fruit weight, FL: Fruit length, FD: Fruit diameter, SW: Seed width, NS: Number of seeds, SW: Seed weight, SL: Seed length

Evaluating and characterizing fruit quality represents a significant goal within the context of melon breeding (Paris et al., 2003). Similar results were found in previous studies on melon accessions and varieties (De Amorim et al., 2016; Yusuf et al 2020; Chikh-Rouhou et al., 2021).

The colour of melon flesh is a significant quality characteristic typically characterized by metrics such as L*, a*, b*, hue angle, and chroma value (Saftner et al., 2006 Saftner and Lester, 2009). The values for colour reflectance (L*, Hue, and Chroma) can be found in Table 4. According to the results in Table 4, there was a statistically significant 5% difference between the subjects in terms of L*, h^{o}, and C* values in the fruits obtained from the experiment subjects. For the fruit flesh, colour values L*, h^{o}, and C* were found to be in the range of 16.31-74, 13; 76.56-87.47, and 15.97-68.11, respectively. Chikh-Rouhou et al. (2021) reported that the findings for melon colour parameters, including L*, hue, and chroma angle, exhibited a range of 51.82–73.88, 9.91-49.46 and 76.24-117.0 respectively. As per the findings from Park et al. (2018), melon exhibited L*, hue, and chroma values ranging from 64.8-75.5, 73.3-114.2, and 18.7-40.7, respectively.

The concentration of total soluble solids (TSSC) is likely the primary quality parameter routinely evaluated by researchers due to its strong correlation with sugar concentration in fruits. In all melon varieties, the TSCC ranged from 5.2 to 15.9%, and statistically significant differences were observed among the varieties (p<0.05). In accordance with the USA grading standards for melons, the minimal TSSC values stand at 9% for cantaloupe and 8% for honeydew melon, as indicated by Lester and Shellie in 1992. However, the varieties we utilized in our study belonged to different groups, and climatic conditions may have influenced the TSCC. Titratable acidities obtained in the fruit are given also in Table 4. As seen in Table 4, statistically significant differences were found between the varieties in terms of titratable acidity values (p<0.05). The titration acidity values ranged from 0.10 to 0.43%, and the highest titration acidity was detected in the Albella hybrid melon variety. In previous studies, it has been argued that titration acidity is inversely related to pH, with high pH values associated with low titration acidity (Burger et al., 2003; Simandjuntak et al., 1996). In the conducted study clearly shows that among the 22 varieties examined, those with higher pH values also had lower titration acidity. These findings were consistent with the results of our study, as the 'Sweety' variety exhibited the highest pH and the lowest titratable acidity (TA) compared to all other cultivars (Table 4). Sweet melons are generally characterized by their low acidity levels, typically ranging from 0.12% to 0.2% (Roy et al., 2012). The taste of sweet melons is amplified by their sugar/acid ratio. This reduced acidity contributes to their gentle and sweet flavour, making them a preferred choice for individuals who appreciate fruits with a sweeter taste profile.

Table 4. Colour values, total soluble solids content, titratable acidity, ratio of total soluble solids content/titratable acidity and pH of 22 melon varieties

Varieties	**Lightness (L*)**	**Hue (hº)**	**Chroma (C*)**	**TSSC (%)**	**TA (%)**	**TSS/ TA ratio**	**pH**
Adahan	60.93 I	76.56 A	40.25	11.5 EF	0.14 HIJ	82.8 CD	6.30 DE
Alabanda	65.67 E	-75.89 G	21.90	10.6 GH	0.33 BC	32.1 FG	6.26 DE
Alara	65.61 E	-70.67 C	21.35	8.5 JK	0.23 EFG	37.0 FG	5.21 H
Albella	74.13 A	-72.41 DE	25.38	6.7 L	0.43 A	15.6 G	5.40 GH
Asude	54.43 N	-76.50 HI	18.48	6.0 L	0.25 DEF	24.2 G	5.60 FG
Sarıkız	66.40 CD	-79.32 J	18.04	8.3 K	0.35 ABC	23.9 G	5.82 F
Chems	66.84 C	-76.82 I	26.10	12.5 CD	0.10 J	125.1 AB	6.40 B-E
Dağhan	69.14 B	-81.57 K	25.75	10.8 FG	0.14 IJ	77.3 CDE	6.30 DE
Duru	66.53 CD	-76.09 GH	31.04	13.3 C	0.19 FGH	70.0 DE	6.21 E
Hanzade	59.18 K	75.80 B	38.94	9.6 I	0.30 CDE	32.4 FG	6.29 DE
Işıl	57.98 L	-81.77 K	15.97	9.2 IJ	0.41 AB	22.8 G	6.20 E
Man 15025	64.74 F	-83.18 L	18.89	8.8 JK	0.31 CD	28.4 FG	6.26 DE
Merlin 4300	66.10 DE	-76.71 I	23.35	9.8 I	0.35 ABC	28.0 G	6.67 ABC
Mertcan	68.54 B	-74.94 F	16.50	5.2 M	0.33 BC	15.8 G	5.20 H
Napolyon	25.49 P	-87.36 N	64.78	12.2 DE	0.12 J	101.7 BC	6.60 A-D
Nasa	26.86 O	-87.47 N	68.11	14.2 B	0.12 J	119.5 AB	6.68 AB
Natal	64.44 F	-76.48 HI	18.82	12.2 DE	0.11 J	111.8 AB	6.31 CDE
Nefise	16.31 Q	-86.42 M	62.54	12.1 DE	0.12 J	100.9 BC	6.71 AB
Paşa	63.65 G	-72.24 DE	18.74	8.7 JK	0.29 CDE	30.1 G	5.33 GH
Sweety	60.23 J	-72.76 E	31.31	15.9 A	0.12 J	132.6 A	6.93 A
VCR 601	61.70 H	-79.60 J	23.33	9.9 HI	0.18 GHI	55.6 EF	6.60 A-D
Zeynep	56.54 M	-72.00 D	29.81	11.8 DE	0.32 CD	37.4 FG	6.60 A-D
CV (%5)	0.35	-0.26	0.30	2.40	4.58	13.24	1.42
Prob > F	<.0001	<.0001	<.0001	<.0001	<.0001	<,0001	<.0001

*Different letters in the same column indicate statistically significant differences between varieties (p< 0.05)
TTSC: total soluble solids content, TA: titratable acidity, TTS/TA: total soluble solids/ titratable acidity

The sugar content of the melons is presented in Table 5. The samples underwent comprehensive analysis to detect the presence of sugars, including sucrose, fructose, and glucose, and the results were determined to be statistically significant at the 5% confidence level. All melon varieties exhibited significant sugar content, with sucrose, glucose, and fructose being the predominant sugars, consistent with prior reports (Lamikanra et al., 2020; Ercan et al., 2023). Among the melon varieties, the highest sucrose content was observed in Nasa and Sweety varieties. As shown in Table 5, the glucose content in the melon varieties ranged from 1.02 to 3.80 g per 100g, while fructose content ranged from 1.21 to 3.70 g per 100g. The Alara melon variety stood out notably, displaying significantly higher levels of both glucose and fructose when compared to the other melon varieties. The relative proportions of the different sugars examined in the study may account for differences in taste, for equal amounts of total sugars. Sugars exhibit varying levels of sweetness due to their distinct weights (Albuquerque et al., 2006). In the context of our research on melon fruits, it is noteworthy that despite the variation in sugar levels observed among different cultivars, this parameter not only exhibits a positive correlation with sweetness but also appears to be associated with flavour. This dual correlation is intriguing and may necessitate further evaluation through sensory assessments.

Table 5. Sugar and ascorbic acid content of melon varieties

Varieties	Sucrose (g/100g)	Glucose (g/100g)	Fructose (g/100g)	Ascorbic acid (mg kg-^{1})
Adahan	5.23 I	3.38 B	2.85 B	504.19 C
Alabanda	5.06 IJ	2.84 C	2.70 B	108.96 O
Alara	1.01 M	3.80 A	3.70 A	59.17 R
Albella	4.10 K	1.42 JK	1.21 I	32.69 U
Asude	2.82 L	1.59 IJ	1.62 GH	192.27 K
Sarıkız	4.02 K	2.18 E	2.13 CD	60.98 Q
Chems	8.59 D	1.90 FGH	2.03 CDE	97.68 P
Dağhan	7.00 G	1.99 FG	1.82 E-H	548.01 B
Duru	9.45 B	1.78 H	2.02 CDE	338.79 E
Hanzade	7.08 G	1.49 JK	1.02 I	705.06 A
Işıl	7.17 G	1.02 M	0.97 I	109.84 N
Man 15025	6.37 H	1.18 LM	1.21 I	256.29 G
Merlin 4300	7.59 F	1.19 L	0.99 I	156.27 M
Mertcan	0.38 N	2.59 D	2.19 C	48.63 T
Napolyon	9.18 C	1.41 K	1.60 H	208.33 J
Nasa	11.05 A	1.10 LM	2.04 CDE	165.86 L
Natal	8.50 D	1.85 GH	1.82 E-H	191.98 K
Nefise	8.17 E	2.04 EF	1.89 D-G	253.63 H
Paşa	4.94 J	1.78 H	1.96 C-F	56.90 S
Sweety	11.15 A	2.54 D	2.18 C	480.26 D
VCR 601	6.43 H	1.73 HI	1.74 FGH	292.17 F
Zeynep	7.46 F	2.42 D	1.94 C-F	224.11 I
CV (%5)	1.18	2.86	4.85	0.09
Prob > F	<.0001	<.0001	<.0001	<.0001

*Different letters in the same column indicate statistically significant differences between varieties (p< 0.05)

Additionally, it should be noted that sugar levels could be influenced by climate conditions. Indeed, as Beaulieu et al. (2003) suggested, melon varieties may exhibit slight variations in total sugar content, potentially attributed to differences in genotypes and the agroclimatic conditions of their respective harvesting regions.

Ascorbic acid, which is a soluble vitamin in water, can also contribute to the tartness or sourness in fruits (Manchali et al 2021). Statistically significant ($p<0.05$) differences were found for ascorbic of the fruits studied at different varieties (Table 5). In the case of the examined melon varieties, the ascorbic acid content varied between 32.69 to 705.06 mg kg^{-1}, with the Hanzade variety exhibiting the highest ascorbic acid content. The presently determined concentration of ascorbic acid in melon varieties was quite comparable with that investigated by Evana and Barek (2021) and Fundo et al. (2018).

Eighteen traits of all melon varieties were analysed to establish principal components. Presented data, clustered by UPGMA (Fig. 1), showed that the melon varieties were grouped into two clusters, mainly reticulatus subgroups (Man 15025, Merlin 4300) or inodorus and cantaloupes subgroups (Adahan, Alabanda, Alara, Albella Asude, Sarıkız, Chems, Dağhan, Duru, Hanzade, Işıl, Mertcan, Napolyon, Nasa, Natal, Nefise, Paşa, Sweety, VCR 601 and Zeynep) The cophenetic analyses, which involved comparing the UPGMA cluster analysis with the simple matching similarity matrix, revealed a strong correlation of r=0.82, suggesting that the dendrogram effectively represented the data within the matrix.

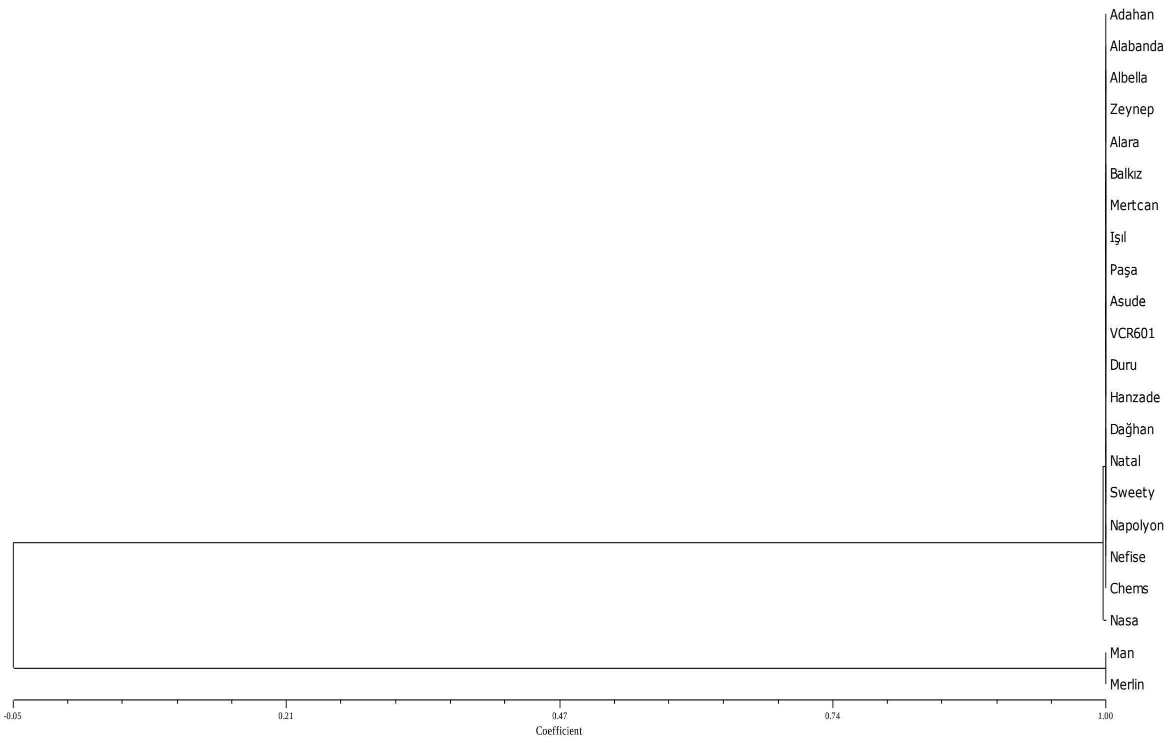

Figure 1. Dendogram of melon varieties obtained from cluster analysis of eighteen components

CONCLUSION

In conclusion, our study of 22 melon varieties encompassing the cantalupensis, inodorus, and reticulatus subspecies has revealed significant genetic diversity along with variations in various quality parameters. Variations were evident in fruit weight, fruit length, fruit diameter, and flesh thickness in the longitudinal section with notable distinctions among specific varieties. The study also unveiled a meaningful relationship between 1000-seed weight and seed count highlighting the impact of both genetics and environmental factors.

The assessment of fruit quality, encompassing traits like flesh colour, total soluble solids content (TSSC), titratable acidity, and sugar composition, demonstrated variations influenced by subspecies and climate. Additionally, differences in ascorbic acid content, contributing to fruit sourness, were consistent with previous research. Finally, principal component analysis and clustering revealed two main clusters of melon varieties, mainly reticulatus subgroups and inodorus-cantalupensis subgroups.

Overall, this study provides valuable insights into the genetic diversity and quality characteristics of diverse melon varieties. These findings hold significance for melon growers, breeders, and stakeholders, aiding in informed decision-making related to cultivation and market strategies.

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THE CHARACTERISTICS OF THE FOX GRAPE (*VITIS LABRUSCA* L.) AND ITS PLACE IN THE VITICULTURE OF THE BLACK SEA REGION

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ABSTRACT

Vitis labrusca L. has a homeland that extends from Southeastern Canada, the Northeastern and Eastern United States to Georgia (Caucasus) and also takes in Western India. It is distinguished from other types of *Vitis* by its intermittent tendril structure, which is one of its most distinctive features. Another trait of *V. labrusca* that aids ampelographers in identification of vines and hybrid varieties descended from the species is the large, thick leaves of the vines that have a hairy underside with dense brown or white hairs. Vines belonging to this species have strong development, can reach up to the highest points of their trees. It is more resistant to diseases and damages against the foxy grape than the varieties included in the *vinifera* type. In general, it is large, black, blue-black, pink, copper red and white in color, thick-shelled and foxy. In addition, the fruit flesh can be easily separated from the skin. Varieties belonging to this species are used commercially for the production of fruit juice, selective, jelly and wine. There are many varieties of Catawba, Concord, Delaware, Kyoho, Niagara, Steuben, Cayuga White in different parts of the world, especially in America.

Turkey has a large number of grape varieties and types due to the ecological conditions it has. Viticulture in the Black Sea region, which ranks last in terms of grape production, is intensively carried out with varieties of the *Vitis vinifera* species in the provinces of Tokat, Çorum and Amasya. However, since the coastal areas of the Black Sea Region contain high humidity, the cultivation of varieties consumed as table food and included in the *Vitis vinifera* L species is not easy due to fungal diseases (powdery mildew and mildew). There are foxy grape types that have adapted well to these conditions and grow naturally in the region. Along the coastline from Artvin to Sinop, *Vitis labrusca* L. species with a foxy (strawberry) aroma naturally hybridized are high-quality and productive types. Five grape varieties with excellent properties that grow easily in the humid, rainy climate of the Black Sea Region, do not get sick even if not sprayed, have a thick crust and a floral smell taste (foxy), have been registered and included in the National Variety list with the names ‘Rizessi’, ‘Çeliksu’, ‘Ülkemiz’, ‘Rizellim’ and ‘Rizpem’. With the introduction of these varieties to our country, the way for commercial grape cultivation has now been opened in the coastal parts of the Black Sea Region.

Keywords: Fox grape, *Vitis labrusca* L., Black Sea Region, viticulture

INTRODUCTION

The *Vitaceae* family, which includes grapes, comprises more than 10 genera, over 80 species, and thousands of varieties (Çelik, 2007). Geographically, it spans across Asia, Europe, and the Americas. There are 29 identified grapevine species in Asia, while in the Americas, there are 34 species. Both Asia and the Americas have high genetic diversity, with species adapted to different environmental conditions (Blembeel et al., 2020). The southeastern region of North America is rich in *Vitis* species, with approximately 70% of species belonging to this genus. The most significant species on the European continent is *Vitis vinifera* (Çelik et al., 1998). Numerous grape varieties are grown for various purposes, including table grapes, raisins, and wine production. The world's most widely cultivated grape species is *V. vinifera*, known as European grape. *V. labrusca* is the second most cultivated species (Toaldo et al., 2015).

Anatolia, one of the ancestral homelands of many plant species, is also considered one of the homelands of the grapevine. Grape cultivation has been practiced in Anatolia since ancient times (Karabat, 2009), and it is documented that grapevines have been present in this region since the dawn of civilization. In Anatolia, abundant remnants related to grapevines and wine from the Hittite period exist. Archaeological excavations have revealed that viticulture was highly developed in Anatolia during the years 1800-1550 BC, and grapes and grape juice were consumed abundantly in daily life (Oraman, 1965; Akşit, 1981; Karabulut & Akyürek, 2022). Throughout history, grapevines have spread across Anatolia, leading to a rich diversity of varieties. Due to favorable climatic conditions and abundant grapevine types and varieties, Turkey has a rich heritage and culture of viticulture, boasting an extensive genetic potential for grapevines (Ağaoğlu & Çelik, 1985). In our country, more than 1500 grape varieties are cultivated (Candar et al., 2020). All regions in Turkey have suitable ecological conditions for grape cultivation (Çelik et al., 1998).

When considering grape production in our country in terms of geographical regions, it is observed that the highest production, accounting for 51.28% (2,135,849 tons), occurs in the Aegean Region. The Mediterranean Region follows this with 15.78% (657,416 tons), and the Southeastern Anatolia Region with 14.01%. The Black Sea Region ranks last with a production quantity of 2.07% (86,009 tons) (Figure 1) (TUIK, 2023).

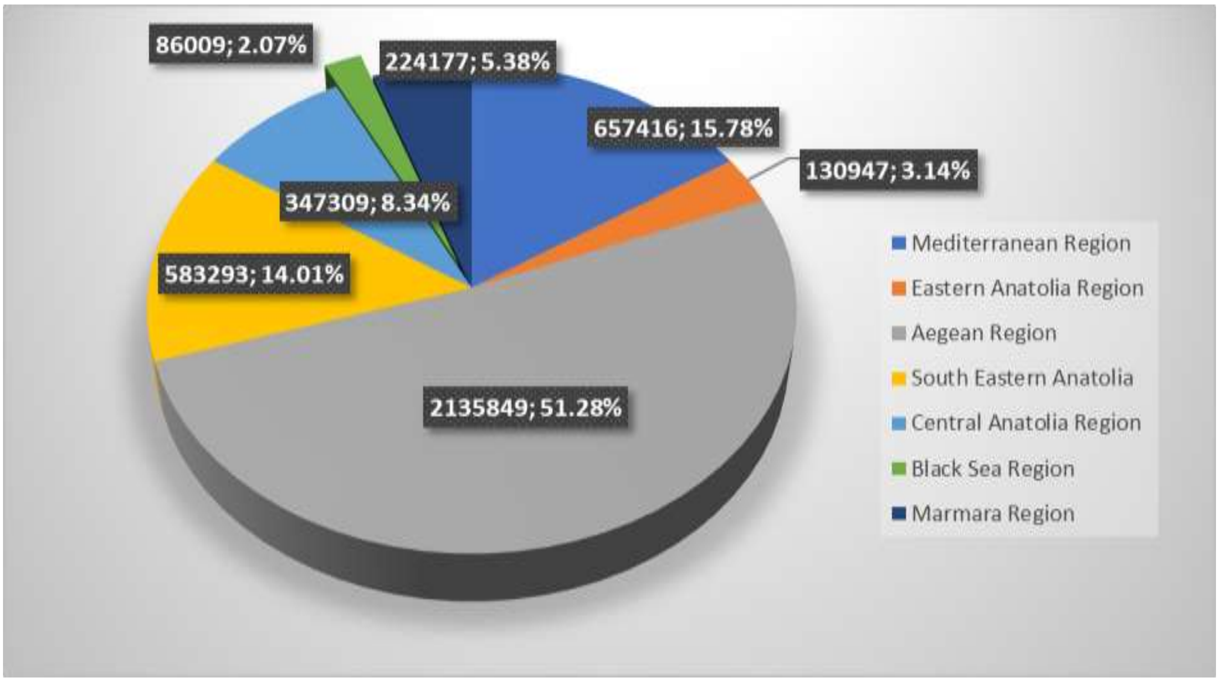

Figure 1. Grape production quantity by regions (tons and %) (TUIK, 2023)

When examining the distribution of vineyard areas by region, it is again observed that the highest production area is in the Aegean Region, accounting for 34.90% (1,342,052 da). The Southeastern Anatolia Region follows with a share of 25.93% (997,232 da), and the Central Anatolia Region ranks third with 13.84% (532,011 da). The Black Sea Region, with 3.05% (117,434 da) of vineyard area, is in the last place (Figure 2) (TUIK, 2023).

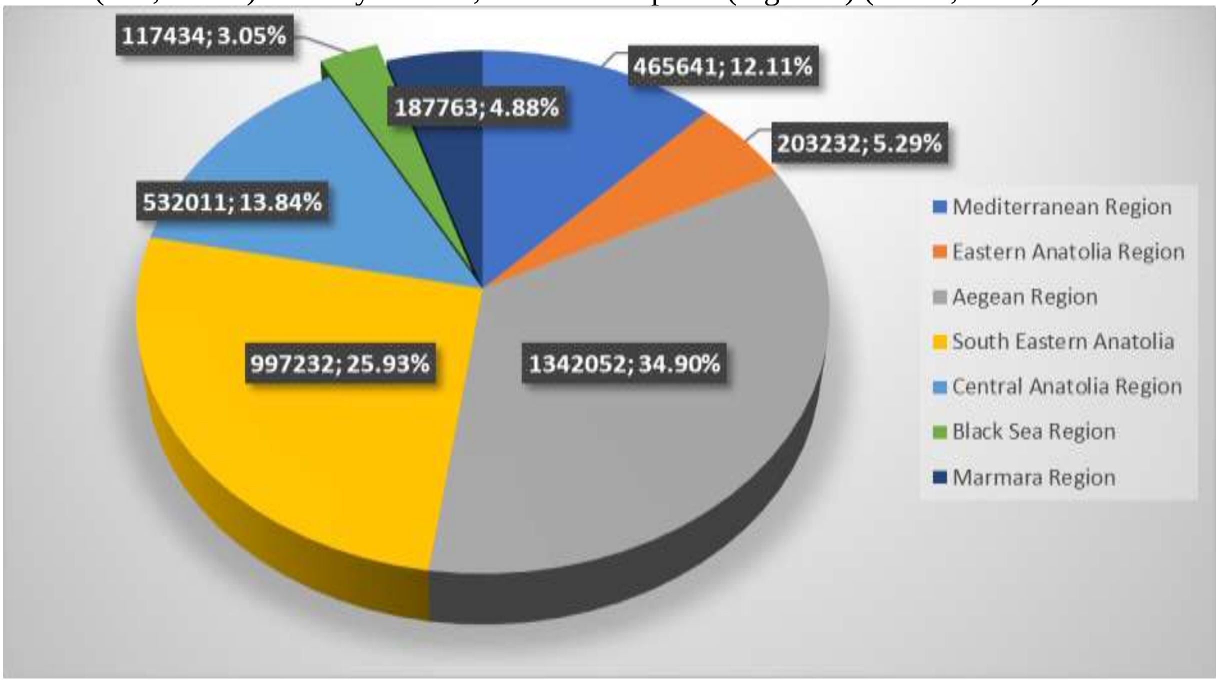

Figure 2. Grape production area by regions (da and %) (TUIK, 2023)

The grape production quantities and areas in the Black Sea Region, broken down by provinces, are provided in Table 1. The highest grape production quantity and grape production areas are respectively Tokat with 61.74% (53,105 tons) and 57.99% (68,097 da), Çorum with 17.83% (15,333 tons) and 26.60% (31,236 da), and Amasya with 12.62% (10,854 tons) and 6.89% (8,089 da) (TUIK, 2013). A common characteristic of these three provinces is that they do not have boundaries along the Black Sea coastline. In other words, the humid and rainy climate of the Black Sea does not affect these provinces. Therefore, the cultivation of *V. vinifera* is carried out in the inland provinces of the Black Sea Region. When looking at the provinces located in the coastal region of the Black Sea, it can be observed that grape production quantity and vineyard area are significantly lower. Especially in the eastern parts of the Black Sea Region, the annual rainfall ranges from 1200 to 1600 mm, and humidity levels are very high, increasing fungal diseases. Since *Vitis vinifera* L. varieties are sensitive to fungal diseases, their cultivation is not widespread in this region, especially in the coastal areas. Therefore, in terms of grape production and provincially, cities located along the coastline of the Black Sea Region rank at the bottom.

Table 1. Grape production amount and area by provinces in the Black Sea Region (da; %) (TUIK, 2013)

Cities	Production Quantity (ton)	Production Quantity (%)	Production Area (da)	Production Area (%)
Amasya	10854	12.62	8089	6.89
Artvin	1881	2.19	2033	1.73
Bartın	3	<0.01	2	<0.01
Bolu	949	1.10	1250	1.06
Çorum	15333	17.83	31236	26.60
Düzce	1	<0.01	1	<0.01
Giresun	2	<0.01	2	<0.01
Karabük	807	0.94	757	0.64
Kastamonu	2540	2.95	5198	4.43
Ordu	15	0.02	19	0.02
Rize	101	0.12	196	0.17
Samsun	392	0.46	500	0.43
Sinop	15	0.02	40	0.03
Tokat	53105	61.74	68097	57.99
Zonguldak	11	0.01	14	0.01

In the eastern part of the Black Sea Region, especially along the coastline, grape varieties are available to the *Vitis labrusca* L. species. These grapes resemble a strawberry aroma (foxy), have thick skins that easily separate from the flesh, and contain seeds. Grapes of this type can be found in various colors such as blue-black, black, white, pink, and coppery-red. They are typically grown by training them on pergolas or trees (Çelik, 2004). Local people in the region consume these grapes according to their local needs, using them for table consumption, making jam, molasses, pickles, preserves, or fruit juice (Çelik & Köse, 2006; Çelik et al., 2008; Köse et al., 2014). Grapes of this species, native to North America, have vigorous growth and can reach the tops of trees. Their distribution ranges from Georgia to Southeastern Canada, including India. These grapevines belonging to *V. labrusca* L. species have the characteristic of having a tendril on each node after the last cluster, distinguishing them from grape varieties of the *V. vinifera* L. species. They have a distinct aroma that may seem unusual to most people (Keller, 2015). Grapes belonging to the *V. labrusca* L. species are of American origin. They have adapted to various climate conditions, ranging from humid to dry climates (Creasy & Creasy, 2018). Grape varieties belonging to the *V. labrusca* L. species, crucial for expanding viticulture in the Black Sea Region, are also highly beneficial for health. They are rich in phenolic compounds, antioxidants (Burin et al., 2014; Rockenbach et al., 2011; Santos et al., 2011), and resveratrol, which is known to halt events that can lead to cancer (Çelik, 2001).

Due to the exceptionally high humidity in the coastal areas of the Black Sea Region, it is considered impossible to cultivate white grapes for table consumption. However, the region's natural grapes, known as foxy grapes (strawberry grapes or black grapes), are abundantly consumed for household needs. The thick skins are typically discarded when consumed as table grapes, and only the flesh is eaten. When processed into grape juice, molasses, or marmalade, the substances from the skins can transfer to these products. It is recommended that these grapes be consumed with their skins to maximize the intake of resveratrol, a compound found in the skins, which can offer greater health benefits to the human body. Grapes, when consumed fresh with their skins, are considered a true brain food, and they are known for their beauty-enhancing and weight-loss properties. Grapes are a summer staple and a source of healing. They contain essential B vitamins (B1, B2) that support brain and nerve metabolism, amino acids, and minerals such as potassium, magnesium, and iron, and they also provide natural fructose, which aids in the rapid storage of expended energy (Çelik, 2004).

V. labrusca species are known for producing highly aromatic and fragrant fruits called "foxy." The mature fruits are easily separated from the cluster, and when squeezed with fingers, the flesh detaches easily from the fruit skin. This characteristic has led to these grape varieties being called "slip-skin." Typically, the grapes of this species are black, and their leaves are large and thick, with a dense layer of white and brown hairs on the lower surface. Grape varieties such as Concord and Catawba belong to the *V. labrusca* species and are known as the most common hybrids in North America (Robinson, 2015). This species' variety is generally characterized by high productivity and disease resistance. Furthermore, grape varieties belonging to the *V. labrusca* species are known for their remarkable flavor and aroma characteristics. Consumers prefer them for fresh consumption and wine and fruit juice production. Some *V. labrusca* varieties, such as Concord and Niagara, are commercially grown in the United States to produce fruit juice, jam, jelly, and wine. These varieties are generally tolerant to cold and resistant to powdery mildew and root-knot nematodes. However, they are sensitive to diseases like black rot and Pierce's disease and have low tolerance to lime (Keller, 2015).

Some Foxy Grape Varieties Cultivated in the World

The crossing of the American grape species with the European grape species *Vitis vinifera* has resulted in the development of numerous hybrid varieties such as Catawba, Kyoho, Cayuga White, Buffalo, Venus, Norton, Concord, Niagara, Herbemont, Bordo, Mars, Steuben, Delaware, and many others (Çelik, 2004). The parent species for these hybrids are *V. vinifera* and *V. labrusca*. These varieties are used in wine production, for making unfermented grape juice and jelly, or as table grapes. The fruit typically has a strong aroma, and those accustomed to the taste of *V. vinifera* varieties often find the distinct "foxy" character of many American grapes sharp and unpleasant (Morton, 1985; Reisch et al., 2012). The native American grape *V. labrusca* was initially cultivated in America and is valuable for its resistance to phylloxera and tolerance to cold temperatures. Over time, these foxy grapes have naturally hybridized with other grape species, giving rise to second-generation new varieties (Robinson, 2015). Information on some foxy grape varieties cultivated in the world is as follows;

Catawba

The complete origin and parentage of Catawba grapes remain unknown (Pinney, 1989). Sometime between 1807 and 1816, this variety was propagated along the banks of the Catawba River in Washington DC and Maryland, hence the name Catawba. The first wine made from Catawba grapes was produced in 1822 by Major John Adlum in Georgetown, Washington DC. Several years later, Adlum sent cuttings to Nicholas Longworth in Cincinnati, who further developed and popularized the cultivation of Catawba grapes in the western region to make dry, sweet, or sparkling wines. It is vigorous, hardy, and productive. However, it matures very late and thus prefers regions with an extended vegetation period. It has large fruits and forms medium-sized clusters (Figure 3). Susceptible to black rot, downy mildew, and, to a lesser extent, powdery mildew (Robinson et al., 2013). The fruit's skin is copper-red and is used to produce sweet, white, red, and rosé (pink) wines. Wines made from

Catawba grapes typically have a distinct "foxy" taste that sets them apart. New York's winery uses this grape variety to produce a significant amount of sparkling wine. The delightful iced wine made from Catawba is extremely popular. It is more susceptible to fungal diseases compared to the Concord variety. Besides being considered a table grape, Catawba is also used in the grape juice industry (Çelik, 2004).

Figure 3. Cluster and shoot view of Catawba grape (VIVC, 2023)

Delaware

The origin of this variety is unknown, but it began to spread in Ohio in 1849. This variety, characterized by its dark pink skin, is quite popular in New York. It has also gained widespread cultivation in Korea and Japan (Robinson, 2015). In these regions, Delaware is a highly important grape variety used in the production of ice wine (Çelik, 2004). It is an early-ripening variety with a less pronounced "foxy" aroma than other American varieties. Its wines are typically white or light pink, with a simple but fruity taste. Delaware grapes grow in small, thin-skinned clusters with pink fruit (Figure 4). They have low yields and are susceptible to fungal diseases, especially downy mildew and black rot, powdery mildew, and botrytis bunch rot. Delaware grapes are not resistant to phylloxera, requiring grafting onto phylloxera-resistant American rootstocks (Robinson et al., 2013).

Figure 4. Cluster and shoot view of Delaware grape (VIVC, 2023)

Concord

Concord, the most commonly grown grape variety in New York in the eastern United States, has most of its genes derived from *V. labrusca*. Concord originated in nature by chance. Its juice has a characteristic "foxy" aroma. It was first obtained in 1843 when Ephraim W. Bull of Massachusetts planted seeds from a wild vine, and it was named Concord (Figure 5). It is significant for producing grape juice, wine, and grape jelly. It is known for its resistance to cold winter conditions. It is vigorous and productive. It ripens in the middle to late season and produces large clusters with sizable grapes. In warmer climates, it may exhibit uneven ripening. Concord is highly susceptible to black rot and phomopsis but less susceptible to downy mildew, botrytis bunch rot, and anthracnose (Robinson et al., 2013). This grape variety can also be consumed fresh and is used in making jams and jellies. However, its usage for fresh consumption is somewhat limited due to the tendency of the skin to crack, excessive separation of skins from the berries before harvest, and the presence of many seeds (Çelik, 2004).

Figure 5. Cluster and shoot appearance of Concord grape (VIVC, 2023)

Kyoho

Kyoho, a grape variety that is a local hybrid of the American Concord variety, is currently the most widely cultivated grape variety in Japan, constituting 35% of the vineyard area (Robinson, 2015). Kyoho was initially bred in 1945 by a private breeder named Y. Ohinoue to create a variety with large fruits due to its tetraploid structure. Kyoho is an American hybrid with a foxy aroma (similar to Concord) popular in East Asia. Its fruits are dark purple or almost black, with large seeds, juicy flesh, high sugar content, and mild acidity (OIV, 2017) (Figure 6).

Figure 6. Cluster appearance of Kyoho grape (VIVC, 2023)

Niagara

American hybrid bred in 1866 by Claudius L Hoag and B Wheaton Clark, private breeders in Lockport, Niagara County, New York (Robinson et al., 2013). It is cultivated extensively in New York and Brazil. Known as the white version of Concord, one of its parent varieties, this grape variety has a strong foxy aroma (Figure 7). It is vigorous, productive, and well-suited to low temperatures (Robinson, 2015). Being a white grape variety, it is used to produce white wines with a distinctive grape flavor (Çelik, 2004). It forms large to medium clusters with thin skin. It is highly susceptible to black rot, downy mildew, and phomopsis. It ripens in the middle to late season and is one of the varieties sensitive to copper damage (Robinson et al., 2013).

Figure 7. Cluster and shoot appearance of Niagara grape (VIVC, 2023)

Bordo (York Madeira)

The American grape variety Bordo (*Vitis labrusca*) is one of the most important grape varieties used in wine or juice production (de Souza et al., 2014). It is a variety cultivated in Brazil and is genetically close to American hybrids Concord and Niagara. It is productive and resistant to anthracnose and, to a lesser extent, various types of rot (Robinson et al., 2013). Bordo grapes are intensely red-purple (Lago-Vanzela et al., 2011) (Figure 8).


Figure 8. Cluster and shoot appearance of Bordo (York Madeira) grape (VIVC, 2023)

Cayuga White

This hybrid variety, first named Cayuga White in 1972, was developed by John Einset and Willard B. Robinson in 1945 at the New York State Agricultural Experiment Station (NYSAES) in Geneva, New York. These hybrid vines are vigor. The clusters are medium to large, long, and slightly pointed. The fruits are medium-sized, nearly round, and resistant to cracking (Einset & Robinson, 1972). Cayuga White is primarily grown in the eastern United States, particularly New York. When harvested early, it is suitable for making sparkling wine, but as it ripens, its foxy aroma becomes more pronounced (Robinson et al., 2013) (Figure 9).


Figure 9. Cluster and shoot appearance of Cayuga White grape (VIVC, 2023)

Foxy Grape Varieties of Turkey

Çelik et al. (2008) pointed out that many of the grapes grown in the northeastern region of Anatolia, which is the genetic center of the grapevine, belonging to the species *Vitis labrusca* L. and possessing a foxy aroma, have been lost in modern times. These researchers, over ten years, they researched the provinces of Artvin, Rize, Trabzon, Ordu, Giresun, Samsun, and Sinop, identifying 86 different aromatic grape types. Furthermore, an ampelographic study conducted in Trabzon in 2003-2004 by Cangi et al. (2006) identified ten different grape types with hermaphroditic flower structures characteristic of *Vitis labrusca* L. As a result of these studies, Ondokuz Mayıs University Faculty of Agriculture, in 2016, identified superior varieties among the different types specific to the region and registered five grape varieties belonging to the species *Vitis labrusca* L. ("Ülkemiz", "Rizellim", "Rizpem", "Rizessi", and "Çeliksu") for protection (Çelik et al., 2018). The characteristics of these grape varieties are as follows;

'Rizessi'

The 'Rizessi' variety has black-colored, seeded fruits. The shoot tip is fully open, and it possesses a hermaphrodite structure. It typically has 2-3 flower clusters, with very short cluster lengths and moderate cluster density. The fruits are large, round, and have thick skins. The tendrils are short, and the leaves are three-lobed. This variety has a high juice yield and carries a foxy character. The upper part of young leaves is yellowish-green in color. The fruits ripen in the mid-season (Çelik et al., 2018; Karabulut & Çelik, 2022; Uray et al., 2023) (Figure 10).

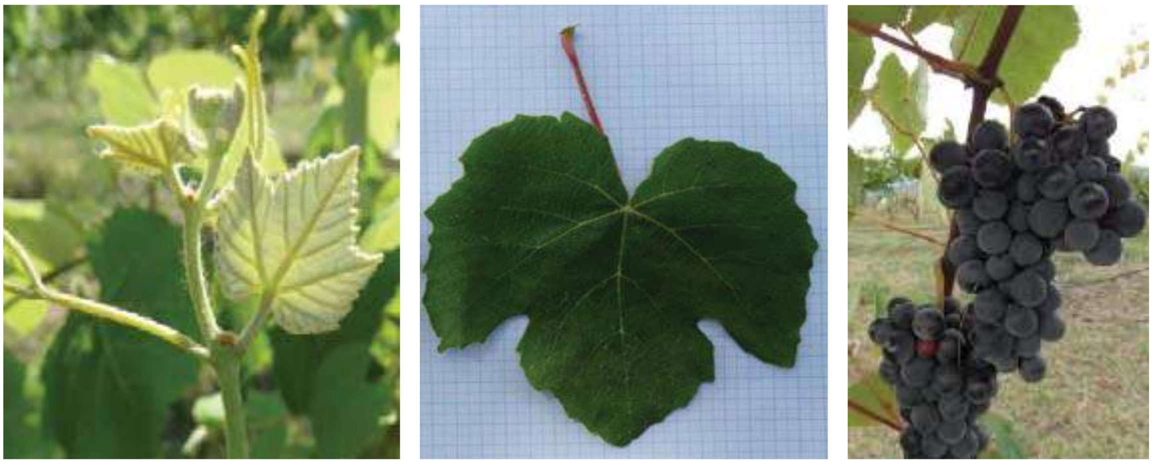

Figure 10. Shoot, leaf and cluster appearance of 'Rizessi' grape (Photo: H. ÇELİK)

'Çeliksu'

The 'Çeliksu' variety has blue-black colored fruits with seeds and a hermaphrodite structure. It possesses fruit with a foxy taste and a high juice yield. The leaves of the vines belonging to this variety are three-lobed. There are typically 2-3 flower clusters, with short cluster lengths and tendrils. The shoot tip is open. The fruit grains are large, round, and have very thick skins. Young leaves of this variety are yellow-bright green in color. The fruits ripen in the late season (Çelik et al., 2018; Karabulut & Çelik, 2022; Uray et al., 2023) (Figure 11).

Figure 11. Shoot, leaf and cluster appearance of 'Çeliksu' grape (Photo: H. ÇELİK)

‘Ülkemiz’

The 'Ülkemiz' grape variety is blue-black. Its fruits have a foxy taste and contain seeds. It has a high juice yield. This variety has a hermaphrodite structure, and the number of flower clusters is typically 1-2, with moderate tendril length. The shoot tip is broad and open. The cluster size is tiny, and the cluster length is short. The grains are medium-sized, round, and have thick skins. The leaves of this variety do not have lobes. The young leaves are green and anthocyanin-rich. This grape variety ripens in the mid-season (Çelik et al., 2018; Karabulut & Çelik, 2022; Uray et al., 2023) (Figure 12).

Figure 12. Shoot, leaf and cluster appearance of 'Ülkemiz' grape (Photo: H. ÇELİK)

‘Rizellim’

This variety has a blue-black fruit skin and contains seeds. Its flower structure is hermaphroditic, capable of forming 2-3 flower clusters. The cluster size is very small, and the cluster and tendril are short, with leaves being three-lobed. The shoot tip is fully open. The fruit grains are medium-sized, round, and have a very thick skin. The fruits are foxy and have a high juice yield. The upper side of the young leaves is yellow-green. The fruits exhibit late ripening (Çelik et al., 2018; Karabulut & Çelik, 2022; Uray et al., 2023) (Figure 13).

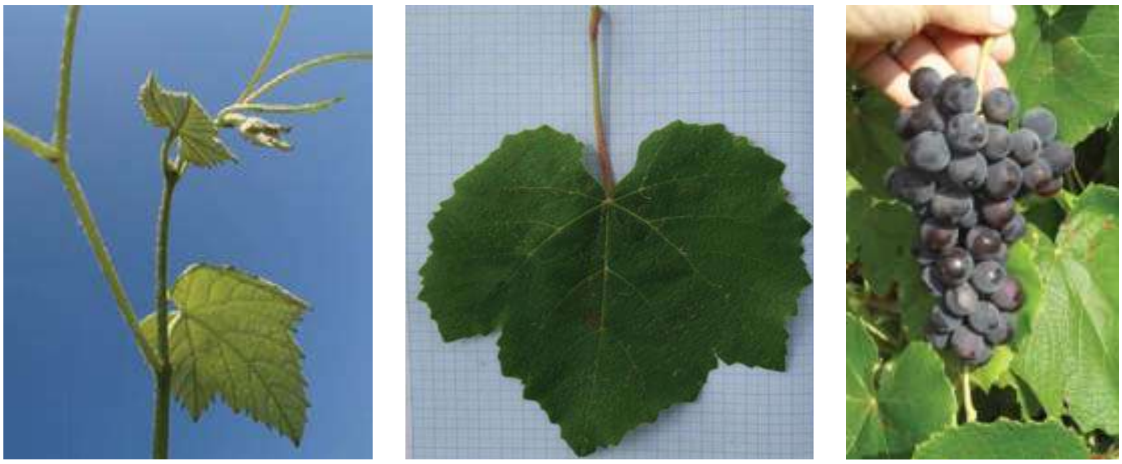

Figure 13. Shoot, leaf and cluster appearance of 'Rizellim' grape (Photo: H. ÇELİK)

‘Rizpem’

The fruits of this variety are pink and have a foxy taste. Its flowers are hermaphroditic, and it produces seeded fruits. It forms 1-2 flower clusters. It has a fully open shoot tip. The clusters are short and produce very small clusters. The tendrils are short, and the leaves do not have lobes. The grain size is medium, round, and has a thick skin. It has a high juice yield. The upper side of young leaves is light copper-reddish. The fruits ripen late (Çelik et al., 2018; Karabulut & Çelik, 2022; Uray et al., 2023) (Figure 14).

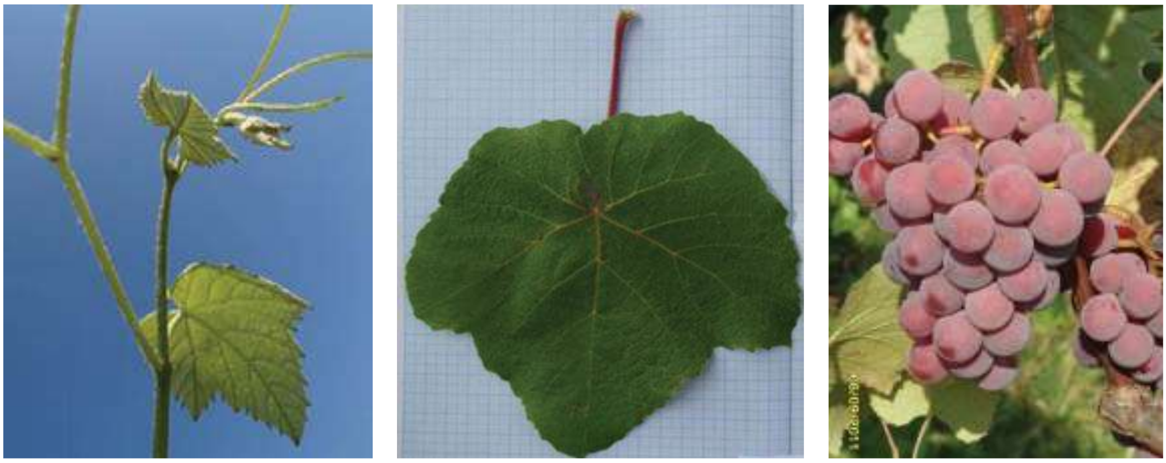

Figure 14. Shoot, leaf and cluster appearance of 'Rizpem' grape (Photo: H. ÇELİK)

Conclusion

Viticulture has been practiced in Turkey since time immemorial, and is one of the homelands of the grapevine. However, the Black Sea region has lagged in grape cultivation. While viticulture is practiced in the inland areas of the Black Sea region, especially in Tokat, Çorum, and Amasya, it is insufficient. Along the coastal areas, grape cultivation is either very limited or nonexistent. This is due to the excessive humidity and rainfall in the region, which makes it challenging to protect grape varieties of the *Vitis vinifera* species from fungal diseases. To increase grape production and cultivation areas in these regions, emphasis should be placed on cultivating varieties of the *Vitis labrusca* species, also known as American grapes. Especially in the Americas, grapes from these varieties are used in various fields, such as table grapes, wine production, and fruit juice. In the Black Sea region, natural hybrids have the foxy aroma, which has emerged through natural crossbreeding. Through research and collection of these types, studies have identified five superior grape varieties that thrive easily in the humid and rainy climate of the Black Sea region, remain disease-resistant even without spraying, have thick skins, and possess a foxy aroma. These varieties have been registered under the names 'Rizessi,' 'Çeliksu,' 'Ülkemiz,' 'Rizellim,' and 'Rizpem' as a result of these efforts (Çelik et al., 2018).

New and superior foxy grape varieties, obtained through the first-ever selection breeding in Turkey, are located in the vineyard area of Ondokuz Mayıs University's Faculty of Agriculture. With these indigenous and foxy grape varieties, initial steps have been taken to establish new vineyards, especially in the coastal regions of the Black Sea, for grape cultivation, aiming to produce seedlings and process them for table grapes or the fresh-sweet grape juice industry. Through new R&D projects, the goal is to develop vineyards in some provinces of the Black Sea region under breeding and modern trellising systems to improve viticulture in the region. This way, in addition to tea and hazelnuts, which are the primary products of the Black Sea region, foxy grapes will also contribute to product diversity. Furthermore, these vineyards will provide products for the agricultural industry based on grapes and grape products, enhancing the region's agricultural potential and increasing viticulture's contribution to the national economy, especially in the grape juice industry. Efforts have also been initiated to crossbreed these indigenous and aromatic grape varieties, known for their resistance to biotic and abiotic stress factors such as fungal diseases, cold, and phylloxera, with other grape varieties to obtain new cultivars. This development is crucial for advancing viticulture in the Black Sea region.

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THE EFFECT OF ADDITION OF GRAPEFRUIT OIL TO THE DIET ON PERFORMANCE AND EGG QUALITY IN AGED QUAILS

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ABSTRACT

The aim of this study was to determine the effects of supplementation of different levels of grapefruit oil (0, 250, 500 and 750 mg/kg) to laying aged quail diets on performance and egg external and internal quality. In the ten-week trial, a total of 96 female Japanese quails, 32 weeks aged, were distributed to 4 trial groups with 6 replications each containing 4 quails. The addition of grapefruit oil to the diet did not statistically affect performance parameters except for feed intake (P>0.05). Compared to the control group, the addition of 750 mg/kg grapefruit oil to the diet significantly reduced the feed intake of quails (P<0.05). Yolk a* value decreased statistically (P<0.01), but other egg quality parameters were not affected in laying quails (P>0.05) with the addition of 500 and 750 mg/kg grapefruit oil to the diet. According to these results, it was determined that the addition of 500 mg/kg grapefruit oil to the diet did not affect the performance in laying quails and reduced the yolk a* value, but feed intake decreased with the addition of higher grapefruit oil.

Keywords: Quail, grapefruit, performance, egg quality

INTRODUCTION

Studies on the use of medicinal and aromatic plants as additives in poultry nutrition have increased in the last 20 years. The phenolic compounds contained in these plants can be used as antibiotic (Çimrin and Demirel, 2016), performance enhancing (Kaya and Turgut, 2012), and egg production with high antioxidant capacity (Kaya and Turgut, 2012; Goliomytis et al., 2018) in poultry. In addition to medicinal and aromatic plants, plant parts that are not suitable for human consumption have an important role in animal nutrition as natural additives due to the phenolic compounds they contain. One of the by-products rich in phenolic compounds and antioxidant substances is grapefruit by-products, which are from the citrus family grown in our country.

Grapefruit (*Citrus paradisi*) is an evergreen tree belonging to the Rutaceae family. Grapefruit is used in traditional medicine as a natural antibacterial, antifungal, and antiviral preparate. Rich in flavonoids, carotenoid, citric acid, limonoid, vitamin C, tocopherol, and naringin grapefruit is a good antioxidant and antibacterial (Krajewska-Kułak et al., 2001; Bae, 2002; Vanamala et al., 2006; Fuselli et al., 2008). Vlaicu et al. (2021) reported that the performance of broilers fed with 2% grapefruit pulp was adversely affected.

The aim of this study is to examine the effect of adding grapefruit oil to laying quail diets on performance and egg quality.

MATERIAL AND METHODS

In the present experiment was conducted with a total of 96 female quails at the 32 weeks of age obtained from a commercial company. A completely randomized design was used for this experiment. The study was conducted in 4 experimental groups consisting of 6 replicates, each containing 4 female quails. The quails housed in clean and disinfected battery cages (30 cm wide 45 cm long). Quails were maintained in a well-ventilated room with a lighting program of 16 hours. A temperature of 20 ± 2.0°C and a relative humidity of 55 ± 5% were arranged in each pen. Each pen was provided with individual feeder and drinker to allow *ad libitum* intake.

Table 1. Basal diet and calculated nutrient contents

Ingredients	g/kg	Nutrient compositions	g/kg
Corn	549.6	Metabolizable energy, kcal/kg	2900
Soybean meal	339.0	Crude protein	199.91
Soybean oil	35.8	Calcium	24.99
Limestone	56.0	Available phosphorus	34.90
Dicalcium phosphate	11.5	Lysine	10.81
Salt	3.5	Methionine	4.51
Premix[1]	2.5	Methionine + cystine	8.53
Methionine	2.1		
Total	1000.0		

[1]Premix provides the 80 mg manganese (manganese oxide), 60 mg iron (iron carbonate), 5 mg copper (copper sulphate pentahydrate), 1 mg iodine, 0.15 mg selenium, 8800 IU vitamin A (trans-retinol acetate), 2200 IU vitamin D3 (cholecalciferol), 11 mg vitamin E (tocopherol), 44 mg nicotinic acid, 8.8 mg Cal-D-Pan, 4.4 mg Vitamin B2 (riboflavin), 2.5 mg thiamine, 6.6 mg vitamin B12 (cyanocobalamin), 1 mg folic acid, 0.11 mg biotin, 220 mg choline to per kg of diet.

Quails were fed for 10 weeks with 4 treatments with the addition of grapefruit oil at 0, 250, 500, and 750 mg/kg levels. The basal diet was formulated according to the NRC (1994) to supply requirements layer quails (Table 1).

At the beginning of the experiment, the quails were randomly allotted to the six trial groups, body weight and body weight change were weighing the groups at the beginning and final of the experiment with precision weighing balance (±0.01 g). Experimental diets were given by weighing to each subgroup, and subsequently feed intake was calculated as the daily feed intake per quail. At the same time of each day (at 10:00 am) eggs were collected and recorded. Egg production was determined by dividing the number of eggs obtained in a day by the number of quails and multiplying by 100 and it was given as a percentage (%).
Egg weight was determined by weighing one by one of all eggs collected in the last three days of the experiment with a precision weighing balance (±0.01 g). From these data, egg mass

was calculated as daily egg weight per quail according to the following equation: *(egg production x egg weight) x 100*. Finally, feed conversion ratio was determined according to next equation: *feed intake / egg mass.*

During the experiment, broken, cracked, and damaged eggs were recorded and calculated as percentage of the number of eggs. Egg internal and external quality parameters were determined at room temperature and at Selcuk University, Faculty of Agriculture, Egg Quality Laboratory from all eggs collected in the last three days of trial. Eggs were broken on a clean, glass surface, and after the residues in the eggshell were cleaned, the shells were dried at room temperature for three days and weighed, and relative weights were calculated as a ratio (%) of the egg weight. Eggshell thickness was calculated by averaging the measurements obtained from three sections (equator, blunt, and pointed parts) of the eggshell using a micrometre (Mitutoyo, 0.01 mm, Japan). Albumen and yolk heights were measured with a height gauge and their length and width were measured with a 0.01 mm digital calliper. The parameters calculated from these data and the equations used are follows. Albumen index using the next equation: *(albumen height / ((albumen width + albumen length)/2) x 100.* To determinate yolk index the following equation was used: *(yolk height / yolk diameter) x 100.* Finally, Haugh unit for each egg was calculated using data of egg weight and albumen height according to the next equation proposed by Haugh (1937): $100 \times \log(\text{albumen height} + 7.57 - 1.7 \times \text{egg weight}^{0.37})$. For colorimetric analysis, samples were deposited on the flat surface of Petri dishes, and all analyses were performed to maintain the integrity of the egg yolks. According to Titcomb et al. (2019), egg yolks were subjected to a previously calibrated Konica Minolta digital colorimeter (Minolta Chroma Meter CR 400 (Minolta Co., Osaka, Japan) for the measurement of the parameters L* (lightness), a* (redness), and b* (yellowness).

Data were analysed by one-way ANOVA using the Minitab (Minitab, 2000) using the cage mean as an experimental unit. Duncan multiple comparison test performed in the determine the differences among treatment groups.

RESULTS AND DISCUSSION

The effect of adding grapefruit oil to laying quail diets on performance is presented in Table 2.

Table 2. The effect of adding different levels of grapefruit oil to the diet on performance parameters in laying quails

Parameters	**Grapefruit oil, mg/kg**				**SEM***	***P*-value**
	0	**250**	**500**	**750**		
Initial body weight, g	274.20	278.17	272.33	277.80	8.105	0.947
Final body weight, g	290.83	288.67	286.67	295.80	7.304	0.838
Body weight change, g	16.63	10.50	14.33	18.00	4.863	0.742
Feed intake, g/day/quail	30.58^{a}	30.26^{ab}	30.35^{ab}	28.41^{b}	0.524	0.037
Egg production, %	88.60	90.80	91.94	82.39	2.388	0.133
Egg weight, g	11.90	12.17	12.94	12.21	0.340	0.892

Egg mass, g/day/quail	10.53	11.03	10.97	10.15	0.413	0.585
Feed conversion ratio, g feed/g egg	2.91	2.75	2.79	2.92	0.123	0.810

*****Standard error means
[a,b;] Means with in the same rows without common superscripts are significantly different (P<0.05).

The effects of adding grapefruit oil to the diet on body weight, egg production, egg weight, egg mass, and feed conversion ratio were statistically insignificant (P>0.05). Feed intake of quails was statistically affected by the inclusion of grapefruit oil (P<0.05). The addition of 750 mg/kg grapefruit oil to the diet compared to the control group caused a decrease in feed intake, but this effect did not observed at other levels. There is no study in the literature on the effect of grapefruit oil on feed consumption. There is no study in the literature on the effect of grapefruit oil on feed intake. However, it was stated that the addition of naringin (0.50-0.15 g/kg), one of the active ingredients in grapefruit, did not affect the feed intake of laying hens (İskender et al., 2017; Goliomytis et al., 2019). Similar results were reported in broiler breeders with the supplementation of naringin at 0.1, 0.2, and 0.4 levels (Li et al., 2022).

The effect of adding grapefruit oil to laying quail diets on performance is demonstrated in Table 3.

Tablo 3. The effect of adding different levels of grapefruit oil to the diet on egg quality in laying quails

Parameters	**Grapefruit oil, mg/kg**				**SEM***	***P*-value**
	0	**250**	**500**	**750**		
Damaged egg rate, %	1.23	1.10	0.00	0.68	0.523	0.499
Relative eggshell weight, %	8.34	8.12	8.33	8.11	0.227	0.837
Eggshell thickness, µm	228	232	231	227	3.97	0.802
Albumen index	4.67	4.28	4.51	5.28	0.321	0.215
Haugh unit	61.42	61.81	60.33	65.78	3.314	0.682
Yolk index	43.84	42.53	44.07	42.81	1.353	0.816
L*	50.73	49.99	49.70	50.51	1.029	0.902
a*	12.11^{A}	12.50^{A}	7.49^{B}	6.47^{B}	0.982	<0.001
b*	38.33	35.56	35.52	36.35	1.347	0.492

*****Standard error means
[A,B;] Means with in the same rows without common superscripts are significantly different (P<0.01).

The effect of adding grapefruit oil to the diet on the damaged egg rate, relative eggshell weight, eggshell thickness, albumen index, yolk index, Haugh unit, and L* and b* values was insignificant (P>0.05). The administration of grapefruit oil to the diet affected the yolk a* value, and the a* value of the yolk significantly decreased with the 500 and 750 mg/kg levels (P<0.01). Unlike this study, İskender et al. (2017) and Goliomytis et al. (2019) reported that the addition of naringin (0.50, 0.75, and 1.5 g/kg), one of the active ingredients of grapefruit, to the diet did not affect yolk color in hens.

There is no similarity between the limited studies examining the effects of grapefruit products in poultry in previous years and the current study in terms of performance and egg quality. It can be said that the use of quail as animal material and grapefruit oil as additive in the current study and the use of hen and active substance (naringin) in other studies are the main reasons for these differences. According to the results of this study, it can be said that the addition of grapefruit oil at the level of 750 mg/kg reduces the feed intake of quails and it is not necessary to add grapefruit to the diet, but more studies are needed in terms of the level used and the parameters examined.

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THE ROLE OF STERILE INSECT TECHNIQUE IN PEST MANAGEMENT

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ABSTRACT

Pests and diseases in crop production cause significant economic losses. Various chemicals are used to prevent economic losses caused by these pests. These chemicals have negative effects on the environment and human health. Due to these negative effects, new solutions are sought in agricultural control. Recently, genetic research has been carried out on alternative control methods. The sterile insect method is one of these alternative control methods. The Sterile Insect Technique (SIT) is based on the sterilization of male individuals raised in laboratory conditions by radiation and mass release into nature. This sterilization method does not change the mating behaviour of the pest. Reproduction does not occur when sterilized male individuals mate with the wild female individuals. Thus, the target pest population in the new generation is suppressed. One of the most important advantages of this technique is that the application is species-specific and interaction doesn’t occur with other species. The sterile insect technique is applied for controlling various pests in many parts of the world, including our country. The first application of the sterile insect technique in our country was made against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), which is considered one of the most important agricultural pests in the world with more than 250 hosts. The Mediterranean fruit fly which is an invasive and intolerant pest in the EPPO A2 (European and Mediterranean Plant Protection Organization) quarantine list. The pest causes rotting, crushing, and collapse in this fruit. In this study, information about the sterile technique against this pest was given and its application areas, advantages, and disadvantages in Türkiye were evaluated.

Keywords: Sterile Insect Technique (SIT), *Ceratitis Capitata*, Pest Management, Biotechnological Control Method.

INTRODUCTION

Chemical control is undoubtedly one of the leading methods used for controlling pest organisms that cause significant product yield and quality losses in crop production. The reason why chemical control is preferred so much in practice is that it gives fast and effective results as well as farmer habits. Although chemical control gives fast and effective results in pest control, it has many negative aspects on human health and the environment. The most important of side effects of pesticides are residues in agricultural products, pollution of water soils resources, decrease in the populations of non-target beneficial insects, causing various health problems of producers and consumers, affecting other living organisms and animals in the sprayed area and disruption of the ecological balance, water and soil resources. Due to these known negative effects of chemical control, alternative control methods have been sought for sustainable agriculture in recent years. One of these methods is the Sterile Insect Technique (SIT), which is a biotechnological control method.

This promising technique date back to the early 20th century. In 1937, the American entomologist Edward F. Knipling investigated and arithmetically analysed the population control potential of SIT (Knipling, 1955). In 1955, Knipling studied the biology and mating behaviour of *Cochliomyia hominivorax,* the twisted wound fly (Sönmez and Mamay, 2109). In recent years, the development of sustainable vector control methods has become one of the most popular and important topics for the vectorization of anthropogenic diseases such as malaria, dengue, chikungunya or to reduce the impact of agricultural pests such as fruit flies (Angelov et al., 2020). One of the greatest successes of this method in the past was undoubtedly its use for controlling *C. hominivorax* (Viktorov, 2021).

The sterile insect technique involves the mass rearing of large numbers of sterilized insects in the laboratory (Knipling, 1955), and the mass release of these sterile insects to reduce or eliminate subsequent generations of the target pest (Knipling, 1979). In this technique, sterilization is achieved by radiation, chemosterilants, and gene transfer. The sterilization process is based on causing damage to the reproductive system without damaging sperm viability. There is no change in the biology and mating behaviour of insects subjected to this procedure. Mass releases of males sterilized by irradiation are carried out. The released sterile males mate with fertile females in the wild, and since fertilization does not occur, the female is unable to lay eggs, or although she lays eggs, no development of individuals occurs (Maiti et al., 2006; Ami et al., 2009), thus causing a gradual decline of the targeted population. Sterilization with chemosterilants such as tetramine, triazine, and folic acid (Sönmez and Mamay, 2019) causes breaks in insect chromosomes and the resulting mutations prevent zygote formation or inhibit or suppress the release of hormones that play an important role in the reproductive system (Anonymous, 2019). In the sterilization method by gene transfer, individuals with certain characteristics are produced by making changes in certain gene regions in insects. However, there is not enough studies in the world including Türkiye (Birişik et al., 2013).

The Sterile Insect Technique has been applied in many parts of the world to control different pest species. It has been successfully applied in large areas, especially against dipterous pests such as olive fly, Mediterranean fruit fly, and cherry fly, which cause significant economic damage. In addition to these pests, the same method has been applied against Lepidoptera species such as apple borer and pink bollworm (Schetelig and Wimmer, 2011; Henneberry, 2007). This technique is applied not only against agricultural pests but also against some mosquitoes that adversely affect public health (Vreysen, 2001). In our country, various researches have been carried out on the radiation sterilization method against some corn pests (Aksoy et al., 2014; Avan Aksoy, 2014), especially a few fruit flies (Tephritids) (Genç et al., 2016) and the warehouse pest crushing weevil (Azizoğlu et al., 2010) have been used successfully against many pest species (Handler, 2002). The first release trial against Mediterranean fruit flies was conducted in 1983 in the Çeşme district of İzmir. The first mass release was carried out in 2021 by the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policy against the Mediterranean Fruit Fly (*Ceratitis capitata*), which causes significant economic losses in citrus fruits, in the mandarin orchard in Izmir province where the population is very high (Anonymous, 2021). The sterilization process of these released insects is carried out by the Türkiye Energy, Nuclear, and Mining Research Institute (TENMAK) and General Directorate of Agricultural Research and Policy.

The fruits that have an important share in exports are mainly citrus fruits, figs, pomegranates, apricots, persimmon, quince, peach, and avocado. Mediterranean fruit fly is distributed along the Mediterranean and Aegean coasts including Central Anatolian region. This pest can cause damage in both adult and larval stages. Females cause damage by laying eggs in the fruit. While larvae feeds inside fruits and gives quality damage. In the area where the pest lays its eggs, yellowish-brown strike spots are formed. The larvae, which cause the main damage, feed on the flesh of the fruit and cause depression, dents, and softening (Birişik et al., 2013). It is forbidden to export fruits infected with med fly, because it is on the list of many countries including EPPO members as a quarantine pest and there is no tolerance for entrance. For this reason, the control of this polyphagous pest, is very important for our country as Türkiye producer of many fruits which are hosting med fly. Since there is no any identified biological control agent against the Mediterranean fruit fly in our country, biological control could not be implemented. However, some cultural measures and partially poisoned bait branch spraying methods are applied. In addition to these methods, sterile insect release has been practiced since 2021. This method, which has just started to be applied in our country, can be integrated with other control methods.

In this technique, several studies and survey results should be done to see the effect of sterile insect properly. First, the biology of the pest should be well known. It is necessary to have sufficient information about the mating behaviour of the pest, and its ability to survive and spread in nature. The fact that the female mates once in her lifetime and the male preferably mates several times is one of the factors that can increase the success of the application. It is very important that the males to be released mass reared can easily spread and be mobile in nature. It is also very important for the applicability of this method that these individuals can be easily produced mass rearing. At the same time, the number of released individuals should be higher than the number of pests in nature. If the population of the pest is high in the application area, efforts should be made to reduce the population with other control methods before releasing SIT insects. After the mass release, the pest population should be monitored and the appropriate method should be selected to determine the population (Birişik et al., 2013; Özbek and Pande, 1992).

The sterile insect release technique has some advantages and disadvantages. One significant advantage is that it is specified for certain pest species and does not interact with other species so that the ecological balance is not disturbed. Another advantage is that it can be applied in an integrated manner with other control methods, reducing the use of chemicals and thus avoiding problems such as resistance. At the end of a properly conducted and monitored mass release, the population can be suppressed. The disadvantages of this method are that it does not obtain a result as quickly as chemical control, therefore, rerelease many times it is necessary to in certain periods and it can be applied in limited areas.

RESULTS AND DISCUSSION

In conclusion, this method is a promising biotechnological control method in terms of being environmentally friendly, alternative to chemical pesticides, reducing production inputs, and suppressing pest populations. Like other methods, this method also has some disadvantages, but these disadvantages can be reduced with a good theoretical analysis during field studies. The application areas of the SIT technique can be increased in the controlling the Mediterranean fruit fly, which causes significant economic losses in our country. In addition, SIT can be used to suppress the populations of main pests such as olive fly and corn borer. Investigation about in our country is not at a sufficient level and need to be developed. In short, for the vicious insect release technique to become widespread and provide success in practice, application studies need to be expanded and tested on different species.

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STUDIES ON THE DISTRIBUTION OF STOMATA IN VINE AND THE IMPORTANCE OF STOMATA

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ABSTRACT

It is very important to increase the high quality and yield level in viticulture and to determine the plant-water level for successful cultivation. In addition to having a vital effect on the physiology, adaptation, and productivity of plants, stomata are also of indispensable importance for the survival of plants. There is a large number of stomata on the vine that carry out the passage of gases necessary for photosynthesis on the lower surfaces of the leaves and water loss by evaporation. Even in vine species or varieties that are morphologically similar to each other, serious differences are observed in the number of stomata, size, and leaf blade distributions. Apart from morphological differences, different factors also have a significant effect on the number and density of stomata. These factors include carbonization (C02), the water content of the leaf, temperature, light, air, and soil moisture, wind, cultural practices, internal growth agents, enzymes, and vitamins. In the beginning, the most important features of stomata are the changes in the stoma character that occur under stress conditions. Many studies conducted in the field of viticulture have also shown that stomata protect the plant against drought stress by reducing the rate of transpiration and photosynthesis under dehydrated conditions. In a similar way, it has been found that they have fewer stomata in varieties where cold resistance has been determined to be high compared to sensitive varieties. For this reason, determining the number and distribution of stomata, as well as their structural characteristics, will make a great contribution to the development of viticulture, which is negatively affected by climate change.

Keywords: Stomata, Grape, Stoma Density, Draught Stress, Stress Factors

INTRODUCTION

Regulation of plant-water relations is extremely important for high yield and quality in plants and successful cultivation. Stomata are organs in the leaves and are gateways for gas exchange between the internal tissues of the plant and the external environment. Stomata adjust transpiration by opening and closing according to various conditions. Thanks to stomata, plants continue their lives without losing much water (Yanmaz and Eriş, 1984; Vardar, 1969).

Stomata are small pores that allow CO^2, O^2, and water vapor to enter and exit the plants and are densely located in the above-ground organs of plants, especially in the leaf epidermis (Winkler et al., 1974). The gap between the stomatal cells, which opens and closes, is called the stomatal aperture (ostiol), and the thin-walled cells next to them are called neighboring cells (Akman, 1985). On the lower surfaces of grapevine leaves, many stomata regulate the gas exchange required for photosynthesis and allow water to escape as vapor, and the grapevine (Vitis vinifera L.) can maintain its vital activities through the stomata on its leaves. The stomata, which can open and close regularly, open when necessary to allow the gas exchange necessary for photosynthesis and close to prevent unwanted water losses (Eriş, 1979; Nicotra et al., 2011; Wang et al., 2019).

Based on their stomatal control behavior under varying environmental conditions, plants are grouped into isohydric and anisohydric. Isohydric plants have a tight arrangement of stomata that allows the maintenance of nearly constant leaf water potential in plants exposed or not exposed to drought. In contrast, anisohydric plants show lower stomatal control, leading to large fluctuations in leaf water potential under soil water deficit and evaporative demand (Prieto et al., 2010).

The first studies on stomatal numbers in grapevine started in the late 19th century. In Müller-Thurgau's 'Ampalographische Berichte' published in 1882, it was reported that there were 186 stomata per 1 m^2 on the lower surface of the 'Riesling' grape variety (Oraman, 1972).

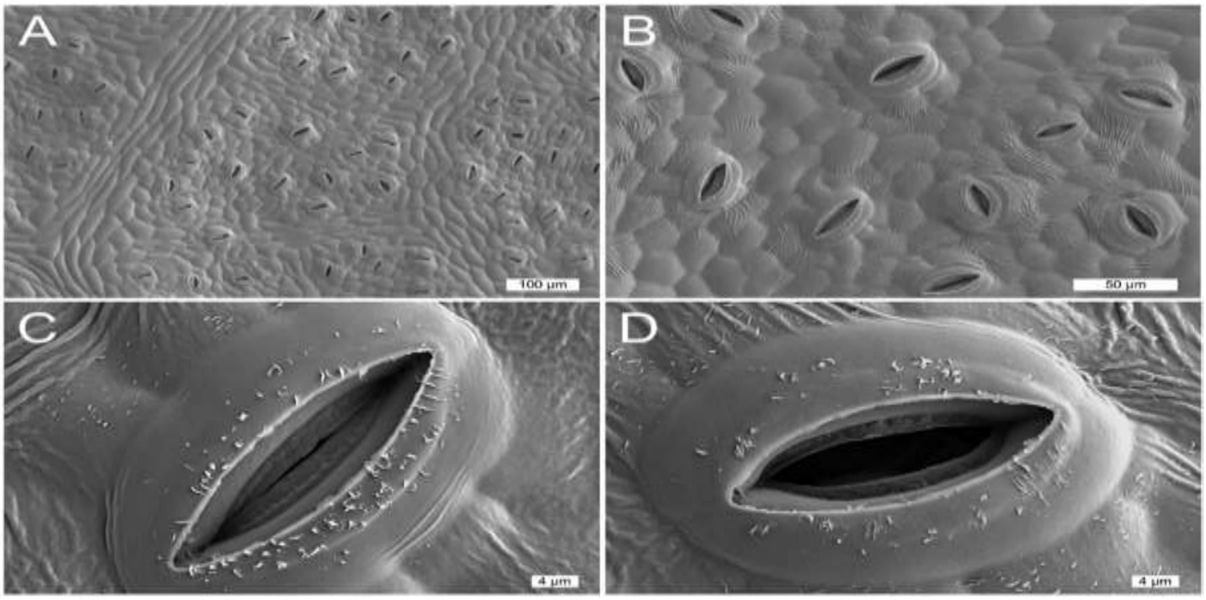

Figure 1. Cryo-scanning electron microscopy images of the underside of a grapevine (Vitis rupestris) leaf at various magnifications. Islands of stomata (A) are visible in between leaf vascular traces, and there is ample aperture diversity (B) of stomata in more closed (C) or open (D) states (Gambetta et. al. 2020).

Stomata are mostly located on the leaf. Their location on the leaf may differ between plant species. While in many species they can be found on both the upper and lower surfaces of the leaves (amphistomatic), most species, including grapevine, are grouped as hypostomatic, and stomata are located on the lower surface of the leaves (Lebon et al., 2006; Alonso-Villaverde et al., 2011).

It is known that there are differences between Vitis species and cultivars in stomatal morphology mechanisms of opening and closing and their participation in physiological processes (Franks and Farquhar, 2007; Rogiers et. al., 2011).

Stomatal morphology and the mechanisms of their involvement in physiological processes have been studied extensively, especially recently. The relationship between the density and size of stomata and vineyard myrtle (Plasmopara viticola) (Dai et. get., 1994; Lu et. get., 2010; Boso et. get., 2016), the variation of stomatal characteristics according to the location on the leaf surface, age and origin of the shoot (Palliotti et. al., 2000; Yildirim and

Dardeniz, 2017), the relationship between stomatal number and leaf characteristics (Santiago et. al, 2007; Workman et al., 2015), effects of atmospheric CO2 variation on stomatal number and density (Moutinho-Pereira et al., 2009; Rogiers et al., 2011; Kunter et al., 2015), relationship between stomatal conductance (gs) and stomatal number and stomatal aperture ratio (Franks and Beerling, 2009), water stress (Marasalı and Aytekin, 2002; Costa et al, 2012; Candar et al., 2021; Herrera et al., 2022), soil temperatures (Rogiers et al., 2011; Damiano et al., 2022), different cultivation practices (Atik and Dardeniz, 2018), wind (Gökbayrak et al., 2008), effects of different doses of plant nutrients (Diligent and Patience, 2022), interactions of microorganisms on leaf surfaces with stomata (Konnlecher and Sauner, 2016), genetic differences of rootstocks and cultivars (Gargin, 2009; Workman et al., 2015; Bekisli and Gürsöz, 2016; Uyak et al., 2016; Dardeniz and Yildirim, 2017).

Studies on Stomatal Density and Number of Stomata in Grapevine

Plants growing in different environments undergo some anatomical and morphological changes in that environment (Mert et al., 2009). Some intrinsic and extrinsic factors, sometimes alone and sometimes in combination, affect the density and movement of stomata. These factors include carbon dioxide (C02), leaf water content, temperature, light, air and soil moisture, wind, cultural practices, rootstocks, internal growth substances, enzymes, and vitamins (Şahin, 1989; Gökbayrak et al., 2008).

The stomata density of grapevine leaves can present a large variation. A range of 50 to 400 stomata per mm^2 was reported by Keller (2010), while Monteiro et al., (2013) observed a range of 207 to 286 stomata per mm^2 and Teixeira et al., (2018), 170 to 250 stomata per mm^2 . Amongst the cultivars ‘ Grenache Noir’ and ‘Syrah’, significant differences in stomatal density with pot grown grapevines have also been noted, showing greater differences than field grown plants (Gerzon et al., 2015).

The number of stomata in mm^2 is one of the effective factors in resistance to abiotic stresses. İşçi et al. (2015) found that the number of stomata in varieties grafted on 110R rootstock, which is among the rootstocks with high drought resistance, was higher than those grafted on 41B rootstock. It is thought that drought-resistant plants have a higher number of stomata (Gindel, 1969).

Düzenli and Ağaoğlu (1992) found that the number of stomata of Razakı grape variety, which has a high level of drought resistance, was higher than other grape varieties. In another study, it was found that the number of stomata per mm2 was lower in drought-sensitive Çavuş, Amasya, Tarsus Beyazı, and Sultani seedless varieties than in drought-resistant Yapıncak and Balbal varieties (Eriş and Soylu, 1990).

The size and density of stomata vary according to plant species and varieties and growing conditions. In the Round Seedless grape variety grafted on drought-resistant rootstocks such as 99R and 110R, the number of stomata per mm2 was 284.4 and 294.8, respectively, and was higher than those grafted on other rootstocks (Kara and Özeker, 1999).

Atik and Dardeniz (2018) examined the effects of different crown management practices on stomatal width, stomatal length, and stomatal density in the Yalova İncisi grape variety and obtained the highest stomatal density in the treatment with the tip cut 10 cm above the first vine wire.

Clemens et al., (2022), reported VvEPFL9-1 Knock-Out via CRISPR/Cas9 Reduces Stomatal Density in Grapevine. In the study conducted, it was determined that stomatal density in transgenic plants was lower than the control. They also obtained an increase in water-use efficiency and stomatal length compared to the control.

Investigation of the Effects of Abiotic and Biotic Stress Factors on Stomata in Grapevine Leaves

Drought Stress

One of the early responses of plants to drought conditions is the closure of stomata and reduction of water loss through transpiration (Mahajan and Tuteja 2005). The plant hormone ABA (Abscisic acid) (Mahajan and Tuteja 2005) and changes in calcium concentration play a role in stomatal closure (Wilkinson et al., 2001). Closure of stomata reduces Rubisco activity, which in turn reduces CO2 uptake and decreases photosynthesis rate (Bota et al., 2016).
Under drought conditions, leaf area tends to decrease with decreasing available water (Scienza ve Boselli, 1982; Bierhuizen and ark., 1984; Cramer et al., 2009). On the other hand, small leaves are known to have lower leaf surface resistance due to their morphological structure and lower leaf temperatures, providing better adaptability to drought conditions (Leigh et al., 2017).

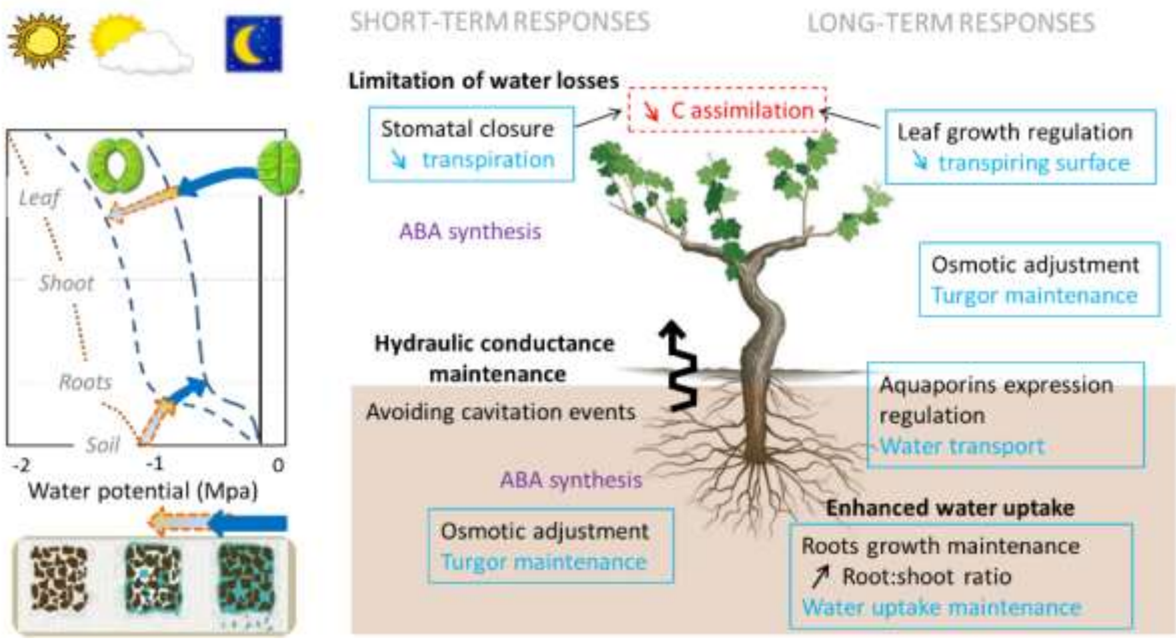

Figure 2. Short and long term effects of drought on grapevine (Simonneau et. al., 2017).

Soil-plant water relations were investigated in three cultivars (Carignane, Merlot, Shiraz) subjected to long-term and short-term water stress under field conditions. It was reported that stomatal regulation or limitation of leaf area played an important role in both daily and long-term water stress; in the short term, physiological adaptation to water stress through stomatal control was much better developed in the Carignane cultivar. In the long term, it was observed that the Shiraz variety adapted to water stress by reducing leaf area (Winkel and Rambal 1993).

In 110R (*V. berlandieri* x *V. rupestris*) rootstock, which is known for its drought tolerance, how stomatal conductance is regulated under different physiological conditions created by water stress and re-irrigation was analyzed and the effect of water stress on effective water use. It was found that water stress significantly induced stomatal closure and increased effective water use (Martorell et al. 2015).

In a study investigating the effects of some organic fertilizers on the stomatal characteristics of grafted and ungrafted 'Michele Palieri' grapevines grown under calcium stress in a soilless culture medium, it was found that stomatal density increased significantly (Gayretli and Sabır, 2022).

OST1 (open stomata 1, Ser/Thr protein kinase deficient) mutants show hypersensitivity to freezing, while transgenic plants overexpressing OST1 show high tolerance to freezing. In addition to ABA, H2S, a gas signaling molecule, is involved in the closure of stomata as well as many physiological and developmental events (García-Mata and Lamattina, 2013). Recent studies show that ABA greatly increases the amount of other compounds such as Methyl Jasmonate, Salicylic acid (SA) and Polyamines (Pas) (Alcázar et al., 2010). Compounds modulated by draught stress are shown in Figure 3.

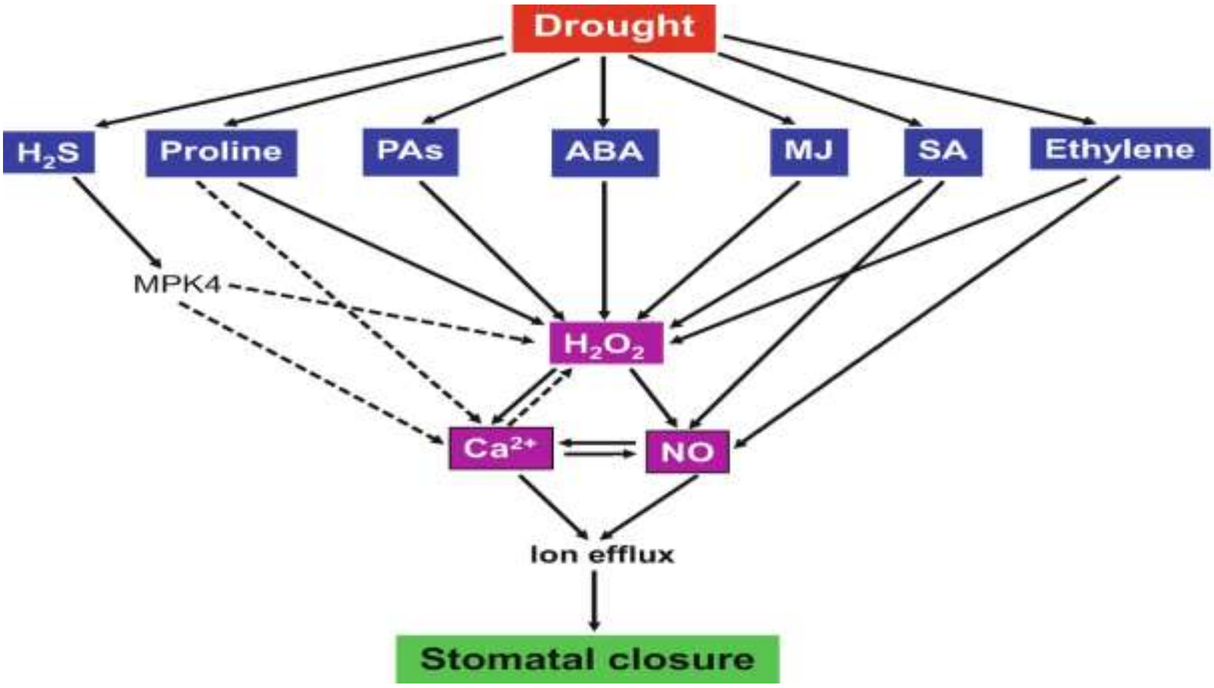

Figure 3. A diagrammatic representation of hormonal/metabolite signal transduction mechanism under drought stress in stomatal guard cells during stomatal closure (Agurla et al., 2018).

Drought stress was applied to 'Grenache' known to be tolerant to drought stress and Semillion cultivars known to be sensitive to drought stress and various parameters were investigated. During the 10-day drought stress period, 'Grenache' cultivar was found to have lower pre-dawn and mid-day root water potential values than Semillion cultivar. Grenache cultivar was also found to maintain lower stomatal conductance and transpiration than Semillion cultivar (Rogiers et al. 2011).

Cold Stress

During cold stress, H2S accumulation up-regulates mitogen-activated protein kinase 4 (MAPK4). Stomatal development is affected, leading to a reduction in stomatal density under cold conditions (Hetherington and Woodward 2003; Vatén and Bergmann 2012). This will reduce transpiration and prevent water from evaporating and being lost. In addition, stomata are activated at the onset of drought or cold stress due to various events. Under these conditions, stomatal closure is a common response due to the accumulation of compounds such as ABA, methyl jasmonate (MJ), ethylene (ET) and brassinosteroids (BS). Some other compounds modulated by cold stress in many plants and their effects on stomatal function are shown in

Figure4.

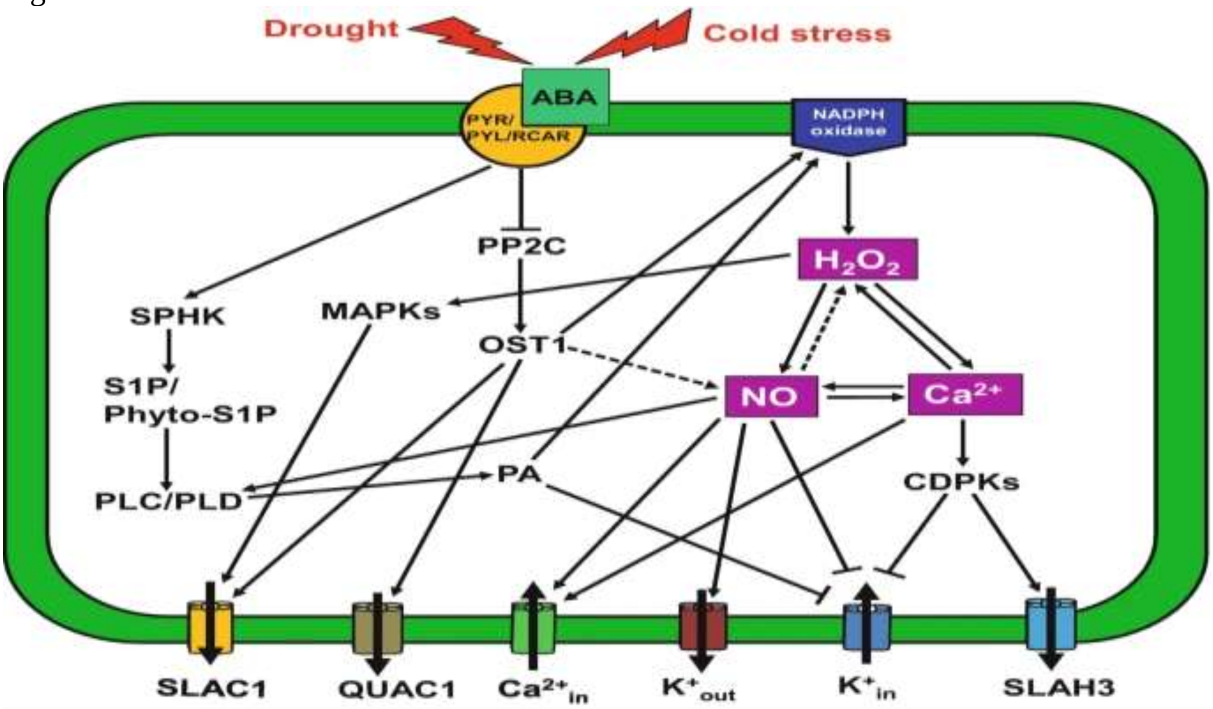

Figure 4. A scheme of signal transduction mechanism under abiotic (drought/cold) stress was mediated by several secondary messengers in guard cells during stomatal closure (Agurla et al., 2018).

Stomatal Responses to Disease Agents

The infection starts 2-4 hours after the disease agent enters through the stomata of the leaves of the susceptible genotype, haustoriums are formed after 72 hours and sporangiphores are formed after 96 hours, while in the resistant genotype, the grass tube is formed 24 hours after inoculation, but sporulation stops due to the closure of the stomata (Alonso-Villaverde, Voinesco, Viret, Spring, & Gindro, 2011; Gindro, Pezet, & Viret, 2003).

Although the number of stomata on grapevine leaves varies according to environmental conditions and genotypes (Rogiers, Hardie, & Smith, 2011), it has been reported that there is a positive correlation between the number of stomata and myrtle infection and that determining the developmental stages of the agent in the stomata is an important criterion for resistance (Gindro et al, 2003; Gindro, Spring, Pezet, Richter and Viret, 2006; Gómez-Zeledón, Kaiser, M. and Spring, O., 2017; Kortekamp, Wind and Zyprian, 1998; Paolocci, Muganu, Alonso-Villaverde and Gindro, 2014; van Leeuwen, Roby, Alonso-Villaverde and Gindro, 2013).

Gindro et al. (2006) tested the resistance of 42 grapevine cultivars to P. viticola by determining sporangium density 6 days after inoculation of sporangium suspension of the pathogen by spraying on leaf discs of the cultivars, callose accumulation in the stomata after 48 hours, δ-viniferin and ε-viniferin levels. The researchers grouped 42 cultivars according to sporangium density as very resistant, resistant, less sensitive, sensitive, and very sensitive. As a result, the study reported that callose accumulation and ε-viniferin in the stomata were very high in very resistant varieties (>100 µmol/mg TA) followed by δ-viniferin (>80 µmol/mg TA) and it is suggested that such tests will contribute to the reduction of time in breeding studies.

In the studies conducted, it was reported that the number of infected stomata in genotypes with a low percentage of infected areas 24 hours after inoculation was lower than in susceptible ones (Paolocci et al., 2014).

Stomata in Mutation Breeding

With mutation breeding, it is possible to change one or more characteristics of grape varieties with high consumer demand. Mutation refers to a sudden and hereditary change in genetic material. It is possible to obtain new varieties that are partially or completely different from the parent plant by separating the mutated part from the parent plant and vegetatively propagating it (Çoban, 2003). However, while a long time is required for classical breeding studies, mutation breeding studies give results in a shorter time (Ahloowalia and Maluszynski, 2001).

Radiation application is mostly used as a mutagen in plant mutation breeding studies due to the ease of obtaining direct mutant varieties, easy access to target cells due to its high permeability, and no toxic effects and damage (Değirmenci, 2006).

Ekbiç (2010) reported that colchicine applications caused a decrease in stomatal number and an increase in stomatal width and length depending on the dose increase.

Kunter et al. (2015) examined stomatal density, size, and structural characteristics of chimeric genotypes obtained by ionizing radiation applications for mutation breeding in Kalecik Karasi, Sultani Seedless, and Uslu grape varieties. The leaves used in the study were collected from individuals irradiated with 20 Gy, 25 Gy, 30 Gy, and 45 Gy and selected for their chimeric characteristics. As a result of the study, stomatal density was found to be lower in all doses of ionizing radiation treatments compared to the control groups, whereas stomatal size (length/width) was found to increase. Among the genotypes, the largest stomata were obtained from 25 Gy and 20 Gy treatments of Sultani Seedless cultivar. It was observed that stomata were generally embedded in the epidermis in chimeric plants.

CONCLUSION

Stomata, which have extremely important effects on the physiology, adaptation and productivity of plants, are also an indispensable part of the life chains of plants. For this reason, the structural characteristics and movements of stomata, as well as their number and distribution on the leaf surfaces have been the subject of various studies due to both morphological and physiological reasons.

Stomatal density and size in grapevine leaves can be highly affected by grape varieties, study period, natural conditions, cultural practices, biotic and abiotic stress factors and genetic differences. Therefore, it is thought that with the help of new studies on stomata, it is possible to contribute to the completion of the missing aspects in this subject.

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TOMATO RESISTANCE GENES *MI* AGAINST TO THE ROOT KNOT NEMATODE (*MELOIDOGYNE* SPP.) AND MOLECULAR APPROACHES.

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ABSTRACT

Meloidogyne spp. was first detected in the UK but is now a worldwide problem for tomato and other Solanaceae crop production, threatening production both in open fields and greenhouses. If appropriate control measures are not taken 15- 85 % yield losses can take place. Tolerances of the plants themselves, as well as chemical spraying and biological agents, are of great importance for damage prevention. Understanding and engineering these gene mechanisms is of great importance for development of tolerant varieties against *Meloidogyne* spp. Plant resistance(R) proteins recognize pathogen virulence (Avr) determinants and trigger plant defense mechanism. Then the carefully organized dynamic defense regularly emerges as a Hypersensitive Response (HR) and the defense becomes active. As a result of these changes, new studies identified new components of *Mi-1*-mediated resistance to the nematodes. In this study we review the molecular mechanisms of tolerance against *Meloidogyne spp.* in tomato.

Key words: *Meloidogyne* spp., defense mechanisms, host response, *Solanum lycopersicum, Mi-1* genes

ÖZET

Meloidogyne spp. ilk olarak İngiltere’ de tespit edilmiştir. Ancak artık domates ve diğer *Solanaceae* familyasına ait mahsul üretimi için dünya çapında bir sorun haline gelmektedir. Hem açık tarlalarda hem de seralarda üretimi tehdit etmektedir. Mücadelesinde uygun önlemler alınmaz ise üretimde %15-85 verim kaybına sebep olmaktadır. Kimyasal mücadele ve biyolojik mücadelenin yanı sıra bitkilerin kendi toleransları da zararı önlemede büyük önem taşımaktadır. *Meloidogyne* spp.'ye karşı dayanıklı çeşitlerin geliştirilmesi için bu gen mekanizmalarının anlaşılması ve mühendisliği büyük önem taşımaktadır. Bitki direnci(R) proteinleri, patojen avirülans (Avr) belirleyicilerini tanır ve bitki savunma mekanizmasını tetiklemektedir. Daha sonra dikkatlice organize edilmiş dinamik savunma, düzenli olarak bir Aşırı Duyarlı Tepki(HR) olarak ortaya çıkarak ve savunma aktif hale gelmektedir. Yapılan araştırmalar sonucunda nematodlara karşı dayanıklılığın *Mi-1* geni ile sağlandığı tespit edilmiştir. Bu çalışmada ise domateste *Meloidogyne* spp.'ye karşı moleküler dayanıklılık mekanizmalarının gözden geçirilmesi amaçlanmıştır.

Anahtar Kelimeler: *Meloidogyne* spp., dayanıklılık mekanizması, konukçu yanıtı, domates, *Mi-1* geni

Introduction

Tomato is one of the most important vegetables grown in the world. They also contain high levels of lycopene, an antioxidant that reduces the risks associated with many cancers and neurological diseases. The homeland of the tomato includes Chile, Peru and Ecuador in western South America. In addition, it was determined that there are 2 endemic wild tomato species in Galapagos Island (Darwin et al. 2003). *Solanum peruvianum* L. is the most common and polymorphic wild tomato species. It has been stated that the possible ancestor of the tomato, which is an annual plant, is the wild cherry tomato (*Solanum lycopersicum* var. cerasiforme) (Jenkins 1948; Akkurt et al. 2013). It has been reported that in ancient Mexico, the tomato was grown for food and was called “tomati” (Boswell 1937). Tomatoes are used fresh or in various forms such as peeled, chopped, frozen, canned, tomato paste, ketchup, pickles in the food industry (Causse et al. 2016).

After it became widespread in the European Mediterranean countries in the 16th century, it is cultivated in many parts of the world in the 20th century (Yazgan and Fidan 1996). 189.1 million tons of tomatoes were produced around the world in 2021. China ranked first in tomato production and harvested area in 2021. Türkiye ranked 3 rd in world tomato production in 2021.

Tomato production; 8.7 tons in 2021/22. According to the 1st Estimation of Crop Production by TURKSTAT for 2023, tomato production is expected to be 13.5 million tons in 2023.Considering these data, the importance of tomato in the country's agriculture is clearly seen.

There are many pests and diseases that cause yield loss in tomatoes. Root-knot nematodes, which are plant parasitic nematodes that feed as endoparasites, also cause serious damage to tomatoes (Bleve-Zacheo et al. 2007). Root-knot nematodes (*Meloidogyne* spp.) are spread all over the world and have a wide host range (Sasser 1980, Karssen and Moens 2006). The four most important pests worldwide are *Meloidogyne javanica* (Treub) *Chitwood*, *Meloidogyne arenaria* (Neal) *Chitwood*, *Meloidogyne incognita* (Kofoid et White) *Chitwood* and *Meloidogyne hapla Chitwood* (Netscher and Sikora 1990). Root-knot nematodes are obligate parasites that feed only on the cytoplasm of living plant cells (Willamson and Hussey 1996). They become adults after four larval stages (Luc 1990). It is a second instar larva (J2) that penetrates the root and moves to an area near the vascular tissue to create a permanent feeding site (Williamson and Hussey 1996). After J2 enters the root, it moves between the cells in the vascular cylinder and fixes itself when it determines the feeding area (Abad and Williamson 2010). They cause the formation of giant cells in the area where they feed. The swellings that occur on the root surface as a result of growth in giant cells are called galls or galls (Williamson and Hussey 1996). The resulting galls significantly restrict the nutrient and water uptake of the roots from the soil. Then, they cause yellowing, wilting, stagnation in growth, deterioration in fruit quality and decrease in yield. In addition, they increase the formation of diseases by causing soil-borne pathogens to enter through the wounds they open.

It has been reported that *Meloidogyne* spp. causes an annual loss of 157 billion dollars worldwide (Abad et al. 2008). In addition, disease severity increases as a result of co-infection with soil-borne pathogens (Lambert and Bekal 2002). That's why it's so important to management. Cultural measures, physical control, biological control and chemical control methods are used. Chemical control is the most used method for controlling root-knot nematodes (Gowen et al. 2007). Despite this, the use of nematicides is decreasing in some regions of the world (Nyczepir and Thomas 2009). In addition, the prohibition of widely used fumigant such as methyl bromide (methyl bromide) has increased the search for alternatives in chemicals. Studies are carried out on alternative methods of struggle that will not cause the stated results. One of them is the use of biological organisms. The difficulty of adaptation of these organisms to environmental conditions and the cost of the preparations limit their use. Therefore, the use of resistant varieties comes to the fore (Rotino et al. 2002; Toppino et al. 2008). However, host resistance, which is one of the cultural methods, is known as the most effective and environmentally friendly method against root-knot nematodes (Devran and Söğüt 2014).

Resistant varieties provide ease of application and provide an environmentally friendly solution without the need for special tools and equipment (Lopez-Perez et al. 2006; Cortada et al. 2009; Verdejo-Lucas et al. 2009). Resistance prevents the reproduction or development of the nematode through the resistance genes it carries or keeps it at a very low level (Boerma and Hussey 1992, Roberts 2002). Resistant varieties suppress root-knot nematode and reduce the need for chemical control (Williamson 1999).

1. Resistance to Root Knot Nematodes

Resistance to root-knot nematodes was reported for the first time in a wild tomato species, *Solanum peruvianum* (Bailey, 1941). This resistance gene, called the *Mi-1* gene, was transferred to *S. esculentum*, the cultivar of tomato, by embryo rescue technique (Smith, 1944). Today, commercially developed root-knot nematode resistant cultivars carry this gene (Yaghoobi et al., 2005). Many genes (*Mi-2* to *Mi-9*) have been identified against the root-knot nematode, except the *Mi-1* gene (Capnet al., 1993; Yaghoobi et al., 1995; Veremis & Roberts, 1996a; Veremis & Roberts., 1996b; Milligan et al., 1998; Ammiraju et al., 2003). Knowing the characteristics of resistance genes and their responses to nematodes is important for breeding and control.

Plants have developed different defense mechanisms to protect themselves from diseases and pests. Resistance, which is one of these mechanisms, has been defined as the ability of the plant to prevent, eliminate or reduce the attacks of disease agents and pests (Wingard, 1953). For entomologists, the "hardy" plant is less affected by the same population of the pest (Painter, 1951). In general, a nematode-resistant plant is one that can inhibit the growth of the nematode compared to a non-resistant one (Cook & Evans, 1987; Trudgill, 1991; Barker, 1993).

Plants first show a passive response consisting of physical barriers to protect themselves from the pathogen. Thickening of the cell wall as a result of lignin accumulation is one of these barriers (Tör, 1998). Important plant hormones such as salicylic acid, jasmonic acid and ethylene play a role in defense (Kunkel & Brooks, 2002). Another defense mechanism is the hypersensitivity reaction (Hypersensitivity Reaction-HR) created by the resistance genes (Willamson & Hussey, 1996).

The emergence of resistance in plants occurs when the resistance gene (R) in the host and the avirulence gene (avr) products of the pathogen match each other (Flor, 1955). Resistant plants prevent the reproduction or development of the nematode through the genes they carry (Roberts, 2002). These plants protect the plant from nematode damage and reduce the nematode population (Lopez-Pérez, 2006). Tolerant plants, on the other hand, cannot suppress the growth of nematodes, but prevent yield loss (Gonzalez, 2009).

Root-knot nematodes cannot form a feeding zone in a resistant plant (Milligan et al., 1998). In order to create a feeding zone, a hypersensitive reaction occurs immediately in the cell to which it inserts its stylet. In the incompatible interaction of the plant with the nematode, O is produced enzymatically outside the cell and is converted to hydrogen peroxide (H2O2), a compound that can pass through the cell membrane (Bleve-Zacheo et al., 2007). H2O2 begins to accumulate rapidly in the cells, and oxidative combustion occurs along with it. The first symptoms of the hypersensitive reaction resulting from the incompatible relationship appear approximately 12 hours after the nematode inoculation (Dropkin, 1969a; Milligan et al., 1998; Bird & Kaloshian, 2003). As a result, the nematode dies before it can form a feeding place (Verdejo-Lucas et al., 2012). In case of a compatible interaction between the nematode and the plant, H2O2 is produced 12 hours after the nematode enters the plant, but after 48 hours H2O2 cannot be detected. The reason why H2O2 could not be determined is the activity of the genes responsible for the enzymes that prevent oxidative combustion. As a result, structures called giant cells are formed (Apel & Hirt, 2004; BleveZacheo et al., 2007).

Resistance to root-knot nematodes in tomato is provided by the *Mi-1* gene. In tomato, it is a dominant gene called *Mi-1* that provides resistance against *M. incognita*, *M. javanica* and *M. arenaria*. It was named after the nematode species (*M. incognita*) used in tests to determine the resistance status of plants (Gilbert & McGuire, 1956). *Mi* gene was found in *S. peruvianum* (PI128657) and hybrid plant was obtained using embryo rescue technique since it could not be hybridized with culture forms using conventional breeding methods (Smith 1944). The widely used *Mi-1* gene against root-knot nematodes comes from this source (Ammati et al., 1986). Mi-1 gene is 7 homologous genes (*Mi-1.1*, *Mi-1.2*, *Mi-1.3* and *Mi-1.4*, *Mi-1.5*, *Mi-1.6*, *Mi-1.7* 2 clusters in the 650 kb region of the short arm of the 6th chromosome of tomato) are available as. Of these homologues, *Mi-1.3* and *Mi-1.5* are pseudogenes. As a result of studies carried out in plants to which homologous genes are transferred, it has been determined that resistance is provided by *Mi-1.2* (Milligan et al., 1998) (Table 1). The cytoplasmic protein encoded by *Mi-1.2* consists of 1257 amino acids. This resistance gene motif is called CC-NBS-LRR. The nucleotide binding site of this structural motif is called NBS (Nucleotide Binding Site), the LRR portion with leucine amino acid-rich repeats (Leucine Rich Repeat) and the helical motif at the amino end of these proteins is called CC (Coiled-coil) (Milligan et al., 1998; Hwang & Williamson, 2003).

Mi-1.2 gene was found to be resistant to *Meloidogyne* species as well as some biotypes of potato aphid [*Macrosiphum euphorbiae* (Thomas)] and cotton whitefly [*Bemisia tabaci* (Gennadius)] B and Q biotypes (Nombela et al. 2003).

Table 1: Characteristics of genes providing resistance to root-knot nematode (*Meloidogyne* spp.) in tomato

Gene	Source	Resistant Species	Temperature	Chromosomal Location	Literature
Mi-1 (Mi)	*S.peruvianum* PI128657	*M. incognita* *M. javanica* *M. arenaria*	<28°C	6	Milligan et al., 1998
Mi-2	*S. peruvianum* PI270435-2R2	*M. incognita*	32°C	-	Cap et al., 1993
Mi-3	*S. peruvianum* PI126443-1MH	*M. incognita*	32°C	12	Yaghoobi et al., 1995
Mi-4	*S. arcanum* LA1708-I	*M. arenaria*	32°C	-	Veremis & Roberts, 1996a
Mi-5	*S. peruvianum* PI126443-1MH	*M. incognita*	32°C	12	Veremis & Roberts, 1996b
Mi-6	*S. peruvianum* P1270435-3MH	*M. incognita*	32°C	6	Veremis & Roberts, 1996b
Mi-7	*S. peruvianum* PI270435-3MH	*M. incognita*	<28°C	6	Veremis & Roberts, 1996b
Mi-8	*S. peruvianum* PI270435-2R2	*M. incognita*	<28°C	6	Veremis ve Roberts, 1996b
Mi-9	*S. arcanum* LA2157	*M. incognita* *M. javanica* *M. arenaria*	32°C	6	Ammiraju et al., 2003

2. Naturally Resistant Resources

Several *Mi*-genes have been detected in some tomato lines, genotypes, and cultivars. These genes confer resistance against root-knot nematodes. Many resources of resistance have been discovered since 1944. Which resistance genes some of these plants contain is still not known. The preferred and safest method for controlling RKNs is in the discovery of new resistant plants. It is important to perform an extensive evaluation of tomato plants whose resistance has not been determined.

2.1. The Mechanism of Natural Resistance

Tomatoes, like all plants, undergo several modes for protection and immunity. The plant has an innate immune system that can recognize pathogen-associated molecular patterns. PAMP-triggered immunity (PTI) is the first defense line of response of the plant to pathogens. The extra cellular receptor proteins, receptor-like kinases (RLK), and receptor-like protein (RLP) are initiation factors and activators of the first defense line. The second defense line is triggered by intracellular proteins that contain a nucleotide-binding site (NBS), a toll-like interleukin receptor (TIR), which is not found in the *Mi-1* gene, and leucine-rich repeats (LRRs). During the second-line defense, there are two modes of pathogen interaction: direct and indirect.

The first pathway depends on a gene-for-gene interaction. In this mode, the receptor protein of tomato directly interacts with the nematode effectors. According to Flor's theory, the inheritance of both resistances in the tomato and the RKN's ability to cause disease are controlled by pairs of matching genes. The first gene, like the *Mi-1* gene, is in the tomato, and the other one is in RKNs and is called a virulence (Avr) gene. One of the responses of this type of defense is localized programmed cell death (PCD), one of the most important responses. This is a type of hypersensitive response (HR) (Figure 3). After the nematode enters the root of the plants; the nematode Avr genes produce effectors that trigger the production and the expression of plant *Mi*-resistant genes in an incompatible interaction. The result, because of this theory, is that no feeding site (giant cell) is formed. The second defense mode is not a direct gene-for-gene interaction, but an alternative mode called the guard hypothesis. The mechanism in this theory consists of pathogen effectors that trigger the virulence factors/protein of the plant, which finally induces R-gene. In these cases, the virulence factor of nematodes (Avr genes) interacts with tomato accessory protein, resulting in some modification of this accessory protein, which allows for the recognition by plant NBS-LRR proteins that monitor for infection. The last result of this indirect interaction is the prevention of the production and growth of nematodes by the inhibition of the formation of feeding sites.

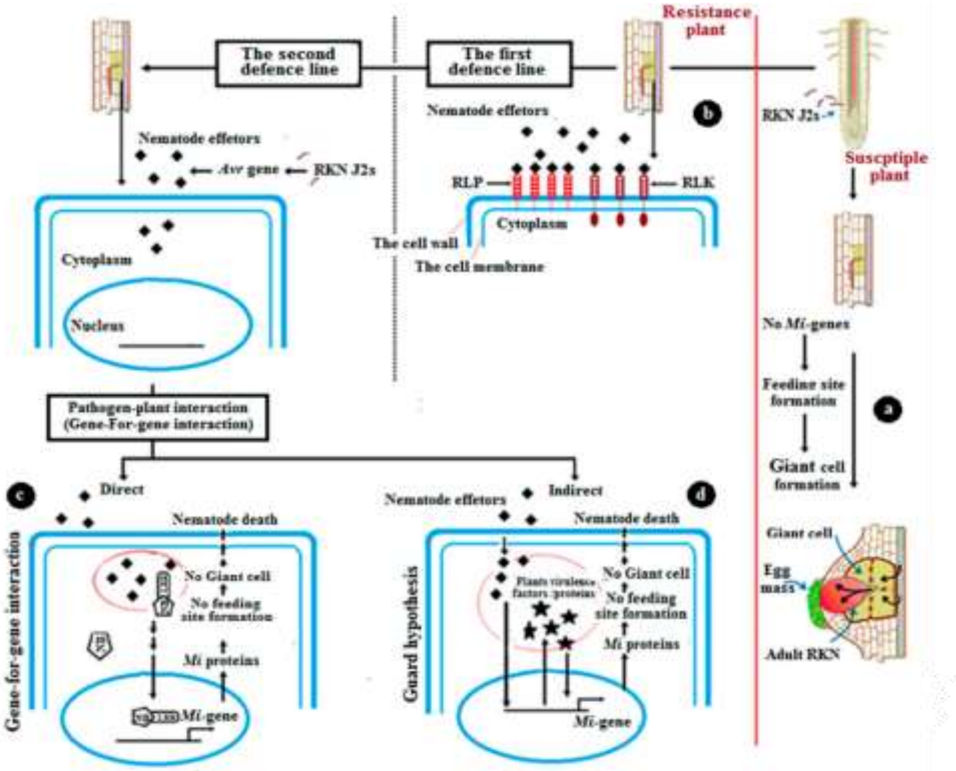

Figure 2. The mechanism of natural resistance against the root-knot nematode (RKN). (a) In susceptible plants, where there are no *Mi*-genes, the nematode completes its life cycle in the root by forming giant feeding cells. (b) In the resistance case, the plant undergoes the first defense line against RKN penetration by the interaction between extracellular receptor proteins, receptor-like kinases (RLK), receptor-like protein (RLP), and nematode effectors. (c) The plant then begins the second defense line, which includes direct gene-for-gene interaction. This theory depends on direct interaction between the receptor protein of tomatoes and nematode effectors, producing *Mi*-proteins, which prevent the nematode from feeding. No giant cell formation is observed. (d) The other second defense line is an indirect pathway, which is referred to as the guard hypothesis. In these cases, the virulence factor of the nematode (Avr genes) interacts with tomato accessory protein.

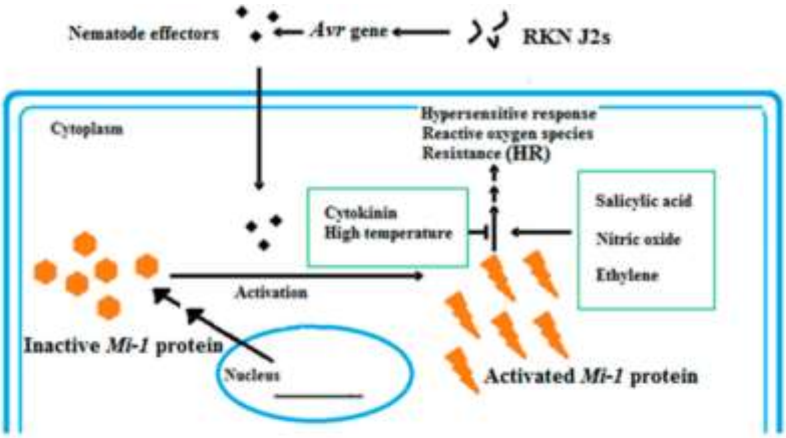

Figure 3. Hypersensitive response of *Mi-1* after nematode infection. The nematode Avr genes trigger the tomato *Mi-1* resistance gene(R-gene) to be active under the salicylic acid pathway with inhibition by both cytokinin and high temperature

3. Different Approaches to Strengthening Natural Resistance

3.1. Marker-Assisted Selectionin Breeding Programs

Marker-assisted selection (MAS) means the use of a binding pattern of linked molecular (DNA) markers for indirect selection in the desired plant phenotype. MAS is based on the concept that the presence of a marker that is tightly linked to the gene of interest indicates the presence of that gene.The improvement of new resistance plants has many benefits. The two most important benefits of using molecular breeding are first that it is less harmful to the environment than pesticides, and second that it is less expensive. Tomatoes are considered one of the most optimal plants for using molecular markers in commercial breeding. Moreover, molecular markers linked to the *Mi*-1 gene have enabled the rapid screening of resistance alleles, without requiring nematode inoculation. The use of molecular marker technologies in sync with new breeding techniques is promising for the a dvancement of tomato breeding.

3.1.1 Genetic Engineering in Controlling RKN

Although molecular breeding is the method that is most applied to achieve resistance against root-knot nematodes in tomato plants, genetic engineering is a future aspiration for further increases in resistance

3.1.2 Transfer Resistance Genes

This strategy is based on two foundations. The first is the transfer of a resistance gene from other plants to tomatoes. The second is the transfer of the *Mi* resistance gene from resistant varieties to susceptible one with high production qualities. Several resistance genes from different plants have been successfully transferred to tomatoes. These tomatoes transformed with new genes reduce diseases in transformed plants. Transgenic tomatoes with these genes would be novel sources for resistance against root-knot nematodes. Moreover, cloned Mi-1 is a good candidate for transfer to susceptible plants. There are more difficulties in understanding the mechanism of R-genes in other plants of the sames pecies or plants of another family. There have been many contradictions in previous studies in the case of other transformed solanaceous plants with the *Mi-1* gene. Transgenic tomato plants showed reduced chitin content and retardation in embryogenesis in nematode eggs.

3.1.3 Resistance Effectors

Proteinase inhibitors (PIs) are one of the most promising methods for managing nematodes. Proteinase inhibitors are protein molecules secreted by pathogens, which inhibit the function of proteinases.Different types of proteinase have been identified in tomatoes.

Conclusions

Considerable potential has been developed in recent years for improving rootknot nematode resistance in tomato and other crops. The *Mi* gene of tomato has provided effective resistance to three root-knot nematode species for many years. The availability of a clone of *Mi* will allow introduction of this gene into selected varieties and possibly other crops, further expanding its use. However, *Mi* will not solve all root-knot nematode problems; it is not effective against all species or isolates of this nematode. In addition, the failure of *Mi* at high temperature can be a problem in the field. It is possible that in vitro modifications of the cloned gene will improve the range of nematodes controlled by *Mi*. For example, it may be that the partial resistance against *M. hapla* can be improved or the temperature sensitivity can be reduced by modifications in the structure, expression, or signal transduction of *Mi*. Other resistance genes into cultivated tomato using classical or marker-assisted breeding may also broaden the basis of root-knot nematode resistance. As technology advances, cloning of these genes directly from the wild species may be a faster route than conventional breeding for transferring the gene to elite cultivars or other species. However, even now there are virulent root-knot nematode isolates that can infect all currently identified sources of resistance. Continued searches of germplasm are needed to identify new sources of resistance. Artificially engineered resistance based on antisense technology or expression of anti-nematode proteins may be an additional source of resistance. Strategies to best use *Mi* and other genes to maximize their useful lifespans need to be developed. The gene Mi, which confers resistance to several species of root-knot nematode, is present in many modern tomato cultivars. Recent cloning of this gene revealed that it encodes a member of the plant resistance protein family characterized by the presence of a putative nucleotide binding site and a leucine-rich repeat. Although highly effective in many conditions, Mi fails to confer resistance at high soil temperature, and Mi-virulent nematode isolates have been identified in many areas of the world. These findings have stimulated efforts to identify new sources of root-knot nematode resistance. Resistance genes that differ from Mi in properties and genetic position have been identified in Lycopersicon peruvianum. These genes, as well as the cloned Mi gene, provide a resource for broadening the base of root-knot nematode resistance in tomato and other crops.Is pyramiding several resistance sources in selected elite cultivars the best solution or will it promote the spread of supervirulent nematodes? Getting a better understanding of nematode virulence is an important consideration for developing control strategies. As chemical control is reduced, the need for better understanding and implementation of host resistance and pathogen virulence will continue to increase.

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ASSESSMENT OF THE ECOPHYSIOLOGICAL STATUS AND PRODUCTIVITY OF TOMATOES - EARLY FIELD PRODUCTION IN THE AREA SAEDINENIE VILLAGE, BULGARIA

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ABSTRACT
Tomato plants are influenced by a number of environmental factors in their growth and development. Agro-climatic conditions are the basis of their physiological status, which influences their overall productivity and yield.
The main aim of this paper is to estimate the ecophysiological status and productivity of tomato plants grown under open field conditions by analyzing the main agro-climatic factors, soil characteristics, photosynthesis and transpiration parameters, biomass accumulation and yield.
Keywords: tomato, *Solanum lycopersicon*, photosynthesis and transpiration parameters, productivity of plants

INTRODUCTION

Worldwide, tomatoes are the most important horticultural crop, with total production and area under cultivation estimated at 164 million tonnes and 4.76 million hectares respectively. China is the main producer with 31.0% of total world production and 20.6% of total cultivated area, and Spain is the country with the highest average tomato yield (81.3 t-ha-1) (Rodríguez-Ortega et al. , 2019). For Bulgarian vegetable production, they are also the first crop with the largest production areas and highest yields. According to the Department of Agro Statistics of the Ministry of Agriculture for 2021, the total production of tomatoes is 116.4 thousand tons, the cultivated area is 2427 hectares and average yields are 23650 kg/ha (https://www.mzh.government.bg/).

The development and productivity of plants depends on many environmental factors such as temperature, rainfall, soil type, etc (Cholakov, 2009).

Many scientists use the physiological behaviors of plants as indicators to assess their productivity and vigour. The eco-physiological status of plants under water stress was studied by Nawata, and Sakuratani, T. (1999) in four different tomato cultivars. Changes in photosynthetic parameters and antioxidant activity under stress conditions were investigated Camejo et al. (2006). Arena et al. (2020) conducted an eco-physiological screening of different tomato genotypes in response to high temperatures, combining field and laboratory analyses to create an "eco-physiological identity card" for different genotypes. According to the authors, the relationship between leaf gas exchange and crop yield has been extensively studied in tomato. Leaf transpiration was used as an indicator to predict yield. Ahmadpour and Armand (2020) determined the ecophysiological status of tomato in dependence on the use of organic fertilizers. Qasim et al. (2023) studied the morphological behaviors and physiological parameters of tomato after application of combined fertilization with organic and chemical fertilizers. In most cases, the design and conduct of scientific experiments evaluate different stress effects on plants or the impact of different agronomic practices on their development. The results obtained are based on precisely conducted field trials and/or laboratory analyses.

In this paper, we aimed to use the tools of scientific research to assess the ecophysiological status of plants from a manufacturing plantation, by analyzing the main agro-climatic factors, soil characteristics, photosynthesis and transpiration parameters, biomass accumulation and yield.

MATERIAL AND METHODS

The monitored cultivated plantation is located in Saedinenie village, Bulgaria. It has a total area of 4,6 dka.

The tomatoes were grown according to the conventional early field production technology in Bulgaria (Cholakov, 2009). The cultivar used was Pink Magic.

For characterizing the phenological development of tomato plants is uses the BBCH scale (Meier, 2001, 2018). The basic phases of monitoring of tomato plants are presented in Table 1.

Таблица 1. The basic phases of development of tomato на развитие на доматите

	BBCH code	Phase
1	69 610	Full flowering
2	73 703	full fruit formation
3	85 805	full fruiting

Soil variation is pseudopodzolic, powerfully humusy, clayey-sandy soils (Dystric Planosols) (IUSS Working Group).

The basic climate indicators are determined by a meteorological station Meteobot® Pro measuring the indicators in dynamics (https://meteobot.com/meteostancii/) .

The basic physico-chemical parameters of the soil are determined in depth 40 - 60 cm. Soil pH: determined potentiometrically in the field using Soil pH meter, PCE-PH20S (PCE Instruments, United Kingdom) and in laboratory conditions (H2O, KCl) using pHotoFlex Set, 2512000 (WTW, Germany). Soil moisture: determined indirectly (in the field) by Soil Humidity Meter TR 46908 (Turoni, Italy). Mechanical composition of the soil - determined according to ISO 11277; Content of organic matter in the soil - determined according to Tyurin's methodology; Humus content: determined according to ISO 14235:02; Nitrogen, phosphorus and potassium content (mobile forms): determined according to ISO/TS 14256-1:2003 and GOST 26209:1991.

Plant and soil samples are taken in a randomized method in five test fields. The sites of test fields have an area of 50 m^2 with 125 plants each.

Transpiration (E, µmol m-2 s-1) was measured on three fully developed, undamaged leaves of the same physiological age, taking at least 50 values for each individual leaf. It is expressed as the amount of water transpired (in grams or milliliters) per unit mass or leaf surface per unit time Q-box CO650 Portable Photosynthetic System, Quibit Systems Inc., Canada; Camspec M108 spectrophotometer, UK. Intensity of photosynthesis (A, µmol m-2 s-1) is measured on three fully developed, undamaged leaves of the same physiological age, taking at least 50 values for each individual leaf. It is expressed as the amount of CO_2 absorbed per unit mass or leaf area per unit time (Q-box CO650 portable photosynthetic system, Quibit Systems Inc., Canada; Camspec M108 spectrophotometer, UK).

To assess productivity, in phases of fruit formation and fruiting by analyzing 25 plants for each phase were determined following indicators (Georgiev et al., 1980): Stem height -

cm; Number of leaves; Fresh leaf-stem weight, as the sum of stem weight and leaf weight - g; Number of fruits per plant; Leaf area per plant; Leaf area index, as the ratio of leaf area to the area on which plants are grown (Ahmedova, 2013); Total fresh biomass kg per 1 da; Total dry biomass kg per 1 da; (drying of leaf, stem and fruit samples was carried out under laboratory conditions to constant weight).

RESULTS AND DISCUSSION

The Saedinenie village and its adjacent territories belong to the municipality of Saedinenie. It is situated in the Thracian valley northwest of the town of Plovdiv. It covers an area of 297 721 hectares. The average altitude is about 200 m. The relief is mostly flat. The municipality has a transitionally continental climate. The annual average rainfall amounts measured here are among the lowest in southern Bulgaria (495 l/m^2). In this sense, the pronounced spring and summer droughts as well as the high temperatures define the area as less favorable in climatic terms. According to the meteorological station, snow fell earliest in the second half of November and persisted until the second half of March at the latest. The snow cover is very thin and the duration of the snow period averages about 80 days. It is at its lowest in the second ten days of November and the third ten days of January. The first frost in the region occurs on average on 27 October and the last on 9 April. There is therefore a fairly long growing season for frost-free plants, which ensures that a large number of crops can be grown https://saedinenie.bg/ .

The agro-climatic characteristics of the tomato growing season show that for much of the period, plants develop under conditions at the limit of their agrobiological maximum. During the first ten days of June, agrometeorological conditions are determined by intense rainfall (Fig. 1). Comparatively low temperatures during this period are a prerequisite for slow plant development and pathogen expansion.

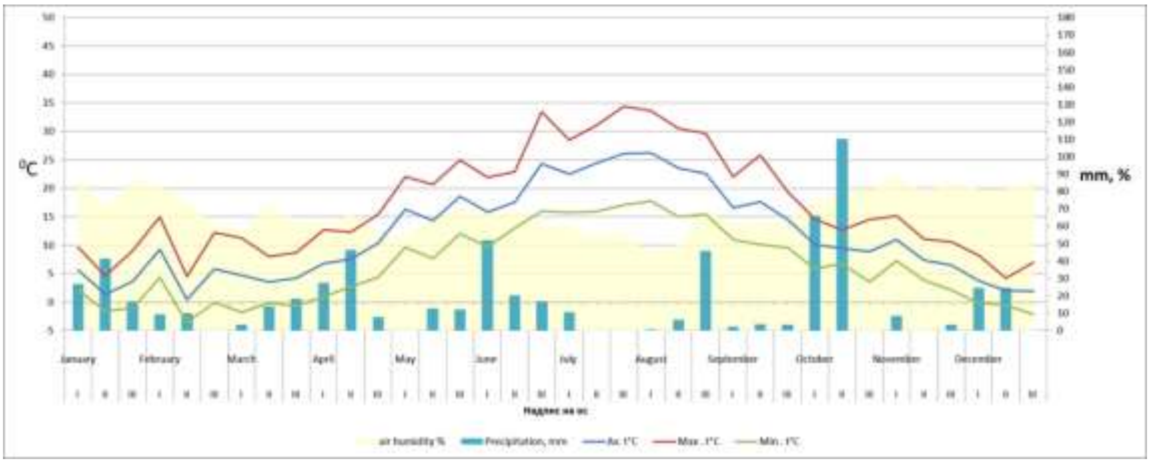

Figure 1. Climatic characteristics of the region of Saedinenie village for the year 2021

Improvement of agro-climatic indicators is observed in the third ten days of the month. The average temperature increased by 7°C compared to the previous period and maximum temperatures reached the agrobiological maximum of tomatoes. After the rainy month of June, almost no rainfall was recorded in July. There is a deficit in soil moisture, which has to be compensated by regular irrigation. High temperatures combined with low atmospheric humidity have an impact on flowering and pollination of tomato flowers.

Daily temperatures, however, are in the range providing active photosynthesis (Zou et al., 2016). After the prolonged heat wave in July and in August, the warm weather continued.

The extremely high temperatures recorded during this period disrupt the normal course of plant physiological processes and stress conditions such as e.g. poor pollination, poor fruit set may be recorded. On the other hand, temperatures higher than optimal for this vegetable species lead to a decrease in the length of periods between the different phases.
In agrometeorological terms, September provides conditions closer to the biological requirements of plants. The dry and warm weather in the second and third ten days of the month helps ripening, increasing the number of harvests and increasing the total yield. Tomatoes have good ecological plasticity (Cholakov, 2009). Due to this quality, unfavorable agro-climatic conditions are compensated by regular irrigation and fertilization. An additional positive effect is achieved by regular harvesting of ripe fruit and application of green pruning.

The results of agrochemical analyses, showed that the mineral nitrogen content ($\sum$N-$NH_4^+NO_3^-$), values at flowering stage of tomato averaged 29.91 $mg.kg^{-1}$ (Tabl.2).

Table 2. Agrochemical parameters of soil in the different phases of development of tomatoes.

Phase	pH H_2O	N-NH_4 $mg.kg^{-1}$	N-NO_3 $mg.kg^{-1}$	P_2O_5 $mg.100g^{-1}$	K_2O $mg.100\ g^{-1}$	Organic matter $g.kg^{-1}$	Humic %
flowering	6.56	17,72	12,19	47,62	111,21	17,46	3,1
fruit formation	7,24	18,46	20,95	64,48	122,6	14,1	2,43
fruiting	7,35	21,52	3,99	54,94	113,81	14,82	2,55

In terms of plant available nitrogen at this phenophase, the soil nitrogen availability was found to be medium, which was attributed to the fertilization applied to obtain good yields. The available forms of phosphorus P_2O_5 averaged 47.62 mg.100 g^{-1}, and the data indicate high availability. The average value of available forms of potassium is 111.21 mg.100 g^{-1}, based on which the stock of mobile forms of potassium is defined as very high. Soil organic carbon content and humus availability can be defined as medium to high. The soil reaction of the studied field ranges from slightly acidic to neutral as pH /H2O/. The average value is pH=6,56, and it is typical for the soil type - pseudopodzolic soils.

Results in the fruit formation phase show similar trends. The soil reaction ranges from neutral to slightly alkaline as pH /H2O/ - pH=7,24. The values of mobile nitrogen (especially N-NO_3) and mobile phosphorus are increasing, which can be explained both by the migration in depth of the profile and by increased mineralization of soil organic matter. The values of mobile potassium are decreasing, as are the contents of soil organic carbon and humus, as a consequence of active plant vegetation. Similar trends are observed in the fruiting phase of tomatoes in terms of organic carbon and humus content. Differences are observed in the mobile forms of nitrogen, with ammonium nitrogen increasing and nitrate nitrogen decreasing more than 5 times within 30 days. The mobile forms of phosphorus and potassium also decreased, although to a lesser extent. The soil reaction is slightly alkaline with pH / H2O of pH=7.35.

The moisture content values at the different stages of tomato development are within the optimum range(tabl.3.).

Table 3. Soil moisture at the main stages of tomato development.

Depth cm	Soil moisture, %		
	flowering	fruit formation	fruiting
0-10	16,37	19,83	24,81
20-30	19,68	24,48	19,70
40-60	25,03	24,79	10,03

The relatively good moisture content during the growing season is due to the rainfall in the first half of June and the periodic irrigation.

Increasing of photosynthetic pigments may be due both - to their intensive synthesis, and their more slowly degradation (Tabl.4). The ground for this admission we have a comparatively constant levels of carotenoids in the all three phases of development. It is well known that the main function of the yellow pigments is chlorophyll prevention of oxidation. Any decrease in the content of carotenoids would break the stability of the green pigments in pigmentprotein complex (Haytova, 2015).

Table 4 Photosynthetic pigment content at the main stages of tomato development

Phase	Content, mg/g				Ratio	
	chl a	chl b	chl a+b	car	chl a/b	chl a+b/car
flowering	29,73	17,26	46,99	16,60	1,72	2,83
fruit formation	48,01	30,93	78,95	19,96	1,55	3,95
fruiting	44,81	30,32	75,13	20,57	1,48	3,65

Increase of photosynthetic pigments content can be reason for the higher photosynthetic rate. Berova and Karanatsidis (2008) made similar conclusions by studying these physiological parameters in pepper. According to Klamkowski et al. (2011) this fact could be explained as an adaptation of photosynthetic apparatus to nutrient regime and environmental conditions.

Increasing of the rate of photosynthesis increasing transpiration and stomatal conductivity were observed (Tabl.5.).

Table 5. Parameters of photosynthesis and transpiration of plants in the main phases of tomato development

Phase	Photosynthetic rate, mol m^{-2} s^{-1}	Transpiration rate, mol m^{-2} s^{-1}	stomatal conductivity, mol m^{-2} s^{-1}
flowering	4,524	0,513	43,26
fruit formation	14,618	0,983	44,66
fruiting	7,792	1,61	170,93

The processes of photosynthesis are most intensive during fruit formation. The intensity of transpiration and stomatal conductivity are increase in parallel. This phase is a combination of still intensive vegetative growth and increasing reproductive behavior.

This feature is probably due to a better water status of the treated plants. According to Berova and Karanastidis (2008) the improved leaf gas exchange is a precondition for higher productivity of the plants.

No significant variation was observed in the evaluation of plant growth behaviors (Table 6).

Table 6: Vegetative characteristics and productivity of tomatoes

phase	Stem height /cm/	Number of leaves	Fresh leaf-stem weight per plant /g/	Number of fruits per plant	Leaf area per plant /cm^2/	Leaf area index	Total fresh biomass kg per da;	Total dry biomass kg per da;
fruit formation	137,8	34	734	4	6779,28	1.02	2309,4	277,01
fruiting	170,52	89	1214	5	10892,58	1,63	3194,28	396,424

At fruiting stage, increase in stem height, number of leaves, fresh leaf-stem weight was recorded. Also the leaf area was able to cover the growing area of the plants over1.5 times. An increase is also recorded in the total fresh and dry biomass formed per 1 harvest area. There is regular fruit set, and their number remains constant in both phases of plant development. This fact is probably based on the polycarpic nature of fruiting in this vegetable species (Cholakov, 2029). The visual assessment we made did by direct observation of the plants not reveal any visible signs of stress, lack of nutrients, attack by nematodes or pathogen development.

CONCLUSIONS

All obtained results give us reason to consider that, in despite of a climatically variable year, the plants are able to maintain their vigour and express their biological potential without any drastic variations in their productivity. The ecophysiological status of the plants can be used for a complex assessment of the conditions for plant development and the effectiveness of the applied technology. Precise application of cultivation technology, irrigation, fertilization helps to eliminate the negative effect of unfavorable environmental conditions.

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DETERMİNATİON OF CARBON SEQUESTRATİON İN BİOMASS ACCORDİNG TO THE PHENOLOGİCAL PERİODS OF SOME C3, C4 PLANTS (AGROPYRON CRİSTATUM (L.) GAERTN. SUBSP., ARTEMİSİA SCOPARİA WALDST. & KİT., BASSİA SCOPARİA (L.) A.J.SCOTT) DUE TO CLİMATE CHANGE - FİRST DETECTİON

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Abstract

Biodiversity and shifting climate zones are emphasized as indicators of climate change. Determining the response of natural vegetation to changing climatic conditions will guide the relevant branches of science and humankind regarding biodiversity and the adaptation of species and their ability to continue their life cycle. Three species found in natural vegetation: Agropyron cristatum (L.), Gaertn subsp Artemisia scoparia Waldst & Kit and Bassia scoparia (L.) AJScott were selected in this study. The flower-maturity-seed periods of the species were monitored for four years in the Konya Karapınar General Directorate of Combating Desertification and Erosion Protection Area. Biomass carbon retention was determined separately in aboveground and subsoil parts during these periods.

The plants were compared with the C3 plant wheat as a control subject. Carbon sequestration of Agropyron cristatum (L.), Gaertn subsp Artemisia scoparia Waldst & Kit and Bassia scoparia (L.) AJ Scott plants was recorded for the first time in this study. While determining the carbon retention of species, monthly, daily, hourly and annual highest, lowest and average values were taken into account. Air temperature, precipitation, surface temperature, soil depth profile temperatures, wind and relative humidity are the climate parameters evaluated. Under changing climatic conditions, the volume of vegetative and generative parts of plants and their carbon retention also varied from year to year. In the study, each year was evaluated under its own conditions for 4 years, thus the carbon content of the plants and their responses to changing conditions were measured. The plants in the study will be suitable to be used to create a carbon sequestering biological environment. From the results obtained from the study, it was observed that the economic value of feed and medicinal aromatic plants may change with climate change. It was concluded that the plants studied would be suitable to be used to create a carbon sequestering biological environment.

This information will guide the pharmaceutical and livestock industry's adaptation policies to climate change. Additionally, In the study, different results were also obtained regarding the control plant wheat. it has been determined that wheat can compete with other C3 plants. Thus, it was concluded that agricultural production should not be considered entirely as an emission source and agricultural carbon footprint and carbon budget calculations should be reviewed. With these results, the study serves as a basis for many future studies.

Keywords: climate change, biomass carbon, carbon sequestration, agricultural carbon budget, carbon footprint, Agropyron cristatum, Artemisia scoparia, Bassia scoparia

1.INTRODUCTION

Climate change has become visible and acutely felt, especially in recent years, such as shifts in climate zones, erosions, changes in the form of precipitation and increased intensity. Today, this change has reached a limit that will greatly affect the survival and quality of life of humanity. Considering the entire ecology, the increase in carbon dioxide emissions not only causes the atmosphere to become a warmer layer, but also affects all fauna, flora and biological activities, which are a cycle. Therefore, when evaluating the effects and consequences of climate change, it would be the most rational approach to explain it by taking many factors into account. Humanity has reached international consensus and signed agreements in order to take a common stance on this issue. Awareness of climate change in the world started in the early 1990s. This awareness continued to rise until 2022.

Since the 1992 Rio Conference, awareness of climate change has increased in the world. After many years of negotiations, all countries are expected to present their National Contributions (INDCs) at the Paris Climate Summit, the 21st Conference of the Parties, in 2022, based on the Principle of Common But Differentiated Responsibility and mitigation capacity (Karakaya ve Sofuoğlu, 2015).

Turkey became a party to the United Nations Framework Convention on Climate Change (UNFCCC) in 2004, the Kyoto Protocol in 2009 and finally the Paris Climate Agreement in 2022. However, although Turkey has started to plan action to combat climate change since the 1990s, it did not assume any responsibility during the Second Commitment Period of Kyoto, which started in January 2013, and no concrete steps were taken until 2022 (Anonymous, 2022). Türkiye has officially become a party to the Paris climate agreement as of 2022. In accordance with the agreement, all participating countries have the responsibility to reduce carbon emissions according to their carbon responsibility and development levels. The justification for emission reduction is to keep the warming rate in the atmosphere below 2^{o}C. Emission reduction efforts and actions will be possible by developing or restoring carbon sinks and pools.

Carbon sinks play an important role in the global carbon cycle. By absorbing CO_2 from the atmosphere, they help slow down the rate of climate change (Şentürk et al. 2023; Mısır et al. 2011). The more carbon is stored in carbon sinks, the less CO_2 is present in the atmosphere, thus reduing the greenhouse effect in the atmosphere.

A carbon sink is a natural or artificial reservoir that absorbs and stores atmospheric carbon through physical and biological mechanisms. Forests, oceans, soil and atmosphere are important carbon reservoirs (Tuğluer and Oğuz, 2022; Mısır et al. 2011).

Carbon stored in carbon sinks can remain there for hundreds or thousands of years, helping to mitigate the effects of climate change. The most important natural carbon sinks are oceans, soil, forests, pastures and agricultural areas (Tuğluer and Oğuz, 2022; Akalın, 2014; Güllü and Akdağ, 2022; Akgün et al.2023).Oceans absorb CO_2 from the atmosphere through a variety of processes, including the dissolution of carbon dioxide gas into seawater and the formation of marine carbonate sediments. Soil is the largest carbon sink after the oceans. Soil contains a lot of organic matter, which consists of the remains of dead plants and animals. When organic matter decomposes, some of the carbon is released back into the atmosphere as carbon dioxide. However, some of the carbon is also stored in soil, where it can remain for thousands of years. Forests are also an important sink with tree and underwood plant forms. Trees and other plants absorb CO2 from the atmosphere through photosynthesis, and the carbon is stored in the trees' wood and leaves. When forests are cleared, the carbon stored in trees is released back into the atmosphere. When trees die, their remains are buried in the soil where carbon is stored for long periods of time (Lenton, 2000; Kheshgi et al. 1996; Reay, et al. 2008; Heinze et al. 2015).The atmosphere is also a carbon sink. However, it is a much smaller sink than oceans or forests. The atmosphere contains about 0.04% carbon dioxide, and this amount is relatively constant. This is because carbon dioxide released into the atmosphere from human activities is absorbed by oceans and forests. In addition to natural carbon sinks, there are also a number of artificial carbon sinks that have been developed.

• Carbon capture and storage (CCS): CCS is a technology that captures CO2 from power plants and other industrial plants and stores it underground.

• Reforestation: Reforestation is the process of planting trees in deforested areas. This helps restore the natural carbon sink function of forests.

• Biochar: Biochar is a type of charcoal produced from biomass such as wood, agricultural waste or manure. Biochar can be added to soil where it helps store carbon and increase soil fertility (Anonymous, 2023a).

Pastures and farmland are both important for carbon sequestration, but they do so in different ways. Pastures tend to have higher soil organic carbon (SOC) levels than farmland, meaning they can store more carbon. This is because pastures are typically not tilled, which helps maintain soil SOC. Additionally, the roots of pasture grasses help anchor the soil and prevent erosion, which helps maintain SOC. On the other hand, agricultural lands can also sequester carbon, but they do so through different mechanisms. For example, crops such as corn and soybeans can remove carbon dioxide from the atmosphere during photosynthesis. Additionally, agricultural soils can store carbon in the form of crop residues and manure. However, agricultural land is also more likely to be plowed, which can release SOC into the atmosphere (OECD, 2023).

Table 1. The main differences between pastures and agricultural lands in carbon sequestration (OECD (2023)

Factor	Pasture	Agricultural Lands
Soil Organic Carbon (SOC)	High	Lowering
Soil	No-till	Plowed
Erosion	Less likely	More likely
Carbon dioxide removal	Less	More
Crop residues and fertilizer	Less	More
Carbon absorbing potential	High	Low

The reason why pastures can generally store more carbon is; It has deeper soils than agricultural lands. The roots of plants in pastures also help improve soil structure and drainage, which can further increase carbon storage. In addition, the vegetation of pasture plants mostly contains C4 plants as well as C3 plants. C3 and C4 plants are very important in reducing the impact of climate change or developing compatible ecological and technological solutions. It is possible to reduce the effects of climate change by taking advantage of the carbon sink and sink properties of vegetation.

Since the first compound that occurs in some of the plants in the warm regions of the world is 4-carbon, plants with a system called 4-carbon photosynthesis system are called C4 plants (Türk and Çelik, 2006). C3 and C4 plants produce energy by using carbon dioxide and water through photosynthesis. C3 plants take in CO_2 directly and perform photosynthesis. C4 plants first combine CO_2 and then photosynthesize. CAM plants, on the other hand, take in CO_2 at night and perform photosynthesis during the day. C3 plants perform better in low light conditions, while C4 plants perform better in high temperature and low humidity conditions (Rowen, 2014). The evolution of C4 plants has been associated with changes in the world's climate. The decrease in atmospheric CO2 levels and the drying of the climate promoted the evolution of C4 plants (Ehleringer and Cerling, 2002). C4 energy plants are plants that do not depend on fertile agricultural lands, have a very short growth period, require little maintenance, are more resistant to diseases and drought, and therefore can provide high yields at low cost. (Anonymous, 2023b; Rowen, 2014; Keerberg et al. 2014; Hirst, 2022). The transformation of C3 plants into C4 plants occurs when high temperature and low humidity conditions promote the evolution of C4 plants (Rowen, 2014). C4 photosynthesis is a biochemical modification of the C3 photosynthesis process. In C4 plants, the C3-style cycle occurs only in inner cells within the leaf. (Ehleringer and Cerling, 2002).

It is known that only 3% of plants in nature are C4 plants. C4 plants; Forage plants are materials with high economic value, cosmetically and pharmacologically, with their medicinal and aromatic contents. It is used as raw material and also as bioenergy sources. As a gene source; They are of great importance in genetic studies and plant breeding units. For example; Agropyron cristatum (L.) Gaertn., one of the most important wild relatives of wheat (2n = 4x = 28, PPPP), which was also the study material. Wheat contains many genes that are desirable for genetic improvement. Wheat with superior agronomic properties-A. The development of cristatum translocation lines facilitates the genetic improvement of wheat (Oi et al. 2021).

C3 and C4 plants have economic value in many sectors, as well as ecological potential as carbon sinks. Natural carbon sink areas are under pressure due to human activities. Deforestation, for example, reduces the amount of carbon forests can absorb. Burning fossil fuels releases CO2 into the atmosphere, which can suppress the ability of carbon sinks to absorb it.

This study focused on the carbon sequestration and emission reduction potential of C3 and C4 plants. It was carried out to reveal the potential of these plants to be used in climate change adaptation and mitigation studies. The studied area was selected as an arid and marginal area, and the carbon contents of three plants (Agropyron cristatum (L.) Gaertn. subsp. Artemisia scoparia Waldst. & Kit., Bassia scoparia (L.) A.J.Scott's) were associated with their phenological periods, and for the first time, this recorded in the study.

2. MATERIALS and METHODS

2.1. Conceptual Foundations:

• Photorespiration is a path that starts with the action of the Calvin cycle enzyme rubisco on oxygen, not on carbon dioxide, and is not a very economical pathway.

• The majority of plants (97%) are C3 plants that do not have the ability to cope with photorespiration. Plants that do not have photosynthetic adaptations to reduce photorespiration are called C3 plants.

• C4 plants minimize photorespiration by separating the initial CO2 fixation and the Calvin cycle and performing these steps in different cell types.

• Crassulacean acid metabolism (CAM) plants minimize photorespiration and store water by separating these steps in time between day and night (Hrist, 2022; Anonymous, 2023a; 2023b; 2023c).

In the first step of the Calvin cycle, rubisco fixes carbon. Plants that use this "standard" mechanism to fix carbon are called C3 plants due to the three-carbon compound 3-PGA formed by the reaction. In C4 plants, the Calvin cycle and its light-dependent reactions are physically separated: The light-dependent reactions occur in the mesophyll cells, a spongy tissue located in the middle part of the leaf, while the Calvin cycle occurs in special cells around the leaf veins. These cells are called bundle sheath cells. CO2 present in the atmosphere is fixed in mesophyll cells to form oxalo acetate, a simple 4-carbon organic acid. This step is carried out by a non-rubisco enzyme called PEP carboxylase, which has no tendency to bind O2. Oxaloacetate is then converted to a similar molecule that can be transported to the bundle sheath cells. In the bundle sheath cells, malate is broken down and a CO2 molecule is released. This CO2 is fixed by rubisco and converted into sugar by the Calvin cycle, just like in C3 photosynthesis (Anonymous, 2023c; Walker, Berkeley et al., 2016; Reece et al., 2011; Türk and Çelik, 2006) (figure 1.)

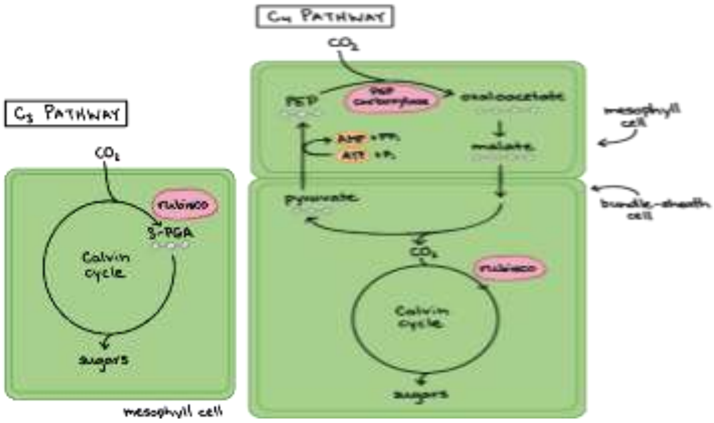


Figure 1. Schematic representation of the C3 and C4 photosynthesis pathway (Anonymous, 2023c).

2.2. Material

2.2.1 Research Area Description and Soil Properties

Karapınar district of Konya province is located between 37°42' North latitude and 33°33' East longitude. The distance to the city center is 102 km. The average altitude of the district above sea level is 1,026 meters. The soils in Karapınar are generally of alluvial origin, formed on old lake deposits. Soils are mostly defined as Inceptisol and Entisol orders according to soil classification (Soil Survey Staff, 1999).

The distance of Karapınar district of Konya province to the city center is 102 km. The average altitude of the district above sea level is 1,026 meters. The district has Aksaray province in the north, Karaman province Ayrancı district in the south, Karatay and Çumra districts in the west, and Ereğli district in the east. The surface area of the district is 2,939,17 km² (MEVKA, 2019).

2.2.2 Plant Material: Three plants were selected in the study and their carbon contents were compared with wheat plants grown in the same area.

a) Agropyron cristatum (L.) Gaertn. subsp. pectinatum (M.Bieb.) Tzvelev var. pectinatum – Grassland weed:

It is a meadow plant belonging to the Poaceae family. It is one of the three grassland species growing within the borders of Türkiye. Distribution in Turkey: Thrace (Istranca Region), Eastern Black Sea Region, Y. Sakarya, O. Kızılırmak and Konya Sections, Y. Fırat, Erzurum-Kars and Y. Murat-Van Sections (Güner et al., 2012). It is a species that can adapt to many different ecosystems in Turkey. It is one of the most durable forage plant species in pastures.

It is found in the natural vegetation of steppe pastures. It is a cool climate grass plant that is included in natural species mixtures in lawn production. In this respect, it has great economic importance. It is one of the important species, especially for the evaluation of saline and alkaline soils. It is one of the alternative species that can be produced in fallow areas (Acar et al., 2020). It is also one of the showy natural grassaceae members that can be used in landscaping applications in xeric areas (Çorbacı and Özyavuz, 2017). Medicinally, in some regions, all plant parts are used in the treatment of hemorrhoids and diabetes. There are publications indicating that rhizomes are used as a diuretic and laxative in the Artvin region (Gürhan and Ezer, 2004; Sarıkaya et al., 2010; Cesur and Yüksel, 2018; Eminağaoğlu, 2003).

b) Artemisia scoparia Waldst. & Kit. - Black broom:

It is a perennial herbaceous species belonging to the Asteraceae family. Distribution in Türkiye: Çatalca-Kocaeli and Ergene Regions; B. and O. Black Sea Sections; Central Anatolian Section; Central Anatolia Region; Y. Fırat, Y. Murat-Van and Hakkari Departments; O. Fırat Department (Güner et al., 2012). The Artemisia genus is generally used in the literature with its Turkish Scientific Names Yavşan or Pelin otu (Güner et al., 2012). It is one of the species that prevents erosion and adapts to drought. The genus Artemisia scoparia is represented by approximately 500 species in the world. 27 species grow naturally in Turkey (Güner et al., 2012; Kürşad et al., 2018). Some species of the genus Artemisia scoparia form plant associations in xeric and eroded areas in Central Anatolia. They are noticeably distributed in a significant part of the floristic composition (Geven et al., 2015). Some species of Artemisia scoparia are used medicinally as an appetite stimulant, antipyretic, strengthening agent and dewormer. Studies have found that their species exhibit a wide range of bioactivity (antifungal, anticancer, antioxidant, antimicrobial and insecticidal, etc.). Additionally, "Artemisin", used in the treatment of malaria, is an alkaloid obtained from the plant. Essential oils obtained from the aerial parts of the plant are used in the perfume and cosmetic industry (Coşgel and Şenkal 2017).

c**) Bassia scoparia (L.) A.J.Scott's:**

It is a perennial herbaceous plant known as Kochia (old synonym name). It has been transferred to the genus Bassia through molecular studies and phylogenetic classification studies carried out in recent years. It is a member of the Amaranthaceae family. Three species of this genus are naturally distributed in Turkey (Güner et al., 2012). Distribution in Türkiye: W. Black Sea Region; Y. Sakarya, O. Kızılırmak and Konya Departments; Y. Fırat

Department (Güner et al., 2012). It is a branched perennial herbaceous species that can grow up to 20-150 cm tall. They can spread up to 1200 m above sea level, on the edges of waste dumps and cultural areas. It has a body that turns from green to bright red at the end of the vegetation period. It grows in regions connected to Europe and Asia in the world. Plant form varies in species growing near cultural areas (Davis, 1976). In North America, the predictable feature of the emergence period of the vegetative part of the Kochia scoparia plant in spring has been found to have the potential to be used in weed control studies (Schwinghamer & Acker, 2017). It is a typical halophyte plant. The continuation of the vegetation period of the plant in late summer and autumn is very important in terms of competition with other plants (Khan et al, 2001). While saline waters and soils in desert ecosystems are used for animal husbandry, the salinity-resistant Kochia scoparia species can be used as animal feed in emergency situations (Nabati et al., 2011).

The control wheat plant is a variety of Kızıltan durum wheat grown conventionally (with chemical fertilization) in the production area.

2.3 METHOD

Determination of Carbon in Biomass: The underground (root) and above-ground (stem) parts of the plants were taken separately and their carbon content was determined. Plant samples were taken three times for each plant species, on the dates determined. The number of sampling varied according to the length of the detected periods, and the samples taken during the observation corresponding to the same period were recorded in the relevant period and averaged. Thus, recurrence in plant samples increased during the period. While determining the carbon contents, the moisture content of the plant root and surface samples was determined by making the air dry. Then, the samples were burned at 375 ^{o}C and carbon readings were taken (Tüzüner 1990).

C3 and C4 pasture plants were compared with C3 plant wheat, which is a cultivated plant corresponding to the same period as natural vegetation. It is assumed that it has not developed any adaptation mechanism since its needs such as fertilizer and irrigation are met in order to grow wheat.

However, since the change of the other three plants is a matter of control and their cultivation has high economic value and is widespread, revealing the contribution of agricultural production to carbon sequestration has been considered as a secondary aim.

Statistical Analysis Method: A three-factor trial design statistical model was used to evaluate the data. Results were evaluated by ANOVA analysis of variance with LSD multiple comparisons.

3. RESULTS and DISCUSSION

In this section, the carbon contents of the plants examined in the examined periods were determined and evaluated.

3.1. Evaluation of climate data of the Research Area

During the study, the climate parameters of the Karapınar region between 2018 and 2021 are average temperature, maximum temperature, average relative humidity, total precipitation, average wind speed 2m, lowest above-ground minimum temperature of 5cm, lowest underground minimum temperature of 20cm, underground average temperature of 20cm. was examined and the change was monitored. Temperature in the study area in Karapınar during 2018-2021; It varied between 11.2 oC -36.4 oC in 2018, between 9.5-36 oC in 2019, between 11.1-39 oC in 2020, and between 12.1-37.2 oC in 2021. The temperature averages were 24.2 oC in 2018, 22.3 oC in 2019, from late April to early May, when vegetation begins and continues, until mid-November. 24.4 oC in 2020; It was recorded as 23.9 oC in 2021. During 2018-2021, the average relative humidity in the study area in Karapınar varied greatly with the high fluctuations of the falling precipitation regime, its highest value was determined in the first months of 2020 (87.6%) and its lowest value was determined in August 2018 (35%).

A continuous decrease in precipitation was recorded in 2020. The highest total precipitation in Karapınar in a four-year period was recorded as 142.6 mm at the beginning of 2020. It was observed that the distribution of total precipitation between 2018 and 2021 was the most stable in 2019, and the sharpest and most decreasing decrease was in 2020.

It has been determined that total precipitation in 2021 fell below 2018 during the annual and vegetation periods. In 2019, unlike the other three years, it was observed that rainfall fell in the months of July-August-September.

The average wind speed at an altitude of 2m between 2018 and 2021, the lowest wind speed was recorded in 2019. The wind speed did not exceed 1.9 m/ for four years, but it was determined that it peaked twice in March in 2018, in January in 2019, in February 2020, and twice in April and July in 2021. July was recorded as 1.8;1.8;1.6 m/s for the previous three years. The most stable decrease occurred from September to November in 2019, and was recorded as 0.6 m/s in November.

Between 2018 and 2021, the lowest 5cm above ground temperature was recorded as -15.1 in February 2021, -13 at the end of November 2020, and -12.7 in February. The lowest temperatures in four years were detected in 2021 and finally in 2020. The lowest soil temperature in May was recorded as -4.1 in middle of May (day 15) in 2021. On the same dates, the temperature, which was 6.8 oC in 2018, was recorded as 2.4 oC in 2019, 1.2 oC in 20219, -1.1 oC in 2020, and -0.1 oC in 2021.

June temperatures are 8.6, respectively; 8.6; 7.9; It was determined that it was 9.2 oC. The lowest temperatures tended to increase in four years, and the year with the highest temperature difference was 2021. 11.9 respectively in the last 10-day period in July; 9.5; 8.7; After 2018, when 10.3 oC was recorded, the highest values were seen in 2021.

Between 2018 and 2021, the lowest underground temperature at a profile depth of 20 cm was observed as an average of 1.1 oC in the first 10 days of April 2018. In May 2018, when the phenological periods began, the temperature ranged between 3.2 and 1 oC, while in 2019 the same period varied between 15-13 oC. In 2020, the period of the month is between 24.1 and 25.6; In 2021, it increased to 28.3 oC.

3.2 Carbon Content and Statistical Evaluation of Plants

In 2018, stem carbon accumulations came to the fore, the highest carbon retentions were seen in the stem parts during the flowering period, and carbon contents were found to be close to each other in both periods of the plants. It is thought that the reason for this is that extreme values are not seen in this year's climate data, and the root and stem crown volume reached at flowering is similar in size to maturity and seed stages, and the plants have completed their vegetation period. This year, Bassia scoparia and Artemisia scoparia were significantly separated from other plants. Artemisia scoparia is a C3 plant and its response to climate in vegetation was similar to Bassia scoparia , a C4 plant. It has been determined that Agropyron cristatum and wheat both have C3 plant and stem carbon retentions that are close to each other, while Agropyron cristatum in the root part reaches a lower value than wheat. All interactions were found to be significant in 2018 (Figure**2;3; Table 2;3;4;5**).

In 2019, the wheat control plant showed the lowest carbon retention value and it was determined that the stem part was equivalent to the root part of Agropyron cristatum and both plants were in the same group as C3. In 2019, Bassia scoparia stood out in terms of carbon sequestration. The flower period is significantly separated from other plants and the stem and root parts have reached their highest value. It is thought that the reason for this is that the dry and extreme values of 2019 fluctuated. Bassia scoparia , as a C4 plant, has highlighted its adaptation feature and is distinguished from other C3 plants (Figure**4;5).**

It followed the C3 plant, Artemisia scoparia Bassia scoparia . This year, especially wind speed, root depth, soil temperature differences and extreme decreases and increases were observed, and this negatively affected the carbon retention of C3 plants, especially the wheat plant, which is a cultivated plant. It has been determined that other C3 plants, which are pasture plants, show better carbon retention than wheat. It is thought that the shift in seasonal temperature, relative humidity, wind speed, soil and air temperatures this year caused the difference in carbon retention between the vegetation periods of plants to differ statistically significantly (**Table 6;7;8;9**).

In 2020, wheat was again found to have the lowest carbon retention in comparison with C3 plants. This year, the periods and parts of the plants showed similar values to each other, and although there was no difference between the periods of the plants, it was determined that the periods were significantly different in plant comparisons. 2020 was generally dry and hot.

This caused the plants to reach root depth temperatures early and vegetation to start and end early. Artemisia scoparia bloomed earlier this year and the flowering period lasted longer than previous years. For this reason, carbon sequestration came to the fore this year and was in the first group. This year, Bassia scoparia was in the second group together with Agropyron cristatum . Again, it continued to exist in the vegetation for a long time and provided adaptation. Although Artemisia scoparia , which is a pasture plant, is a C3 plant, it has been determined that it can largely adapt to the conditions that C4 plants can adapt to.

Although wheat did not perform as well as other pasture plants in terms of carbon retention, it was observed that it reached similar values with Agropyron cristatum , one of its oldest ancestors. From the results obtained this year, it was concluded that carbon retention of plants increased in direct proportion to the beginning and length of vegetation, largely depending on the root depth temperature ((Figure **6;7, Table 10;11;12;13**).

2021 is a year in which seasonal precipitation has moved forward by at least two months, and the highest differences in air temperature, surface and root depth temperatures are observed, that is, extreme values are frequently observed. For this reason, all carbon sequestrations and interactions of plant parts and plant species were significantly different. This year all interactions were found to be important. Due to the long flowering period, the carbon retention of the stem parts of the plants has come to the fore. Bassia scoparia showed the highest adaptation and the longest vegetation and flower period after 2018 this year. Again, Agropyron cristatum showed higher values than wheat control in terms of adaptation and carbon sequestration ((Figure **8;9, Table 14;15;16**).

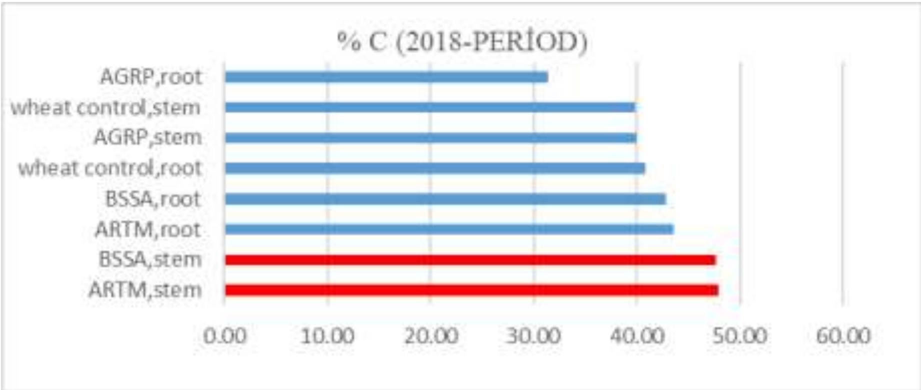
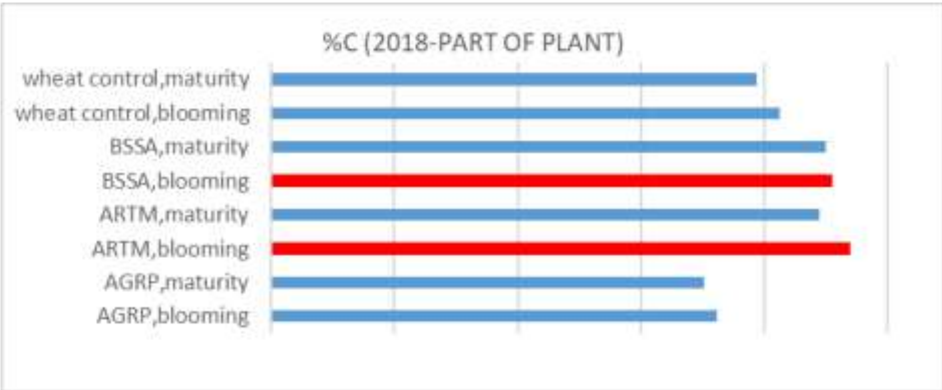
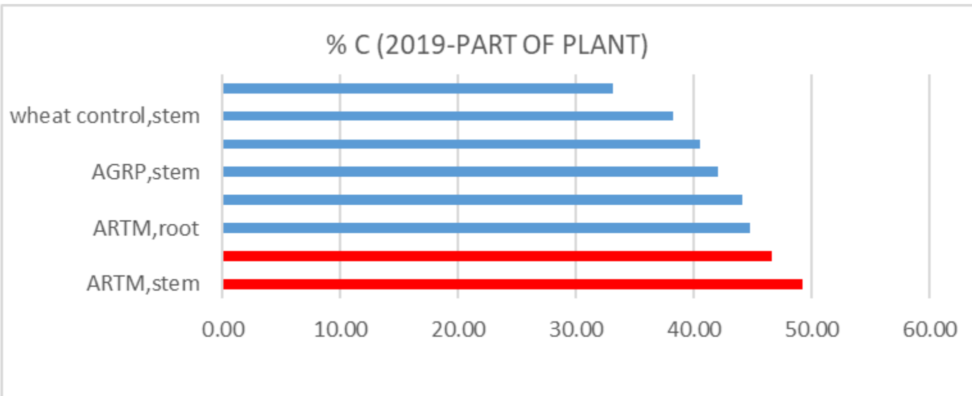


Figure 2. 2018 carbon sequestration rankings of plants according to their



periods

Figure 3. 2018 carbon sequestration rankings of plants according to their



parts

Figure 4. 2019 carbon sequestration rankings according to plant parts

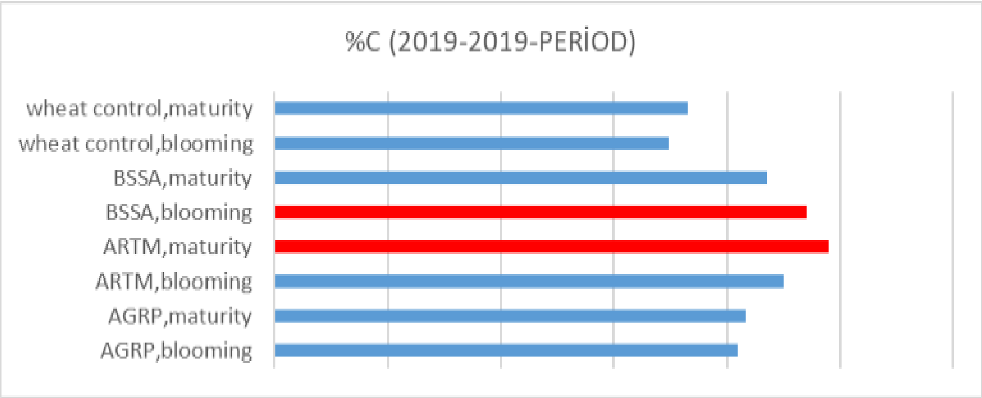


Figure 5. 2019 carbon sequestration rankings of plants according to their periods

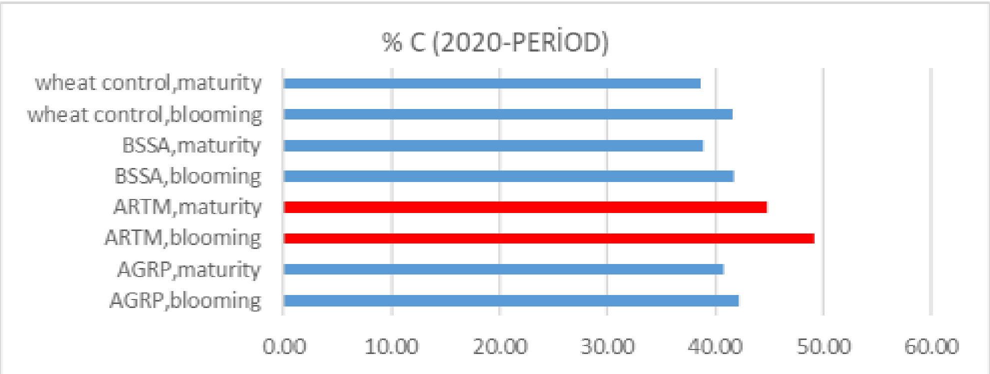


Figure 6. 2020 carbon sequestration rankings of plants according to their periods

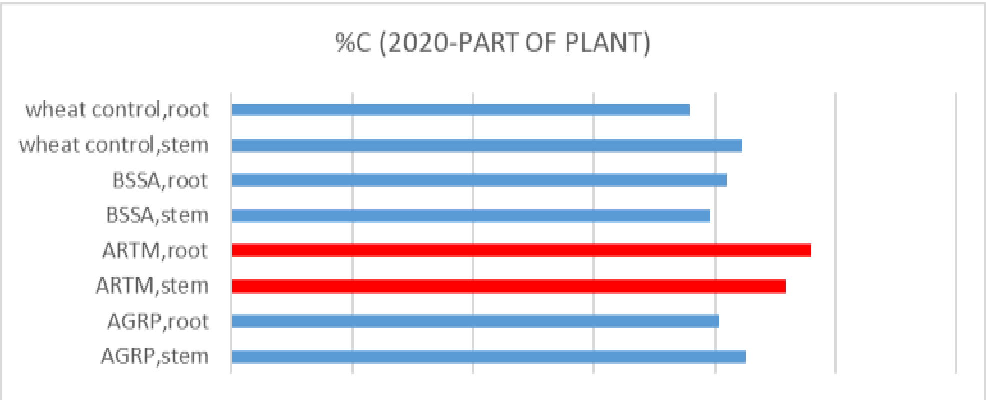


Figure 7. 2020 carbon sequestration rankings according to plant parts

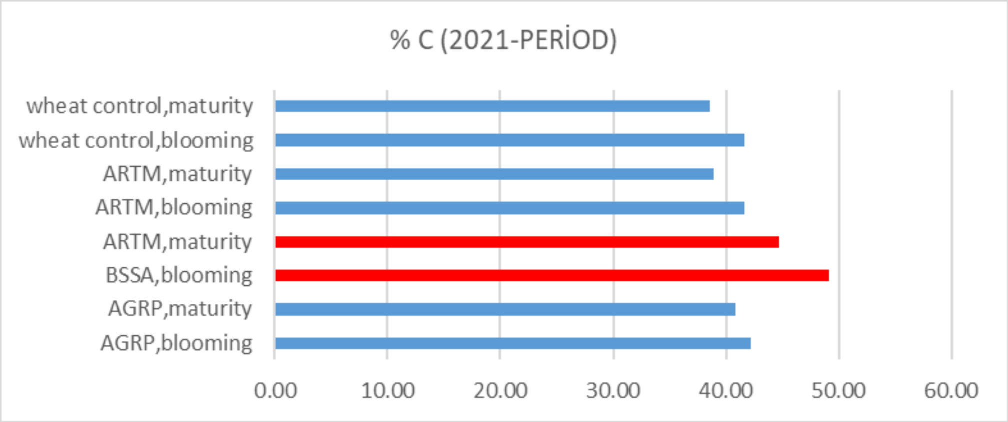


Figure 8. 2021 carbon sequestration rankings of plants according to their periods

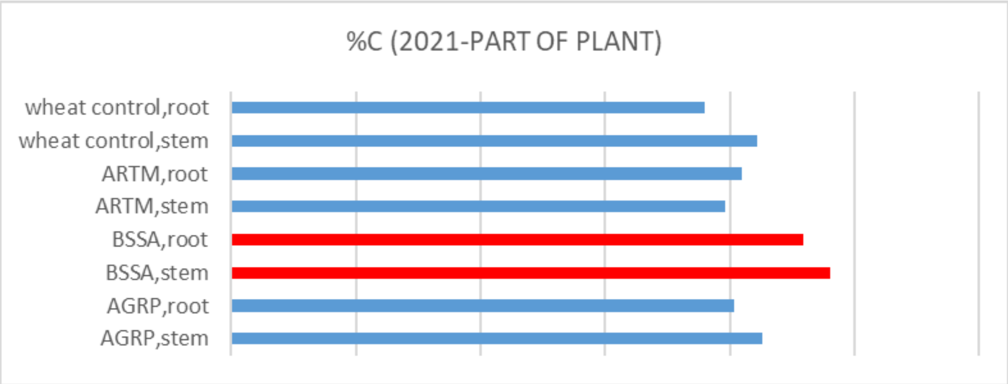


Figure 9. 2021 Carbon sequestration rankings according to plant parts

Table 2. Carbon measurement Variance analysis table for 2018

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	15	1292,4722	86,1648	32,8999
Error	32	83,8079	2,6190	**Prob > F**
C. Total	47	1376,2801		<,0001*

Table 3. Carbon measurement in 2018 Statistical evaluation model- interactions

Term	Std Error	T Ratio	Prob>|T|
Intercept	0,233586	178,73	<,0001*
Plants[AGRP]	0,404583	-15,05	<,0001*
Plants[ARTM]	0,404583	9,81	<,0001*
Plants[BSSA]	0,404583	8,73	<,0001*
Plant Parts[Stem]	0,233586	9,03	<,0001*
Period[Blooming]	0,233586	3,06	0,0044*
Plants[AGRP]*Plant Parts[Stem]	0,404583	5,35	<,0001*
Plants[ARTM]*Plant Parts[Stem]	0,404583	0,19	0,8496
Plants[BSSA]*Plant Parts[Stem]	0,404583	0,79	0,4331
Plants[AGRP]*Period[Blooming]	0,404583	-0,56	0,5780

Term	Std Error	T Ratio	Prob>|T|
Plants[ARTM]*Period[Blooming]	0,404583	1,31	0,2006
Plants[BSSA]*Period[Blooming]	0,404583	-1,21	0,2337
Plants[AGRP]*Plant Parts[Stem]*Period[Blooming]	0,404583	4,80	<,0001*
Plants[ARTM]*Plant Parts[Stem]*Period[Blooming]	0,404583	-4,74	<,0001*
Plants[BSSA]*Plant Parts[Stem]*Period[Blooming]	0,404583	1,01	0,3199
Plant Parts[Stem]*Period[Blooming]	0,233586	1,85	0,0735

Table 4. Impact evaluation Carbon in 2018 measurement significance test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Plants	3	3	807,70511	102,8008	<,0001*
Plant Parts	1	1	213,78339	81,6280	<,0001*
Period	1	1	24,59080	9,3894	0,0044*
Plants*Plant Parts	3	3	136,37508	17,3572	<,0001*
Plants*Period	3	3	7,30099	0,9292	0,4379
Plants*Plant Parts*Period	3	3	93,75082	11,9322	<,0001*
Plant Parts*Period	1	1	8,96603	3,4235	0,0735

Table 5. Average values of carbon measurement in 2018 (%C)

Level	Least Sq Mean	Std Error	Mean
AGRP	35,661667	0,46717205	35,6617
ARTM	45,719444	0,46717205	45,7194
BSSA	45,282957	0,46717205	45,2830
Buğday kontrol	40,334839	0,46717205	40,3348

Table 6. Carbon measurement Variance analysis table for 2019

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	15	1202,9924	80,1995	6,5694
Error	32	390,6558	12,2080	**Prob > F**
C. Total	47	1593,6482		<,0001*

Table 7. Carbon measurement in 2019 Statistical evaluation model - interactions

Term	Std Error	t Ratio	Prob>|t|
Intercept	0,504315	83,91	<,0001*
Plants[AGRP]	0,873499	-1,23	0,2294
Plants[ARTM]	0,873499	5,35	<,0001*
Plants[BSSA]	0,873499	3,45	0,0016*
PLANT PARTS[stem]	0,504315	3,35	0,0021*
PERİOD[Blooming]	0,504315	-0,71	0,4812
Plants[AGRP]*Plant Parts[Stem]	0,873499	-1,09	0,2857
Plants[ARTM]*Plant Parts[Stem]	0,873499	0,65	0,5182
Plants[BSSA]*Plant Parts[Stem]	0,873499	-0,54	0,5957
Plants[AGRP]*Period[Blooming]	0,873499	0,01	0,9904
Plants[ARTM]*Period[Blooming]	0,873499	-1,89	0,0682
Plants[BSSA]*Period[Blooming]	0,873499	2,44	0,0205*
Plants[AGRP]*Plant Parts[Stem]*Period[Blooming]	0,873499	-0,63	0,5311
Plants[ARTM]*Plant Parts[Stem]*Period[Blooming]	0,873499	0,28	0,7813
Plants[BSSA]*Plant Parts[Stem]*Period[Blooming]	0,873499	-0,30	0,7670
Plant Parts [Stem]*Period[Blooming]	0,504315	1,42	0,1666

Table 8. Carbon measurement in 2019 Statistical evaluation model - interactions

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Plants	3	3	910,71890	24,8667	<,0001*
Year	0	0	0,00000	.	.
Plant Parts	1	1	136,70357	11,1979	0,0021*
Period	1	1	6,20093	0,5079	0,4812
Plants*Plant Parts	3	3	25,91365	0,7076	0,5546
Plants*Period	3	3	89,90112	2,4547	0,0811
Plants*Plant Parts*Period	3	3	9,09841	0,2484	0,8618
Plant Parts*Period	1	1	24,45579	2,0033	0,1666

Table 9. Average values of carbon measurement in 2019 (%C)

Level	Least Sq Mean	Std Error	Mean

Level	Least Sq Mean	Std Error	Mean
AGRP	41,248810	1,0086292	41,2488
ARTM	46,991667	1,0086292	46,9917
BSSA	45,336111	1,0086292	45,3361
Buğday kontrol	35,700000	1,0086292	35,7000

Table 10. Carbon measurement Variance analysis table for 2020

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	15	610,73264	40,7155	12,5475
Error	32	103,83705	3,2449	**Prob > F**
C. Total	47	714,56969		<,0001*

Table 11. Carbon measurement in 2020 Statistical evaluation model - interactions

Term	Std Error	t Ratio	Prob>|t|
Intercept	0,260004	162,30	<,0001*
Plants[AGRP]	0,450341	-1,59	0,1228
Plants[ARTM]	0,450341	10,53	<,0001*
Plants[BSSA]	0,450341	-4,26	0,0002*
PLANT PARTS [stem]	0,260004	1,40	0,1697
PERİOD[Blooming]	0,260004	5,54	<,0001*
Plants[AGRP]*Plant Parts[Stem]	0,450341	1,60	0,1198
Plants[ARTM]*Plant Parts[Stem]	0,450341	-3,20	0,0031*
Plants[BSSA]*Plant Parts[Stem]	0,450341	-2,34	0,0257*
Plants[AGRP]*Period[Blooming]	0,450341	-1,68	0,1030
Plants[ARTM]*Period[Blooming]	0,450341	1,71	0,0970
Plants[BSSA]*Period[Blooming]	0,450341	-0,14	0,8873
Plants[Agrp]*Plant Parts[Stem]*Period[Blooming]	0,450341	-0,38	0,7098
Plants[Artm]*Plant Parts[Stem]*Period[Blooming]	0,450341	1,93	0,0620
Plants[Bssa]*Plant Parts[Stem]*Period[Blooming]	0,450341	-1,45	0,1561
Plant Parts[Stem]*Period[Blooming]	0,260004	2,52	0,0171*

Table 12. Carbon measurement in 2020 Impact assessment significance test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Plants	3	3	373,25041	38,3422	<,0001*
Year	0	0	0,00000	.	.
Plant Parts	1	1	6,40382	1,9735	0,1697
Period	1	1	99,66950	30,7157	<,0001*
Plants*Plant Parts	3	3	82,21109	8,4451	0,0003*
Plants*Period	3	3	14,05149	1,4434	0,2485
Plants*Plant Parts*Period	3	3	14,61018	1,5008	0,2331
Plant Parts*Period	1	1	20,53616	6,3287	0,0171*

Table 13. Carbon measurement average values table in 2020 (%C)

Level	Least Sq Mean	Std Error	Mean
AGRP	41,485000	0,52000864	41,4850
BSSA	46,939107	0,52000864	46,9391
ARTM	40,279643	0,52000864	40,2796
Buğday kontrol	40,091429	0,52000864	40,0914

Table 14. Carbon measurement Variance analysis table for 2021

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	15	1672,5855	111,506	9,8933
Error	32	360,6665	11,271	**Prob > F**
C. Total	47	2033,2520		<,0001*

Table 15. Carbon measurement in 2021 Statistical evaluation model - interactions

Term	Std Error	t Ratio	Prob>|t|
Intercept	0,484571	82,11	<,0001*
Plants[AGRP]	0,839301	-2,04	0,0493*
Plants[ARTM]	0,839301	3,79	0,0006*
Plants[BSSA]	0,839301	0,57	0,5718
PLANT PARTS[Stem]	0,484571	4,34	0,0001*
Period[Blooming]	0,484571	4,65	<,0001*
Plants[AGRP]*Plant Parts[Stem]	0,839301	6,58	<,0001*

Term	Std Error	t Ratio	Prob>|t|
Plants[ARTM]*Plant Parts[Stem]	0,839301	-1,31	0,2010
Plants[BSSA]*Plant Parts[Stem]	0,839301	0,08	0,9335
Plants[AGRP]*Period[Blooming]	0,839301	-3,79	0,0006*
Plants[ARTM]*Period[Blooming]	0,839301	1,85	0,0732
Plants[BSSA]*Period[Blooming]	0,839301	0,94	0,3560
Plants[AGRP]*Plant Parts[Stem]*Period[Blooming]	0,839301	0,47	0,6445
Plants[ARTM]*Plant Parts[Stem]*Period[Blooming]	0,839301	-1,42	0,1651
Plants[BSSA]*Plant Parts[Stem]*Period[Blooming]	0,839301	0,69	0,4967
PLANT PARTS[Stem]*Period[Blooming]	0,484571	4,20	0,0002*

Table 16. Carbon measurement in 2021 Impact assessment significance test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Plants	3	3	205,34040	6,0729	0,0022*
Plant Parts	1	1	212,50079	18,8541	0,0001*
Period	1	1	243,79350	21,6305	<,0001*
Plants*Plant Parts	3	3	622,77516	18,4185	<,0001*
Plants*Period	3	3	166,09170	4,9121	0,0064*
Plants*Plant Parts*Period	3	3	23,49507	0,6949	0,5620
Plant Parts*Period	1	1	198,58891	17,6197	0,0002*

SONUÇ ve ÖNERİLER

It has been determined that Agropyron cristatum is very sensitive to changing climate conditions and that this sensitivity is directly related to its carbon content. This plant can be used as a control and indicator plant in areas where the effects of climate change are observed. Wheat plant was used as a control plant in the study and it was determined that it had a higher carbon content than other C3 plants in the first year of the study.

The carbon sequestration of the wheat plant was similar to the C4 plant, Bassia scoparia , in the first year of the study and was statistically significantly higher than the other two C3 plants.

Throughout the study, it was observed that wheat plant was in competition with Artemisia scoparia and Agropyron cristatum , which are also C3 plants. It has been determined that the wheat plant has high carbon content not only in the harvested stem part but also in the root part, which has shown significant differences over the years with C3 and C4 plants in the same groups, which is not expected from a control plant in statistical evaluation.

One of the most important results of the study is that the data obtained from the control subject in the study show statistically significant different values regarding carbon retention. It has been determined that the control plant, wheat, has the same carbon sequestration ability as C3 plants in the pasture, even though it is grown in the production area with chemical fertilization. The results of the study showed that crop production in agriculture does not fully contribute to carbon emissions, but should also be considered as a sink and should be included on the carbon sequestration side in carbon budget calculations.

The plants monitored in the study are plants that are used in pasture improvement, are added to the plant composition in grazing areas with their high nutritional values, are used in livestock farming due to their benefits that facilitate the digestion of animals and increase protein synthesis, and also contain active substances used in many diseases such as stomach diseases, malaria and cancer.

The plants examined in the study have high economic values and their ecological roles are also very important. Considering the economic and ecological values of plants, the results obtained from the study showed that the amount and quality of extractable active substances of plants may change with climate change.

With these data, it is possible that with the change in the habitats where the species have existed for a long time, they have evolved with their adaptation abilities and gone beyond their usage patterns and purposes until today, and their economic value may be lost.

This possibility is an unforeseen risk for the food, pharmaceutical and cosmetic industries. This risk is ignored in all climate change projections and action plans. This situation should be included in national climate change action plans, and current analysis results and possible active ingredient content changes of plants used for this and similar purposes should be checked.

Planting trees, reducing deforestation, protecting wetlands, reducing dependence on fossil fuels, and investing in renewable energy sources to help protect carbon sink areas have been recommendations for years.

Today, in addition to these measures, it has been revealed that we can help reduce the effects of climate change by improving pastures and soil health in urban and rural areas, as well as creating carbon sink areas with the help of C4 plants. Afforestation can be an effective solution for carbon sinks, but not every area is suitable for afforestation.

It will be possible to use marginal areas that are not suitable for agriculture and urbanization as carbon sinks through C3 C4 plants. Thus, marginal areas can turn into an economic and ecological opportunity. The use of C3 and C4 plants for marginal areas will bring benefits in protecting natural resources such as creating carbon sink areas and pasture and soil reclamation.

A number of measures can be implemented for carbon sinks:

- No-till agricultural activities, which have been emphasized in many studies before,
- Covering and planting Class 2 and 3 lands, which are not suitable for agricultural production, with C4 plants,
- To prevent and improve the degradation of natural areas by saving water by growing C4 plants,
- Focusing on the more widespread study of C4 plants as landscape and genetic resources,
- Restoring natural and artificial pastures with C4 plants and increasing the carbon sink potential
- Development of new technologies in this field along with ecological approaches such as the use of C4 plants
- Carbon budget calculations regarding the carbon sequestration of agricultural areas should be reviewed and studies should be carried out to ensure that they are not fully included in the emission calculation and are considered as a sink.

- Increasing studies on the continuation of the gene pool and product quality by monitoring the habitat requirements and adaptation characteristics of plants with economic value in the medicinal and aromatic plant class under climate change.

- Organic farming is a recommended form of production in every aspect. However, determining the carbon sequestration values of agricultural production areas not only with organic methods but also in areas where existing conventional agriculture is carried out is of vital importance in terms of international carbon quota commitments.

The data and results recorded in this study should be evaluated urgently regarding "national net zero carbon emission" commitments.

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FARMERS WHO ARE PARTNERS IN THE AGRICULTURAL IRRIGATION COOPERATIVE IN ANTALYA PROVINCE REVIEW OF IRRIGATION PRACTICES

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ABSTRACT

In arid countries, irrigation is the most important factor in increasing productivity in agriculture. In these countries, the construction of an irrigation facility involving a single enterprise (drilling a well, installing a water pump or motor) can be done by a private firm or a multi-purpose cooperative. In this case, the job of the firm or the cooperative ends after the facility is completed and the cost is paid.

If the irrigation facility requires that many farmers benefit from the same water source (river, pond, canal, artesian, well, etc.), then there is a need to establish an irrigation cooperative in order to regulate both the amount of water each farmer will use and the order of water intake. If these issues are not regulated through cooperatives, it will lead to constant and inexhaustible fights, especially in arid countries where water is scarce and vital. Cooperative is the gathering of economic forces of individuals by means of solidarity in order to do the things that they cannot do alone or that are beneficial for them to do together, in the best way and at a cost price.

In this study, it is aimed to reveal the practices and thoughts of the farmers who are partners in the agricultural irrigation cooperative. In the study, a questionnaire was applied to the farmers in order to determine the common cooperative relations in terms of irrigation management and the data obtained were evaluated in the SPSS package program. The number of partners to be surveyed was determined by using the current information in the irrigation cooperatives in the research area. In the research, a questionnaire was applied to 61 farmers who are partners in the agricultural irrigation cooperative; 43% of this is made up of the farmers in Dosemealti and 57% in Korkuteli district. The survey application was carried out in December 2021. The data obtained from the survey application are summarized in the charts as scale, ratio and percentage values.

Keywords: *Cooperative, Agricultural organization, Demographic characteristics, Irrigation, Antalya*

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INTRODUCTION

Many countries, especially those located in arid and semi-arid regions, face significant water problems. Population growth and financing problems are at the forefront of the factors that create the water crisis. The most important aim of the agricultural sector is to get more products per unit area to feed the rapidly increasing population. The most intense water demand occurs in agricultural uses. Turkey is not among the countries suffering from water scarcity in the light of the worldwide evaluations in terms of water resources. However, considering the increasing water consumption values due to population growth, urbanization and industrialization, it is clear that there is a decrease in renewable fresh water resources in terms of quantity (Süheri ve Topak, 2005).

Among the water user sectors in Turkey, agriculture ranks first as the most water user sector. For this reason, the use of tools and techniques that ensure efficient water use in agriculture should be among the priority targets. If the current use of water in agriculture continues unchanged, it is highly likely that water scarcity will turn into a water crisis in Turkey. Precautions should be taken to ensure that the agricultural sector is affected by a possible water problem at the minimum level and due importance should be given to irrigation management. Therefore, the things to be done in agriculture are as follows: Making limited irrigation, designing irrigation systems according to pressurized irrigation methods, developing alternative water resources (recovery of waste water, directing surface water to areas where water is scarce, developing water-saving irrigation methods and techniques, opportunities to benefit from waste water and drainage water), dissemination of closed pipe systems instead of open systems in order to minimize water distribution losses, re-evaluation of the area, monitoring and evaluation of irrigation water quality, establishing the infrastructure for the transition to price-based pricing, ensuring inter-institutional coordination, giving more importance to farmer education and disseminating the trainings (Çakmak ve ark., 2008).

One of the obligatory options for increasing agricultural production in Turkey, where arable land is at the limit, is to buy more products per unit area. It is a fact that the expected production increase in irrigated agriculture in Turkey has not been achieved today. The factors affecting this situation start from the planning of the resources and continue their effects until the end use area. Problems encountered in this field; organization, personnel, financial opportunities, irrigation application problems, legal problems and business problems (Yurteri, 2011).

When the forms of irrigation management in Turkey are examined, it is seen that there are five types of management: public irrigation management, local governments irrigation management, irrigation union management, irrigation cooperatives management and public irrigation management (Sarı, 2017). In order to obtain the expected benefit from irrigation, the necessary care must be taken during the distribution and use of water. One of the most important organizations that ensure the distribution of water in Turkey, in other words, the delivery of water to the end user, is irrigation cooperatives (Cengiz, 2018).

Cooperative is the gathering of economic forces of individuals by means of solidarity in order to do the things that they cannot do alone or that are beneficial for them to do together, in the best way and at a cost price (Mülayim, 2013). According to the Cooperatives Law No. 1163, the cooperative; It is defined as partnerships with variable partners and variable capital, which are established by real and legal persons in order to provide and protect certain economic interests of their partners, especially their professional and livelihood needs, as having legal personality, through mutual assistance, solidarity and surety with their labor and monetary contributions.

Irrigation cooperatives are organizations formed by those who want to benefit from underground and surface resources, by combining their economic powers, according to Law No. 1163. The aim of irrigation cooperatives is to obtain the water needed by the farmers who want to benefit from water resources in an organized manner for their agricultural production, to make a fair distribution and to ensure its effective use (Ünver, 2016). Irrigation Cooperatives; It was established in 1963 under the name of "Soil-Water Cooperatives" in accordance with the main contract of association prepared by the General Directorate of Soil-Water affiliated to the Ministry of Rural Affairs. Later, these cooperatives became irrigation cooperatives and the Ministry of Agriculture and Rural Affairs had a new main contract of association prepared for irrigation cooperatives. According to the new main contract of association, the purpose of irrigation cooperatives; It has been determined as "to establish agricultural irrigation facilities such as land leveling, field head canals, in-field irrigation and drainage related to the use of water to be taken from irrigation facilities supplied or to be supplied by the state or to be extracted from agricultural fields in any way, or to operate, operate, maintain and have the irrigation facilities established".

Some of the studies conducted in Turkey on irrigation practices, behaviors, participation of producers in irrigation investments and similar issues in agricultural production can be listed as Aydin (2006), Uzunlu (2008), Sayın (2011), Yurteri (2011), Demir (2014), Buyukbas (2015), Unver (2016), Sari (2017), Cengiz (2018), Fisekcioglu (2018), Patlar (2018), Buz (2019), Candan (2020).

In this study, irrigation practices and behaviors of producers who are partners in agricultural irrigation cooperatives in Antalya province were investigated.

MATERIAL AND METHOD

Primary and secondary sources were used in the study. The primary material of the study consisted of the data obtained from the survey application made by face-to-face interview method with the producers who are partners in the Antalya province Agricultural Irrigation Cooperatives. Secondary data were obtained from sources such as national and international research reports, articles, theses, statistical data on similar subjects.

The data obtained from the questionnaires applied to determine the common cooperative relations in terms of irrigation management were evaluated in the SPSS package program. The number of partners to be surveyed was determined by using the current information in the irrigation cooperatives in the research area. The total number of Agricultural Irrigation Cooperatives in Antalya is 76, and the total number of partners is 11,879. In the districts of Döşemealtı and Korkuteli, a survey was conducted with the producers who are partners in the irrigation cooperative. In the research, a total of 61 partners were surveyed; 43% of this includes the district of Dosemealti and 57% of Korkuteli (Table 1). These data are summarized in the tables as scale, ratio and percentage values. In the study; a survey was conducted with the face-to-face interview method in Korkuteli and Dosemealti districts in December 2021 for the producers who are partners of the Irrigation Cooperative.

Table 1. Survey Application by District and Village

County Name	*Person*	*%*	*Village Name*	*Person*	*%*
Dosemealti	26	42.6	Karatas	8	**13.1**
			Karaveliler	2	**3.3**
			Kovanlik	9	**14.8**
			Yagca-Çiglik	7	**11.5**
Korkuteli	35	57.4	Bozova	10	**16.4**
			Sulekler	8	**13.1**
			Yelten	8	**13.1**
			Yesilyayla	9	**14.8**
Total	***61***	***100.0***	***Total***	***61***	***100.0***

Information about the Research Area

Antalya province is a center of tourism and agriculture in the south of Turkey, located on the Mediterranean coast, with an area of 20,177 km² and a population of 2,548,308 as of the end of 2020. The province of Antalya is between 29° 20'-32° 35' east longitudes and 36° 07'- 37° 29' north latitudes in the southwest of Turkey. It is surrounded by the Mediterranean Sea in the south and the Taurus Mountains in the north, and borders with Mersin, Konya and Karaman in the east, Isparta and Burdur in the north, and Mugla in the west. The surface area of the province corresponds to 2.6% of Turkey's surface area. Antalya province, located in the west of the Mediterranean region, constitutes 17.6% of the region's surface area.

Antalya province ranks 5th in Turkey in terms of population size, 2nd place with 156,395 farmer families and 9,53 billion TL agricultural production values, ranks 1st with 6.79% of Turkey's plant production value, and is the locomotive of Turkey's agriculture. Agricultural structure, land distribution and distribution of agricultural areas of Antalya province are given in Table 2. In the province of Antalya, 50,667 hectares of open and greenhouse vegetables, 551 hectares of ornamental plants, 74,787 hectares of fruit growing, 184,867 hectares of field crops are cultivated, of the 3,617,072 hectares of agricultural land.

Table 2. Agricultural Structure in Antalya Province (2019)

Number of Districts (unit)		**19**
Total Number of Neighborhoods (units)		**909**
Total Number of Farmers (person)		**156,395**
Number of Farmers Registered with CKS (person)		**39,533**
Average Size of Farm Registered with CKS (da)		**32**
Land distribution	***Antalya (da)***	***(%)***
Area	***20,177,000***	
Agricultural Land	3,617,072	**17.9**
Irrigated Agricultural Land	523,580	**2.6**
Meadow-Pasture Area	2,044,630	**10.1**
Forest Area	11,417,020	**56.6**
Agricultural Land		
Field Crops	1,789,048	**49.5**
Vegetable (Open Field)	214,643	**5.9**
Vegetable (Covered)	286,522	**7.9**
Ornamental Plants	5,944	**0.2**
Fruit Garden	797,144	**22.0**
Fallow	523,771	**14.5**
Total	***3,617,072***	***100.0***

Source: Anonymous, 2022.

Agriculture in Antalya is carried out in 4 agricultural basins and the product variety differs according to the basins (Figure 1).

In the Coastal Aegean Basin, the land starts from sea level and rises inland. Kas district is located in the Coastal Aegean Basin.

In the Coastal Mediterranean Basin, the land structure starts from sea level and rises up to 800-1000 m in the interior. This basin has coast and plateau sections. It is a region where coastal tourism is common. It is the basin with the best market opportunity for agricultural products. As the overseas connection points (port and airport) are in this region, it is in an advantageous position especially for export-oriented agricultural production. Aksu, Dosemealti, Kepez, Muratpasa, Konyaalti, Kemer, Kumluca, Finike, Demre, Serik, Manavgat, Alanya and Gazipasa districts are located in the Coastal Mediterranean Basin.

The Aegean Plateau Basin is a region with a plateau feature, located in the Western Taurus Mountains at an altitude of over 1000 meters topographically, with rich water resources. In this basin, the amount of agricultural land in the total land surface is the highest compared to other sub-regions. Korkuteli and Elmalı counties are located in the Aegean Plateau Basin.

The Lakes Basin has a mountainous terrain that forms the high plateau of the Taurus Mountains. This basin is limited in terms of agricultural land and is covered with forested areas. It starts from 800 meters above sea level and goes up to 1300 meters. Akseki, İbradi and Gundogmus districts are located in the Lakes basin.

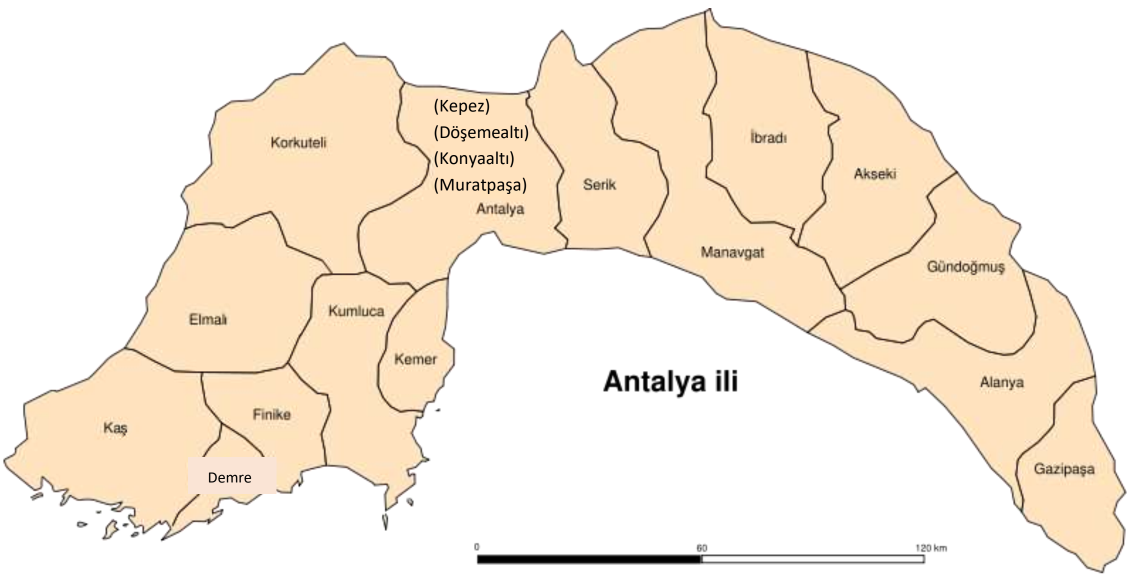

Figure 1. Districts of Antalya Province

2.8% of the 2451 Agricultural Irrigation Cooperatives in Turkey in 2020 are located in Antalya. Although the number of irrigation cooperatives in Antalya in the 2013-2020 period varies in this period, it is 69 in 2020. When irrigation cooperatives are examined by districts in Antalya province, Korkuteli district ranks first, followed by Elmali, Dosemealti and Alanya districts, respectively (Table 3).

Table 3. Irrigation Cooperatives by Districts in Antalya Province (number)

Districts	*2013*	*2014*	*2015*	*2016*	*2017*	*2018*	*2019*	*2020*	*2020 (%)*
Akseki	1	1	1	1	1	1	1	1	**1.4**
Alanya	8	8	8	8	8	7	7	7	**10.1**
Demre	3	3	3	3	3	3	3	3	**4.3**
Dosemealti	8	10	10	10	10	9	9	9	**13.0**
Elmali	12	13	13	13	13	13	13	13	**18.8**
Finike	2	2	2	2	3	3	3	3	**4.3**
Gazipasa	1	1	1	1	1	1	1	1	**1.4**
Kas	3	4	4	4	4	3	3	3	**4.3**
Konyaalti	1	2	2	2	2	2	2	2	**2.9**
Korkuteli	21	21	21	21	21	21	21	21	**30.4**
Kumluca	3	3	3	3	3	3	2	2	**2.9**
Manavgat	4	5	5	6	5	4	3	3	**4.3**
Serik	0	1	1	1	1	1	1	1	**1.4**
Total	***67***	***74***	***74***	***75***	***75***	***71***	***69***	***69***	***100.0***

Source: Anonymous, 2022.

Research Results

Demographic Characteristics of the Investigated Farms

The findings regarding the gender, educational status, age and experience of the farmers in the farms surveyed in the research are given in Table 4. Of the 61 farmers participating in the survey, 59 are male and 2 are female farmers. 71% of the farmers are primary school graduates. In the surveyed farms, 73.8% of the farmers are in the 50 and over age group. The average age is 56.

Table 4. Characteristics of Farmers in the Investigated Farms

	Person	*%*
Gender		
Female	2	3.3
Male	59	96.7
Total	***61***	***100.0***
Education Level		
Not Literate	1	1.6
Primary School Graduate	43	70.5
Secondary School Graduate	7	11.5
High School Graduate	7	11.5
College/University Graduate	3	4.9
Total	***61***	***100.0***
Age		
15-49	16	26.2
50 +	45	73.8
Total	***61***	***100.0***
Average age	56	
Average Experience in Agriculture (years)	34	

34% of the farmers participating in the research are engaged in plant production, 23% in animal production, and 43% in both plant and animal production. 5% of the farmers work in agricultural jobs outside the enterprise, 26% work in non-agricultural jobs, and 95% have their own social security (Table 5).

Table 5. Employment Information of the Farmer in the Investigated Farms

	Person	***%***
Production Activity		
Plant Production	21	34.4
Animal Production	14	23.0
Plant and Animal Production	26	42.6
Total	***61***	***100.0***
Off-Farm Agricultural Work		
Yes	3	4.9
No	58	95.1
Total	***61***	***100.0***
Working in Non-Farm Works		
Yes	16	26.2
No	45	73.8
Total	***61***	***100.0***
Social Security		
There is social security.	58	95.1
There is no social security.	3	4.9
Total	***61***	***100.0***
Type of Social Security		
Social Insurance Institution	20	34.5
Bagkur	26	44.8
Pension Fund	4	6.9
Social Security Institution	1	1.7
Social Insurance Institution Pension	7	12.1
Total	***58***	***100.0***

In the research, within the scope of the survey application, the opinions of the farmers on organization were also included. In the survey conducted to determine the partner-cooperative relations of the study, the target group is the irrigation cooperative partners. 14.8% of the producers are agricultural development partners and 39.3% are Agricultural Credit Cooperative partners (Table 6).

Table 6. Organizational Status (N=61)

Become a partner/member in an organization	***Person***	***%***
Agricultural Development Cooperative	9	14.8
Irrigation Cooperative	61	100.0
Agricultural Credit Cooperative	24	39.3
Chamber of Agriculture	20	32.8
Chamber of Commerce	1	1.6
Agricultural Producer Association	2	3.3
Organic Agriculture Association	1	1.6

* More than one answer has been received.

Irrigation Practices in Investigated Farms

In this part of the research, the demographic characteristics of the farmers who are partners in the irrigation cooperative, their practices on irrigation, their thoughts, etc. were examined.

In Table 7, the interest, knowledge and satisfaction levels of the farmers regarding irrigation are given. 52.5% of the cooperative partners are highly interested in irrigation. 36.1% of the farmers have high satisfaction with the irrigation application, and 26.2% have a medium satisfaction level. The rate of farmers who state that they have a high level of knowledge about irrigation is 44.3%.

Table 7. Opinions of Farmers on Irrigation in Investigated Enterprises (N=61) (%)

	Very low	*Low*	*Medium*	*High*	*Very high*	*Total*
Level of interest in irrigation	1.6	8.2	31.1	52.5	6.6	100.0
Satisfaction level with irrigation	4.9	23.0	26.2	36.1	9.8	100.0
Knowledge level about irrigation	3.3	8.2	31.1	44.3	13.1	100.0

In the examined farms, 47.5% of the farmers determine the irrigation time by looking at the plant, 34.4% according to their experience, 14.8% by controlling the soil and 11.5% according to the irrigation order (Table 8).

Table 8. How Farmers Determine Irrigation Time in Investigated Plants (N=61)

How to Determine Irrigation Time	*Person*	*%*
By controlling the soil	9	**14.8**
By looking at the plant	29	**47.5**
According to their experience	21	**34.4**
According to the irrigation order	**7**	**11.5**

* More than one answer has been received.

The ways of determining the amount of water to be given to the field by the farmers who are partners in the cooperative are shown in Table 9. 62.3% of the farmers stated that they determined the amount of water according to the plant characteristics, and 42.6% according to their experience.

Table 9. How the Producers Determine the Amount of Water to be Given to the Field in Investigated Farms

How to Determine the Amount of Water	*Person*	*%*
Soil feature	9	**14.8**
Plant feature	38	**62.3**
Experience	26	**42.6**
By looking at the moisture in the root zone	1	**1.6**
With soil moisture meter	1	**1.6**
Calculating plant water consumption	**3**	**4.9**

* More than one answer has been received.

The soil analysis practices of the farmers who are partners in the irrigation cooperative in the examined farms are given in Table 10. 65.6% of the farmers who are partners in the irrigation cooperative did not have a soil analysis. The rate of farmers who have soil analysis done in farms is 34.4%. The reasons for the farmers to have a soil analysis were to learn the fertilizer requirement of the product they will plant (85.7%) and to obtain diesel-fertilizer support (14.3%). 55.7% of the farmers find the soil analysis reliable.

Table 10. Soil Analysis Practices in Investigated Farms

	Person	*%*
Soil Analysis Status		
Yes, I did	21	**34.4**
No, I didn't	40	**65.6**
Total	***61***	***100.0***
Reason for Soil Analysis		
Mandatory to get diesel-fertilizer support	3	**14.3**
To find out the fertilizer needs of the plant	18	**85.7**
Total	***21***	***100.0***
Reliability of Soil Analysis		
Yes, reliable	34	**55.7**
No, it's not reliable	9	**14.8**
No idea	18	**29.5**
Total	***61***	***100.0***

The way in which the farmers who are partners in the cooperative determine the fertilizer requirement of the soil is shown in Table 11. 82% of the producers estimate the fertilizer need of the soil.

Table 11. How to Determine the Fertilizer Need of the Soil in the Investigated Farms (N=61)

How to Determine the Fertilizer Need of the Soil	*Person*	*%*
According to the results of soil analysis	7	**11.5**
Estimated	50	**82.0**
By asking my friends	**6**	**9.8**

* More than one answer has been received.

The findings related to the irrigation source in the farms surveyed in the research are given in Table 12. 60.7% of the farmers use drip irrigation. Groundwater is used for irrigation in 88.5% of the farms.

Table 12. Irrigation Source in Investigated Farms

	Person	*%*
Drip Irrigation Usage Status		
Yes, I'm using.	37	**60.7**
No, I do not use it, but I intend to.	17	**27.9**
No I do not use; because it is an expensive irrigation method.	4	**6.6**
There's no need	1	**1.6**
No, there is no system.	2	**3.3**
Total	***61***	***100.0***
Groundwater Use		
Yes I'm using.	54	**88.5**
No I do not use.	7	**11.5**
Total	***61***	***100.0***

Table 13 shows the findings regarding whether the farmers agree with the idea that "the more I irrigate, the more yield I get" in the farms examined within the scope of the research. 77% of the farmers stated that they did not agree with this idea and stated that water should be given as much as the plant needs. While 67.2% of the farmers knew the harms of giving too much water to the soil, 26.2% stated that they did not know. The rate of farmers who partially know the harms of giving too much water to the soil is 6.6%.

Table 13. State of Participation in Over-Irrigation and Over-Efficiency in the Investigated Farms

	Person	***%***
State of Participation in Over-Irrigation-Over-Efficiency Thought		
Yes, with a lot of irrigation, a lot of yield is obtained.	14	**23.0**
No, water should be given as much as the plant needs.	47	**77.0**
Total	***61***	***100.0***
Do You Know the Harms of Giving Too Much Water to the Soil?		
Yes, I know.	41	**67.2**
I partially know.	4	**6.6**
No, I do not know.	16	**26.2**
Total	***61***	***100.0***

In the examined farms, 80.3% of the farmers stated that they did not know the quality of the irrigation water, 13.1% stated that they knew, and 6.6% stated that the quality of the irrigation water was not important (Table 14).

Table 14. Information of the Famers on Irrigation Water Quality in the Investigated Farms

Water Quality	***Person***	***%***
Yes, I know its quality.	8	**13.1**
No, I don't know the quality.	49	**80.3**
The quality of the water does not matter.	4	**6.6**
Total	***61***	***100.0***

In the study, the opinions of the farmers about the cost of irrigation in the examined farms were investigated. Of the farmers surveyed, 83.6% stated that the electricity consumed for irrigation was expensive, 55.7% said that they irrigated economically, 67.2% stated that irrigation water was expensive. 63.9% stated that the irrigation fee should be calculated according to the hour (Table 15).

Table 15. Opinions on the Cost of Irrigation in the Investigated Farms

	Person	*%*
Electricity Fee Paid for Irrigation		
Expensive	51	**83.6**
Not expensive	9	**14.8**
No idea	1	**1.6**
Total	***61***	***100.0***
Economic Aspect of Irrigation		
I am irrigating economically	34	**55.7**
I do not make economically appropriate irrigation.	25	**41.0**
No idea	2	**3.3**
Total	***61***	***100.0***
Irrigation Water Fee		
Expensive	41	**67.2**
Normal	20	**32.8**
Total	***61***	***100.0***
How to Calculate Irrigation Fee		
Calculated According to Irrigation Hours	39	**63.9**
It should be calculated according to the irrigation flow	11	**18.0**
Calculated by Area	9	**14.8**
No idea	2	**3.3**
Total	***61***	***100.0***

In the farms examined within the scope of the research, 78.7% of the farmers stated that they irrigate at night, while 21.3% stated that they do not irrigate at night (Table 16).

Table 16. Night Irrigation Situation in Investigated Farms

Night Irrigation	*Person*	*%*
Yes I do.	48	**78.7**
No, I don't.	13	**21.3**
Total	***61***	***100.0***

75.4% of the farmers surveyed do not have information on the useful water capacity of the soil (Table 17).

Table 17. Information of the Farmers on the Useful Water Capacity of the Soil in the Investigated Farms

About Useful Water Capacity of Soil	*Person*	*%*
Yes, I have information.	15	**24.6**
No, I don't know.	46	**75.4**
Total	***61***	***100.0***

The availability of irrigation water for the farmers at any time in the examined farms is shown in Table 18. While 77% of the farmers buy irrigation water when it is their turn, 14.7% buy it whenever they want. The rate of farmers who cannot get irrigation water whenever they want is 6.6%.

Table 18. Availability of Farmers to Supply Irrigation Water at Desired Time in the Investigated Farms

Ability to Supply Irrigation Water	*Person*	*%*
I can take it whenever I want.	9	**14.8**
I can't get it whenever I want.	4	**6.6**
I get it when it's my turn.	47	**77.0**
I get delayed from time to time.	1	**1.6**
Total	***61***	***100.0***

Drip irrigation (73.8%) is the most suitable irrigation method according to the farmers in the enterprises included in the study, followed by sprinkling (18%) and surface irrigation (8.2%), respectively (Table 19).

Table 19. The Most Appropriate Irrigation Method According to the Farmers in the Investigated Farms

Irrigation Method	*Person*	*%*
Sprinkler irrigation	11	**18.0**
Drip irrigation	45	**73.8**
Surface irrigation	5	**8.2**
Total	***61***	***100.0***

Within the scope of the survey application in the research, the opinions of the producers about the irrigation organization that best meets their needs were determined. While 72.1% of the farmers stated that the organization that best met their irrigation needs was the irrigation cooperative, this was followed by DSI (19.7%) and the Irrigation Association (8.2%) (Table 20).

Table 20. Irrigation Organizations to Meet the Needs of the Farmers in the Investigated Farms

Irrigation Organization	*Person*	*%*
Irrigation Cooperative	44	**72.1**
Irrigation Association	5	**8.2**
State Irrigation Management (DSI etc.)	12	**19.7**
Total	***61***	***100.0***

The opinions of the farmers regarding the water distribution program application of the irrigation cooperative in the examined enterprises are given in Table 21. While 49.2% of the farmers stated that the water distribution program of the irrigation cooperative was determined according to the decision of the managers, 23% stated that it was made according to the products grown, 4.8% according to the water reserves, 4.9% according to the order.

Table 21. Water Distribution Program Implementation of the Irrigation Cooperative According to the Farmers in the Investigated Farms (N=61)

Water Distribution Program Application	*Person*	*%*
According to the number of farmers demanding water	12	**19.7**
According to the decision of the managers	30	**49.2**

According to the grown products	14	**23.0**
According to water reserves	9	**14.8**
By order	**3**	**4.9**

* More than one answer has been received.

The characteristics of the irrigation organization according to the farmers in the examined farms are shown in Table 22. According to this; 59% of the farmers said that the state should supervise and help, and 26.2% said that the democratic participation of the farmers should be essential.

Table 22. Characteristics of the Irrigation Organization According to the Farmers in the Investigated Farms

Irrigation Organization Features	*Person*	*%*
The democratic participation of the farmers should be essential.	16	**26.2**
The government should supervise and assist	36	**59.0**
Government should not interfere	1	**1.6**
Must be public enterprise	8	**13.1**
Total	***61***	***100.0***

In the research, the status of the farmers receiving technical information support from agricultural engineers in the surveyed farms was examined. 83.6% of the farmers did not receive technical information support from agricultural engineers on irrigation (Table 23).

Table 23. The Status of Obtaining Technical Information Support from Agricultural Engineers
for Irrigation in the Investigated Farms

Technical Information Support	*Person*	*%*
Yes, I am getting information.	6	**9.8**
I rarely get information.	4	**6.6**
No, I have not received any information.	51	**83.6**
Total	***61***	***100.0***

In Table 24, the participation of farmers in any seminar or meeting on irrigation and fertilization in the investigated farms was examined. Accordingly, 85.2% of the partners stated that they did not attend a seminar or meeting on irrigation and fertilization.

Table 24. Participation of Farmers in the Meetings on Irrigation and Fertilization in the Investigated Farms

Participation in a Seminar or Meeting on Irrigation and Fertilization	*Person*	*%*
Yes, I joined.	9	**14.8**
No, I did not participate.	52	**85.2**
Total	***61***	***100.0***

In the study, the information sources of the farmers on irrigation were examined in the farms where the survey was applied, and the findings are given in Table 25. Friends (47.5%) take the first place among the information sources of farmers on irrigation. This is followed by Agriculture and Forestry Provincial-District Directorates (34.4%), television programs

(24.6%), own experience (23.0%) etc. information sources.

Table 25. Information Sources of the Farmers on Irrigation in the Investigated Farms (N=61)

Information Sources	Person	%
From the Provincial and District Directorates of Agriculture	21	**34.4**
From private institutions	3	**4.9**
From printed publications on agriculture	3	**4.9**
From television programs	15	**24.6**
From friends	29	**47.5**
From the internet	5	**8.2**
according to my own experience	**14**	**23.0**

* More than one answer has been received.

In the study, the state of being aware of the state's support for irrigation methods was examined. 67.2% of the farmers are not aware of the support on this issue. The rate of farmers who are aware of the irrigation supports but cannot benefit is 24.6% (Table 26).

Table 26. Awareness of the Farmers of the State's Support for Irrigation in the Investigated Farms

About State Support for Irrigation Methods	*Person*	*%*
Yes, I know, I'm using it.	5	**8.2**
Yes, I know, but I don't use it.	15	**24.6**
No, I don't know.	41	**67.2**
Total	***61***	***100.0***

Opinions of farmers to benefit from credit support for irrigation systems in the examined farms are presented in Table 27. 65.6% of the farmers stated that they intend to benefit from credit support.

Table 27. Opinions of the Farmers on Benefiting from the Credit Support for the Irrigation System in the Future in the Investigated Farms

Benefiting from Credit Support	*Person*	*%*
Yes, I'm thinking of using it.	40	**65.6**
No, I don't intend to use it.	20	**32.8**
No idea	1	**1.6**
Total	***61***	***100.0***

In the farms included in the research, who performed the maintenance and repair services for irrigation is given in Table 28. 86.9% of the farmers stated that the maintenance and repair services were carried out by the cooperative they are a partner of, 11.5% of the farmers stated that they carried out the maintenance of the canals in their own land and 1.6% stated that the maintenance and repair was done by the state.

Table 28. Maintenance and Repair Services for Irrigation in the Inspected Enterprises

Maintenance and Repair Services	***Person***	***%***
I maintain the canals on my land myself.	7	**11.5**
Maintenance and repairs are done by the state.	1	**1.6**
Maintenance and repair is done by the cooperative.	53	**86.9**
Total	***61***	***100.0***

CONCLUSION

In this study, irrigation practices and behaviors of producers who are partners in agricultural irrigation cooperatives in Antalya province were investigated.

In the farms examined within the scope of the research, 70.5% of the producers are primary school graduates and the agricultural experience period is 34 years. Crop production is carried out in 77% of the examined farms. 26% of farmers work in non-agricultural jobs. The rate of farmers with social security is 95%. All farmers in the farms are partners in the irrigation cooperative, followed by partnerships in the agricultural credit cooperative and agricultural development cooperative.

In the study, 54% of the farmers stated that they were not satisfied with irrigation. According to 74% of the farmers, the most suitable irrigation method is drip irrigation. According to 72% of the farmers in the examined farms, the irrigation organization that will meet the needs is Irrigation Cooperatives. The rate of farmers who share the opinion that the state should supervise and support the irrigation organization is 59%. In the farms included in the research, 84% of the farmers stated that they did not receive technical support from agricultural engineers on irrigation, 85% did not attend meetings on irrigation, and 67% stated that they were not aware of the state's support for irrigation methods.

In regions where the place of agriculture in the economy is important, rational operation and continuity of irrigation facilities are of great importance in order to develop water and soil resources and increase their contribution to the national economy (Süheri and Topak, 2005). Irrigation methods also play an important role in the effective and economic use of existing resources and ensuring their sustainability. Improper, unplanned and inappropriate management of irrigation networks prevents the expected benefit from these systems. The most important application of increasing irrigation efficiency is to reduce water losses during transmission and application. Thus, as the losses in irrigation networks can be reduced, the amount of irrigated area will also increase (Sarı, 2017).

In order to increase production in the agricultural sector and to ensure rural development, first of all, it is necessary to develop soil and water resources and determine the principles of benefiting from them. In the development of soil and water resources; It is important to establish agricultural infrastructure, rational management of resources and to carry out studies for effective use. On the other hand, in order to ensure the sustainability of utilizing soil and water resources, besides the planning and construction of irrigation investments, it is necessary to rationally operate irrigation networks and ensure full economic and social participation of farmers in irrigation management (Patlar, 2018).

In particular, information training on water management such as soil-plant-water relations, irrigation time, irrigation methods should be provided to water users through applied training and extension programs to be organized by irrigation cooperatives and agricultural stakeholders on agricultural issues.

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EVALUATING THE EFFECTS OF *Eucalyptus camaldulensis* LEAF EXTRACTS ON *Meloidogyne incognita:* LABORATORY AND GREENHOUSE EVALUATIONS FOR NEMATODE CONTROL

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ABSTRACT

Root-knot nematodes are among the most damaging nematode groups to plants worldwide. While there are numerous species of nematodes belonging to the *Meloidogyne* genus, *Meloidogyne incognita* is one of the most common root-knot nematode species globally. Chemicals are commonly used to control root-knot nematodes. However, the tendency of environmentally friendly application methods that control nematodes increased in recent years. Nevertheless, the effects of *Eucalyptus camaldulensis* leaf extracts on root-knot nematodes have not been fully understood yet. Therefore, in this study, laboratory and greenhouse applications were conducted against the root-knot nematode *Meloidogyne incognita*, using the leaf extract of *Eucalyptus polybractea* diluted at 1%, 5%, 15%, 30%, 40%, Nematode+water, Non-nematode (control), and Nematicide. The effects of *Eucalyptus camaldulensis* leaf extracts on nematode and plant parameters were determined under laboratory and greenhouse conditions. The results revealed that, following the nematicide application, the highest larval mortality of 70% was observed in the 40% diluted leaf extract. The final nematode number significantly decreased with the application of the 40% diluted leaf extract, following the nematicide treatment. Based on these results, although the application of *Eucalyptus camaldulensis* leaf extracts shows promising results in controlling root-knot nematodes, the effectiveness can be better understood after field trials are conducted.

Keywords: *Eucalyptus camaldulensis, Meloidogyne incognita, Leaf extract, nematode*

INTRODUCTION

Nematode species belonging to the *Meloidogyne* genus exhibit an endoparasitic feeding behavior, causing damage to a wide range of plant species worldwide through the formation of various-sized root galls (Jones et al., 2013; Bozbuga, 2017). Root-knot nematodes (*Meloidogyne* spp.) not only result in crop losses but also impact plant physiology by affecting water and nutrient uptake, leading to wilting. Moreover, they facilitate the entry of other pathogens into plant roots, posing a significant threat due to their status as quarantine organisms (Moens et al., 2009). *M. incognita*, *M. hapla*, and *M. chitwoodi* are among the most common species (Hunt and Handoo, 2009). Root-knot nematodes undergo through various life stages, including eggs, first-stage juveniles, second-stage juveniles (J2), third-stage juveniles, fourth-stage juveniles, and adults, with J2 larvae possessing infective capabilities (Karssen and Moens, 2006).

Resistance genes against root-knot nematodes have been identified (Milligan et al., 1998; Williamson, 1998, 1999), and varieties carrying these resistance genes are preferred because they exhibit resistance to infection and provide an environmentally friendly option (Lopez-Perez et al., 2006). Infection by root-knot nematodes modifies the root's suitability, making it more conducive to subsequent reinfection by these nematodes over time (Kihika et al., 2020). Root-knot nematodes stand out as the group causing the most damage to plants among plant-parasitic nematodes. Their direct damage leads to crop loss, and they also have indirect effects due to quarantine regulations (Perry and Moens, 2013).

In the initial studies related to root knot nematodes in our country, the presence of the *Meloidogyne incognita* species was identified in Malatya and Elazığ (Öztüzün, 1970). In the Central Anatolia region, the rate of contamination with root-knot nematodes ranged from 10% to 94%, with *M. incognita* being the most prevalent species (Enneli, 1980). Additionally, it has been demonstrated that the root-knot nematode *M. incognita* alters the molecular structure of the cell wall, resulting in variations in infection rates (Bozbuga et al., 2018). Root-knot nematode J2s bypass the root endodermis barrier by traveling to the root's elongation zone near the tip, avoiding the Casparian strip, and then proceeding upward through the cortical tissues to establish feeding sites (Grundler et al., 1992). Second-stage larvae of root-knot nematodes induce the formation of large cells within plant roots, and males and second, third, and fourth-stage larvae of this species, which are freely present in the soil, live endoparasitically within the roots (Mıstanoğlu and Devran, 2015).

In recent years, there has been an increasing trend towards the use of environmentally friendly methods in nematode control. However, the effects of *Eucalyptus camaldulensis* leaf extracts on root-knot nematodes have not yet been fully understood. Therefore, this study investigates the impact of *Eucalyptus camaldulensis* leaf extracts on the root-knot nematode *M. incognita* and its morphological effects on the plant.

MATERIAL AND METHOD

This study included main materials such as tomato plants, pure culture of root-knot nematode (*Meloidogyne incognita*), potting soil mix, and laboratory equipment.

a- Measurement of Plant Root and Shoot Height

Plant sizes, including both root and shoot lengths, were measured using a ruler, and the longest root and shoot lengths were recorded in centimeters.

b- Plant Fresh Weight

Plant fresh weight was measured in grams by weighing the roots and stems separately using a precision scale.

c- Plant Dry Weight

Plant dry weight was determined by placing the plants in paper bags, allowing them to place in an oven at 80°C for 24 hours, and then measuring them with a precision scale.

d- Nematode Count

As an initial population, 1.5 nematodes/gram of soil were added to each pot. For nematode counting, 200 grams of soil, along with plant roots, were collected. Nematodes were extracted using the Baermann funnel method in the laboratory, nematodes was counted under microscope and the final nematode population was calculated.

e- Preparation and Application of *Eucalyptus camaldulensis* Extract

Leaves of *Eucalyptus camaldulensis* were collected and dried in the laboratory. The dried leaves were crushed into a powder, and this powder was soaked in an Erlenmeyer flask containing distilled water for 24 hours. The concentration of this solution was considered 100%, and it was diluted with tap water to prepare different concentrations (1%, 5%, 15%, 30%, 40%), which were then applied to the plants in the pots for two months. Additionally, applications were made with nematicide, nematode+water, and non-nematode plants. In the laboratory setting, to assess its effect on nematodes, 100 live second-stage nematode larvae were placed in Petri dishes, and *Eucalyptus camaldulensis* extracts were added. After 24 hours, the viability of the nematodes was determined by counting them under a microscope.

f- Application of Nematodes and Tomato Plants

A pure culture of *Meloidogyne incognita* species was used. The experiment was repeated five times, with negative control plants used for each one. The study was conducted with a tomato variety susceptible to root-knot nematodes. Twenty-five-day-old seedlings were planted in pots containing sterile soil, peat, and sand mixture and were grown in a greenhouse at a temperature of 27°C with 16 hours of light and 8 hours of darkness. Each plant was inoculated with 1.5 J2s/gram soil, and after two months, the plants were uprooted, and their gall index and other parameters were observed and evaluated.

g- Gall Index

After two months of inoculation, the plants were removed, washed, and their root condition was evaluated using the gall index method (Hartman and Sasser, 1985).

h- Statistical Analysis

The data were used to one-way ANOVA and Duncan multiple comparison tests.

RESULTS

a- Plant height

In terms of total plant height (root+shoot), the effect of the 40% *Eucalyptus camaldulensis* extract application was the highest, followed by the nematode+water and nematode-free+water applications, which recorded the tallest tomato plant height. The lowest total plant height was observed in plants treated with 1% *Eucalyptus camaldulensis*. Regarding plant stem height, the tallest shoot was observed in plants treated with nematode+water and 5% *Eucalyptus camaldulensis* extract. The shortest shoot height was found in plants treated with 5% *Eucalyptus camaldulensis* extract. Concerning plant root height, the greatest root height was determined in plants treated with 40% *Eucalyptus camaldulensis* extract, while the shortest root height was observed in plants treated with 5% *Eucalyptus camaldulensis* extract (Figure 1).

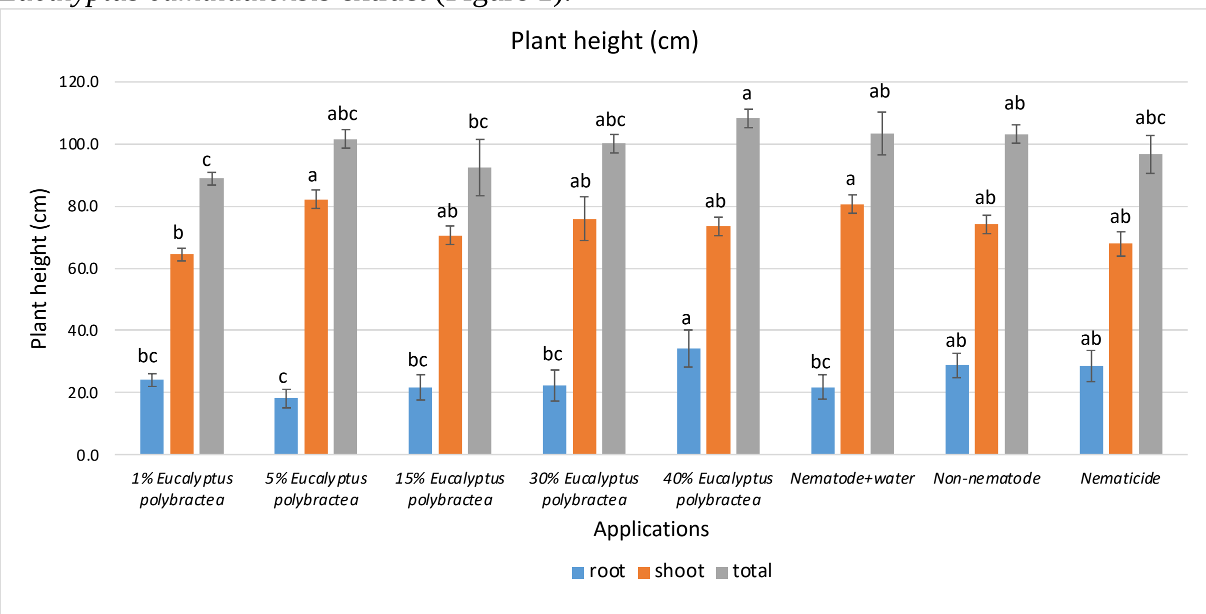

Figure 1. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the root, shoot, and total plant heights of tomato plants. The statistical analysis was performed separately for each group (root, stem, and total) within each group.

b- Plant Fresh Weight

In terms of total plant fresh weight (root+shoot), the effect of the 1% *Eucalyptus camaldulensis* extract application was the highest, followed by the non-nematode application, which recorded the highest tomato fresh weight. The lowest total fresh weight was observed in plants treated with 40% *Eucalyptus camaldulensis*. Regarding plant fresh weight, the highest shoot weight was observed in non-nematode and 1% *Eucalyptus camaldulensis* extract-treated plants. The lowest stem fresh weight was found in plants treated with 40% *Eucalyptus camaldulensis* extract. Concerning plant root fresh weight, the greatest fresh weight was determined in plants treated with 1% *Eucalyptus camaldulensis*, while the lowest root fresh weight was observed in plants treated with nematode+water (Figure 2).

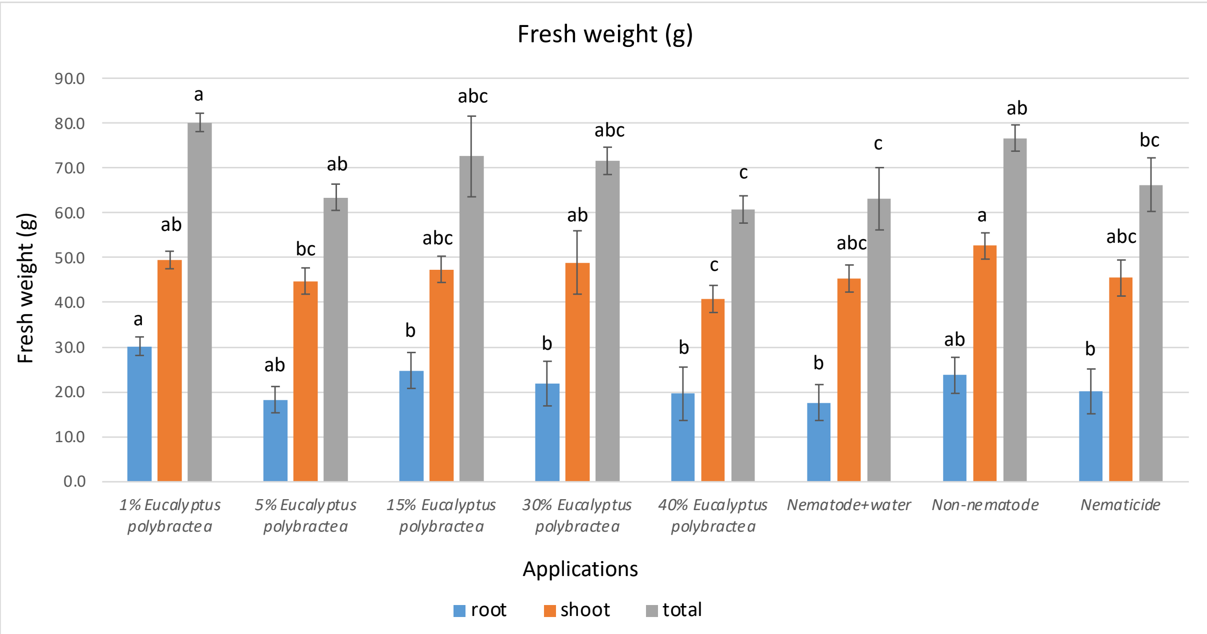

Figure 2. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the root, shoot, and total fresh weights of tomato plants were evaluated. Statistical analysis was conducted separately for each group (root, stem, and total). Letters indicate differences between means in the treatments (P<0.05).

c- Plant Dry Weight

In terms of the total plant dry weight (root+shoot), the effect of the 1% *Eucalyptus camaldulensis* extract application was the highest, followed by the non-nematode application, which recorded the highest tomato dry weight. The lowest total dry weight was observed in plants treated with 5% *Eucalyptus camaldulensis*. Regarding plant dry weight, the highest shoot weight was observed in non-nematode and 1% *Eucalyptus camaldulensis* extract-treated plants. The lowest stem dry weight was found in plants treated with 5% and 40% *Eucalyptus camaldulensis* extract. Concerning plant root dry weight, the greatest dry weight was determined in plants treated with 1% *Eucalyptus camaldulensis*, while the lowest root dry weight was observed in plants treated with nematode+water (Figure 3).

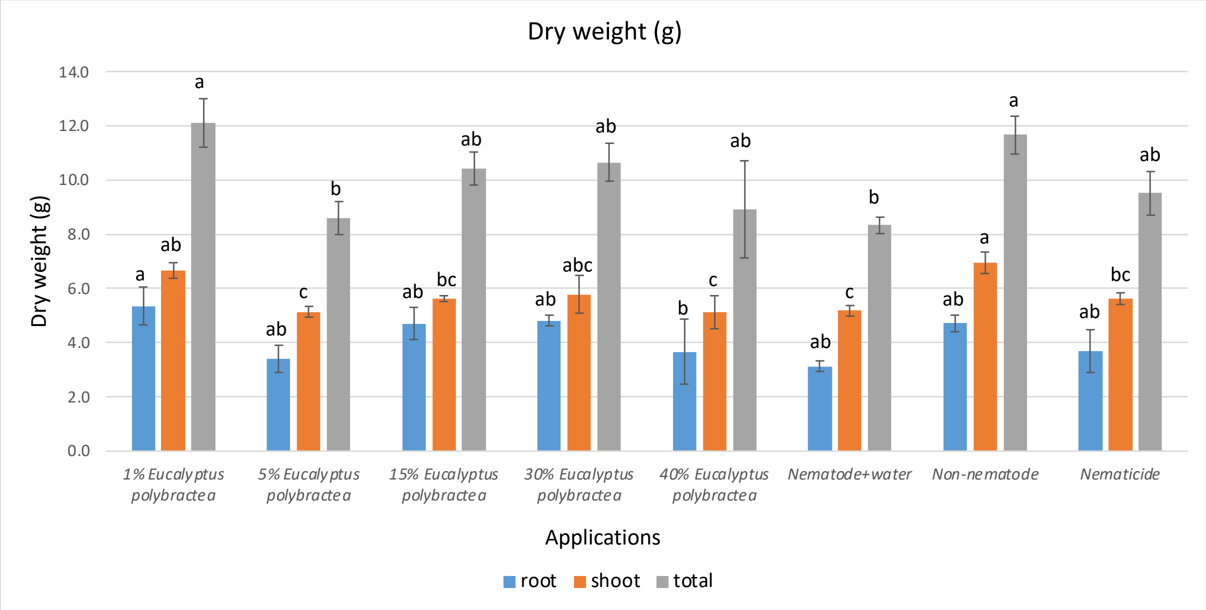

Figure 3. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the root, shoot, and total dry weights of tomato plants were assessed. Statistical analysis was performed separately for each group (root, stem, and total). Letters indicate differences between means in the treatments (P<0.05).

d- Root Gall Index

The lowest gall index in tomato roots caused by nematode infection was found in the nematocide treatment. There was no statistically significant difference in the gall index among the *Eucalyptus camaldulensis* applications of 1%, 5%, 15%, 30%, and 40% (Figure 4).

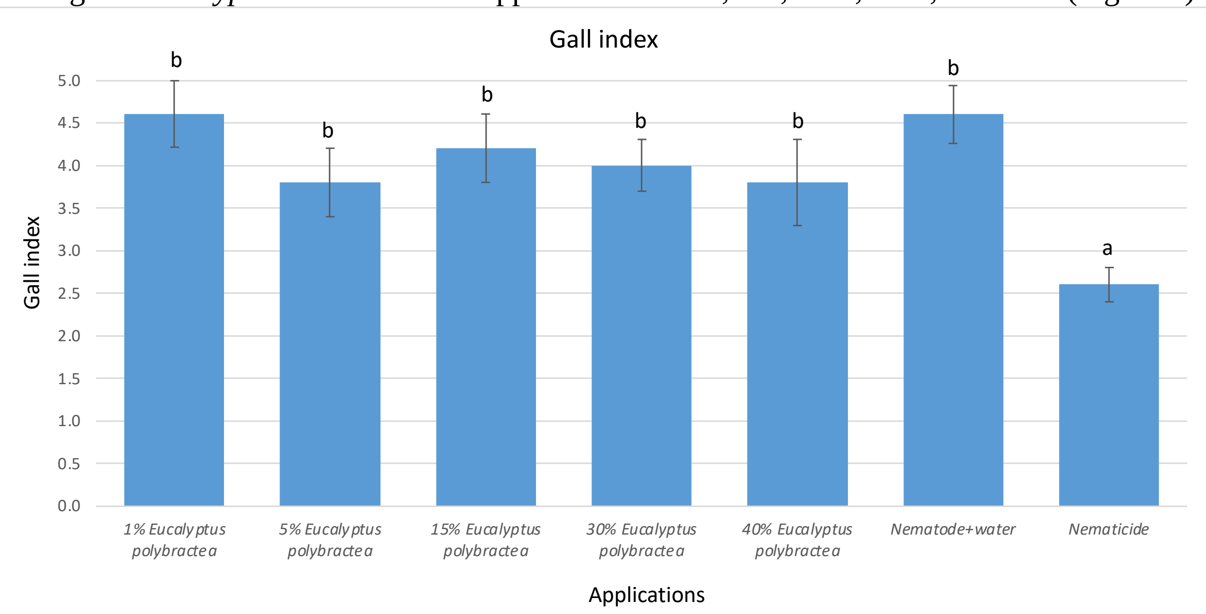

Figure 4. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the nematode gall index (0-5) in tomato plant roots were evaluated. Letters indicate differences between means in the treatments (P<0.05).

e- *Eucalyptus camaldulensis* Effect on Larva Death in Lab Condition

In the laboratory-based study, the lowest larval mortality rate was found in the water and 1% *Eucalyptus camaldulensis* applications, with the least larval mortality rate being a percentage.

The highest larval mortality rate was observed in the nematicide treatment, followed by the 40% *Eucalyptus camaldulensis*, 30% *Eucalyptus camaldulensis*, 15% *Eucalyptus camaldulensis*, 5% *Eucalyptus camaldulensis*, and 1% *Eucalyptus camaldulensis* applications, respectively (Figure 5).

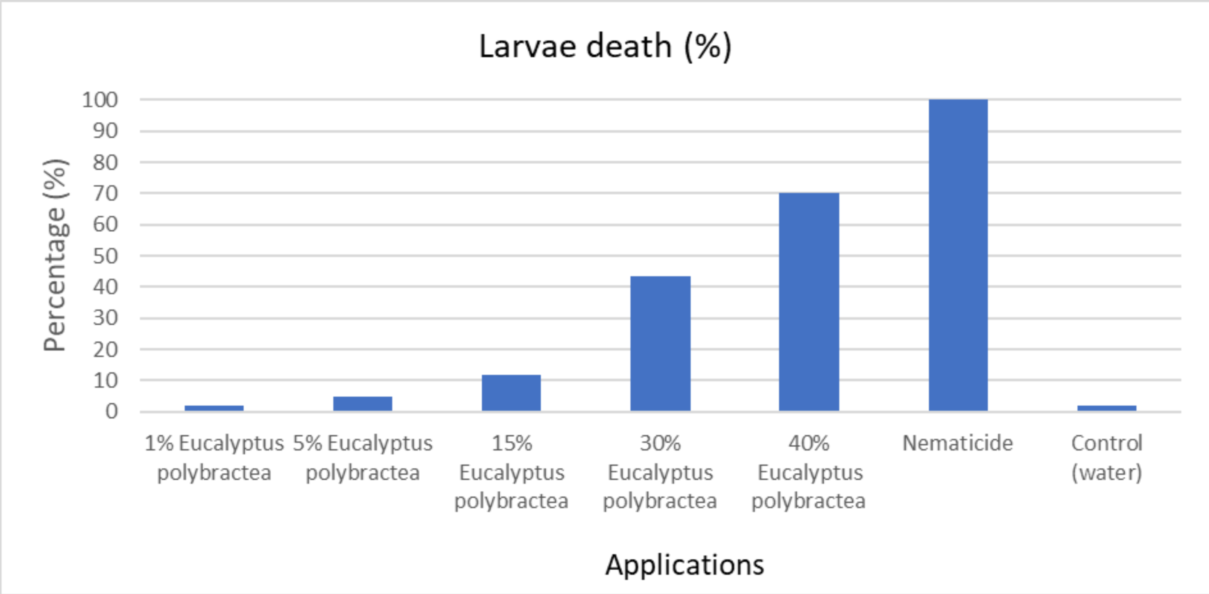

Figure 5. The effects of *Eucalyptus camaldulensis* leaf extracts and other treatments on second-stage larvae of the root-knot nematode *Meloidogyne incognita*.

f- *Eucalyptus camaldulensis* Effect on Nematode Population in Greenhouse

The study aimed to determine the effects of *Eucalyptus camaldulensis* leaf extracts and other treatments on the population of the root-knot nematode *Meloidogyne incognita*. It was found that nematode populations were reduced in the nematode+water, 5% *Eucalyptus camaldulensis*, and 1% *Eucalyptus camaldulensis* applications. In other applications, including 40% *Eucalyptus camaldulensis*, 30% *Eucalyptus camaldulensis*, and 15% *Eucalyptus camaldulensis*, the nematode population decreased, but the lowest nematode population was observed in the nematicide treatment (Figure 6).

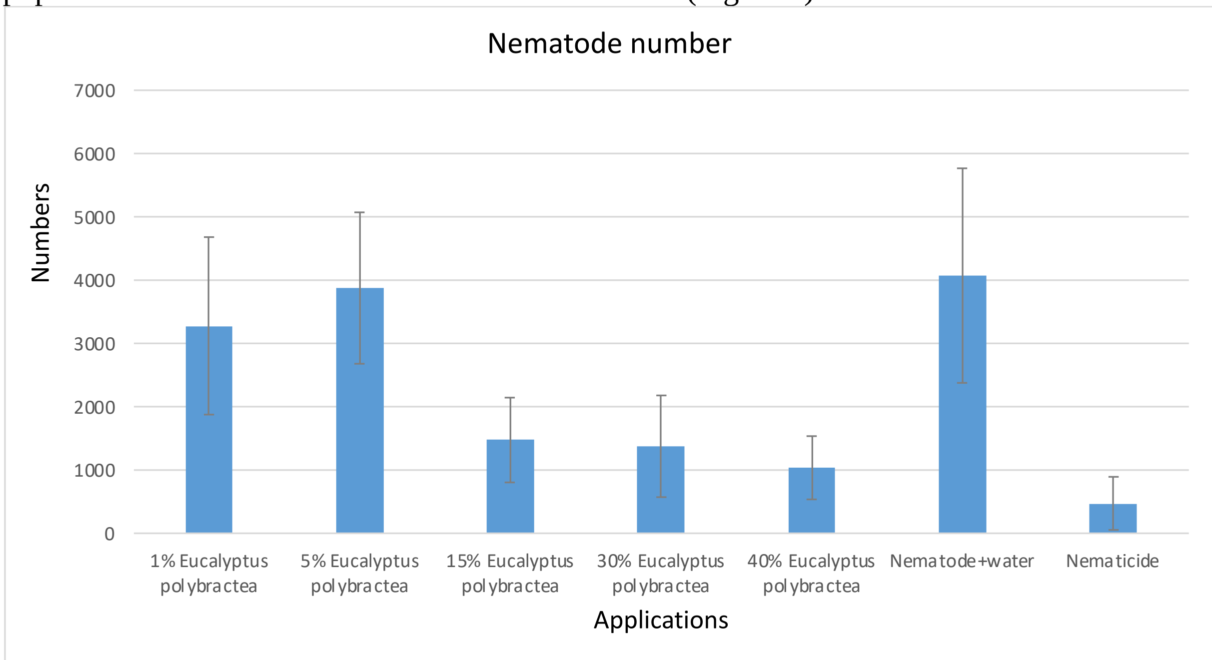

Figure 6. The effects of *Eucalyptus camaldulensis* leaf extracts and other treatments on the populations of the root-knot nematode *Meloidogyne incognita*

DISCUSSION

In the world, there are over 100 species of root-knot nematodes, with *Meloidogyne incognita* being one of the most common and harmful species (Moens et al., 2010). Worldwide, *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are reported as common species (Sasser and Carter, 1985). In the Central Anatolia region, the rate of infection with root-knot nematodes ranges from 10% to 94%, with *M. incognita* being the most common species (Enneli, 1980). In this study, the most common root-knot nematode species, *M. incognita*, was selected. Nematodes belonging to the *Meloidogyne* genus have a permanent endoparasitic feeding habit, causing various sizes of galls in the roots of their hosts, and they harm a wide range of plant species worldwide (Bozbuga, 2017). In this study, after the inoculation of second-stage larvae of root-knot nematodes, galling occurred in the roots, and according to the gall index, no statistical difference was found in the application of *Eucalyptus camaldulensis* leaf extracts (Figure 4). Although larval mortality was successful in laboratory applications (Figure 5), it is thought that the soil is influenced by various factors. Additionally, the root-knot nematode *M. incognita* alters the molecular structure of the cell wall, resulting in differences in the infection rate of the nematode (Bozbuga et al., 2018).

Plant defenses in response to pathogens are influenced by phytohormones like Saliclic acid and jasmonic acid, and less-studied hormones, such as auxin, cytokinin, gibberellic acid, and abscisic acid, are also involved in plant-nematode interactions and defense (Gheysen and Mitchum, 2019). Plant extracts can exhibit nematicidal effects on nematodes. For example, the application of *Lantana camara* extract on *Meloidogyne incognita* larvae caused high mortality (Begum et al., 2000). In this study, the nematode population decreased in the applications of 40% *Eucalyptus camaldulensis*, 30% *Eucalyptus camaldulensis*, and 15% *Eucalyptus camaldulensis* (Figure 6). Similarly, the extract of *Lantana camara* plant reduced root-knot nematode populations and caused high mortality in *Anguina tritici* (Dura et al., 2019). The effect of *L. camara* ferment on *M. javanica* in tomato plants was reported (Malahlela et al., 2021). Strengthening the endodermal tissues in roots not only reduces RKN penetration but also diminishes the development of feeding sites and galls, indicating that reinforcing the endodermis enhances resistance to Root knot nematodes across the nematode's entire life cycle (Rutter et al., 2022).

In the study of *Eucalyptus camaldulensis* leaf extract applications on plant fresh weight, dry weight, and plant length (Figure 1, 2, 3), the high values of certain parameters at low doses are also considered as a possible fertilizing effect on the plant. The failure of some parameters to increase at high doses suggests a potential toxicity effect. In future studies, working with more complex and detailed dosage values is believed to provide a more comprehensive understanding of the effects of this plant's extracts on nematodes.

CONCLUSION

Based on the results of the studies, the application of 40% *Eucalyptus camaldulensis* leaf extracts is considered to have the potential for nematode control. Additionally, conducting field studies and investigating the molecular mechanism in the future is believed to be essential to understand the plant extract-nematode host relationship.

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ETHANOL TREATMENT ALLEVIATES ADVERSE EFFECTS OF DARKNESS STRESS IN PEPPER

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ABSTRACT

Light is both the main energy source for plants and an essential regulator throughout the plant's life cycle. Plants are photosynthetic organisms. For this reason, more or less lighting affects many metabolic and physiological processes, especially photosynthesis. Absence of light or darkness stress can cause morphological, physiological and biochemical response on plants. In this study, the changes in plant growth parameters, pigment and total phenolic contents of pepper seedlings exposed to darkness stress and the effects of exogenous ethanol application on these changes were investigated. According to our findings, extended darkness stress (5 days) caused a decrease in number of leaves, fresh and dry weights of plant, as well as carotenoid content in plants. 20 mM ethanol pre-treatment alleviated the negative effects of darkness stress on these parameters.

Keywords: Pepper, Darkness, Ethanol, Plant Growth Parameters, Chlorophyll, Carotenoids, Total Phenolic Compounds

INTRODUCTION

Throughout their lifespan, plants have the potential to encounter varying environmental circumstances. The growth and development of plants might be adversely affected by the stress caused by the rapid fluctuations in environmental circumstances. Light stress is a common stressor experienced by plants. Light is a crucial climatic component that has significant importance in the growth and development of plants. Plants, like all living things, get the energy they need to exist from the sun. Plants are photosynthetic organisms. Light is one of the most significant factors influencing the photosynthetic parameters and phytochemical content of plants. Light is of significant importance in several physiological processes, particularly in the photosynthetic electron transport (PET) chain. This chain is responsible for converting the energy obtained from light into biochemical components like ATP, NADPH, and facilitating the absorption of CO_2 in chloroplasts. Plants are exposed to high or low light due to fluctuations in light intensity. The physiological metabolic processes of plants, such as antioxidant mechanism, C-N fixation capacity, and photosynthetic activity, are negatively influenced by variations in lighting conditions.

Light stress induces changes in the cellular, biochemical and molecular structures of plants. (Szymańska et al., 2017; Yang et al., 2019). Low light intensity is about 40-50% of natural light. It triggers stress conditions due to insufficient energy supply to the energy-rich photons, which are the energy source of the photosystem and the regulator of photosynthesis. Low light intensity causes growth and productivity losses in plants. The dark period is an important source of stress for the plant and causes significant changes in plant metabolism. It was determined that the dark environment caused a decrease in root and stem length and biomass yield of plants (Akgül, 2018).

Plants can cope with stress by creating a variety of defensive responses. In addition, exogenous application of some compounds increases stress tolerance of plants. Plant growth regulators play an important role in increasing yield and quality in plants and reducing abiotic stresses. Recently, various researchers have emphasized that ethanol may be an alternative to plant growth regulators. Ethanol (EtOH) has been proposed to increase seed germination and biomass yield in some plant species (Smits et al. 1995). Rowe et al. (1994) reported that exogenous application of ethanol (up to 20%) to plants increased biomass yield.

This research aimed to investigate the impact of darkness stress on plant growth parameters, as well as the levels of photosynthetic pigment and total phenolic content. Additionally, effects of exogenous ethanol application on these changes were examined.

MATERIAL AND METHOD

In this study, pepper (Capsicum annuum sp.) (Üç Burun Cv.) seedlings were used as plant material. The pepper, native to Central America and Mexico, belongs to the Capsicum genus of the Solanacea family and is an annual plant in temperate climates and a perennial plant in tropical climates (Kanal et al., 2021). The pepper plant, which is widely used in Turkey, is rich in vitamins. The pepper has 160 mg of vitamin C per 100 g, making it particularly vitamin C-rich.

The present study was carried out in the Gazipaşa Vocational School of Alanya Alaaddin Keykubat University. Seedlings were grown in pots containing a 3:1 mixture of peat:perlite (v/v) under natural conditions with an average temperature of 30 °C and an average humidity of 65%. At about the sixth week of growth, 20 mM EtOH was applied as foliar for some plants (daily for five days). EtOH concentrations were determined according to the literature (Das et al. 2022). Tween 20 at a concentration of 0.01% was sprayed on the plants that were not treated with EtOH.

On the fifth day after EtOH application, independent of EtOH pre-treatment, some of the plants were placed in a darkened room for seven days under controlled conditions. Treatment groups are shown in Table 1. Regardless of dark application, all plants were harvested on the 7th day of darkness stress. Plant growth parameters (plant height, root length, number of leaves as well as leaf, shoot and root fresh weight /dry weigh), pigment and total phenolic contents were analyzed in harvested plants.

To determine the total chlorophyll and carotenoid contents, 1 g of leaf tissue was homogenized in 50 mL acetone and then centrifuged. The absorbance values of the samples were measured at wavelengths of 662, 645 and 470 nm using a spectrophotometer (De Kok and Graham 1980; Lichtenthaler and Welburn 1983). Total phenolic compound determination was made according to the Folin-Ciocaltaeu method (Singleton et al., 1999).

Groups	Treatments
1	Control
2	Dark
3	20 mM EtOH
4	Dark + 20 mM EtOH

Table 1. Treatment groups

Experiments were repeated three times and statistical analyzes were performed with SPSS software 20.0. The differences between the treatment groups were determined according to the Tukey test ($p < 0.05$).

Figure 1. Pepper seedlings treated with dark and EtOH

RESULTS AND DISCUSSION

A. Morphological parameters

Darkness stress decreased plant height, root length and number of leaves in all treatment groups regardless of EtOH applications. However, plant height, root length and number of leaves were found to be higher in plants treated with darkness stress + 20 mM EtOH compared to plants exposed to dark stress ($p < 0.05$) (Table 2).

Treatments	Plant height	Root lengt	Number of leaves
Control	28,16±0,16 a	25,33±1,20 a	27,67±1,45 a
Darkness	26,50±0,28 a	23,33±0,33 a	20,67±1,20 b
20 mM EtOH	27,00±1,1 a	26,33±0,66 a	26,33±1,45 a
Darkness + 20 mM EtOH	30,16±0,44 a	24,16±0,44 a	23,00±1,15 ab

Table 2. The effects of EtOH and darkness stress treatments on plant height, root lenght and number of leaves. The different lowercase letters are significantly different from each other (P<0.05) among different treatment groups according to Tukey test.

According to our results, the fresh weight of roots, shoots and leaves decreased in plants exposed to darkness stress compared to other treatments. However, the fresh weights of roots, shoots and leaves were higher in plants treated with darkness stress + 20 mM EtOH compared to plants exposed to darkness stress alone ($p < 0.05$) (Table 3).

Treatments	Shoot FW	Root FW	Leaves FW
Control	10,76±0,41a	12,67±0,39 a	15,10±1,08 a
Darkness	8,82±0,77b	11,66±0,37 a	13,64±0,88 b
20 mM EtOH	10,89±1,01a	13,92±0,95 a	15,68±0,61 a
Darkness + 20 mM EtOH	11,82±1,46a	14,09±0,54 a	14,10±0,22 ab

Table 3. Effects of EtOH and darkness stress treatments on FW of root, shoot and leaves. The different lowercase letters are significantly different from each other (P<0.05) among different treatment groups according to Tukey test.

The data presented in Table 4 illustrates the effects of treatments including EtOH and darkness stress on the dry weight of roots, shoots, and leaves. seedlings. Combined application of EtOH and dark stress increased root, shoot and leaf dry weight compared to dark stressed plants alone ($p < 0.05$).

Treatments	Shoot DW	Root DW	Leaves DW
Control	1,25±0,10 a	1,15±0,07 a	1,71±0,10 a
Darkness	0,96±0,05 b	0,97±0,11 b	1,51±0,12 b
20 mM EtOH	1,22±0,11 a	1,08±0,03 ab	1,82±0,07 a
Darkness + 20 mM EtOH	1,21±0,11 a	1,13±0,03 a	1,61±0,10 ab

Table 4. Effects of EtOH and darkness stress treatments on DW of root, shoot and leaves. The different lowercase letters are significantly different from each other ($P<0.05$) among different treatment groups according to Tukey test.

B. Physiological parameters

The lowest Chl a content was observed in 20 mM EtOH application, while the lowest Chl b content was measured in darkness stress applied plants. The carotenoid concentration was found to be lowest in plants exposed to darkness stress. However, a substantial increase in carotenoid content was detected when plants were exposed to a combination of 20 mM EtOH and darkness stress ($p < 0.05$). (Tables 5 and 6).

Treatments	Chl a	Chl b	Chl a/b
Control	12,12±0,05 a	1,96±0,008 b	6,16±0,05 a
Darkness	12,01±0,03 a	1,98±0,006 b	6,05±0,03 a
20 mM EtOH	10,62±0,46 b	2,25±0,009 a	4,75±0,04 b
Darkness + 20 mM EtOH	11,51±0,04 b	2,09±0,007 a	5,45±0,04 ab

Table 5. Effects of EtOH and darkness stress treatments on Chl a and b contents and ratio of Chl a/b in pepper The different lowercase letters are significantly different from each other ($P<0.05$) among different treatment groups according to Tukey test.

The change in the amount of total phenolic compounds in the control and application groups is given in Table 6. Darkness stress reduced the total phenolic content compared to control group. EtOH pre-treatment increased the total phenolic content of plants exposed to darkness stress.

Treatments	Total Chl	Carotenoid	Total Phenolic
Control	14,09±0,04 a	6,30±0,09c	14,24±0,06ab
Darkness	14,00±0,02 a	5,43±0,07d	13,70±0,05c
20 mM EtOH	12,88±0,37 b	6,75±0,04b	14,61±0,10a
Darkness + 20 mM EtOH	13,60±0,03 ab	7,78±0,05a	14,01±0,11bc

Table 6. Effects of EtOH and darkness stress on contents of Total Chl, Carotenoid and Total Phenolic in pepper The different lowercase letters are significantly different from each other ($P<0.05$) among different treatment groups according to Tukey test.

CONCLUSIONS

Darkness stress causes changes in the physiological and morphological structures of plants. Light has a direct effect on numerous chemical and physiological processes. The length of the plant's shoot and roots, the number of leaves, the plant's fresh and dried weights, and the total chlorophyll, carotenoid, and total phenolic substances decreased in plants exposed to darkness stress. The use of EtOH as a pre-treatment mitigated the adverse impacts of darkness stress on plant.

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ASSESSING THE TRANSFORMATIVE IMPACT OF NUCLEUS ALBANIA'S INNOVATIVE APPROACH IN THE AGRICULTURAL SECTOR A SURVEY-BASED ANALYSIS

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ABSTRACT

This paper aims to assess the impact of Nucleus Albania's innovative approach in the agricultural sector. Nucleus Albania, a not-for-profit organization at the forefront of innovation, provides collaborative platforms and introduces the nuclei approach to support the growth of agricultural businesses and foster sustainability in the sector. A mixed-methods research design is employed for an accurate assessment, primarily relying on survey data collected through a structured questionnaire administered to participants across all nuclei (all participants have been part of the survey for two consecutive years). Literature review, secondary data analysis and the data collected from participants within the nuclei provide a comprehensive overview and information to assess the impact of the NA innovative approach on agricultural business development, production, diversified product supply, access to finance, investments, employment, and overall business performance. Findings suggest an overall positive impact of the NA's innovative approach in various aspects of the agricultural businesses. By harnessing the power of collaboration, businesses experience accelerated growth and heightened productivity, propelling them towards sustainable development. The nucleus approach has proven instrumental in fostering innovation and knowledge-sharing, empowering farmers and agribusiness owners to adopt state-of-the-art practices and advanced techniques, thereby significantly contributing to overall sectoral development. This validation highlights the approach's robustness and effectiveness in addressing the sector's challenges, offering valuable insights for stakeholders, policymakers, and other organizations seeking to foster sustainable growth in agriculture.

Keywords: Albania, sustainable development, agriculture, nucleus approach, innovation, SDG2

INTRODUCTION

Agriculture, the cultivation of plants and the raising of animals for food, fibre, medicinal plants, and other products, has been and remains one of the fundamental activities for human survival and progress throughout history. The importance of this sector extends far beyond simple food production. Agriculture is crucial in providing food, ensuring economic stability, promoting environmental sustainability, and fostering social development worldwide. The United Nations has included agriculture in Sustainable Development Goal No. 2: *"End hunger, achieve food security and improve nutrition, and promote sustainable agriculture"* (UN, 2015).

Agriculture in Albania has a long history and strong tradition. This sector has played and continues to play an essential role in the country's economy and the life of the population, particularly that of rural areas. Agriculture is essential for food production and job creation, particularly in rural areas. Various agricultural products, including other crops such as wheat, corn, beets, olives, vegetables, and medicinal plants, characterize the agricultural sector. Also, Albanian farmers produce dairy products, meat, eggs, and tree products.

The agricultural sector (in this case, we include agriculture, livestock, hunting, fishing, and other activities related to rural development) is of particular importance for a country like Albania. Based on the National Strategy for Development and European Integration 2030 (draft version for public consultation, page 100), the vision for agriculture and rural development is *"to make possible an efficient, innovative and sustainable agro-food sector in Albania, which can better withstand the pressures of the national and international market and respond to the challenges of climate change and sustainable management of natural resources, while contributing to improving the quality of life of the inhabitants of rural areas and further increasing the attractiveness to exercise economic activity that guarantees sufficient income to live there and provide products and services to agricultural and non-agricultural consumers visiting rural areas and beyond rural areas."*

Fulfilling this vision for the sector requires addressing several critical economic, environmental, and social challenges to enable agricultural development, fishing activity and business development, especially in rural areas. Also, it is important to guarantee gender equality in the engagement of young entrepreneurs in the agricultural sector (National Strategy for Development and European Integration). Quantitatively (based on data from the Institute of Statistics), the contribution of agricultural activities to the gross value added (GVA) shows a significant weight. The data show that from 2000-2008, the agricultural sector contributed about 23% of the total GVA created in the economy. In 2009-2018, the weight of this sector in GVA decreased to about 22%, and in 2018-2021, its weight was 21%.

Despite its essential weight and role in the local economy, the sector is dominated by small-sized farms (about 85% of the total and the family type), according to NSDEI 2030. According to the same source, the average size of the farm is 1.2 ha, a factor that has influenced the reduction of the weight of agriculture in GVA (in 2021, the weight was 21%). The small size, combined with the fragmentation and ambiguity of property rights, hinder the growth and competitiveness of the agricultural sector in the region and beyond. However, in the case of Albania, agriculture has an important role in providing food for households and alleviating extreme poverty in rural areas. Notwithstanding, the agricultural sector in Albania encounters various issues and difficulties that impede the growth and endurance of the sector, including:

- Land fragmentation: After the privatization of land in the 90s, many agricultural plots were fragmented into small and inefficient production environments. This has influenced the reduction of productivity and added difficulties in implementing modern technologies in agriculture.
- Lack of investment in agricultural infrastructure: Albania needs more investment in agricultural infrastructure, such as the irrigation network, rural transport, and agricultural trade infrastructure. This limits the possibilities of production and export of agricultural products.
- Lack of modern technology and tools: Albanian agriculture still uses a significant part of traditional technologies and needs modern tools in agricultural production, limiting productivity and efficiency increase in agriculture.
- Dependence on natural factors: Agriculture is exposed to natural factors such as the harshness of the climate, strong wind, irregular rainfall, and climate change. These factors can lead to huge losses of crops and agricultural wealth.
- Lack of financing: Agriculture in Albania needs help accessing financing and loans for investment in production. Financial institutions offer few opportunities for agricultural loans, and the conditions for financing are difficult for most farmers.
- Lack of sustainable agricultural policies: The lack of sustainable agricultural policies and the lack of government support in agriculture affect the agricultural sector's development. Sometimes interruptions in financial support occur, and the lack of long-term planning

creates difficulties for the sustainable future of agriculture (as was the case of the EU-funded project IPARD).

Nucleus Albania is a project which, since 2015, has provided support services for micro and small enterprises in Albania in the agricultural sector, using an innovative approach for Albania. The nuclei approach consists of grouping small enterprises (farmers and livestock keepers) operating in different regions (such as artisans, beekeepers and others) according to the focus of their activity and organizing them into nuclei (e.g. beekeepers nuclei or nuclei of artisans), at the district level.[1] In the nucleus approach, participants receive services and activities such as technical information on new methods of agricultural cultivation and livestock breeding, specific training, and personalized consulting services from qualified international experts (according to the fields covered by the core). These international experts are process facilitators and, together with the nuclei members, aim to improve business activity and the sector where they operate, increasing employment by making them part of the value chain (Nucleus Albania, 2019). This approach is internationally recognized, and its genesis can be found in Latin America in 1991. NA has supported around 1,000 micro, small and medium enterprises by the end of 2019 and continues to support them to date. In general, services are provided in six main sectors: agro-processing, handicrafts, agriculture, livestock, beekeeping, and tourism. Among the results achieved through this approach are listed:

- Establishing networks between members of the same nuclei and between different nuclei.
- Development of successful value chain models and dissemination of this model within the sector and other regions.
- Creating business relations between nuclei and SMEs with group counselling (entrepreneurs help each other), business meetings within the sector and between different regions.
- Transfer of best practices between cores within the region and between regions by exchanging information and participation in national and international fairs.
- Intensive training for various business areas such as marketing, time and human resource management, environmental impact assessment, food safety standards, financial management, agronomic services, value chain, new production methods, experimentation with new materials, etc.

METHODOLOGY[2]

The study employs a mixed methods approach, combining secondary and primary data. The secondary data are drawn from official statistical agencies in Albania and are used to inform agricultural sector developments. The primary data were collected through a structured questionnaire-based survey directed to all participants in the nuclei (the target population). The observed population consists of 529 in 2018 and 1000 members in 2019, distributed among 82 nuclei operating in 9 regions.

The primary data collection technique is two cycles of surveying the target population through a structured questionnaire and direct interviews. In both survey cycles, the same questionnaire was used, including (i) general descriptive questions of the participants in the cores (ii) explanatory questions aimed at evaluating how the members of the nuclei perceived the services and support activities provided. The survey implementation was planned to overlap with the calendar of meetings organized with the nuclei. To facilitate the process, the questionnaire was drafted and used in Albanian language and was administered through direct interviews by Nucleus Albania staff at the beginning of the scheduled meetings. From the content point of view, the questionnaire was structured in 4 different sections, each of them trying to assess specific aspects as follows:

[1] For more information on the approach: www.nucleus.al

[2] The questionnaire and the database are available upon request to the corresponding author.

- Section 1. This section aims to provide some general information on the participating members of Nucleus Albania (gender of the respondent, nucleus group and region, time of participation in the nucleus), how they came into contact and explores the reasons for joining the respective nucleus.
- Section 2. Business Information. This section aims to assess various aspects of doing business over the past 12 months. Most of the questions in this section are symmetrical with three alternatives covering aspects: general business performance, level and typology of investments, expectations regarding investments in the next 12 months and typology, level of employment.
- Section 3. In this section, we assess the impact of the activities and services provided by NA. The participants in the survey assessed the activities and services on a scale from 1 – least important to 3 – very important for each of the activities and services provided by NA. In this section, there are ten questions which aim to assess the impact of NA services regarding the number of customers, products and services, access to raw materials, technology level/production efficiency, access to finance, product portfolio, turnover, number of employees, networking, and collaboration.
- Section 4. Access Nucleus Albania. Since NA has adopted a unique approach to providing its services, it is valuable to explore how members rate it and get some feedback on what they consider to be the most important activities. This section introduced an open-ended question to collect other information not included in the questionnaire.

The questionnaire was coded, and the information was digitized upon completion of the interviewing process. Next, the data file was cleaned of errors and followed by data processing in the SPSS 20 program using (according to the type of question):

- frequencies, the percentage of participants who choose an alternative to the total responses.
- net balances, widely used in surveys for collecting and quantifying information of a qualitative nature. The net balance is calculated as the difference between the percentage frequencies of positive and negative responses, expressed in percentage points
- simple arithmetic mean.

In cases of missing information in any of the questions, the results were scaled to reflect only the valid percentages and the number of participants was reported in each question.

The implementation of the survey through direct interviews is a method which minimizes the problem of reliability of the information obtained. All interviewers were adequately trained on administering the questionnaire, the interviewing process, and the information to be shared with the members of the nuclei subject to interview. The Cronbach Alpha indicator was used to assess the reliability of the information obtained through the questionnaires (Cronbach, 1951). Cronbach's (α) is a widely used indicator to assess the quality of responses of a group of questions with the same structure (having the same rating scale - a symmetrical Likert scale with three alternatives). For survey data, an acceptable range is $0.7 \leq \alpha \leq 0.8$. Lower alpha coefficient values ($\alpha \leq 0.7$) signal low quality of survey responses. In contrast, higher coefficient $\alpha \geq 0.8$ values signal good to excellent reliability of the responses obtained from the survey.

RESULTS AND DISCUSSION

The participation and response rates are satisfactory in both survey cycles. In 2018, the response rate was 81%, and in 2019, it averaged 95% (some of the questionnaires were not 100% complete, Table 1).

Table 1. Response rate

	2018	2019
Total number of members in the nuclei	529	1000
Total number of members interviewed	529	1000
Total number of members completing the questionnaire	430	985
Response rate	81.3%	98.5%

The results of the Cronbach's (α) test suggest a good internal consistency and reliability of the data obtained from the survey in its two cycles (Table 2).

Table 2. Internal consistency test results

	Cronbach's (α)	
	2018	2019
Section 2: P2.1; P2.2; P2.4; P 2.6	0.707	0.701
Section 3: P3.2; P3.3; P3.4; P3.5; P3.6; P3.7; P3.8; P3.9; P3.10	0.958	0.955
Section 4: P4.2; P4.3	0.977	0.955

General information on respondents. In both survey cycles, men predominate among the interviewees, about 79% in 2018 and 75% in 2019 (Figure 1). This may be related to the local tradition in some work typologies, especially those that have intensity relatively high levels of physical work exercised more by men. However, with the expansion of the number of participants in the nuclei, women have gained ground and represented about 25% of the interviewees in 2019.

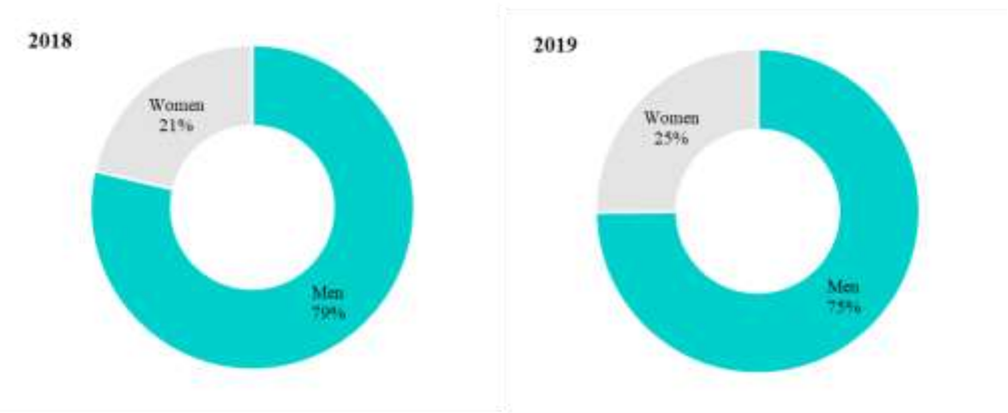

Figure 1. Respondents by gender

Data from more than 80 micronuclei are grouped into 19 primary nuclei. Among them, the nuclei of handicrafts, livestock, olives, and beekeeping have the most significant weight in both survey cycles (Figure 2). These nuclei represent over 50% of the interviewees in both survey cycles.

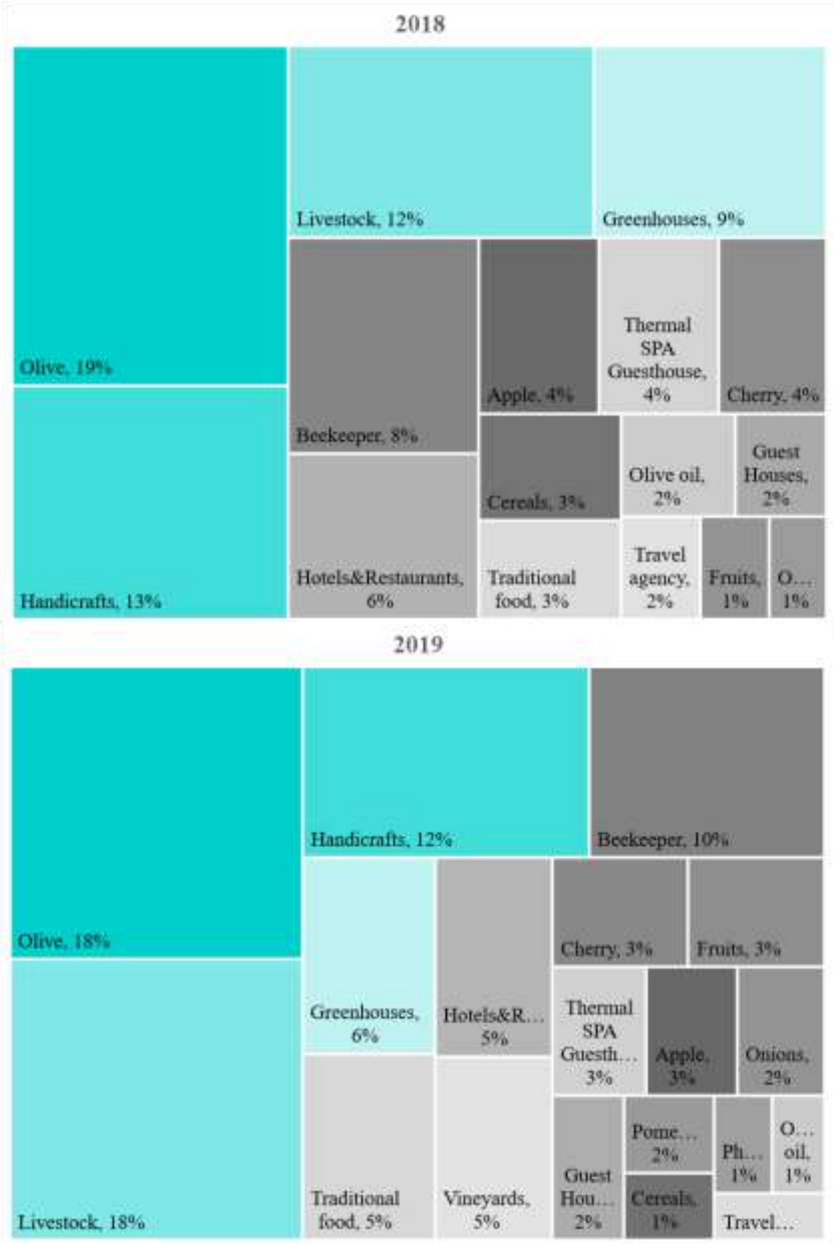

Figure 2. Respondents by nuclei

In terms of territorial coverage, a good distribution of survey participants is observed in both cycles: the highest concentration is observed in the Korça region, in line with the developments and importance of the region in agriculture, livestock and tourist services; furthermore, the interviewees of the nuclei in Shkodër, Elbasan Fier and Berat counties have a lower weight (Figure 3).

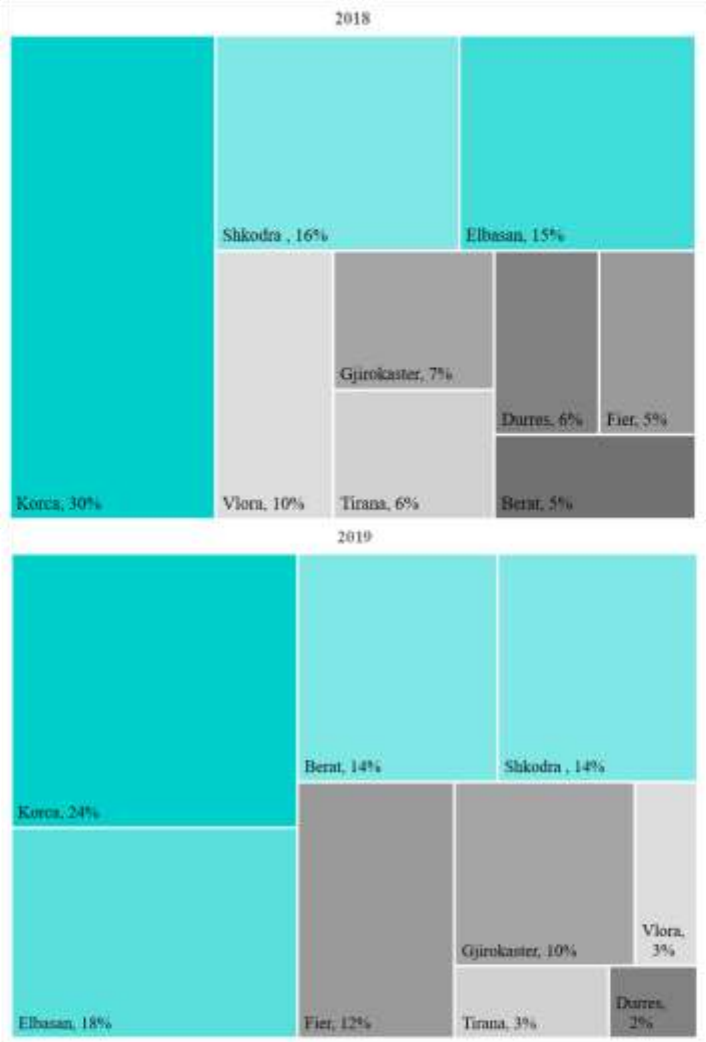

Figure 3. Respondents by regions

In general, the respondents affirm that they have become aware of the services provided through the core approach by the counsellors (affirmed by 86% of the interviewees in 2018 and 91% in 2019). Advertisements and recommendations from partners or colleagues occupy a low percentage. Access through chambers of commerce could be higher, suggesting a lack of orientation regarding development opportunities (Figure 4).

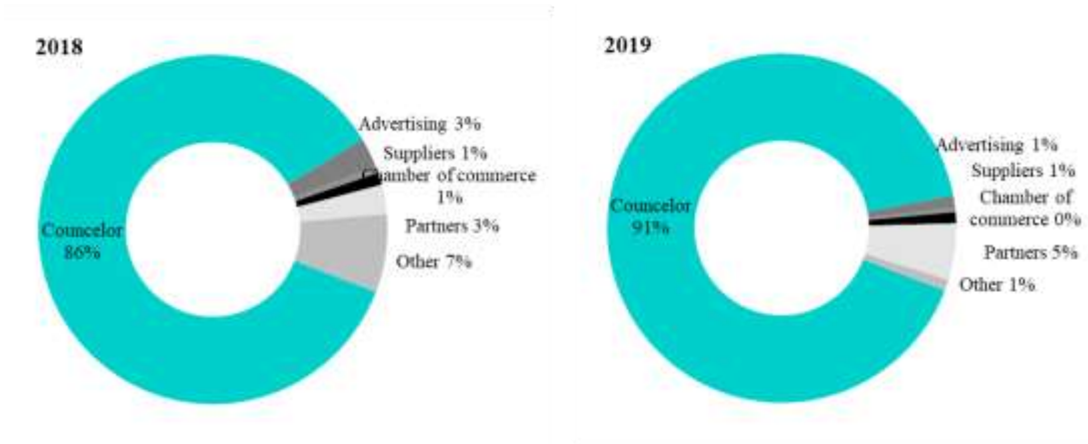

Figure 4. How did the member approach the nucleus

For about 49% in 2018 and about 44% in 2019, the respondents listed networking and sharing experiences as the main reasons for joining the nucleus. In addition to this reason, the curiosity to get to know the approach, as well as the expectation to access financial support (in the form of loans or subsidies), are also among the reasons listed by 32% and 21% of the interviewees, respectively, at the end of the year 2019 (Figure 5).

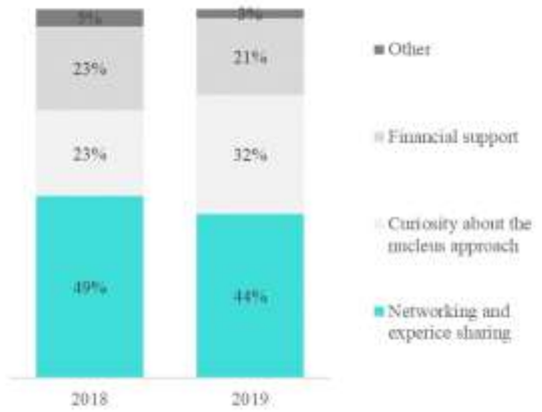

Figure 5. Reasons for joining Nucleus Albania

General information on the businesses. The overall business situation has improved for about 67% of respondents in 2018 and 74% in 2019. Less than 10% of respondents claim a worsening of the situation in both survey cycles (Figure 6)

Figure 6. Overall business situation

Regarding employment level, in both survey cycles, over 50% of the interviewees affirm that it has stayed the same in the last year. Meanwhile, about 45% of respondents in 2018 and about 43% in 2019 affirm an increase in employment during the last year (Figure 7).

Figure 7. Employment level

The improved business situation is reflected in a higher level of investment, affirmed in both survey cycles by 67% of respondents in 2018 and 56% of respondents in 2019 (Figure 8). These investments have mainly concentrated on increases in production capacity (confirmed by 60% of interviewees in 2019) and technological improvements (about 28% of interviewees in 2019). Investment in marketing is affirmed by about 11% of respondents in the 2019 survey cycle.

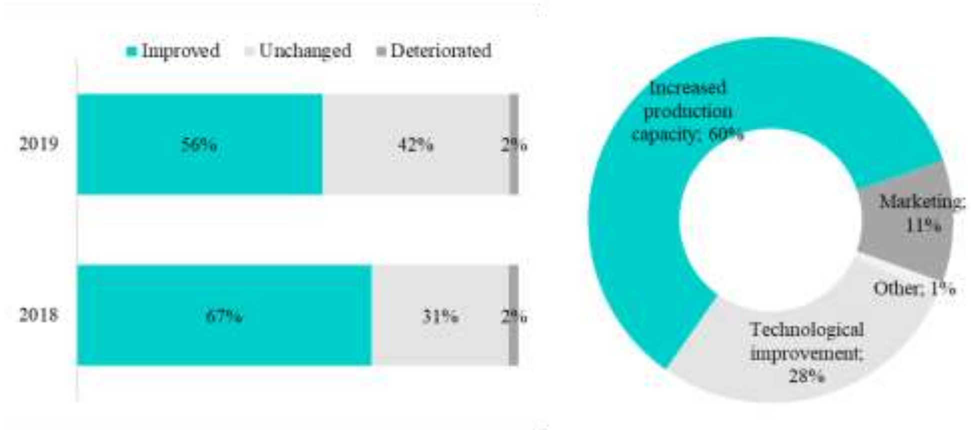

Figure 8. Investment level and typology

Expectations one year ahead for investment levels are optimistic. About expectations one year ahead for investment levels are optimistic. About 81% of the respondents in 2018 and about 69% in 2019 expect to increase investments in the upcoming year (Figure 9). These investments concern the increase of production capacities (about 57% of respondents) and technological improvements (about 26% of respondents)

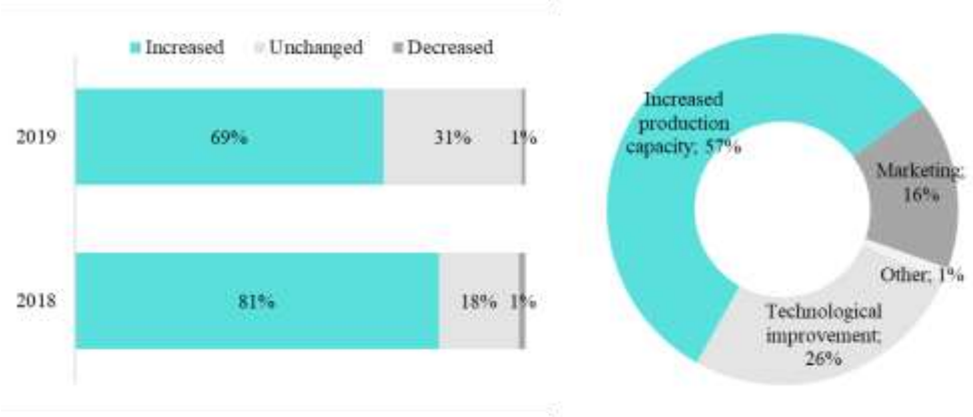

Figure 9. Expectations on investment level and typology

Support from Nucleus Albania. he Nucleus Albania approach embedded a series of services and activities, including consulting, training, technical assistance, study visits or visits to businesses that perform similar activities, participation in fairs, and information on opportunities to access grants. The respondents expressed their assessment on a scale from 1 - least important to 3 - very important on the activities developed during the support given to participating in the nuclei. Based on the respondents' statements in both survey cycles, the highest rating of about 2.9 was for the specific training provided according to the area covered by the nuclei (Table 3). In addition to training, the interviewees evaluate advisory activities, technical assistance, study visits and participation in fairs as very important (average rating of 2.8 for the activities cited).

Table 3. Average assessment of activities and services provided by NA

Activities	2018	2019
Consulting	2.8	2.8
Trainings	2.9	2.9
Technical Assistance	2.8	2.8
Study visits	2.8	2.8
Benchmarking visits	2.7	2.6
B2B meetings	2.7	2.6
Fairs	2.8	2.8
Information sessions for funding opportunities	2.8	2.7
Other	2.6	2.7

In general, participation in the nuclei has positively influenced various aspects of the business, affirmed by the interviewees in both survey cycles, measured by the net balances (almost all positive, Figure 10, 11 and 12). The respondents affirm to have improved the quality of the products and services offered (the net balance marked a value of +69 percentage points in 2019), which may have been determined by the improvement of access to the raw materials used in production (the net balance +46 points percentage in the 2019 survey). The respondents also affirm that they have increased the number of customers for their products and services (net balance +63 in the 2019 survey).

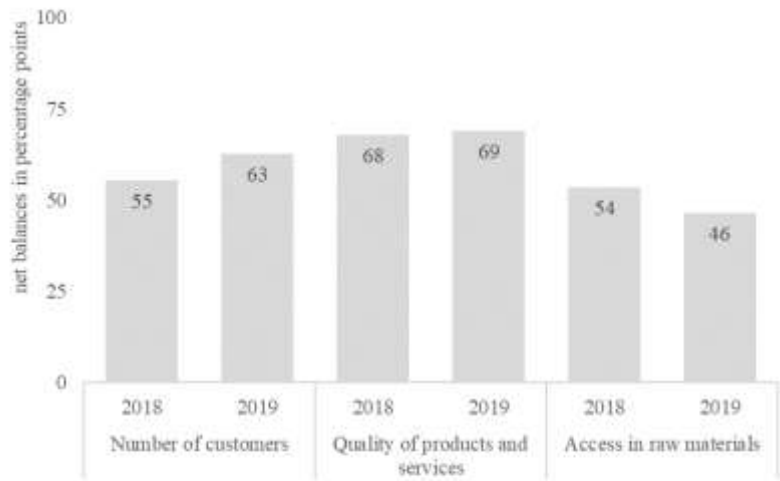

Figure 10. Changes in business activity since participation in NA (1)

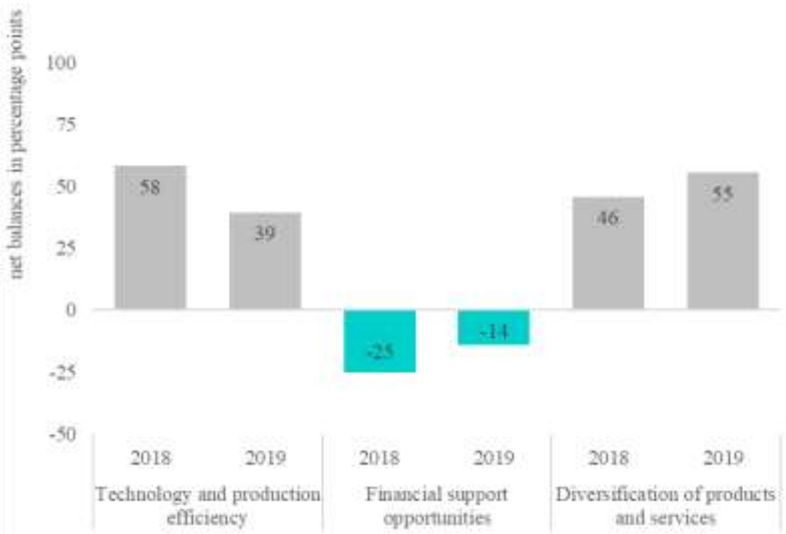

Figure 11. Changes in business activity since participation in NA (2)

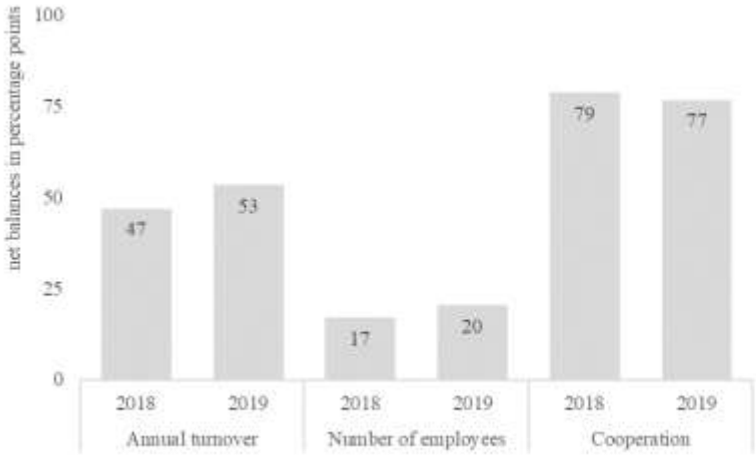

Figure 12. Changes in business activity since participation in NA (3)

The respondents affirm that with the participation in the nuclei and thanks to the activities made available, the variety of products and services offered has increased, the portfolio of products and services has increased (the net balance is positive, and, in the survey of 2019, it marked the value of +55 percentage points) and positive changes in the adopted technology and production efficiency (+39 percentage points in the 2019 survey). In contrast, the respondents in both survey cycles claim that participation in the nuclei has not impacted finding additional opportunities for financial support (access to grants or financing schemes, Figure 11). About this aspect, the net balance shows negative values, and, in the survey of 2019, it recorded a level of about -14 percentage points. Positive developments in the number of customers, technological improvement and increased efficiency are assessed to have been reflected in the increase in the annual turnover (turnover) of the businesses included in the nuclei (the net balance in the 2019 review marked a positive value of +53 percentage points). Similarly, the respondents affirm an increased number of employees in their business (the net balance in the 2019 survey marked a positive value of 20%).

Cooperation and collaboration are aspects of particular importance in business. The respondents affirm improved cooperation with nuclei members (the net balance in the 2019 survey is +77 percentage points). Cooperation with nuclei members improved access to information, finding markets and better access to raw material and/or equipment (Figure 13).

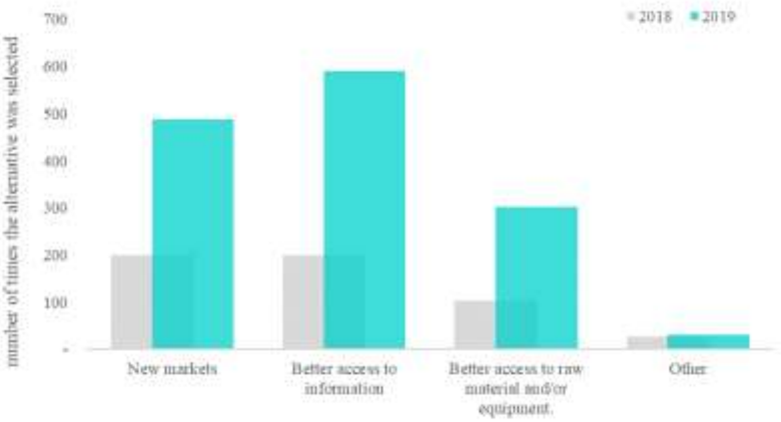

Figure 13. The effects of improved cooperation

In general, the participation in the nuclei has been widely evaluated positively by the respondents (reflected in the positive values of the net balances). The interaction between the participants in the nuclei is perceived as a driving element of cooperation by facilitating access to information, markets, and raw materials. However, access to financing of various categories (grant schemes or other financing opportunities) needs to be improved, significantly limiting the potential for growth of businesses participating in the survey.

The methodology and nuclei participation. The nuclei approach is innovative in the case of Albania. All services offered were open to all members free of charge and without remuneration. In this regard, the respondents affirm to have participated in almost all the activities and services offered throughout the project and in the advisory meetings (the alternative chosen about 958 times in the survey of 2019), followed by training (chosen 764 times) and direct technical assistance (chosen 618 times) (Figure 14).

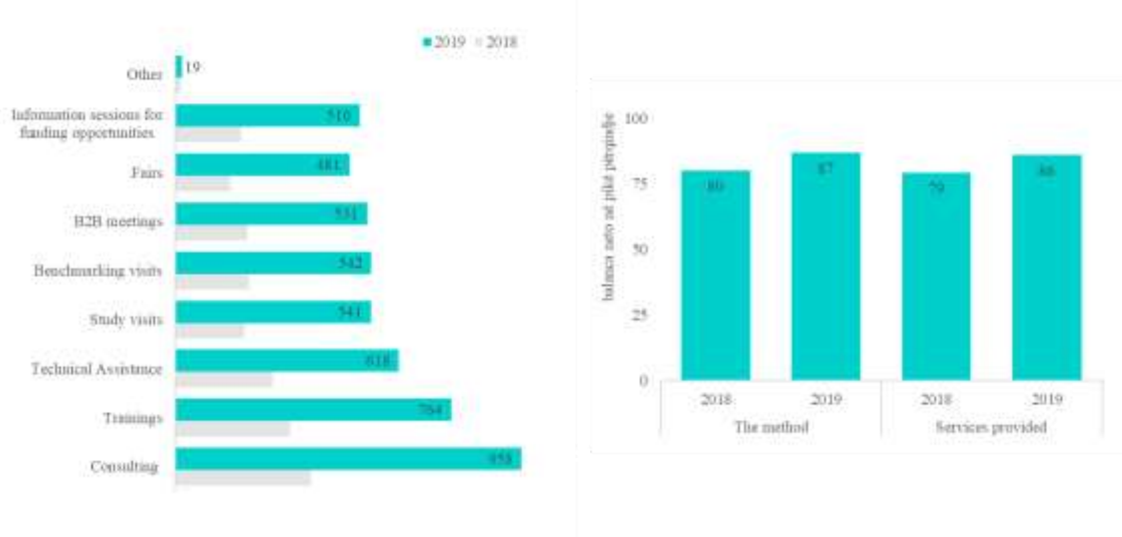

Figure 14. Participation in activities and services provided (left) and the assessment of the method and services provided (right)

CONCLUSIONS

Participation in the Nucleus Albania and access to the nuclei approach increased awareness and attention to the business in the agricultural sector (and other sectors closely linked to it), contributing to its promotion and increased sales. Cooperation and collaboration with other members of the nuclei have built a communication channel for exchanging significant information and experiences, identifying new markets, and providing better access to raw materials. Overall, the nuclei are designed to support participants and provide an engaging and engaging experience for them. If these experiences are successful and positive, they can influence the perception and improvement of business activity.

Direct feedback, evaluation, and data analysis collected with questionnaires show that participation in the nuclei was positively perceived. There is an improvement in the quality of products and services offered and an increase in production capacity. In addition, there are improvements in the number of customers, employment growth, technological progress, increased efficiency, and annual turnover of the businesses involved in the nuclei.

The activities and services provided are assessed to be appropriate to achieve the expected results. The organization of activities and the provision of services must match the interests and expectations of the participants and ensure added value at the end of the project and beyond. The participants have assessed the training and consulting activities as very useful for their businesses, indicating a strong need for these activities that have yet to be carried out. Based on the available data, further investment and innovation are necessary to ensure sustainable growth and development in the agricultural sector in Albania. This is where Nucleus Albania's innovative approach comes into play, providing collaborative platforms and introducing the nuclei approach to support the growth of agricultural businesses and foster sustainability in the sector. The mixed-methods research design employed in this paper provides a comprehensive assessment of the impact of the NA innovative approach on various aspects of agricultural businesses, including production, diversified product supply, access to finance, investments, employment, and overall business performance. The findings suggest that the NA's approach positively impacts these aspects, highlighting the approach's robustness and effectiveness in addressing the sector's challenges and offering valuable insights for stakeholders, policymakers, and other organizations seeking to foster sustainable growth in agriculture. Based on the findings some recommendations are listed as follows:

The innovative nucleus approach to the agricultural sector has the potential to positively impact the agricultural sector and bring about significant changes in the way the sector operates.

Encouraging innovation and technology in agriculture is a must in the present day. The public authorities and relevant organizations should provide incentives and financial assistance for developing new agricultural technologies, such as smart devices, sensors, automatic planting and fertilization systems, and information and communication technology. Improving farmers' access to the latest technologies will allow them to improve agricultural products' efficiency, productivity, and quality.

Promotion of partnerships and cooperation between participants within and between the nuclei. This cooperation and partnership should be extended to include universities, scientific institutions, and businesses in the field of technology, and farmers can bring exchanges of knowledge and experiences, creating suitable conditions for developing and implementing innovative technologies in agriculture. Also, joint investments in technological research and development can encourage the transfer of technology from the agricultural sector to other sectors of the economy.

Diversification of agricultural products and activities is necessary to integrate into value chains (internationally in particular). Farmers can use new technologies and practices to develop the production of alternative products, such as fruit trees, decorative trees, organic products, medicinal plants, and agrotourism services. This diversification of agricultural activities will create added value, reduce dependence on one type of production, and bring competitive advantages to farmers in the domestic and international markets

Establishing and piloting the nucleus approach in the agricultural sector is an excellent opportunity to modernize and transform the agricultural sector and create synergies with other sectors of the economy. Encouraging innovation, using advanced technology, handling and analysing data, promoting partnerships and diversifying agricultural activities are just some recommendations to achieve this innovative approach. The participants positively assessed the nucleus approach to agriculture, and its replication in other sectors of the economy in Albania and beyond would positively contribute to improving practices in the agricultural sector and long-term sustainable development.

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BIOFORTIFICATION IN WHEAT: ENHANCING CLIMATE RESILIENCE AND NUTRITION SECURITY

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ABSTRACT

Biofortification in wheat presents a dual solution to address the challenges of climate change and malnutrition. This review paper comprehensively assesses the potential of biofortified wheat varieties in enhancing climate resilience and improving nutrition security. By targeting essential micronutrients such as iron, zinc, and vitamin A, biofortified wheat offers a sustainable strategy to combat micronutrient deficiencies. Integrating biofortified wheat into agricultural systems has shown promising outcomes in improving health and reducing nutritional deficiencies. Furthermore, biofortified wheat exhibits enhanced adaptability to abiotic stresses associated with climate change, such as drought and heat, contributing to climate resilience. Continued research and investment are crucial to exploit the potential of biofortification in wheat. Future research efforts should prioritize the improvement of biofortification techniques, the development of high-yielding and climate-resilient biofortified wheat varieties, and the assessment of their long-term sustainability. Interdisciplinary collaborations involving researchers, breeders, policymakers, and stakeholders are essential for advancing biofortification initiatives and implementing evidence-based policies that promote the adoption and dissemination of biofortified wheat. Additionally, sustained policy support and investment are vital to create an enabling environment for widescale adoption, ensuring accessibility to nutrient-rich food for vulnerable populations.
Keywords: Biofortification, Wheat, Climate resilience, Nutrition security, Micronutrient deficiencies

INTRODUCTION

Biofortification, an agricultural intervention that has garnered significant attention in recent years, has been recognized for its potential to address the pressing issues of global nutrition security and climate change (Bohra et al., 2015; Nelson et al., 2018). With the continuous growth of the global population and rising concerns about food security and the impact of climate change on agricultural systems, there is an urgent need for innovative strategies to enhance the nutritional value and resilience of staple crops. Wheat, a widely cultivated and consumed cereal grain, is vital in providing essential nutrients to vulnerable populations, particularly in regions where it serves as a primary dietary staple (Arzani & Ashraf, 2017). However, inadequate nutritional levels, such as iron, zinc, and vitamin A, are noteworthy, negatively affecting a considerable population's well-being and general state. In the given environment, the notion of biofortification in wheat emerges as a promising approach to enhance this crucial crop's nutritional profile and resilience in the face of climate change.

The importance of wheat as a fundamental crop cannot be exaggerated. It is a significant energy provider and vital nutrient, encompassing protein, dietary fibre, vitamins, and minerals. Ensuring access to nutritious wheat is of utmost importance in low- and middle-income countries, especially those where wheat-based diets are commonly consumed since it plays a vital role in addressing the issue of malnutrition. Nevertheless, the issue of climate change presents notable obstacles to wheat cultivation, including alterations in precipitation patterns, heightened temperature fluctuations, and the proliferation of pests and diseases (Juroszek & von Tiedemann, 2013; Miraglia et al., 2009; Shahzad et al., 2021). These several causes can potentially compromise agricultural productivity, intensify nutrient insufficiencies, and pose a significant risk to the overall stability of the global food supply. Biofortification presents a climate-smart strategy through the augmentation of the nutritional profile of wheat cultivars, effectively tackling the dual challenge of boosting nutrition and promoting agricultural methods adaptable to climate change (Ebbisa, 2022; Hossain et al., 2021).

Biofortification is a strategy that seeks to enhance the nutritious content of wheat grains through genetic modification. Its primary objective is to enhance the availability and accessibility of vital vitamins and minerals to populations particularly susceptible to micronutrient shortages. In addition, biofortified wheat cultivars have characteristics that facilitate their ability to acclimate to shifting climatic circumstances and surmount related obstacles. This review seeks to comprehensively understand the significance, challenges, and prospects of biofortification in wheat by analysing relevant scientific studies, field trials, and successful implementation cases. The ultimate goal is to contribute to global nutrition security and developing climate-resilient food systems.

Biofortification Strategies in Wheat

Biofortification in wheat comprises a range of approaches that seek to augment the crop's nutritional composition, aiming to mitigate specific widespread nutrient deficits among susceptible populations (Gupta et al., 2020; Yashveer et al., 2015). Conventional breeding and genetic engineering are two critical methodologies utilized in biofortification. Conventional breeding entails deliberately selecting and interbreeding wheat varieties with inherently greater nutritional levels (Vasil, 2007). In contrast, genetic engineering employs sophisticated biotechnological methodologies to insert targeted genes responsible for nutrient accumulation (Sreenikethanam et al., 2022). Implementing these measures has demonstrated potential in augmenting the concentrations of vital micronutrients, including iron, zinc, and vitamin A, within wheat grains. This, in turn, can enhance the nutritional status and promote favorable health outcomes.

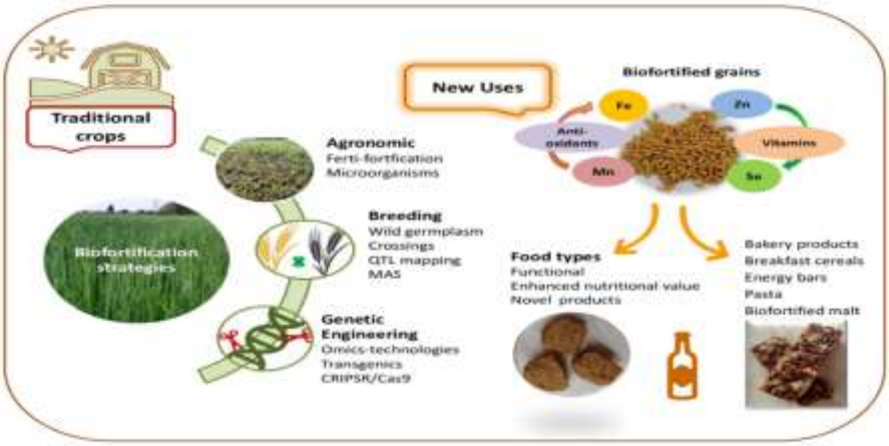

Figure 1. Biofortification strategies in wheat (Sakellariou & Mylona, 2020)

Iron, zinc, and vitamin A have been identified as crucial nutrients for biofortification in wheat, primarily due to their pivotal functions in correcting shortages of micronutrients and the subsequent health problems they cause (Ortiz-Monasterio et al., 2007; Sharma et al., 2021; S. H. Wani et al., 2022). Iron deficiency anemia is a prevalent condition that substantially impacts a considerable proportion of the worldwide populace. This condition is associated with compromised cognitive development and heightened vulnerability to infections. Biofortified wheat types enhanced with iron provide a viable and inclusive approach to addressing the nutritional dilemma (Garcia-Casal et al., 2017; Rao & Annadana, 2017). Likewise, the insufficiency of zinc has significant ramifications on the growth processes, immune system functioning, and the developmental trajectory of children (Monk et al., 2013; A. L. Wani et al., 2017; Younas et al., 2023). Biofortification significantly mitigates zinc deficiency and its associated negative consequences by augmenting the zinc content within wheat grains. Moreover, the insufficiency of vitamin A leads to visual impairments. It compromises the immune system, highlighting the significance of biofortified wheat as a crucial instrument in addressing this prevalent shortage (Bachewe et al., 2023; Dhaliwal et al., 2022).

The efficacy of biofortified wheat in augmenting the crop's nutritional composition and climate adaptability has been substantiated via several research investigations and practical experiments. Multiple studies have demonstrated that biofortified wheat cultivars display elevated concentrations of specific nutrients compared to their non-biofortified counterparts (Dwivedi et al., 2023; Govender et al., 2019; Huey et al., 2022; Hummel et al., 2020). Numerous studies have documented significant enhancements in the iron and zinc levels found in biofortified wheat grains, effectively mitigating nutrient deficits among susceptible populations (Praharaj et al., 2021; Stangoulis & Knez, 2022). Moreover, the biofortified cultivars have exhibited robustness in the face of climate-induced pressures, such as water scarcity and elevated temperatures (M. S. Farooq et al., 2022; Nguyen et al., 2023) . The organisms in question have demonstrated an increased capacity to withstand non-living environmental pressures, leading to a more consistent crop output and fewer losses after harvest. This eventually aids in ensuring food availability and the ability to adapt to changing climatic conditions.

Enhancing Climate Resilience through Biofortification

The utilization of biofortified wheat exhibits significant promise in augmenting the climatic resilience of agricultural systems. The climate change phenomenon presents notable obstacles to wheat cultivation, encompassing elevated temperatures, alterations in precipitation patterns, and the incidence of extreme meteorological occurrences (Motha & Baier, 2005; Thornton et al., 2014). Biofortified wheat cultivars have been genetically engineered to exhibit enhanced tolerance towards abiotic stresses, facilitating their successful growth and development in adverse ecological conditions (Ghorai et al., 2022; Kumar et al., 2020). Biofortification is a method that can enhance the adaptive ability of wheat crops, resulting in increased resilience to adverse environmental conditions such as drought, heat, and salinity. This adaptive characteristic guarantees the long-term viability of crop yields and plays a significant role in mitigating the effects of climate change on agricultural systems.

Table 1. Effect of biofortification with zinc on the adaptation of wheat under stress conditions.

Reference	Fertilizer /stress	Type of fertilizer /trial	Highlighted findings
M. Farooq et al., (2020)	Zn + biochar /Cd stress	Soil/pot trial	Adding charcoal to seeds with high intrinsic zinc levels increased superoxide dismutase and peroxidase activity, proline content, and grain zinc concentration. Conversely, the amount of cadmium dropped.
Adrees et al., (2021)	ZnONPs /Cd + water deficient	Foliar/pot trial	Utilizing a concentration of 100 mg dm^{-3} of ZnONPs yielded the most significant outcomes. The application of NPs resulted in a decrease in electrolyte leakage, a rise in chlorophyll a and b levels in the leaves, and an enhancement of superoxide dismutase and peroxidase activities. Additionally, they achieved a reduction of 81% in the levels of Cd in grain. The drought did not have an impact on the zinc content in cereals.
Rizwan et al., (2019)	ZnONPs FeONPs/Cd	Seed soaking /pot trial	The best results came from giving the most ZnNPs and FeNPs. Under Cd stress, priming seeds with NPs changes plant height, shoot and husk dry weight, chlorophyll and carotenoid content, and Zn/Fe concentration in grain by increasing superoxide dismutase and peroxidase activities.
(Faran et al., (2019)	Zn /Drought stress	Seed soaking /pot trial	The best results came from giving the most zinc (49 mg kg^{-1} seed). Seed seeding with zinc changed how antioxidant activity, plant

			height, wheat grain weight, chlorophyll and carotenoid levels, and leaf area changed after a week of drought stress.
Faran et al., (2019)	Zn /Waterlogging stress	Seed soaking /pot trial	The optimal Zn dosage was found to be 49 mg kg^{1} seed. Seed priming with Zn increased antioxidant activity, plant height, wheat grain weight, chlorophyll and carotenoid contents, and Zn concentration in seeds after a week of waterlogging stress.
Faran et al., 2019)	Zn /Salinity stress	Seed soaking /pot trial	The most excellent Zn dose (49 mg kg^{1} seed) produced the best results. Wheat grain weight, chlorophyll and carotenoid levels, biological yield, and harvest index were all impacted by Zn priming before being subjected to 10 dS m^{-1} salinity stress.

Wheat's adaptation capacity to abiotic challenges related to climate change is greatly aided by biofortification. For instance, drought is a severe problem in many areas where wheat is grown. Biofortified wheat types are more resilient to drought, so their productivity and quality are not compromised even when resources are scarce (Grote et al., 2021; Yashveer et al., 2015). Heat stress also stunts wheat's development and productivity, reducing harvests. Biofortified wheat types are more productive in high-temperature conditions because of their enhanced heat tolerance (Gupta et al., 2020). In addition, salinity is a significant issue in watered areas. Wheat types that have been biofortified have greater salt tolerance, allowing them to grow well in salty soils without compromising harvest yields (Gilliham et al., 2017; J. Singh et al., 2022).

In the face of climate change's effects on food security, biofortification is especially important for bolstering the nutritional status of vulnerable communities. Malnutrition and micronutrient deficiencies can be exacerbated by climate change because of its potential impact on agricultural output and the availability of healthy foods (Nelson et al., 2018; Thompson et al., 2010). Wheat that has been biofortified to include critical micronutrients like iron, zinc, and vitamin A is a long-term answer to these nutritional problems. Incorporating biofortified wheat into meals helps vulnerable groups meet their nutritional needs and improve their health and well-being.

Significance of Biofortified Wheat in Addressing Micronutrient Deficiencies

Millions of people worldwide, especially in poor and middle-income nations, suffer from micronutrient deficiencies, making biofortified wheat an important tool in the fight against this public health crisis. The effects of micronutrient deficiencies, such as those of iron, zinc, and vitamin A, can be devastating for physical and mental health. Since wheat is a staple crop consumed by many people, especially in areas with an everyday wheat-based diet, biofortified wheat provides a sustainable and easily accessible approach to tackling these deficiencies. Biofortified wheat increases specific micronutrients, improving health and food security (Bhardwaj et al., 2022; de Valença et al., 2017; Dhaliwal et al., 2022).

Table 2. List of biofortified wheat varieties for various micronutrients

Variety	Biofortified	Year of Release	Institute	References
HI 8627 (Malav Kirti)	Carotene	2005	Indian Agricultural Research Institute (IARI), India	IARI, (2019)
HD 2932 (Pusa Wheat 111)	Zn	2007	IARI, India	IARI, (2019)
BHU 1, Akshai (BHU3), BHU 5, BHU 6, BHU 17,	Zn	2014	CIAT, CIMMYT, Harvest Plus	HarvestPlus, (2017; Velu et al., (2015)
Abhay (Zinc Shakthi)	Zn	2015	Nirmal seeds and HarvestPlus	(Velu et al., 2015, 2018)
Zincol	Zn	2015	CIMMYT/National Agricultural (2017) Research Center, Pakistan	(R. Singh et al., (2017)
NABIMG-9, ABIMG-10, NABIMG-11	Anthocyanin	2016	National Agri-Food Biotechnology Institute, India	Garg et al., (2016)
Zinc Shakti (Chitra)	Zn	2016	Harvest Plus	R. Singh et al., (2017)
HPBW-01 (PBW 1 Zn)	Fe and Zn	2017	Punjab Agricultural University, India	R. Singh et al., (2017)
WB02	Fe and Zn	2017	Indian Institute of Wheat and Barley Research, India	R. Singh et al., (2017)
BARI Gom 33	Zn	2017	Bangladesh Agricultural Research Institute (BARI) collaborated with CIMMYT	Mottaleb et al., (2019)

Biofortified wheat has been shown to benefit health, particularly in addressing micronutrient shortages and promoting general well-being. For instance, iron deficiency anemia affects a significant portion of the world's population, especially females and young children. Consumption of biofortified wheat, which has a higher iron content, has been demonstrated to help reduce the prevalence of anemia and enhance iron status in at-risk groups (Okwuonu et al., 2021; Siwela et al., 2020). Zinc deficiency, like iron shortage, negatively affects development, growth, and the immune system. Biofortified wheat cultivars treated with zinc are an excellent way to combat zinc deficiency and support normal development and growth. Biofortified wheat, supplemented with vitamin A, has been demonstrated to boost health outcomes, particularly regarding eye and immune system health.

The efficiency of interventions in reducing the prevalence of micronutrient deficiencies is a crucial factor. Compared to other therapies like supplementation and fortification, biofortification has emerged as a more cost-effective technique. A long-term, sustainable strategy for addressing micronutrient deficiencies is incorporating biofortification into agricultural systems. Wheat and other biofortified crops can be distributed through current food systems, eliminating the requirement for new infrastructure. Biofortification also lessens the need for supplementary measures implemented by other parties. Biofortification is an appealing technique for increasing nutrition security and addressing micronutrient deficiencies because of its low cost and high potential for widespread adoption and impact.

Challenges and Barriers to the Adoption of Biofortification in Wheat Production Systems

Several obstacles must be overcome before biofortification programs can be successfully adopted and implemented in wheat production systems. Establishing appropriate regulatory frameworks to guarantee the safety and effectiveness of biofortified wheat varieties is a significant obstacle. Adopting biofortified crops requires establishing regulatory systems and norms for assessing the crops' agronomic performance, nutritional quality, and potential environmental implications. Public opinion also plays an important role. To win public backing and increase consumption of biofortified wheat, it is crucial to dispel myths and address concerns about genetically modified organisms (GMOs) and other perceived hazards linked with the process. Trust and acceptance can only grow via active public participation and open discourse.

The lack of readily available high-quality biofortified wheat seeds is another major obstacle to the widespread implementation of biofortification initiatives. For this innovation to be adopted by farmers, biofortified wheat varieties must be available that are both high-yielding and genetically varied. Cooperative efforts are required to construct seed systems and guarantee biofortified wheat seeds' production, multiplication, and distribution. To maintain optimal availability and accessibility of biofortified seeds, collaboration among breeders, seed producers, and farmers is crucial. In addition, there are logistical hurdles to overcome while expanding biofortification initiatives. For biofortified wheat initiatives to be widely adopted and implemented, it is crucial that plans be developed for mass production and distribution, and that prices remain low.

Policy Support for the Adoption of Biofortified Wheat Varieties

Policy assistance for adopting and distributing biofortified wheat cultivars is essential to increase food and nutrition security and climate resilience. The successful incorporation of biofortification into wheat production systems mainly depends on the policies and regulations put in place by governments and legislators. Research, development, production, and diffusion of biofortified wheat are all helped by the creation of encouraging laws, regulations, and incentives. Seed systems, agronomic techniques, market access, and consumer education are the only facets that should be considered while formulating policy frameworks. To increase nutrition and agricultural sustainability, policymakers should work with research institutions, breeders, farmers, and other stakeholders to develop evidence-based policies that encourage the uptake and utilization of biofortified wheat (Foley et al., 2021) .

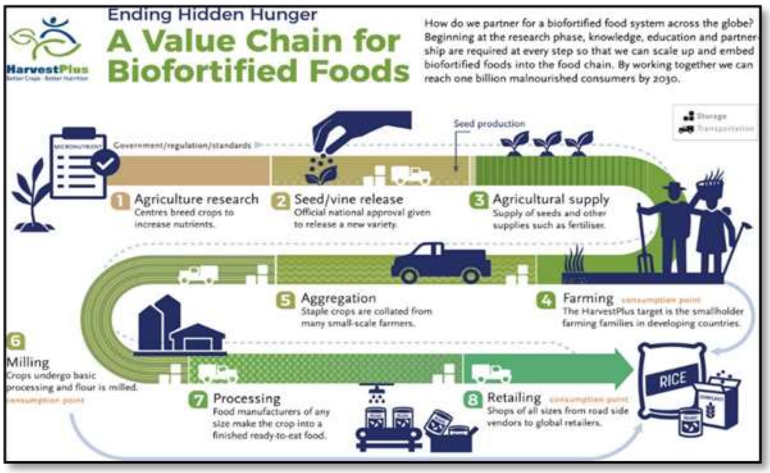

Figure 2. A value chain for biofortified foods (Mitra-Ganguli et al., 2022)

Biofortification methods and breeding high-yield biofortified wheat varieties require continued investment in research and development (R&D). Research and development funds should be consistently allocated to enhance breeding practices, boost the nutrient density of biofortified wheat, and assess their long-term viability. Biofortification techniques require the combined efforts of experts in agronomy, plant breeding, nutrition, and food science. Long-term field trials and monitoring programs are required to evaluate the agronomic performance, nutritional impact, and consumer acceptance of biofortified wheat cultivars. For the long-term success of biofortified wheat, it is crucial to conduct rigorous evaluations of biofortification projects to guide future breeding efforts.

Several suggestions for further study and action are made to help spread the use of biofortified wheat. First, interdisciplinary partnerships among fields such as agriculture, nutrition, health, and policy are required to effectively handle the many difficulties connected with biofortification. By working together, we can share information, strengthen our abilities, and create more thorough plans for expanding biofortification initiatives. Second, it's essential to build initiatives that educate researchers, breeders, extension workers, and farmers on biofortification methods and practices. The third essential factor in boosting the market for biofortified wheat is the implementation of awareness programs aimed at both farmers and consumers. Public involvement and education programs can aid market and consumer adoption of biofortification strategies.

CONCLUSIONS

These results highlight the potential importance of biofortified wheat cultivars in meeting the worldwide nutrition concerns brought on by climate change. Biofortified wheat provides a long-term and easily accessible strategy for addressing micronutrient deficits by boosting essential micronutrients like iron, zinc, and vitamin A. Health outcomes have improved, and nutritional deficits have decreased when biofortified wheat was introduced into agricultural systems. Additionally, biofortified wheat shows increased adaptation capacity to abiotic conditions like drought and heat, which helps make agricultural production more climate-resilient. Improving biofortification methods, producing high-yielding and climate-resilient biofortified wheat varieties, and assessing their long-term sustainability are all areas that need more investigation. Developing evidence-based policies encouraging the widespread use of biofortified wheat requires close cooperation between researchers, breeders, politicians, and other interested parties. Also, vulnerable populations need consistent policy support and investment to establish an enabling environment that encourages the widespread adoption of biofortification projects.

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AEROBIC RICE CULTIVATION: A PROMISING STRATEGY FOR CLIMATE CHANGE MITIGATION

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ABSTRACT

Aerobic rice cultivation is a promising strategy for climate change mitigation in agriculture. This review paper assesses its potential, covering methane emissions reduction, water usage efficiency, soil health improvement, nutrient management, adaptability to climate change, and scalability. Aerobic rice practices reduce methane emissions by up to 50% compared to flooded systems. Water savings of 30-50% contribute to sustainable water management. Improved soil health increases nutrient availability and cycling, enhancing nutrient use efficiency by 20-30% and reducing nitrogen losses. Aerobic rice systems demonstrate heat tolerance, maintaining or improving crop productivity under elevated temperatures. Their flexibility allows for diversification and resilience in cropping systems. Adoption varies, with progress in China and India, but challenges remain, including technical knowledge gaps, economic considerations, and policy support. Recommendations include training programs, economic evaluation, and supportive policies to promote wider adoption.
Keywords: Aerobic rice, climate change, methane, emissions, mitigation

INTRODUCTION

Climate change and its far-reaching repercussions are a pressing global issue that demands prompt attention. The agriculture industry is a significant contributor to the causes of climate change due to its role in releasing greenhouse gases (John et al., 2021; Laborde et al., 2021). Rice farming is essential to pollution because it generates methane in flooded rice systems. Nearly 10% of all anthropogenic methane emissions come from rice growing, as reported by the Food and Agriculture Organization (FAO). The agricultural sector is responsible for around 13% of the world's greenhouse gas emissions (Mboyerwa et al., 2022a; Mrówczyńska-Kamińska et al., 2021; Popp et al., 2010). To prevent these emissions and guarantee food security, novel measures are required.

The production of aerobic rice, characterised by the absence of flooding over the whole growth period, has promise for mitigating greenhouse gas emissions within the agricultural domain. Multiple studies have consistently demonstrated that implementing aerobic rice systems leads to a substantial reduction in methane emissions. Sharma et al., (2016); Surendran et al., (2021); and Suryavanshi et al. (2013) have documented significant reductions of up to 50% when comparing flooded rice production to alternative methods. The necessity of this reduction is rooted in the fact that methane possesses a significant capacity to contribute to global warming. Furthermore, it is worth noting that aerobic rice farming exhibits the capacity to yield significant water conservation benefits in comparison to traditional flooded rice farming practices. Research conducted by Ahmed et al. (2017) and Mallareddy et al. (2023) has demonstrated that aerobic rice farming methods can reduce water consumption by as much as 30-50%. Implementing efficient water management strategies not only alleviates the impacts of climate change but also effectively tackles the problem of water scarcity in regions prone to droughts or experiencing water stress. In addition, aerobic rice systems contribute to promoting sustainable agricultural practices through their facilitation of enhanced nutrient management, improved soil health, and heightened resilience to the impacts of climate change.

The objective of this review article is to offer a thorough evaluation of the pressing necessity to address climate change, with a specific focus on the agricultural sector. Additionally, this research seeks to promote aerobic rice production as a feasible technique for mitigating the impacts of climate change. This review seeks to assess the effectiveness of aerobic rice production in reducing climate change through the synthesis of scientific evidence, research findings, and pertinent data. Moreover, the aim is to emphasise the potential of aerobic rice farming in mitigating greenhouse gas emissions, preserving water resources, and fostering resilient agricultural practices. The amalgamation of data and analysis offered in this comprehensive review will offer significant contributions to policymakers, researchers, and practitioners in their endeavours to delve deeper into and advocate for the implementation of aerobic rice production as a viable and efficient technique for mitigating the impacts of climate change within the agricultural domain.

Aerobic Rice Cultivation and Methane Emissions

The research findings have provided evidence that the growing of aerobic rice significantly reduces methane emissions compared to traditional flooded rice systems. Aerobic rice systems are highly effective in mitigating or eliminating floods, therefore creating aerobic conditions that hinder the synthesis of methane aided by methanogenic bacteria. Much research has continuously presented empirical evidence regarding the possible reduction of methane emissions achieved through aerobic rice growing. Peyron et al., (2016) reported a significant reduction of 47% in methane emissions due to the adoption of aerobic rice farming compared to typical flooded rice systems. In a study by Feng et al., (2013), various methane emissions reductions were recorded in aerobic rice systems, ranging from 35% to 64%.

Table 3. Total cumulative methane (CH_4) emission under flooded rice and aerobic rice cultivation

Location	Year	Treatment	CH_4 emission (kg ha^{-1})	Reference
India	2009	Flooded rice	22.59	Jain et al., (2014)
		Aerobic rice	8.16	
India	2010	Flooded rice	57.9	Sharma et al., (2016)
		Aerobic rice	4.3	
India	2010	Flooded rice	32.3	Suryavanshi et al., (2013)
		Aerobic rice	9.9	
China	2013	Flooded rice	171.4	Chu et al., (2015)
		Aerobic rice	83.6	
Vietnam	2013	Flooded rice	108.1	Pandey et al., (2014)
		Aerobic rice	31.8	

The decrease in methane production reported in aerobic rice conditions can be attributed to two primary factors: a limited availability of organic carbon for methanogenic bacteria and an enhanced activity of methane-oxidizing bacteria (Seo et al., 2014). In the context of aerobic rice systems, it is evident that the abundance of organic carbon, encompassing plant residues and root exudates, is relatively diminished compared to flooded systems. As a result, the accessibility of the substrate for methanogenic bacteria to produce methane is limited. Furthermore, oxygen in aerobic conditions facilitates the metabolic activities of methane-oxidizing bacteria (MOB), leading to efficient methane consumption and subsequent mitigation of its release into the atmosphere. The methane oxidation process by methane-oxidizing bacteria (MOB) transforms methane into carbon dioxide (CO_2), a greenhouse gas that exhibits reduced potency. As a result, this phenomenon contributes to a decrease in the total methane emissions from the system.

As previously stated, the findings highlight the significant potential of aerobic rice growing inefficiently mitigating methane emissions across many environmental settings. Furthermore, it is imperative to acknowledge that extensive research has consistently shown long-term and constant decreases in methane emissions across multiple consecutive cropping seasons. This underscores the durable and reliable nature of this approach.

Greenhouse Gas Balance and Carbon Sequestration

Evaluating greenhouse gas balance in aerobic rice systems involves a thorough analysis beyond the exclusive consideration of methane emission mitigation. It comprises the assessment of various greenhouse gases and explores the possibility of carbon sequestration. While implementing aerobic rice growing has resulted in a significant reduction in methane emissions compared to traditional flooded rice systems, it is imperative to consider the total greenhouse gas profile. The available research suggests that adopting aerobic rice systems has been linked to a decrease in the emission of supplementary greenhouse gases, such as nitrous oxide (N_2O) and carbon dioxide (CO_2). Verhoeven et al., (2018) conducted a study which showed that implementing aerobic rice systems led to a significant reduction of 30-50% in N_2O emissions compared to flooded systems. Furthermore, implementing aerobic rice cultivation can substantially contribute to the carbon sequestration process by augmentation of soil organic carbon (SOC) levels (Gangopadhyay et al., 2022).

Table 4. Total cumulative nitrous oxide (N_2O) emission and global warming potential (GWP) under flooded transplanted and aerobic rice cultivation

Author	Environment	GHG emission	
		N_2O (kg ha^{-1})	GWP (kg CO_2 equivalent ha^{-1})
Tyagi et al. (2010)	Continuous flooded	–	8153.88
	Aerobic condition	–	4816.25
D. Li et al., (2011)	Continuous flooded	132	2920
	Aerobic condition	85	2300
J. Y. Wang et al., (2011)	Continuous flooded	0.47	–
	Aerobic condition	0.38	–
J. Wang et al., 2012)	Continuous flooded	0.22	–
	Aerobic condition	0.13	–
Jain et al. (2014)	Continuous flooded	0.91	888.1
	Aerobic condition	0.61	644.3
X. Li et al. (2014)	Continuous flooded	119	2640

	Aerobic condition	113	2330
Pandey et al., (2014)	Continuous flooded	0.74	2784
	Aerobic condition	0.32	1005
Kumar et al. (2016)	Continuous flooded	1.04	2328.53
	Aerobic condition	0.98	1867.64

Integrating carbon sequestration techniques within aerobic rice systems substantially increases the capacity for mitigating climate change. The implementation of aerobic activities plays a crucial role in the process of carbon sequestration by promoting the accumulation of soil organic carbon. Multiple research studies have shown that aerobic rice systems can increase soil organic carbon (SOC) levels compared to flooded systems. Xue et al., (2015) conducted a study which demonstrated a significant increase in soil organic carbon (SOC) stocks, ranging from 20% to 30%, in aerobic rice fields compared to flooded rice fields. The increase in soil organic carbon (SOC) has a dual purpose: mitigating climate change by sequestering atmospheric carbon dioxide and enhancing soil fertility, water retention capacity, and overall soil health.

The significance of increased quantities of soil organic carbon in aerobic rice systems extends beyond the process of carbon sequestration. The elevated soil organic carbon (SOC) levels have yielded many benefits within sustainable agriculture and climate change adaptation. Research has demonstrated that increased soil organic carbon (SOC) concentrations benefit soil structure, resulting in enhanced water infiltration and reduced soil erosion. Furthermore, it has been observed that higher concentrations of soil organic carbon (SOC) have a beneficial effect on the preservation and availability of essential nutrients, leading to enhanced agricultural output and enhanced efficiency in nutrient uptake. Multiple elements significantly contribute to the increased resilience and adaptation of agricultural systems in the face of climate change. Therefore, the potential of carbon sequestration in aerobic rice systems and its associated improvements in soil health and agricultural sustainability make it a promising strategy for addressing climate change in the agricultural industry.

Water Management and Conservation

Aerobic rice agriculture is a promising strategy for addressing climate change by reducing water usage and removing or minimizing the need for constant flooding. In contrast to traditional flooded rice systems, aerobic rice farming utilises specific strategies to minimise water consumption. Multiple studies have consistently demonstrated significant decreases in water consumption in aerobic rice systems compared to flooded systems. A study conducted by (Maneepitak, Ullah, Paothong, et al., 2019) demonstrated that implementing aerobic rice farming practices can substantially reduce water usage, with potential savings ranging from 30% to 50%. The aforementioned savings substantially contribute to the sustainable management of water resources and have significant implications for mitigating climate change. Proper water resource usage is paramount in resolving the increasingly pressing issues of water shortage, which are intricately connected to the phenomenon of climate change.

Table 5. Water use efficiency (WUE), water productivity, and grain yields of rice under different water management

Country	Approaches	Remarks	References
Thailand	Alternate wetting and drying (AWD)	Compared to CF, AWD enhanced grain yield by 15% during the wet and 7% during the dry seasons. Compared to CF, AWD increased total water productivity by 46% during the rainy and 77% during the dry seasons.	Maneepitak, Ullah, Datta, et al., (2019; Maneepitak, Ullah, Paothong, et al., (2019)
China	Shallow-wet irrigation (SWI), controlled irrigation (CI), intermittent irrigation (II)	Approximately 94.19% of paddy fields in China are suitable for water-saving irrigation (WSI). By thoroughly implementing WSI, China can boost rice yield by 5.39-6.87%. Using WSI, China can save 22.06-26.41% of its irrigation water for paddies. China can minimise nitrogen loss from paddies by 32.11-39.11% by WSI.	Zhuang et al. (2019)
Thailand	Continuous flooding [CF] and AWD	Compared to similar CF plots, CH_4 emissions from RS-B and RS-I plots under AWD were reduced by 36-63% and 37-39%, respectively.	Maneepitak, Ullah, Datta, et al., (2019)
China	Shallow-irrigation and deep-sluice (SIDS), continuous flooding (CF)	SIDS considerably reduced the frequency and amount of irrigation water used compared to FI, leading to a rise of 16.2% in rainfall use efficiency and, as a result, a reduction in the amount of surface runoff and water that had leached.	Qi et al. (2020)
Turkey	Aerobic and anaerobic	Because rice is primarily grown under anaerobic or flooded circumstances, arbuscular mycorrhizae fungi (AMF) inoculums may drop in the rice field.	Iqbal et al., (2020)

Implementing effective water management strategies is paramount in addressing the consequences of climate change and fostering sustainable agricultural practices. Aerobic rice cultivation employs various techniques to maximise water efficiency, including alternate wetting and drying (AWD) or controlled irrigation. Alternate wetting and drying (AWD) is a cultivation technique characterised by intermittent soil exposure to dry conditions followed by subsequent re-flooding, in contrast to the continuous flooding approach. Empirical evidence has demonstrated that this practice yields a noteworthy reduction in water consumption without compromising crop productivity. Implementing water management strategies in aerobic rice systems plays a significant role in mitigating climate change. These practices effectively conserve water resources and alleviate the strain on freshwater availability in agricultural areas.

Soil Health and Nutrient Management

Implementing aerobic rice cultivation techniques, which involve reducing waterlogging and enhancing aeration, holds considerable implications for soil health and the availability of nutrients (Khairul Alam et al., 2020). In contrast to conventional flooded rice systems, aerobic rice cultivation entails establishing aerobic conditions, which facilitate advantageous soil microbial activity and nutrient cycling. Numerous research studies have consistently exhibited notable enhancements in soil health indicators within aerobic rice systems. An investigation by Majumdar et al. (2023) revealed that aerobic rice fields exhibited elevated levels of soil organic carbon content, enhanced soil structure, and increased microbial diversity compared to flooded rice fields. The enhancements mentioned above significantly enhance soil's overall well-being and adaptability, which are imperative for establishing and maintaining sustainable agricultural systems.

Aerobic rice systems offer the capacity to enhance nutrient use efficiency and mitigate nitrogen losses, thereby serving as crucial elements in mitigating climate change. Aerobic rice cultivation effectively mitigates waterlogging, reducing anaerobic conditions that can result in nitrogen losses through denitrification and leaching. Research findings have indicated that aerobic rice systems demonstrate a greater level of nitrogen use efficiency in comparison to conventional flooded systems. An example of a study conducted by Kadiyala et al. (2015); Mboyerwa et al. (2022b); and Ullah et al. (2019) revealed a notable enhancement of 20-30% in nitrogen use efficiency when employing aerobic rice cultivation techniques. The enhanced efficiency discussed in this context not only leads to a reduction in nitrogen losses and the subsequent environmental impacts but also plays a role in improving crop productivity and decreasing the need for fertilizers.

Furthermore, aerobic rice systems have been found to augment nutrient availability by enhancing nutrient mineralization and cycling processes. Aerobic conditions have been found to enhance the activity of advantageous soil microorganisms, which are crucial in facilitating nutrient transformations. Numerous studies have provided evidence indicating a greater availability of nutrients, specifically phosphorus, potassium, and micronutrients, in aerobic rice cultivation systems instead of flooded systems. Jinger et al. (2022); Midya et al. (2021) conducted a study which revealed that aerobic rice fields exhibited enhanced nutrient availability, improved nutrient uptake, and elevated grain nutrient content. As mentioned above, the findings underscore the potential of aerobic rice cultivation in augmenting nutrient management, diminishing dependence on external inputs, and enhancing the overall nutrient status of agricultural systems.

Adaptation and Resilience to Climate Change

The cultivation of aerobic rice exhibits considerable potential in terms of its adaptive capacity to mitigate the impacts of climate change, such as rising temperatures and modifications in precipitation patterns. The resilience and capacity of aerobic rice systems to sustain or enhance crop productivity have been demonstrated in response to increasing temperatures (Farooq et al., 2022). Numerous research studies have provided evidence suggesting that aerobic rice cultivars possess the desirable heat tolerance and adaptability traits, enabling them to endure elevated temperatures more effectively than conventional flooded rice varieties. Jagadish et al. (2015) conducted a study which found that aerobic rice varieties demonstrated superior grain yields in elevated temperatures compared to flooded rice varieties. The capacity of aerobic rice to withstand heat stress offers a significant climate-resilient approach in response to the escalating global temperatures.

Aerobic rice cultivation offers the possibility of diversification and adaptability in cropping systems, thereby bolstering the resilience of agricultural production in the face of climate change. The utilization of the aerobic system facilitates a broader array of crop selections and rotations, thereby creating prospects for diversification and mitigating dependence on monoculture (Farooq et al., 2023; Ijaz et al., 2019; John et al., 2021) . Numerous studies have provided evidence supporting the successful integration of aerobic rice into diversified cropping systems, including rice-legume rotations or intercropping with various other crops (Cai et al., 2018; Kebede, 2021; Sarwar et al., 2022; Xia et al., 2016). The act of diversifying agricultural practices mitigates the potential risks associated with climate variability and improves agricultural production's overall sustainability and stability. Moreover, the adaptability of aerobic rice cultivation, exemplified by its capacity to accommodate fluctuations in water availability, empowers farmers to modify planting schedules and irrigation methods by shifting precipitation patterns. This, in turn, amplifies the resilience of cropping systems.

Adoption and Scaling-Up

Implementing aerobic rice cultivation to mitigate climate change exhibits regional disparities, with some areas demonstrating encouraging progress. Aerobic rice systems have experienced notable adoption and gained momentum in countries such as China, India, and the Philippines. Aerobic rice cultivation has been extensively adopted in China, resulting in approximately 4 million hectares of land dedicated to aerobic rice production (Datta et al., 2017; Seck et al., 2012; Tan et al., 2018). Likewise, the utilization of aerobic rice systems in India has witnessed a notable rise, particularly in regions facing water scarcity (Jabran et al., 2015; Jat et al., 2016; Srinivasa Rao et al., 2016; Srivastav et al., 2021). Nonetheless, the ability to expand aerobic rice cultivation in diverse regions is hindered by obstacles arising from dissimilar agroecological conditions, local farming practices, and socio-economic factors.

The implementation of aerobic rice cultivation as a means of mitigating climate change is impeded by various challenges and barriers. Acquiring technical knowledge and cultivating awareness among farmers and extension services are pivotal factors in facilitating the effective implementation of novel practices. To improve technical proficiency and comprehension of aerobic rice systems, it is imperative to establish training programs, demonstrations, and knowledge-sharing platforms that are sufficient in scope and quality. Economic considerations influence the adoption of aerobic rice cultivation, as farmers must assess the economic viability and profitability of transitioning to this method. Various factors, including the initial investment costs, the availability of appropriate equipment, and the accessibility to markets and value chains influence the economic feasibility of adopting aerobic rice systems. In addition, it is crucial to emphasize the significance of policy support in establishing a conducive atmosphere for the extensive implementation of aerobic rice cultivation. Policymakers can implement various measures such as incentives, subsidies, and support mechanisms to facilitate the widespread adoption of environmentally sustainable practices, such as aerobic rice cultivation.

CONCLUSION

In conclusion, this review paper has emphasized the potential of aerobic rice cultivation as a promising and sustainable approach for addressing climate change in the agricultural domain. Aerobic rice cultivation presents a range of advantages for climate change mitigation and agricultural sustainability, including reducing methane emissions, efficient water utilization, enhanced soil health, improved nutrient management, and the ability to adapt to the impacts of climate change. Numerous studies have consistently provided evidence regarding the efficacy of aerobic rice systems in mitigating methane emissions, preserving water resources, augmenting soil organic carbon levels, optimizing nutrient utilization, and bolstering crop resilience. Implementing aerobic rice cultivation encounters obstacles to technical expertise, economic factors, and policy backing. By effectively tackling these obstacles and facilitating the uptake of aerobic rice cultivation through disseminating knowledge, enhancing skills, providing economic incentives, and implementing supportive policies, the path can be paved for the broader application of this promising approach. By adopting and harnessing the capabilities of aerobic rice cultivation, various actors within the agricultural domain have the opportunity to actively contribute to endeavours aimed at mitigating climate change, bolstering resilience, and fostering a sustainable trajectory for the production of food.

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TURKEY GRAPE FRUIT GENE SOURCES AND MOLECULAR MARKERS AND GENETIC CHARACTERIZATIONS

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ABSTRACT

Turkey has an important place in terms of gene center. Turkey is a country with a very high plant species diversity due to its location, climate and geological differences. Plant gene resources are of great importance for the development of new varieties in order to meet the needs of the increasing population in the world. Our country is quite developed in terms of fruit growing. One of these fruit groups is the berry, which has recently come to the fore with its health benefits, and we have come across a large number of wild fruits in addition to a wide distribution area in our country. Grape fruits are loved and consumed in countries around the world and these fruits, which are evaluated in various ways, are among the fruits of important horticultural crops, the production of which is gradually increasing in Turkey. Until now, breeding has been done by looking at the morphological characteristics in new cultivar development studies in fruits. However, faster and more reliable methods are needed because morphological features are affected by environmental factors. With the use of molecular markers, it is possible to shorten the time in fruit breeding studies that take a long time. At the same time, molecular markers are used to determine, record and protect our gene resources. In this study, the aim is to give information about the berry fruits grown in our country and to examine the characterization studies with molecular markers related to berry fruits that contribute to production.

Keywords: Genetic Characterization, Fruit Gene Resources, Molecular Markers, Berry Fruits

INTRODUCTION

The places where plants first occur and complete their evolutionary processes are defined as gene centers or homeland. 8 gene centers are known in the world that the Russian botanist Vavilov determined. Türkiye is located in these gene centers. Due to the diversity and suitability of the ecological conditions of our country, being on the migration routes, it has a richness of species and variety because it is the gene center for many fruit and vegetable species grown in the world or within its borders (Sarı, 2010). Turkey is located within the natural distribution area of berry fruits and it is possible to encounter different forms and genotypes of one or more species in almost every region. Grape fruits are consumed in the food industry in various ways, as well as consumed as fresh fruit, which is loved and consumed all over the world. These fruits are not very selective in terms of soil preference, they can be used as intermediate plants or hedge plants in the garden, their need for chemical fertilizers is low, they bear fruit in a short time, they are rich in vitamins, mineral substances, phenolic compounds, antioxidant contents, their yields are high. Due to their low production costs, they show superior characteristics compared to other fruits (Ertürk ve Geçer, 2014).

For appropriate variety breeding studies in a region, first of all, it is necessary to apply and benefit from the natural gene resources we have around us (Karakoç, 2011). Gene source in plants provides the diversity of hereditary information in the gene pool of plant species and also indicates genetic diversity (Altındal ve Akgün 2015). Wild species, which have adapted to the region and have a wide distribution in natural conditions for a long time, are mostly resistant to diseases. As these resistance traits are transferred to new varieties, they will become widespread in the region. For this reason, it is necessary to scan and protect our natural vegetation, to define it with different methods and to create gene pools. It is important for the continuity of our natural wealth and the protection of gene resources for future research (Karık vd., 2016). In recent years, there are effective methods developed for the protection of plant gene resources and to be used in breeding programs. With these methods, new opportunities have been provided with methods covering topics such as molecular genetics and tissue culture technologies (Sarı, 2010).

Molecular markers are an important method in identifying genotypes, in genetic resource management, and in revealing the differences between plants that are similar in terms of phenotype. For this reason, the use of molecular markers is very important because they are not affected by the environment, they are fast, reliable and easy. Molecular marker techniques, which reveal the genetic closeness between genotypes, allow the formation of taxonomic classifications, the investigation of the changes of genotypes until this time, and information about phylogenetic relationships. This information is a source for studies in terms of investigating some specific genes in genetic resources. Since morphological characters can be affected by environmental factors, it is very difficult to obtain an accurate result and distinguish between close relatives. For this reason, molecular markers are used more effectively to explain phylogenetic relationships (Türkeli, 2010). It is very important to carry out genetic characterization studies in order to make the diagnosis easy and to provide information for future studies, since they are morphologically similar to each other in species such as berries that are widely distributed (Kuyumcu, 2019).

Turkey Berries and Genetic Resources

Turkey is a country rich in fruit gene resources and has an important place in the world. It is the primary and secondary gene center of many fruits. In terms of fruit growing history and culture, Anatolia is one of the oldest growing areas of berry fruits, as well as many fruit species. It is not possible to fully express the description of berry fruits. Because there is no fixed feature that symbolizes all the species in this group together (Sarıburun, 2009). However, if we express it in terms of botany, it can be defined as semi-shrub or shrub plants with berry fruits, soft flesh, tiny, juicy and edible fruits. Wild ones are found in almost every region of our country and these fruits are known and loved by the public (Yıldız, 2017). At the same time, it has been determined that compounds such as phenolic compounds, organic acids, tannins, anthocyanins and flavonoids in its content have positive effects on human health (Çağlar ve Demirci, 2017). Berry fruits are widely grown in the Mediterranean, Aegean and Marmara regions of our country. Examples of berry fruits are strawberries, grapes, raspberries, blackberries, blueberries, elderberries, mulberries, gilaburu, red/white/black currants, and aronia (Bayizit, 2022).

Since Turkey is within the natural spreading areas of berry fruits, one or more species are encountered in almost all regions. They are important plants because they are easy to cultivate, they produce regular products every year, they are constantly in demand in terms of domestic and foreign trade in our country and can be sold at high prices, and they can also be grown in low socio-economically income regions in our country (Sarıburun, 2009). Berries constitute 25% of the country's total fruit production. Grapes (4,165,000 tons/year) take the first place among the berry fruits that have an important place in the Turkish economy, Strawberry (728.112 tons/year) is the most important fruit in terms of production after grape, followed by raspberry (6.652 tons/year) and blackberry (3,384 tons/year) (TUİK, 2022; Table 1).

Berries	**Production amount in Turkey Year/Ton (2022)**
Grape (Vitis vinifera L.) (seedless), table grape (seed, seedless), wine grape	4.165.000
Strawberry (*Fragaria* L.)	728.112
Raspberry (*Rubus İdeaus* L.)	6.652
Blackberry (*Rubus Fructicous* L.)	3.384

Table 1. Berry fruit production in Turkey in 2022 (tonnes) (TUİK, 2022)

Molecular Markers and Caracterization

Molecular markers are the markers that represent the differences in the base sequence of the DNA that make up the genetic material of the individuals that make up a population, and that can be formed by events such as addition, displacement, deletion, duplication, and that reveal these differences at the same time. Molecular markers represent any gene region or piece of DNA associated with a gene region within the genome (Özcan, 2001).

Molecular markers are very important in terms of being very common in the genome and being safe. Today, using molecular markers to define the correctness of the names of the varieties in a short time has an important place in fruit growing. Because fruit breeding includes studies that require intensive labor and long time. The breeding period of a fruit variety takes about 20-25 years. Therefore, it causes delay in the development of new varieties in fruit growing (Aksu, 2015). In order to characterize genetic diversity, DNA-based marker methods have been used more and more because they can perform the characterization of genetic materials more quickly (Namlı, 2016).

Grape (*Vitis Vinifera* L.) and its Genetic Characterization with Molecular Markers

Turkey has a suitable geography and climate in terms of viticulture and is one of the vine (Vitis vinifera spp.) gene centers. At the same time, looking at the archaeological studies, our country is accepted as the cradle of viticulture culture and its homeland (Sanyürek, 2014). The vine is common in the countries between 20-52 latitudes in the northern hemisphere, and between 20-40 latitudes in the southern hemisphere (Yazıcı, 2019). This special situation has enabled our country to have various grapevine gene resources (Tekdal ve Sarlar, 2016). It has a high adaptability to different climatic and soil conditions, and has a wide range of uses. These grapes, which are the fruit of the vine, can be used in alcoholic and non-alcoholic beverages, in the pharmaceutical and cosmetic industry, with fresh and dried consumption, processed in various ways, while the leaves can be used in the form of brine (Sümbül ve Yıldız, 2022).

Due to the naming differences in the grapevine gene resources, which have very rich local varieties grown in almost every region in our country, and the variations seen in the varieties, variety confusion has occurred and some varieties that are not preferred in production have been in danger of extinction (Aslan, 2018). For this reason, characterization studies of local grape varieties with ampelographic and molecular methods are important in terms of revealing the characteristics and similarities of the varieties (Uysal vd., 2023). For many years, ampelographic analyzes have been carried out and ampelographic analysis criteria have been used to define grape varieties. However, the use of DNA-based marker techniques in molecular studies has come to the fore, due to the fact that these criteria are affected by environmental factors and can vary from observer to observer (Aslantaş, 2010).

If we look at the molecular studies; In the study of Baykul and Söylemezoğlu (2023), genetic identification of 52 grape varieties detected in different districts of Eskişehir was made using 18 SSR primers with high discrimination power. As a result, 171 alleles were obtained and as a result of molecular characterization, 1 synonymous and 4 homonymous groups were obtained. In another study, Güler (2023) examined the genetic relationships between some varieties in Turkey and Europe and the local grapevine population using 8 IPBS markers. As a result of the study, 136 bands were obtained, 106 of which were polymorphic, and as a result of the cluster analysis, the genotypes were divided into three main and seven sub-clusters, and the diversity assessments made with the iPBS markers reported that there was serious differentiation between individuals, and that even individuals with the same name should be examined.

Strawberry (Fragaria L.) and its Genetic Characterization with Molecular Markers

Strawberry belongs to the genus Fragaria, belonging to the class Magnoliophyta, to the Rosineae suborder of the Rosales order, to the Rosaceae family. 24 wild and hybrid species belonging to the genus Fragaria were defined. Fragaria x ananassa variety, which is an octoploid strawberry (2n=8x=56), is a commercially important species (Shimomura et al., 2006). While the Chinese ranks first in strawberry production in the world, this ranking is; ABD, Meksika, follow. Türkiye is in the 4th place (TEPGE, 2021). Strawberry is produced in wide areas around the world, including open and greenhouse cultivation (Kesici, 2009). The demand for strawberries is increasing day by day, the biggest reasons for this are; its aroma and its ability to be offered to the consumer for a long time in the market, to be grown economically in different climatic and soil conditions. Another benefit for the producer is that it is particularly suitable for small family businesses, as the investments made return in the first year. All these reasons have made strawberry the most commonly grown fruit among berry-like fruits (Türemiş vd., 2000; Erdem, 2018).

Strawberry gene resources in our country consist of local and wild species. Although our local varieties 'Ottoman', 'Ereğli' and 'Arnavutköy' have superior taste and aroma, they are low in yield and have small fruits and are generally grown for their aroma and taste. For this reason, hybrid breeding practices have been carried out based on the aroma and flavors of domestic varieties of our country, and the characteristics of foreign varieties such as fruit size, yield amount, earliness, and breeding studies are still continuing (Gündüz ve Bayazit, 2017). Two important problems are considered in strawberry cultivation. The first of these is to increase resistance to diseases, that is, to reduce the use of pesticides, while the second is to increase fruit quality yield. For this purpose, molecular markers have become an important method used by plant breeders to accelerate selection studies. Molecular markers offer fast and efficient approaches to cultivar development in the desired direction (Temel, 2011)

In studies with markers, Serçe et al. (2007) carried out molecular characterization of the samples they collected from all regions in Turkey with SSR and RAPD markers and formed the first core collection in our country with the collected samples. In their study, Makaracı et al., (2017) conducted a characterization study in terms of screening the resistance genes against various biotic and abiotic stresses by using 5 SSR markers of the species belonging to the Fragaria genus adapted to the Thrace natural flora, and as a result, they identified 3 different Fragaria species. Sirijan et al., (2020) determined the differences between cultivars by using the RAPD marker in 6 of the cultivars grown commercially in the northern region of Thailand. Their data showed that RAPD markers can easily distinguish strawberry cultivars with different degrees of genetic relatedness. They concluded that it would facilitate breeding approaches for certain traits in new strawberry cultivars in Thailand. Lu et al (2021), in their study, examined the level of genetic variation within and between 169 F. nilgerrensis individuals and the population using 16 newly developed EST-SSR markers, and based on the 16 developed EST-SSR markers, they identified a total of 71 alleles, with an average of 4.44 alleles per locus. All of the results obtained reported that F. nilgerrensis populations showed abundant genetic diversity and significant genetic differentiation.

Raspberry (Rubus Ideaus L.) and its Genetic Characterization with Molecular Markers

The raspberry fruit, whose Latin name is Rubus idaeus 2n=2x=14 (lim et al., 1998), belongs to the Rosaceae family. Raspberry, which is in the group of berry fruits; It is divided into three as red, black and purple according to the color of its fruits. Red raspberries are the most common in production and consumption. In addition, it is a fruit with many different uses in the food industry and rich in chemical compounds it contains. Shows healing properties on human health (Bayizit, 2022).

Raspberry is the second berry type after strawberry in terms of production in our country. For the first time in 1995, data on raspberry were collected and officially recorded (Sarıburun, 2009). It is known to grow naturally in North America, Europe and South Asia (Aydemir, 2008). For raspberries, airy places with relative humidity of more than 1000 m and generally sunny, protected from the wind, with sufficient soil moisture are the most suitable growing environments (Güneş, 2019).

Polat and Göçmen (2008) characterized the genetic differences of 15 raspberry genotypes selected from the Black Sea region using 11 RAPD markers, and it was concluded that they were genetically different and the variation was high in the Black Sea region. In another study, Grichev et al. (2017) collected the genetic resources of Rubus in order to recreate a raspberry breeding program in Germany. Next, all 82 genotypes were evaluated for genetic diversity and species fitness using 16 SSR markers. A total of 224 different alleles were obtained using 15 of the 16 SSR markers. The marker RhM023 was found to be monomorphic in all cultivars studied, so this marker was excluded from further studies as it was not informative. It has been reported that the markers RhM011, RiM017, RhM021, RhM003 and RiM015, Rubus123a, Rubus285a, Rubus270a are polymorphic for all cultivars. Pinczinger et al., (2020) identified a total of 69 different alleles in six raspberry cultivars of six different origins using 16 SSR markers. It has been reported that the RhM023 marker is monomorphic for all six cultivars, 14 markers have at least two different alleles among the cultivars tested, whereas the RiG001 marker does not produce alleles in the cultivar 'Meeker'.

Blackberry (Rubus Fructicosus L.) and its Genetic Characterization with Molecular Markers

Blackberries in the berry group; It is included in the Rubus L. genus, which is divided into 2 sub-genus as Ideaobatus and Euabatus of the Rosaceae family from the Rosales order. It is in the form of a bush and consists of a compound fruit. Rubus fructicosus is used for the general nomenclature of blackberries (Ağaoğlu ve Gerçekçioğlu, 2013). Blackberries are shrub-like plants and their shoots are like springs. The plant can form many shoots and its shoots can be thorny or thornless (Yıldız, 2017). Growing up to 3 m, the blackberry plant in the form of a bush has white or pink flowers and a black or dark purple fruit. Blackberry, which has an economic life of 15-20 years, is not very selective in terms of climate demands. Although they can be grown in most climatic conditions, they develop better in temperate, moist and acidic soils in places with plenty of sun, protected from winds, no rain at harvest time, sufficient soil moisture and mild winters (Barut, 2004). Although it is mostly consumed fresh, it can be used in industrial applications as frozen or freeze-dried.

Blackberry varieties are mostly of North American origin, but their homeland is Southern, Western and Central Europe (Kırıt vd. 2023). Our country, on the other hand, is located in the regions where blackberry is the gene center and it is widespread in a very wide area (Sarı, 2010) There are differences and variations in plant and fruit characteristics due to genotypic differences and environmental factors in the growing areas (Karakoç, 2011). As a result of breeding studies on blackberries, high yields have been obtained as a result of the development of large-fruited varieties and the application of new growing systems (Zenginbal ve Gündoğdu, 2019). In blackberry breeding, wild blackberries have an important role in the development of today's varieties (Pamukçu, 2019).

Although our country is a gene center in terms of location in blackberry cultivation, the desired studies in molecular terms have not been carried out and sufficient level has not been achieved (Karakoç, 2011). In order to characterize genetic diversity, DNA-based marker methods have started to be used more and more because they can perform the characterization of genetic materials more quickly (Namlı, 2016). Molecular characterization study in blackberry Alan (2019) conducted a morphological and molecular characterization study between some blackberry genotypes collected throughout Turkey and standard blackberry varieties using 16 ISSR markers. According to the data obtained as a result of the study, a total of 293 bands and 292 of these bands were polymorphic and the polymorphism rate was determined as 99.75%. When the resulting pedigree is examined, it is reported that they are divided into 2 main groups and some of them are grouped in the same group, while some are grouped mixed. According to the data obtained in general, it was stated that there was a significant genetic variation. In another study, Medina and Posada (2021) evaluated 13 wild and cultivated plants from the genus Rubus. A molecular characterization was performed using 16 SSR markers that produced positive amplification. As a result, it has been determined that there is a 23% molecular genetic variation and this genetic diversity can be useful in breeding programs where the morphological characteristics of fruits and molecular identification of fruits are taken into account.

RESULT

Due to its location, our country is one of the special countries that has optimum conditions in the production of many fruits and vegetables and can produce quality products, thanks to its wide and highly productive agricultural areas and ecological diversity. Many types of fruit are grown in our country. Among them, there are berry fruits, which have increased in popularity recently. Grape fruits can be consumed fresh, and besides the production of seedlings and saplings, they also make a significant contribution to the country's economy, as they are an important source in the food industry. Our country is location gene origin for a very species. As first be grape fruits, in our country have for very much wild fruits, however, the characterization of this species can not known enough and could not be obtained. For development of new superior variety require that realization genetic variation obtanined in thanks to plant genetic sourch, biocemical, morphological, molecular caracterization of plants in the different area and to determine, identicate with molecular markers. Studies carried out wich related to previous breeding and caracterization used morphological caractersBecause this feature give ncorrect result and low reliability wich they are effected in the climate and enviromental factors. Because of the advantages of molecular markers such as not being affected by the environment, being able to be used at every stage of plant development, and giving faster and more reliable results, it is more appropriate to use them in terms of determining, defining and protecting gene sources. Genetic characterization studies to be carried out on berry fruits, which have been collected and loved for many years in our country, have high adaptability and spread in wide areas, are extremely important in terms of evaluating these genetic materials and creating genetic variations in advanced breeding programs.

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INFLUENCE OF ECOLOGICAL FACTORS TO SEASON DYNAMIC OF GASTROINTESTINAL STRNOGYLIDAE OF SMALL RUMINANTS

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ABSTRACT
The grazing method of breeding enables small ruminants to have constant contact with transitional hosts (oribatids, molluscs, etc.) and eggs and larval forms of parasites, so that there is no sheep that is not infected with at least one parasitic species. Based on research in the world and in our country, diseases of parasitic etiology dominate in sheep and goats both in terms of prevalence and incidence, accompanied by significant morbidity and moderate mortality.There are many factors that contribute to the appearance, maintenance and spread of parasitosis. Among the many factors that influence the spread and maintenance of parasitic infections, there is a major influence of environmental factors on the seasonal dynamics of gastrointestinal strongylids of small ruminants. For these reasons, the aim of our work is to review the influence of abiotic factors on the epidemiology of parasitic infections of small ruminants.

Keywords: small ruminants, helminths, climate condition, ecology

INTRODUCTION

Breeding of small ruminants, sheep and goats, represents a significant branch of livestock production. Despite the fact that the number of sheep and goats in the social and individual sector of production varies from time to time, this branch of the economy and its improvement is given exceptional attention. The reason for this lies not only in tradition, but also in the knowledge that the breeding of small ruminants represents a significant economic item, both due to the production of wool and milk, as well as lamb and goat meat, a highly sought after item on the world market (Ivanović and Pavlović, 2015; Petrović et al., 2021, Pavlović and Ivanović, 2022).

The breeding method, which has been established for centuries in small ruminants, creates a series of conditions that favor the development and maintenance of a large number of diseases of various etiologies. Among them, one of the leading places is occupied by parasitic infections. The grazing diet enables small ruminants to have constant contact with transitional hosts (oribatids, molluscs, etc.) and eggs and larval forms of parasites, so that there is no sheep that is not infected with at least one parasite species. Based on research in the world and in our country, diseases of parasitic etiology dominate in sheep and goats both in terms of prevalence and incidence, accompanied by significant morbidity and moderate mortality (Smith, 1990; Familton and McAnulty,1995; Truong and Baker,1998; Ardeleanu et al.,2007; Stokić-Nikolić et.al, 2013; Pavlović and Ivanović,2015,2018).

The damage caused by parasitic infections in this production is a consequence of the negative pathogenic effects of the parasite on the host organism. The fact is, however, that in most cases, parasitic infections occur subclinically, that is, "imperceptibly" to the eye of the herdsman. Negative economic effects are also present in these situations and are manifested by a decrease in animal production, i.e. a decrease in the production of wool and milk, a poorer upbringing of the young, and a decrease in general body resistance, i.e. an increased susceptibility to agents of other etiologies (Pavlović et al.2003,2009,2012; Bojkovski et al.,2010).

Among the many factors that influence the spread and maintenance of parasitic infections, there is a major influence of environmental factors on the seasonal dynamics of gastrointestinal strongylids of small ruminants. For these reasons, the aim of our work is to review the influence of abiotic factors on the epidemiology of parasitic infections of small ruminants.

MATERIAL AND METHOD

During study performed from 2010 to 2020 we collected fecal samples from 470 herds in various part of Serbia. Samples were colected at monthly intervals and we examined more than 6500 faecal samples. Examination was performed using standard coprological technique with saturated NaCl, $ZnSO_4$ solution and sedimentation (Euzeby,1981). The 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 on the egg rise.

Post mortal examination gave us insight into the types of parasites that were present in the infections. During ten years 738 sheep and goats we were examined by post-mortem examination. Total differential worm counts were done on the entire alimentary tract using the standard paristology necropsy technique described by Pavlović and Rogožarski (2017). Determination of adult parasites and eggs of parasites were done by keys given by Euzeby (1981).

Among the ecological parameters, we monitored the average monthly temperatures, humidity and their influence on the seasonal dynamics of established gastrointestinal helminth species.

RESULTS AND DISCUSSION

During these investigations, the following GI helminths were found in sheep:: *Haemonchus contortus*, *Teladorsagia* (*Ostertagia*) *circumcincta*, *Ostertagia trifurcata*, *Ostertagia ostertagi*, *Ostertagia occidentalis*, *Marshallagia marshalli*, *Trichostrongylus axei*, *Trichostrongylus colubriformis, Trichostrongylus vitrinus*, *Nematodirus filicoliis, Nematodirus spathiger*, *Nematodirus abnormalis*, *Cooperia curticei*, *Cooperia oncophora*, *Cooperia punctata*, *Cooperia zurnabada*, *Skrjabinema ovis, Bunostomum trigonocephalum*, *Oesophagostomum venulosum* and *Chabertia ovina*. In goats, the presence has been established*Ostertagia circumcincta*, *O. ostertagi*, *Ostertagia occidentalis*, *Trichostrongylus axei*, *T. Colubriformis*, *T. capricola*, *Nematodirus spathiger*, *N. filicollis*, *Haemonchus contortus*, *Marshallagia marshalli*, *Skrjabinema ovis*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum* i *Cooperia curticei* (Pavlovic et al.2023).

The intensity of infection and polyparasitsm was monitored in relation to the age of sheep and goats. It was found that in younger animals intensity of infection was lower than that of older animals.

There are many factors that contribute to the appearance, maintenance and spread of parasitosis. Among them are: joint keeping of animals of different age categories, joint grazing of animals of different owners, keeping of large and small ruminants on the same pasture, improper use of pasture, large number of animals on pasture, favorable climatic conditions for the development and survival of preparasitic stages and transitional hosts that are necessary for the development of certain types of parasites in the external environment and therefore the infection of animals, quality of pastures, zootechnical measures that are implemented (or not implemented), etc. (Familton and McAnulty,1997; Pavlovic and Ivanovic2015).

The life cycles of all found helminths species are direct, requiring no intermediate hosts, which applies to all of the economically important strongylid parasites of small ruminants. In these cycles, adult female parasites in the GI tract produce eggs that are passed out with the faeces of the animal. The most fertile female is considered to be H. contortus, which lays 5,000-10,000 eggs per day. The eggs are of the strongylid type, 60-110 μm in size, with 8-16 (32) blastomeres. Eggs of Nematodirus spp. are 2 times larger, 130-260 μm, with 4-8 blastomeres. Development and occurs within the faecal mass, the eggs embryonate and hatch into first-stage larvae (L1), which in turn moult into second-stage larvae (L2), shedding their protective cuticle in the process. During this time the larvae feed on bacteria. The L2 moult into third-stage larvae (L3), but retain the cuticle from the previous moult. The third stage (L3) larvae of this species develop in eggs. In the external environment, embryogenesis, hatching of larvae, their molting and emergence of infective L3 larvae. The L3 constitute the infective stage, and these migrate onto surrounding vegetation where they become available for ingestion by grazing sheep and goats. The development, survival and transmission of the free-living stages of nematode parasites are influenced by micro-climatic factors within the faecal pellets and herbage. These include sunlight, temperature, rainfall, humidity and soil moisture (Vlassoff, 1982; Familton and McAnulty,1997).

From these reasons ecological factors of the external environment represent predisposing and limiting factors in the life of helminths. The development of eggs and larvae, as well as the activity of L3 larvae, depends on temperature, humidity, oxygen and sunlight.

Temperature air is extremely important for the maintenance of parasite larvae on green surfaces. In our environmental condition *H. contortus* optimal temperature for egg embryonation and formation of L3 is 35^{o}C; eggs of *Ostertagia, Trichostrongylus* and *Oesophagostomum* sp. they embryonate at a temperature below 26^{o}C, but for a longer time. They are more resistant to lower temperatures and desiccation - embryonation of eggs and hatching of larvae takes place even at 5oC. Eggs of *Ostertagia* sp. survive 20 days at -5^{o}C. For embryonating the eggs of *Nematodirus* sp. the optimal temperature is 21^{o}C (Pavlovic nad Ivanovic,2015; Pavlović and Rogožarski, 2017)

Humidity and oxygen had strong influence to embryonic development in eggs largely depends on soil moisture, i.e. from atmospheric precipitation (dew). The position of the eggs in the bream, i.e. blocks of faeces, helps the survival of the eggs in many ways. Infectious L3 are negatively geotropic and positively phototropic to moderate sunlight intensity. The humidity of the environment is necessary for their activity and the length of their survival in the external environment depends on it - during the winter and hot summers, the largest number of larvae die. The most infectious larvae are found on plants in April and September, less in May and October, and the least in July and August (Pavlović and Rogožarski,2017).

The climate of Serbia is mostly temperate continental with steppe character. The temperature region in Serbia, which has a mean annual temperature of between 11^{o}C and 12 ^{o}C are plains in the north and northeast, the Morava and the areas with an altitude of less than 500 m. The average annual amplitude of temperature variations in the eastern and northeastern Serbia is 23-24 ° C. The average temperature of air in mountainous areas and in the highlands are amplitude from -4°C to 18 ° C. In lowland areas, river basins and Negotinska Krajina temperature variations is between 0 and -2 ° C in winter to 22 ° C in summer time. Annual precipitation regime has two maximums, primary in late spring and secondary, in late autumn; winter and summer, the drought periods. Annual precipitation sums rise in average with altitude. In lower regions annual precipitation height range in the interval from 540 to 820 mm (Pavlovic and Ivanovic,2015)

In natural conditions, every animal is infected - constant contamination of the pasture. This is contributed by the increased susceptibility of the already infected herd, the introduction of susceptible animals into the infected herd and the increase in the intensity of the infection in the already infected herd. The seasonal dynamics of certain types of parasites, the degree of infection and the occurrence of diseases vary not only in different areas but also in the same area during the year. The parasite-host relationship is complex: physiological state and general condition, method of cultivation and nutrition, time of lambing, configuration and macroclimate of the soil. Immunity develops through continuous infections and then there is the elimination of the present parasites (self cure mechanism), complete or partial inhibition of the development of newly introduced larvae (spring rise) and complete or partial inhibition of the reproductive abilities of female parasites (spring rise) (Soulsby, 1977; Truong and Baker,1998).

The population pressure of helminths in the organism induces the development of an immune response that is sufficient to provide the host with a solid degree of protection in the case of infections with several parasitic species, which is reflected through mechanisms that prevent the outbreak of clinically manifest diseases. In sheep, in the spring (without a new pasture infection), there is an increased elimination of the number of eggs in the feces of the sheep ("spring rise"), and it is maximal in April, after which "self-healing" follows and the number of parasitic eggs drops to a low level. The high temperature and high degree of insolation of the pasture leads to a temporary increase in the number of eggs only in August, as a result of the summer pasture infection (Familton nad McAnulty,1995; Ash and Truong,2003).

Furthermore, the number of eggs tends to remain at a low level, so that in the spring there will be a "spring rise" again. In lambed sheep, "spring rise" is significantly more pronounced and the later the lambing of the sheep takes place, the greater the increase in the number of eggs.

During our examination species in the genus *Ostertagia, Trichostrongylus* and *Nematodirus* were present after the first appearance of those present during the entire study period. Haemonchus contortus is ordered in animals during the warmer and *Marshallagia marshali* during the colder period of the year. Species in the genus *Cooperia*, and *Oesophagostomum. Bunostomum* were often present in lambs sacrificed during all the monitoring period. Species in the genus *Cooperia*, and *Oesophagostomum. Bunostomum* were often present in lambs sacrificed during the monitoring period At the beginning of our research, conducted in March, the real extent of gastrointestinal infections strongilidae was 83.33%, after which he soon reached a level of 100% in the same way and moved to the end of follow-up period.

The dynamics of the first appearance of established gastrointestinal strongylid species in both populations of small ruminants was as follows (figure 1):

- in March, Ostertagia spp., Trichostrongylus spp. and Nematodirus spp.
- in May, an infection with Bunostomum spp. was recorded. and Chabertia spp. (sheep)
- in June was the first finding of Scriabinema spp;
- in July, eggs of Haemonchus spp. (contortus) and Cooperia spp.
- in November, the presence of Marshallagia spp.

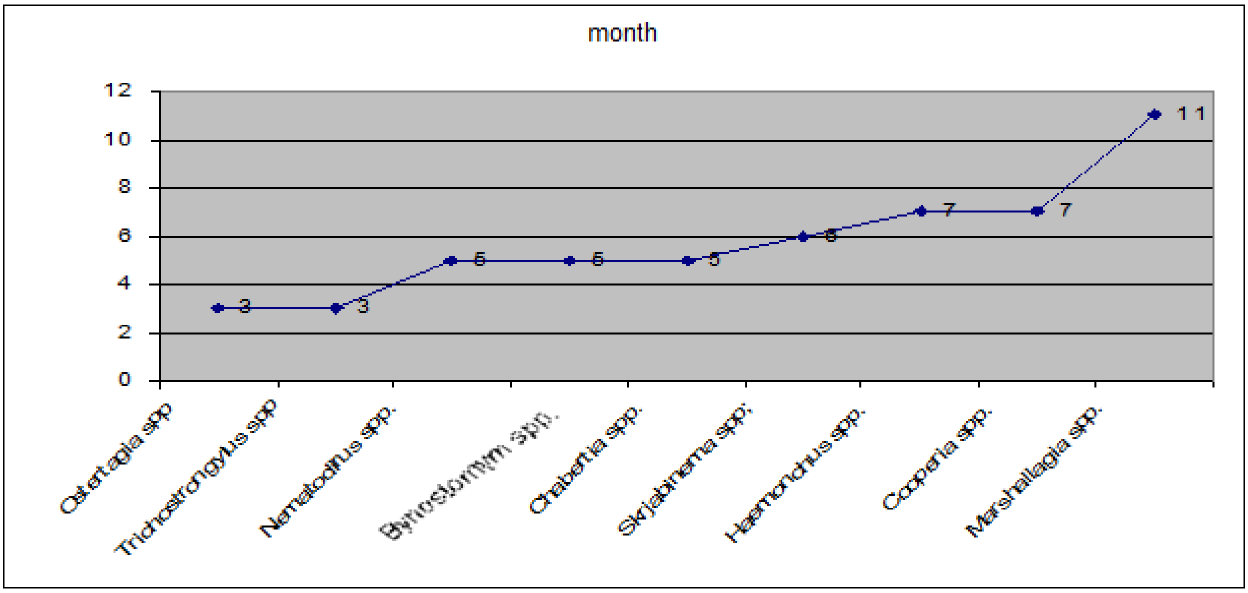

Figure 1. The dynamics of the first appearance of established gastrointestinal strongylid species in both populations of small ruminants

CONCLUSIONS

Impact of climatic conditions play important role on population dynamics and biodiversity of occured helminths species. Related seasonal dynamics and same species of parasites was observed during the investigations performed at goats and sheep breed at other Balkan countries like Montenegro, Romania, Bulgaria, Macedonia or Greek (Georgievski,1991; Zurliiski and Rusev,1990: Theodoropoulos et al.2000; Ardeleanuet al.2007). The data on harmful before and effect of parasitic infections on the sheep and goat performance undoubtedly show that in the anthelminth conditions of rearing high performance animals it is necessary to conduct the measures of prophylactic treatment (Barger et al. 1994; Chartier et al. 2000; Kaplan et al. 2004).

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EVALUATING THE EFFECTS OF *Eucalyptus camaldulensis* LEAF EXTRACTS ON *Meloidogyne incognita:* LABORATORY AND GREENHOUSE EVALUATIONS FOR NEMATODE CONTROL

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ABSTRACT

Root-knot nematodes are among the most damaging nematode groups to plants worldwide. While there are numerous species of nematodes belonging to the *Meloidogyne* genus, *Meloidogyne incognita* is one of the most common root-knot nematode species globally. Chemicals are commonly used to control root-knot nematodes. However, the tendency of environmentally friendly application methods that control nematodes increased in recent years. Nevertheless, the effects of *Eucalyptus camaldulensis* leaf extracts on root-knot nematodes have not been fully understood yet. Therefore, in this study, laboratory and greenhouse applications were conducted against the root-knot nematode *Meloidogyne incognita*, using the leaf extract of *Eucalyptus polybractea* diluted at 1%, 5%, 15%, 30%, 40%, Nematode+water, Non-nematode (control), and Nematicide. The effects of *Eucalyptus camaldulensis* leaf extracts on nematode and plant parameters were determined under laboratory and greenhouse conditions. The results revealed that, following the nematicide application, the highest larval mortality of 70% was observed in the 40% diluted leaf extract. The final nematode number significantly decreased with the application of the 40% diluted leaf extract, following the nematicide treatment. Based on these results, although the application of *Eucalyptus camaldulensis* leaf extracts shows promising results in controlling root-knot nematodes, the effectiveness can be better understood after field trials are conducted.

Keywords: *Eucalyptus camaldulensis, Meloidogyne incognita, Leaf extract, nematode*

INTRODUCTION

Nematode species belonging to the *Meloidogyne* genus exhibit an endoparasitic feeding behavior, causing damage to a wide range of plant species worldwide through the formation of various-sized root galls (Jones et al., 2013; Bozbuga, 2017). Root-knot nematodes (*Meloidogyne* spp.) not only result in crop losses but also impact plant physiology by affecting water and nutrient uptake, leading to wilting. Moreover, they facilitate the entry of other pathogens into plant roots, posing a significant threat due to their status as quarantine organisms (Moens et al., 2009). *M. incognita*, *M. hapla*, and *M. chitwoodi* are among the most common species (Hunt and Handoo, 2009). Root-knot nematodes undergo through various life stages, including eggs, first-stage juveniles, second-stage juveniles (J2), third-stage juveniles, fourth-stage juveniles, and adults, with J2 larvae possessing infective capabilities (Karssen and Moens, 2006).

Resistance genes against root-knot nematodes have been identified (Milligan et al., 1998; Williamson, 1998, 1999), and varieties carrying these resistance genes are preferred because they exhibit resistance to infection and provide an environmentally friendly option (Lopez-Perez et al., 2006). Infection by root-knot nematodes modifies the root's suitability, making it more conducive to subsequent reinfection by these nematodes over time (Kihika et al., 2020). Root-knot nematodes stand out as the group causing the most damage to plants among plant-parasitic nematodes. Their direct damage leads to crop loss, and they also have indirect effects due to quarantine regulations (Perry and Moens, 2013).

In the initial studies related to root knot nematodes in our country, the presence of the *Meloidogyne incognita* species was identified in Malatya and Elazığ (Öztüzün, 1970). In the Central Anatolia region, the rate of contamination with root-knot nematodes ranged from 10% to 94%, with *M. incognita* being the most prevalent species (Enneli, 1980). Additionally, it has been demonstrated that the root-knot nematode *M. incognita* alters the molecular structure of the cell wall, resulting in variations in infection rates (Bozbuga et al., 2018). Root-knot nematode J2s bypass the root endodermis barrier by traveling to the root's elongation zone near the tip, avoiding the Casparian strip, and then proceeding upward through the cortical tissues to establish feeding sites (Grundler et al., 1992). Second-stage larvae of root-knot nematodes induce the formation of large cells within plant roots, and males and second, third, and fourth-stage larvae of this species, which are freely present in the soil, live endoparasitically within the roots (Mıstanoğlu and Devran, 2015).

In recent years, there has been an increasing trend towards the use of environmentally friendly methods in nematode control. However, the effects of *Eucalyptus camaldulensis* leaf extracts on root-knot nematodes have not yet been fully understood. Therefore, this study investigates the impact of *Eucalyptus camaldulensis* leaf extracts on the root-knot nematode *M. incognita* and its morphological effects on the plant.

MATERIAL AND METHOD

This study included main materials such as tomato plants, pure culture of root-knot nematode (*Meloidogyne incognita*), potting soil mix, and laboratory equipment.

a- Measurement of Plant Root and Shoot Height

Plant sizes, including both root and shoot lengths, were measured using a ruler, and the longest root and shoot lengths were recorded in centimeters.

b- Plant Fresh Weight

Plant fresh weight was measured in grams by weighing the roots and stems separately using a precision scale.

c- Plant Dry Weight

Plant dry weight was determined by placing the plants in paper bags, allowing them to place in an oven at 80°C for 24 hours, and then measuring them with a precision scale.

d- Nematode Count

As an initial population, 1.5 nematodes/gram of soil were added to each pot. For nematode counting, 200 grams of soil, along with plant roots, were collected. Nematodes were extracted using the Baermann funnel method in the laboratory, nematodes was counted under microscope and the final nematode population was calculated.

e- Preparation and Application of *Eucalyptus camaldulensis* Extract

Leaves of *Eucalyptus camaldulensis* were collected and dried in the laboratory. The dried leaves were crushed into a powder, and this powder was soaked in an Erlenmeyer flask containing distilled water for 24 hours. The concentration of this solution was considered 100%, and it was diluted with tap water to prepare different concentrations (1%, 5%, 15%, 30%, 40%), which were then applied to the plants in the pots for two months. Additionally, applications were made with nematicide, nematode+water, and non-nematode plants. In the laboratory setting, to assess its effect on nematodes, 100 live second-stage nematode larvae were placed in Petri dishes, and *Eucalyptus camaldulensis* extracts were added. After 24 hours, the viability of the nematodes was determined by counting them under a microscope.

f- Application of Nematodes and Tomato Plants

A pure culture of *Meloidogyne incognita* species was used. The experiment was repeated five times, with negative control plants used for each one. The study was conducted with a tomato variety susceptible to root-knot nematodes. Twenty-five-day-old seedlings were planted in pots containing sterile soil, peat, and sand mixture and were grown in a greenhouse at a temperature of 27°C with 16 hours of light and 8 hours of darkness. Each plant was inoculated with 1.5 J2s/gram soil, and after two months, the plants were uprooted, and their gall index and other parameters were observed and evaluated.

g- Gall Index

After two months of inoculation, the plants were removed, washed, and their root condition was evaluated using the gall index method (Hartman and Sasser, 1985).

h- Statistical Analysis

The data were used to one-way ANOVA and Duncan multiple comparison tests.

RESULTS

a- Plant height

In terms of total plant height (root+shoot), the effect of the 40% *Eucalyptus camaldulensis* extract application was the highest, followed by the nematode+water and nematode-free+water applications, which recorded the tallest tomato plant height. The lowest total plant height was observed in plants treated with 1% *Eucalyptus camaldulensis*. Regarding plant stem height, the tallest shoot was observed in plants treated with nematode+water and 5% *Eucalyptus camaldulensis* extract. The shortest shoot height was found in plants treated with 5% *Eucalyptus camaldulensis* extract. Concerning plant root height, the greatest root height was determined in plants treated with 40% *Eucalyptus camaldulensis* extract, while the shortest root height was observed in plants treated with 5% *Eucalyptus camaldulensis* extract (Figure 1).

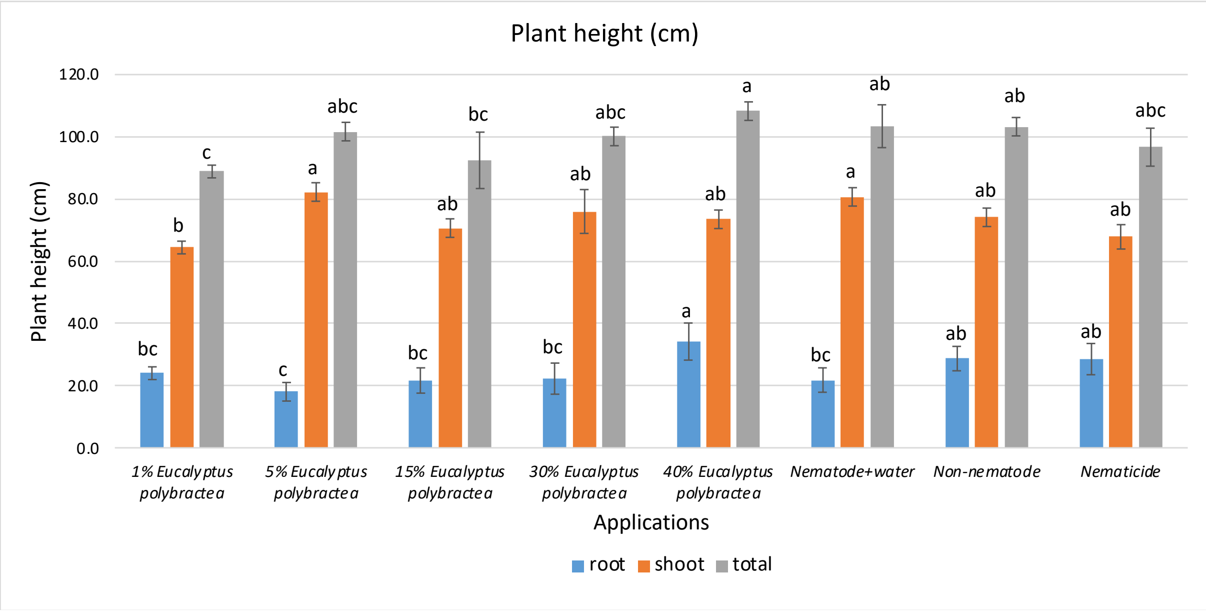

Figure 1. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the root, shoot, and total plant heights of tomato plants. The statistical analysis was performed separately for each group (root, stem, and total) within each group.

b- Plant Fresh Weight

In terms of total plant fresh weight (root+shoot), the effect of the 1% *Eucalyptus camaldulensis* extract application was the highest, followed by the non-nematode application, which recorded the highest tomato fresh weight. The lowest total fresh weight was observed in plants treated with 40% *Eucalyptus camaldulensis*. Regarding plant fresh weight, the highest shoot weight was observed in non-nematode and 1% *Eucalyptus camaldulensis* extract-treated plants. The lowest stem fresh weight was found in plants treated with 40% *Eucalyptus camaldulensis* extract. Concerning plant root fresh weight, the greatest fresh weight was determined in plants treated with 1% *Eucalyptus camaldulensis*, while the lowest root fresh weight was observed in plants treated with nematode+water (Figure 2).

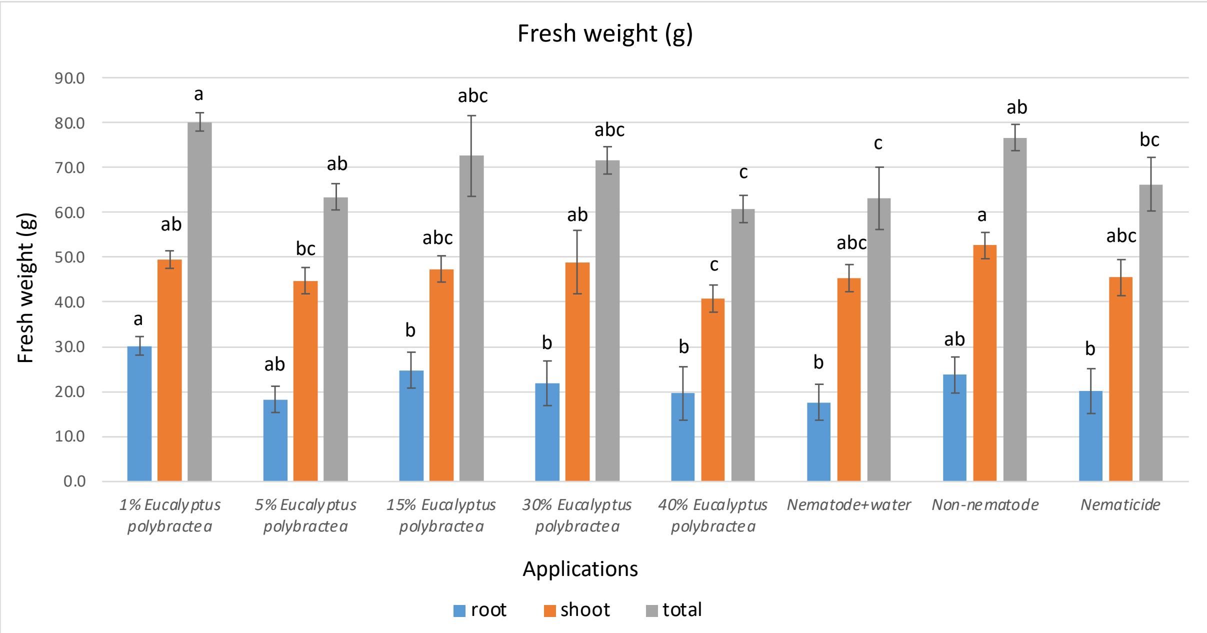

Figure 2. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the root, shoot, and total fresh weights of tomato plants were evaluated. Statistical analysis was conducted separately for each group (root, stem, and total). Letters indicate differences between means in the treatments (P<0.05).

c- Plant Dry Weight

In terms of the total plant dry weight (root+shoot), the effect of the 1% *Eucalyptus camaldulensis* extract application was the highest, followed by the non-nematode application, which recorded the highest tomato dry weight. The lowest total dry weight was observed in plants treated with 5% *Eucalyptus camaldulensis*. Regarding plant dry weight, the highest shoot weight was observed in non-nematode and 1% *Eucalyptus camaldulensis* extract-treated plants. The lowest stem dry weight was found in plants treated with 5% and 40% *Eucalyptus camaldulensis* extract. Concerning plant root dry weight, the greatest dry weight was determined in plants treated with 1% *Eucalyptus camaldulensis*, while the lowest root dry weight was observed in plants treated with nematode+water (Figure 3).

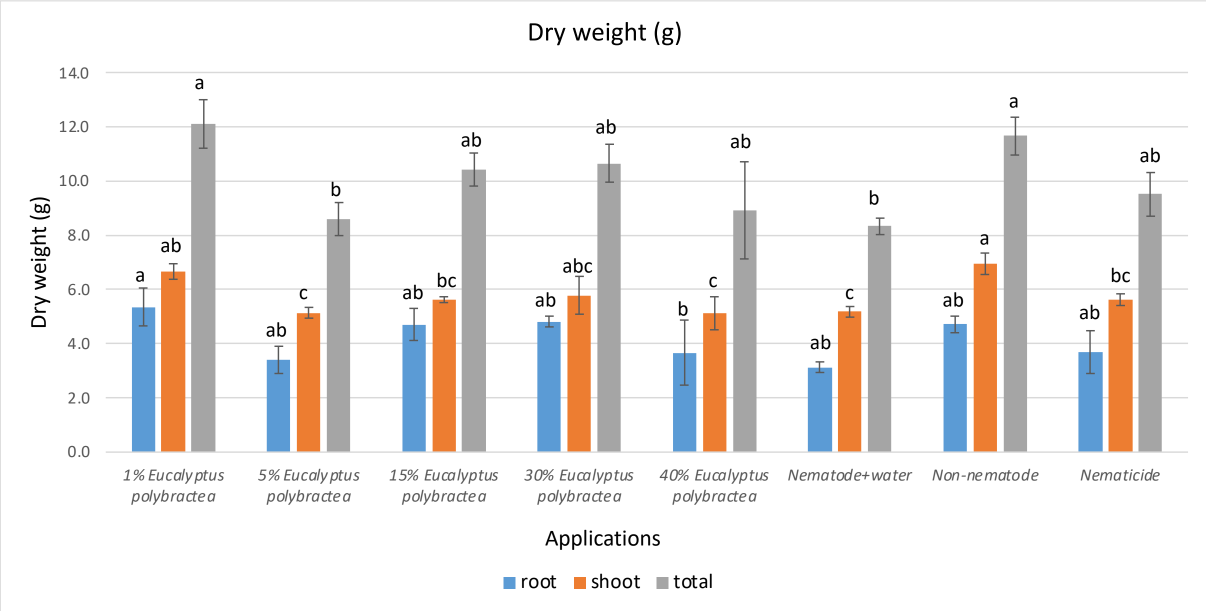

Figure 3. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the root, shoot, and total dry weights of tomato plants were assessed. Statistical analysis was performed separately for each group (root, stem, and total). Letters indicate differences between means in the treatments (P<0.05).

d- Root Gall Index

The lowest gall index in tomato roots caused by nematode infection was found in the nematocide treatment. There was no statistically significant difference in the gall index among the *Eucalyptus camaldulensis* applications of 1%, 5%, 15%, 30%, and 40% (Figure 4).

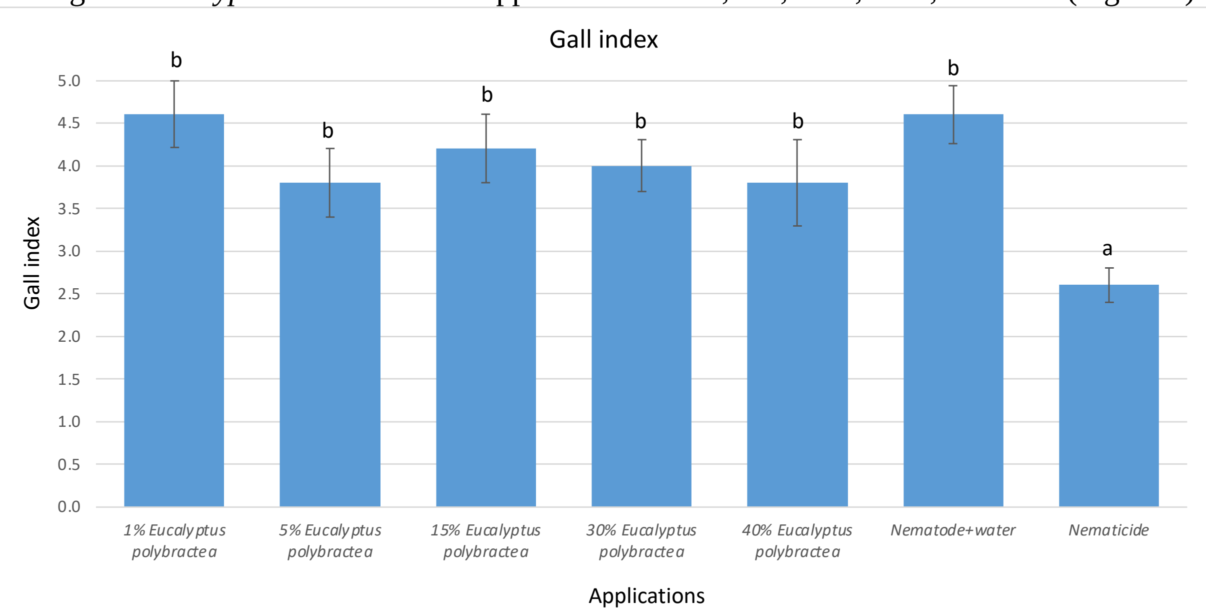

Figure 4. The effects of *Eucalyptus camaldulensis* leaf extract and other treatments on the nematode gall index (0-5) in tomato plant roots were evaluated. Letters indicate differences between means in the treatments (P<0.05).

e- *Eucalyptus camaldulensis* Effect on Larva Death in Lab Condition

In the laboratory-based study, the lowest larval mortality rate was found in the water and 1% *Eucalyptus camaldulensis* applications, with the least larval mortality rate being a percentage.

The highest larval mortality rate was observed in the nematicide treatment, followed by the 40% *Eucalyptus camaldulensis*, 30% *Eucalyptus camaldulensis*, 15% *Eucalyptus camaldulensis*, 5% *Eucalyptus camaldulensis*, and 1% *Eucalyptus camaldulensis* applications, respectively (Figure 5).

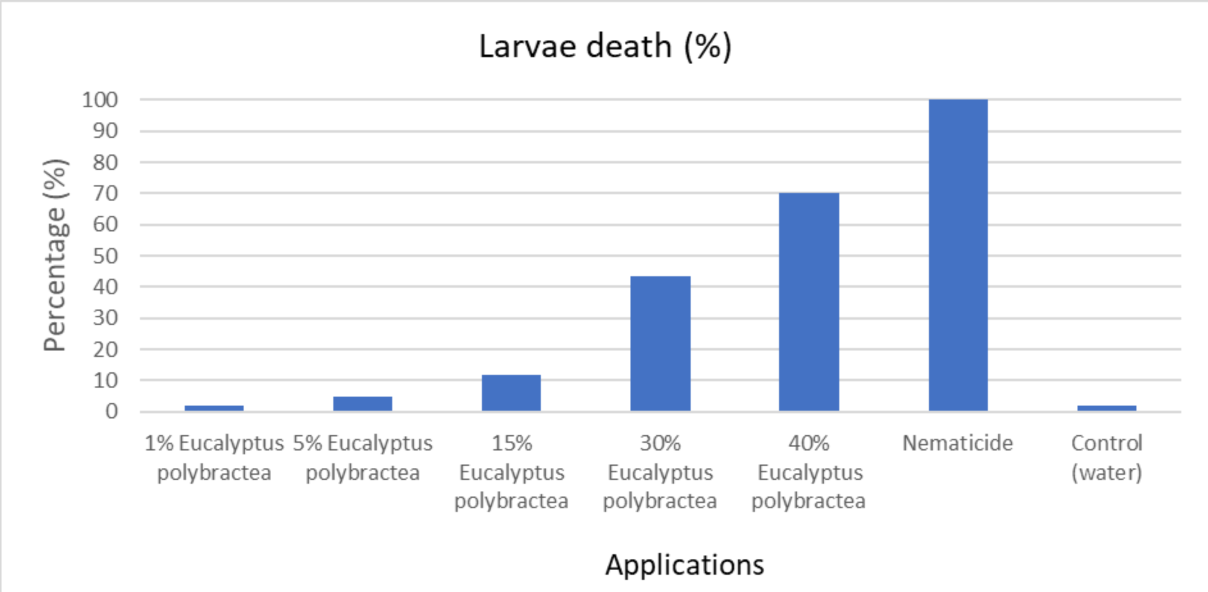

Figure 5. The effects of *Eucalyptus camaldulensis* leaf extracts and other treatments on second-stage larvae of the root-knot nematode *Meloidogyne incognita*.

f- *Eucalyptus camaldulensis* Effect on Nematode Population in Greenhouse

The study aimed to determine the effects of *Eucalyptus camaldulensis* leaf extracts and other treatments on the population of the root-knot nematode *Meloidogyne incognita*. It was found that nematode populations were reduced in the nematode+water, 5% *Eucalyptus camaldulensis*, and 1% *Eucalyptus camaldulensis* applications. In other applications, including 40% *Eucalyptus camaldulensis*, 30% *Eucalyptus camaldulensis*, and 15% *Eucalyptus camaldulensis*, the nematode population decreased, but the lowest nematode population was observed in the nematicide treatment (Figure 6).

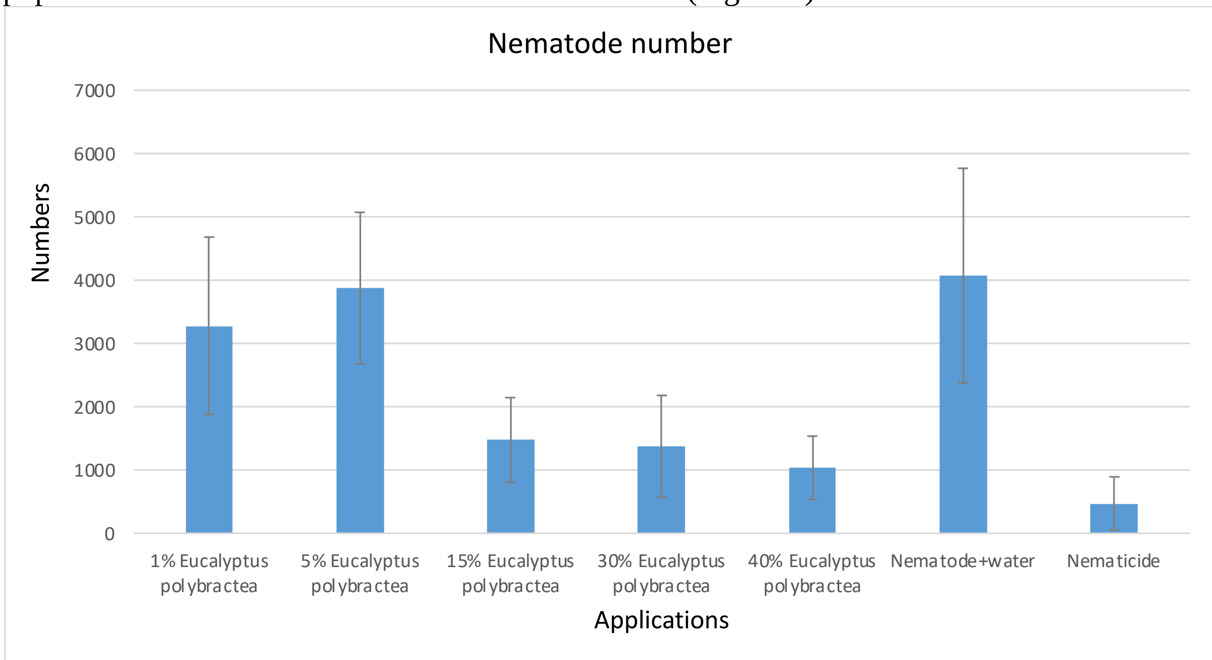

Figure 6. The effects of *Eucalyptus camaldulensis* leaf extracts and other treatments on the populations of the root-knot nematode *Meloidogyne incognita*

DISCUSSION

In the world, there are over 100 species of root-knot nematodes, with *Meloidogyne incognita* being one of the most common and harmful species (Moens et al., 2010). Worldwide, *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are reported as common species (Sasser and Carter, 1985). In the Central Anatolia region, the rate of infection with root-knot nematodes ranges from 10% to 94%, with *M. incognita* being the most common species (Enneli, 1980). In this study, the most common root-knot nematode species, *M. incognita*, was selected. Nematodes belonging to the *Meloidogyne* genus have a permanent endoparasitic feeding habit, causing various sizes of galls in the roots of their hosts, and they harm a wide range of plant species worldwide (Bozbuga, 2017). In this study, after the inoculation of second-stage larvae of root-knot nematodes, galling occurred in the roots, and according to the gall index, no statistical difference was found in the application of *Eucalyptus camaldulensis* leaf extracts (Figure 4). Although larval mortality was successful in laboratory applications (Figure 5), it is thought that the soil is influenced by various factors. Additionally, the root-knot nematode *M. incognita* alters the molecular structure of the cell wall, resulting in differences in the infection rate of the nematode (Bozbuga et al., 2018).

Plant defenses in response to pathogens are influenced by phytohormones like Saliclic acid and jasmonic acid, and less-studied hormones, such as auxin, cytokinin, gibberellic acid, and abscisic acid, are also involved in plant-nematode interactions and defense (Gheysen and Mitchum, 2019). Plant extracts can exhibit nematicidal effects on nematodes. For example, the application of *Lantana camara* extract on *Meloidogyne incognita* larvae caused high mortality (Begum et al., 2000). In this study, the nematode population decreased in the applications of 40% *Eucalyptus camaldulensis*, 30% *Eucalyptus camaldulensis*, and 15% *Eucalyptus camaldulensis* (Figure 6). Similarly, the extract of *Lantana camara* plant reduced root-knot nematode populations and caused high mortality in *Anguina tritici* (Dura et al., 2019). The effect of *L. camara* ferment on *M. javanica* in tomato plants was reported (Malahlela et al., 2021). Strengthening the endodermal tissues in roots not only reduces RKN penetration but also diminishes the development of feeding sites and galls, indicating that reinforcing the endodermis enhances resistance to Root knot nematodes across the nematode's entire life cycle (Rutter et al., 2022).

In the study of *Eucalyptus camaldulensis* leaf extract applications on plant fresh weight, dry weight, and plant length (Figure 1, 2, 3), the high values of certain parameters at low doses are also considered as a possible fertilizing effect on the plant. The failure of some parameters to increase at high doses suggests a potential toxicity effect. In future studies, working with more complex and detailed dosage values is believed to provide a more comprehensive understanding of the effects of this plant's extracts on nematodes.

CONCLUSION

Based on the results of the studies, the application of 40% *Eucalyptus camaldulensis* leaf extracts is considered to have the potential for nematode control. Additionally, conducting field studies and investigating the molecular mechanism in the future is believed to be essential to understand the plant extract-nematode host relationship.

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APPLICATION OF MOBILE FLUORESCENCE SPECTROSCOPY AS A METOD IN THE DETERMINATION OF VARIETAL DIFFERENCES IN RADISH (Raphanus sativus) SEEDS

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ABSTRACT

Standard methods used for the quality of seed are relatively slow and require expensive consumables. A fiber-optic mobile installation for the investigation of radish (Raphanus sativus) seeds has been successfully adjusted and tested. The proposed method includes studies of radish seeds from standard varieties and those from first generation hybrids by dint of fluorescence spectroscopy. The spectral distributions are unique to the seeds of a specific variety or hybrid. This fact gives reason for the use of the installation in recognizing available radish seeds of un-known origin in a non-invasive way with high accuracy. The stability of a breeding line and its common blacks with an established variety of the same species can be monitored by monitoring the signal intensity. The stability and signal intensity level of the hybrid significantly differ from those of the standard varieties. Spectral distribution with reflected emission wavelengths of the studied radish seeds of the standard variety and first generation hybrids show a clear deviation of the hybrid from the characteristic distribution of the standard varieties.

Keywords: Fluorescence spectroscopy, Radish seeds, Standard varieties, First generation hybrid

INTRODUCTION

Radish (*Raphanus sativus*) is an annual root vegetable plant from the Cruciferous family. It originates in Central Asia. It has been cultivated as a vegetable crop since about 1000 BC in China, Japan, Egypt, Rome and Greece. There are two groups of vari-eties - European and Chinese (Kaneko et al., 2007).

Radishes are for fresh consumption. Their widest application is in making fresh vegetable salads. They are not suitable for heat treatment. Radishes are easy to grow root vegetables. They tolerate most soil types and grow rapidly (Perez Gutierrez et al., 2007). Growing radishes from seed is like magic; you throw the seeds on the ground and cover them with a little soil. You water them and watch them grow. It is important to note that radishes always grow from seeds planted directly into the soil where they grow to full maturity (Kyung-Mi et al., 2015).

The development of fast and accurate methods such as optical diagnostics based on non-destructive analysis will help to overcome the barriers in studying and mon-itoring the processes related to seed germination and guarantee quality planting mate-rial and seeds for agro-industry and farmers (Huyan et al., 2018)

The optoelectronic methods for assessing the quality of plant seeds are non-contact, fast-acting, selective, and do not destroy the integrity of the examined sample. On the basis of these, it is possible to create non-invasive methods for the evaluation of radish seeds. Until now, there has been no data on their re-search using the proposed method. Belyakov, 2019 obtained results in the study of cereal seeds. Based on his research, emission excitation wavelengths of 362 nm (Rewatrak, 2020), 424 nm (Zhang et al., 2021) and 485 nm (Singh et al., 2021) were established. In these studies, it was found that during the ripening of seeds of cereal plants (for example, wheat, oats, and corn), the ratio of their excitation levels and changes in radiation for immature seeds is characteristic of the short-wave range, and long-wave prevails in mature seeds. The dependence of the ratio of long-and short-wavelength fluxes on the maturation time increases and can be statistically reli-ably approximated by the linear functions required to create a database.

Belyakov et al., 2021 developed a sensor for determining the level of physiological maturity of seeds, allowing by irradiating seeds with two sources at certain wavelengths and re-cording the photoluminescent flow with appropriate receivers to determine the stage of seed maturation . The maximum luminescence is less pronounced than in the exci-tation spectrum.

The spectral luminescence characteristics of forage plant seeds were meas-ured by scarification during the study. The spectral characteristics of the seeds in-crease, due to the scarification of forage plants. It was established that in the studied seeds with repeated scarification, the observed qualitative changes in the excitation spectrum were related to the appearance of a new maximum at a wavelength of 423 nm. Likewise, for radish seeds from standard varieties and those from first generation hybrids, the obtained results can be used to create a schematic fiber-optic configuration for character-ization of planting material from radish.

The excitation and photoluminescence spectra of seeds of agricultural plants, legumes 10. (Su et al., 2019) and tomatoes (Li et al., 2019) were measured using a previously developed method. The typical excitation spectrum was found to be in the range of 355–500 nm and to have two maxima: the main one at 424 nm and the side one at 485 nm. The emission spectrum is in the range of 420–650 nm and has a maximum in the region of 500-520 nm.

The water contained in the planting material (together with impurities) is expressed as a percentage of its total mass. In addition, with them, it will be possible to evaluate the percentage of their normal seed germination under optimal conditions for germination in a period shorter than the period for which the germination rate is determined, which will determine their germination energy. The application of optoelectronics in the analysis of radish seeds will lead to a rapid and correct determination of the sowing rate, since it will be possible to assess the germination capacity of all clean seeds. A study of radish seeds from standard varieties and those from first generation hybrids was conducted, which aims to develop a non-invasive method for their quality, through the application of the system engineering approaches of modern optoelectronics.

The purpose of the study is to introduce fluorescence spectroscopy as a sensory method for research depending on radish seeds from standard varieties and those from first generation hybrids and the possibilities of its use as a potentially useful tool in the field of agriculture.

MATERIAL AND METHOD

Materials: Seeds of three standard radish varieties and one first generation hybrid variety were investigated

• French breakfast: The variety is suitable for spring and autumn field production. The rhizomes are single and oblong, with a white five around the tail. The fleshy part is white and crispy. The vegetation period is 30 days. The sowing rate is 1.5-2.5 kg per hectare

• Nacional 2: The variety is an early field variety that produces large, round, red roots with white tips. Its flesh is white, delicate in taste and crunchy.

• Red large: The variety is medium early. It is suitable for spring and autumn field production. The fruits are single, deep red, the fleshy part is white with excellent taste qualities. The variety is resistant to cracking.

• Espresso F1: A very early variety with round rhizomes colored red. It is hardy with a fine root, a strong bond with the foliage. It has very good transportability and storage. It is recommended for growing in winter and very early in open areas

Methods

The mobile spectral installation for the study of fluorescence signals was designed specifically for the rapid analysis of plant biological samples. Mobile experimental in-stallation used by fluorescence spectroscopy contains the following blocks:

• Laser diode (LED) with an emission radiation of 245 nm with a supply volt-age in the range of 3V. It is housed in a hermetically sealed TO39 metal housing. The emitter has a voltage drop of 1.9 to 2.4V and a current consumption of 0.02A. The minimum value of their reverse voltage is – 6 V

• Forming optic, which is a hemispherical lens made of N-BAK2 glass. The post-LED forming optics is defined mainly for its refractive, dispersive and ther-mo-optical properties, as well as for its transparency in the UV range [240 280] nm

• Quartz glass area 4 cm2. Its optical properties are to be transparent to visible light and to ultraviolet and infrared rays. This allows it to be free of inhomogeneities that scatter light. Its optical and thermal properties exceed those of other types of glass due to its purity. Light absorption in quartz glasses is weak.

• CMOS detector with photosensitive area 1.9968×1.9968 mm. Its sensitivity ranges from 200 nm to 1100 nm. Its resolution is $\delta\lambda = 5$. The profile of the detector sen-sor projections along the X and Y axes is also designed for very small amounts of data, unlike widely used sensors.

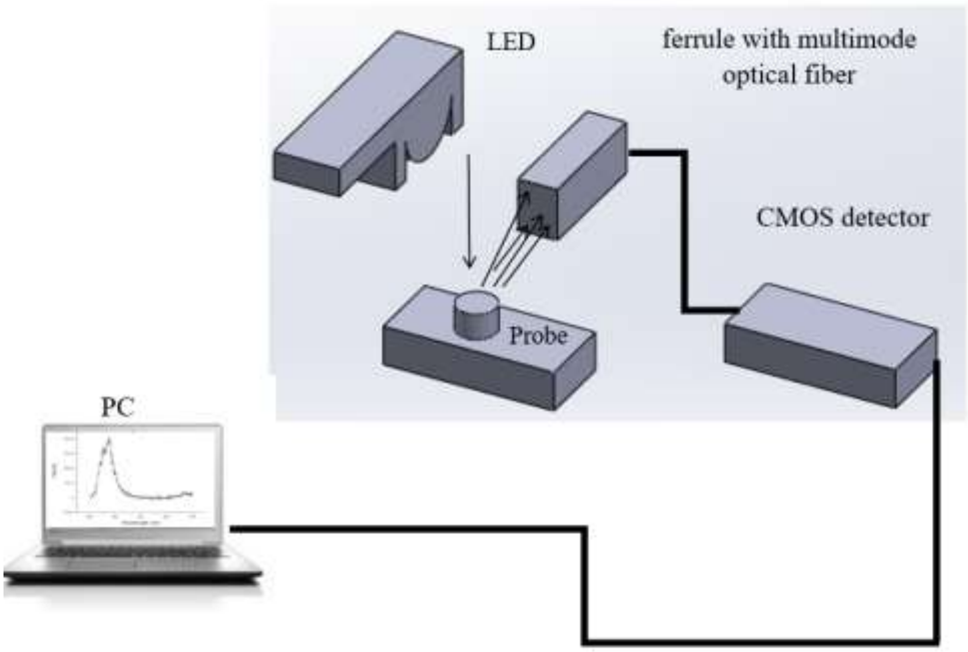

Figure 1. Mobile experimental installation used by fluorescence spectroscopy

The radiation is led from the LED through the forming optics block by means of a quartz fiber. The secondary radiation from the illuminated sample (visible spectrum) – illuminated by the impacting UV radiation is coupled to the CMOS detector by means of light-guide optics. The quartz multimode fiber has a step index of refraction and a numerical aperture of 0.22. In the CMOS detector, the light signal is converted into an electrical-digital signal and, by means of a USB 2.0 wire, it is taken for analysis and downloading of the data to a laptop.

The mobile fluorescence analyzer shown in Figure 1 is a proprietary development. It has been successfully applied for agricultural purposes. Through the application of fluorescence spectroscopy, an accessible and rapid system engineering approach is es-tablished for the analysis of different samples (in the specific case of seeds) with a sin-gle device. Seeds, bulbs, rhizomes and other reproductive organs can be examined equally effectively. The unique advantage of the mobile fluorescence analyzer must be that the sample analysis can be performed on site, without being transported to a la-boratory. The analysis method can be applied to mass tests. The mobile fluorescence analyzer is easy to operate and affordable. Fluorescence analysis by means of the ana-lyzer can replace labor-intensive and very consumable tests in trivial microbiology la-boratories. The three main advantages of the mobile fluorescence analyzer are: the method is fast, does not require consumables and is mobile. The fluorescence method is suitable for early diagnosis of samples, to establish the presence of deviations from standardizations, before their visible occurrence and visualization.

RESULTS AND DISCUSSION

Figure 2 shows a spectral distribution with reflected emission wavelengths of the in-vestigated seeds of standard radish varieties. The moisture content of the seeds de-pends directly on the intensity of the signal, with the highest moisture content being the seeds from French Breakfast, followed by Nacional 2, and the lowest moisture content being those from

Red Large. Figure 3 presents a spectral distribution with re-flected emission wavelengths of a standard radish variety and first generation hybrid seeds (Espresso F1).

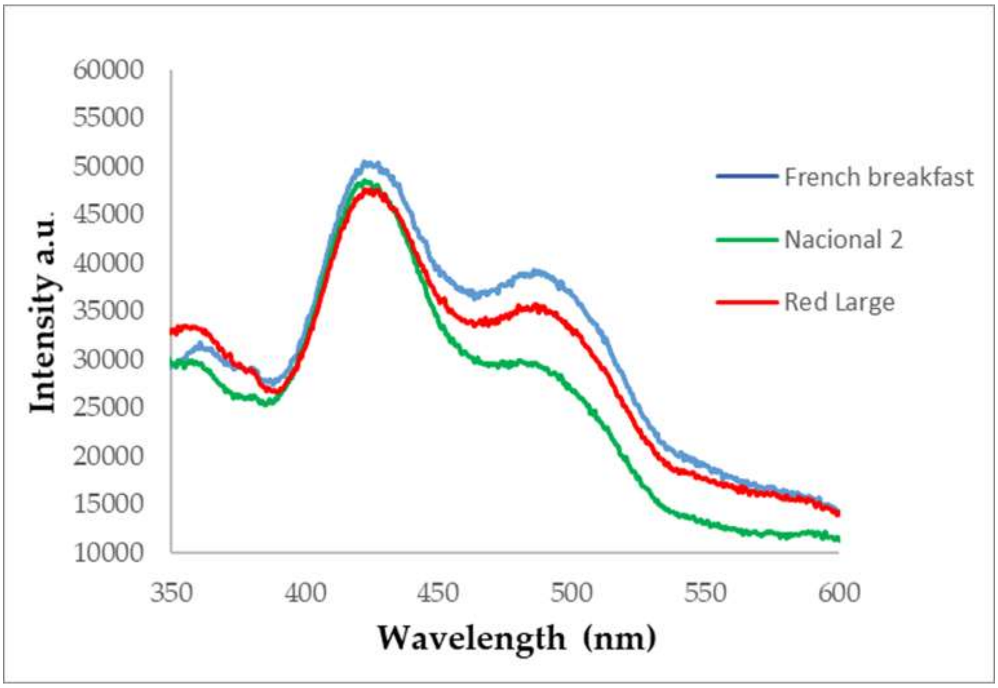

Figure 2. Emission wavelengths of different standard varieties radish seeds

The moisture content of the seeds depends directly on the intensity of the signal, with the higher moisture content of French Breakfast seeds. Figures 2 and 3 present the spectral distributions with reflected emission wavelengths of the investigated standard varieties and first generation hybrid seeds. These distributions are unique to the seeds of a particular variety or hybrid, which justifies the use of the plant in recognizing available radish seeds of unknown origin in a non-invasive way with high accuracy.

Spectral distribution with reflected emission wavelengths of the studied seeds standard variety and first generation hybrid seeds show a clear deviation of the hybrid from the characteristic distribution of the standard varieties, which is observed in figure 2. The lower peak characterizing the radish seeds in the section between 480 and 520nm in the hybrid is not observed. This is due to its production technology, through continuous genetic selection in order to obtain certain desired biological characteristics. In Espresso F1 characteristics and their reproduction among themselves is also due to its much lower intensity at the most pronounced emission wavelength region in radish seeds between 400-445nm.

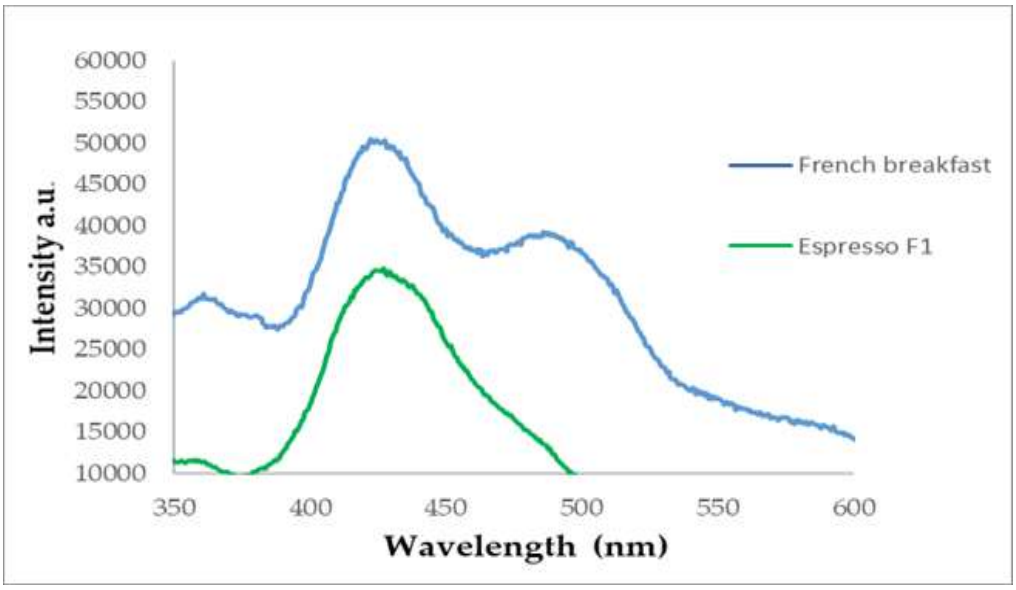

Figure 3. Emission wavelengths of standard variety and first generation hybrid seeds

DISCUSSION

The purpose of conducting the laboratory analysis, which includes studies of radish seeds from standard varieties and those from first generation hybrids, is to adjust a mobile plant for the study of radish seeds with the necessary parameters. During the tests carried out by means of optoelectronic spectroscopy of the seeds, by means of a fiber-optic spectrometer, a clear correlation between their emission signals is visible. This study shows that fluorescence spectroscopy is applicable in the study of radish seeds. The spectral setup based on fluorescence signals allows recording both the emission spectrum and the spectrum of the excitation source. The emission spectrum represents the wavelength distribution of an emission measured for a constant excitation wavelength. The excitation spectrum represents the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength. This spectrum is represented as a dependence of the light wavelength on the light intensity falling on the photodetector, which is part of the installation.

CONCLUSIONS

The systems engineering approach of adjustment (optical setting up) a specialized flu-orescence spectroscopy applied research setup was found to be applicable in the analysis of radish seeds from standard varieties and those from first generation hybrids.

A non-destructive method for evaluating pretreated radish seeds from standard varie-ties and those from first generation hybrids are demonstrated

With a sufficiently well-structured data library, fluorescence spectroscopy can be ap-plied to analyze radish seeds from standard varieties and those from first generation hybrids

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PROBIOTIC PROPERTIES OF *LACTOBACILLUS FERMENTUM* AND *PEDIOCOCCUS PENTOSACEUS* ENCAPSULATED WITH OR WITHOUT HYDROGEL OF CELLULOSE MICROFIBER FROM OIL PALM LEAVES

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ABSTRACT

Probiotics have gained significant attention in recent years because they can improve gut health, boost the immune system, and positively affect human well-being. This study aimed at an *in vitro* characterization of the probiotic properties of *Lactobacillus fermentum* InaCC B1295 (LFB1295) and *Pediococcus pentosaceus* strain 2397 (PP2397) encapsulated with or without cellulose microfiber hydrogel (CMFH) from oil palm leaves (OPL). The experimental design used in this research was an *in vitro* study. The safety of LFB1295 and PP2397 encapsulated with or without CMFH from OPL was assessed, along with the probiotic qualities of auto-aggregation, coaggregation, and hydrophobicity. Hemolytic activity, biogenic amines, cytolysin, gelatinase production, and antioxidant activities (hydroxyl radical-scavenging and DPPH radical-scavenging abilities) were also assessed. The results showed that *in vitro* safety tests showed that PP2397 and LFB1295 cells encapsulated with or without HMCF did not have hemolytic activity and did not produce biogenic amines, cytolysin, or gelatinase. LFB1295 and PP2397, enclosed in CMFH from OPL, compared to free cells, showed higher antioxidant activity. Compared to free cells, LFB1295 and PP2397 encapsulated in CMFH from OPL showed higher antioxidant and auto-aggregation capabilities. LFB1295 free cells, however, exhibited the highest hydrophobicity score. Compared to free cells and cells enclosed in these two LABs, LFB1295 and PP2397 exhibited a higher rate of coaggregation with the harmful bacteria *S. aureus* and *E. coli*. The present finding showed that CMFH-encapsulated LFB1295 and (PP2397 were safe probiotics with outstanding antioxidant activity, autoaggregation, coaggregation, and hydrophobicity.

Key words: Probiotics, *Lactobacillus fermentum, Pediococcus pentosaceus*, Cellulose Microfiber, Oil Palm Leaves

INTRODUCTION

Live bacteria, known as probiotics, can help the host's health when given in sufficient quantities (Kechagia et al., 2013). These helpful bacteria have attracted much interest recently because of their ability to enhance gut health, strengthen the immune system, and have other sound effects on human well-being (Salminen & van Loveren, 2012; Borchers et al., 2009; Galdeano et al., 2019).

Probiotics must pass stringent testing to verify their efficacy and safety before being ingested (Dicks & Botes, 2010; Shi et al., 2016). Several safety tests are carried out to evaluate the potential dangers connected with probiotic strains. These tests involve determining the levels of gelatinase, biogenic amines, and hemolytic activity. The ability of a strain to destroy red blood cells is known as hemolytic activity. Biogenic amines are substances made by bacteria that, in high concentrations, can be poisonous. While gelatinase synthesis is an enzyme that breaks down the creation of gelatin protein, cytolysin is a protein produced by specific bacteria that can harm host cells (Bover-Cid & Holzapfel, 1999; Borges et al., 2013).

Probiotics' different functional qualities are also the subject of research, in addition to safety assessments. These properties significantly impact determining the potential health benefits of probiotic strains. Antioxidant activity, auto-aggregation, coaggregation, and hydrophobicity are a few examples of functional qualities that are frequently examined (Kumar et al., 2020; Xing et al., 2017).

Hydroxyl radicals are free radicals that can cause lipid peroxidation in cell membranes so that cells are damaged. If this condition continues, it will cause an imbalance between free radicals and endogenous antioxidants, called oxidative stress. Hydroxyl radicals can be produced by a biological Fenton reaction, which is a reaction between Fe2+ and hydrogen peroxide under in vitro conditions so that the antioxidant activity of hydroxyl radicals can be measured (Zhang *et al*., 2013). Hydroxyl radicals are highly reactive free radicals formed in biological systems and implicated in lipid peroxidation. Amino acids, phospholipids, and DNA are substances that hydroxyl radicals react within living cells. Lactobacillus can produce endogenous antioxidants as scavengers of hydroxyl free radicals to prevent oxidative stress (Kim et al., 2006).

Probiotic bacteria can group and form clumps, a process known as auto-aggregation. The colonization and adhesion of probiotics to the gut lining, which supports their positive effects, depends on this feature. Contrarily, coaggregation refers to the interaction of probiotics with potentially dangerous bacteria, resulting in aggregation and suppression. The probiotic strains' propensity to attach to hydrophobic surfaces is called hydrophobicity. The colonization and durability of probiotics in the digestive system are greatly influenced by this characteristic (Frese et al., 2012; Priadi et al., 2020).

Other probiotic properties have been looked into in several earlier research. For instance, a study by Kim et al. (2022) assessed a probiotic strain's safety by determining its hemolytic activity, biogenic amine synthesis, and production of cytolysin and gelatinase to ensure its acceptability for human ingestion. In a different investigation, the DPPH and ABTS tests were used to examine the antioxidant activity of several probiotic strains. Their functional features, including antioxidant activity, auto-aggregation, coaggregation, and hydrophobicity, are also intensively researched to ascertain their potential health advantages. This research increases understanding and probiotic strain development for improving human health. (Abushelaibi et al., 2017; Botta et al., 2014).

Encapsulation of probiotics aims to increase the viability of probiotics. Encapsulants can be obtained from various natural and synthetic polymers such as carbohydrates (starch, pectin, sucrose, cellulose, alginate, and chitosan), lipids (wax, paraffin, diglycerides, and monoglycerides) and proteins (milk, gluten, casein, gelatin, and albumin) (Setiarto *et al*., 2018). Probiotic encapsulation using alginate and chitosan can increase the viability of Lactobacillus sp in the digestive tract (Trimudita & Djaenudin, 2021). The survival of *Pediococcus pentosaceus* Li05 encapsulated in microgels was significantly increased after exposure to simulated intestinal fluids (Xie et al., 2021). This study reports the use of cellulose microfiber (CMF) encapsulants from oil palm leaves on the safety and probiotic properties of *Lactobacillus fermentum* InaCC B1295 (LFB1295) and *Pediococcus pentosaceus* strain 2397 (PP2397) *in vitro*.

MATERIALS AND METHODS

This study was conducted experimentally using a completely randomized design (CRD) with four treatments of LAB cells encapsulated and not encapsulated with CMF hydrogel from oil palm leaves, and each treatment was repeated four times, resulting in 16 experimental units. The treatments in this study were WPP (*Pediococcus pentosaceus* Strain 2397 cells without encapsulation), WLF (*Lactobacillus fermentum* InaCC B1295 cells without encapsulation), EPP (*Pediococcus pentosaceus* Strain 2397 cells encapsulated with CMF hydrogel from oil palm leaves) and ELF (*Lactobacillus fermentum* InaCC B1295 cells encapsulated with CMF hydrogel from oil palm leaves).

2.1. Bacterial Propagation and Separation of Cells

Propagation of LFB1295 and PP2397 referred to Pato et al. (2017). Pato et al. (2021) refer to the separation of cells and supernatant. Active cultures of strains LFB1295 or PP2397 were inoculated separately into sterile MRSB medium and incubated for 24 hours at 37°C. This active culture was centrifuged for 15 minutes at 4°C at 4500 rpm after incubation to separate the cells from the supernatant. Then, the cells obtained were washed twice with sterile distilled water until clean cells were obtained from the medium. Furthermore, the cells were removed by adding phosphate buffer with a pH of 7. The addition of phosphate buffer as much as 1:1 with the cells was obtained, then put into a clean container and stored at refrigerator temperature.

2.2. Preparation of Cellulose Microfiber

The preparation of cellulose microfiber (CMF) from oil palm leaves (OPL) refers to Pato et al. (2021). The OPL was cut into small pieces with a length of ± 0.5–1 cm and then dried in an oven at 60°C for 4 hours. The dried OPL was soaked as much as 2.5 kg in 10 L of 6% KOH solution at room temperature for 12 hours. After that, the OPL was washed with water three times. Furthermore, the washed OPL was soaked in a hypochlorite solution for 5 hours. Then, the OPL fiber was filtered and washed with water until the pH was neutral (pH 7). The OPL fiber was then dried in an oven at 60°C for 4 hours, pulverized with a blender, and filtered using an 80 mesh. The sample was processed with CMF by grinding it in a planetary ball mill for 60 minutes at 8,000 rpm. The CMF was obtained by sieving the milling output using a 100 mesh.

2.3. Preparation of Sterile Cellulose Microfiber Hydrogel

Sterile CMF hydrogel (CMFH) was prepared according to Fung et al. (2011). CMF from OPL was mixed with 8% PVA using a ratio of 1:10 while heating with a hot plate and magnetic stirrer until dissolved, then sterilized at 121°C for 15 minutes.

2.4. Preparation of Lactic Acid Bacteria Encapsulation

Lactic acid bacteria (LAB) encapsulation was prepared according to Pato et al. (2021) by adding 40 ml of cell biomass to 40 ml of sterile CMF hydrogel, then stirring using a stir bar until well mixed, and the encapsulated LAB is ready for use.

2.5. Safety Evaluation of Probiotics

Evaluation of the safety of lactic acid bacteria so they can be used as probiotics includes hemolytic activity, cytolysin production, gelatinase, and biogenic amines.

2.5.1. Hemolytic Activity Assay

The hemolytic activity test was conducted according to Pisano *et al.* (2014). LFB1295 or PP2397 cells without or encapsulated by CMFH were streaked on Columbia blood agar (CBA) plates, added with 5% sheep blood agar, and then incubated at 37°C for 24 hours. A clear zone around the colony is a positive result, indicating that the probiotic has hemolytic activity.

2.5.2. Cytolysin Production Assay

Cytolysin production assay referred to Tan *et al.* (2013) with slight modifications. LFB1295 or PP2397 cells without or encapsulated by CMFH were streaked on brain heart infusion (BHI) agar medium, supplemented with 5% (v/v) sheep blood agar. After incubation at 37°C for 24 hours, the clear zone around the colony was considered positive, indicating that the probiotic produced cytolysin.

2.5.3. Gelatinase Production Test

Tan et al. (2013) referred to the gelatinase production test. LFB1295 or PP2397 cells without or encapsulated by CMFH were streaked on BHI agar medium to which 1.5% skim milk was added. The plates were incubated for 24 hours at 37°C. A clear zone around the colony is a positive result, indicating that the probiotic produces gelatinase.

2.5.4. Biogenic Amine Production Test

The biogenic amine production was performed as described (Casarotti et al., 2017). The test used LAB cells without or encapsulated by CMFH that were subcultured twice at intervals of 24 hours in MRS Broth containing 1% of 4 amino acids (AA). Pyridoxal-5-phosphate was introduced as a carboxylase code factor at a concentration of 0.005%. Then, LAB cells were streaked in Duplo on Decarboxylases Agar (DA), which included one of the previous AA and the purple bromocresol. Cultivation took place for 24 hours at 37°C. The control was DA medium devoid of AA. The medium's color shift from brown to purple, which denotes an increase in pH (probiotics make biogenic amines), is a positive outcome.

2.6. Antioxidant Activity

Antioxidant activity measured in this study included DPPH radical scavenging ability and hydroxyl radical scavenging (HRS) activity.

2.6.1. Scavenging Ability of DPPH Radicals

The scavenging ability of DPPH radicals was carried out using the method of Shehata *et al.* (2019) and Tristantini *et al*. (2016). DPPH solution was prepared by dissolving 0.1 g of DPPH in 100 ml of methanol. Furthermore, 1.3 ml of culture supernatant from LFB1295 or PP2397 cells without or encapsulated with CMFH was transferred to a test tube containing 5 ml of DPPH solution with a concentration of 500 ppm, 250 ppm, 125 ppm, and 62.5 ppm in the dark, then homogenized using a vortex mixer. The solution was then incubated for 30 minutes. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 517 nm. The DPPH radical inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs control = absorbance of control solution, and Abs sample = absorbance of the culture supernatant.

Data on inhibition and absorbance values were then processed and analyzed using the linear regression equation y = ax + b. This equation is used to calculate the IC_{50} value.

2.6.2. Hydroxyl Radical Scavenging Activity

HRS activity was carried out using the Fenton reaction method, according to Shehata *et al.* (2019). Fenton solution was prepared by mixing 1.0 mL of 0.435 mM brilliant green, 2.0 mL of 0.5 mM $FeSO_4$, and 1.5 mL of 3.0% H_2O_2, w/v. Lactobacillus fermentum InaCC B1295 encapsulated by CMF hydrogel was grown in MRS broth medium at 37°C for 24 hours. The LAB culture was centrifuged to separate cells and supernatant. The LAB supernatant was mixed with Fenton's solution using a ratio of 1:0, 1:1, and 1:2, then incubated at room temperature for 20 minutes.

Furthermore, the absorbance of the solution was measured at a wavelength of 624 nm using a spectrophotometer. Changes in the absorbance of the reaction mixture indicated the ability to capture LAB strains for hydroxyl radicals. HRS activity is calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{As} - \text{Ao}}{\text{A} - \text{Ao}} \times 100$$

As = sample absorbance; Ao = control absorbance, and A = absorbance without sample or Fenton reaction system

2.7. Autoaggregation Test

Auto-aggregation of LFB1295 or PP2397 cells encapsulated with CMFH from OPL was performed according to the method of (Malik et al., 2013) with slight modifications. LAB cultures were inoculated separately (1%) into MRS broth and incubated at 32°C for 20 hours. Cells were harvested by centrifugation at 5000 rpm for 15 min at 4°C, then washed twice with phosphate-buffered saline (PBS), pH 7.2, and resuspended in PBS. The turbidity of the suspension was standardized (about 10^8 CFU/ml). Cell suspension (4 ml) was vortexed for 10 seconds, and autoaggregation was evaluated at 0 h and 5 hr incubation at 32°C and 37°C. A total of 0.1 ml of the upper suspension was transferred to a tube containing 3.9 ml of PBS, and the absorbance was measured using a spectrophotometer at an absorbance of 600 nm. The percentage of autoaggregation is measured using the following formula:

$$\text{Autoaggregation (\%)} = 1 - (\text{At}/\text{Ao}) \text{ x}100$$

At = absorbance at time t = 5 hours and Ao = absorbance at time t = 0 hour

2.8. Coaggregation Test

The coaggregation test was carried out according to the method of (Kumar et al., 2020). Coaggregation of LFB1295 or PP2397 cells without or encapsulated with CMFH from OPL with *Staphylococcus aureus* (Gram-positive pathogen) and Escherichia coli (Gram-negative pathogen), respectively, was evaluated as a coaggregation test. Each LAB and *E. coli* suspension contains approximately 10^8 CFU/ml). Each cell suspension of 2 ml was mixed in pairs and vortexed for 10 seconds. Each bacterial suspension (4 ml) was used as a control.

This mixture was incubated for 5 h at 37°C, and the absorbance using a spectrophotometer at 600 nm was measured at 0 h and 5 h. The coaggregation percentage is calculated as follows:

Coaggregation (%) = $((Ax + Ay)/2) - A(x + y)\ Ax + Ay/2 \times 100$

Ax and Ay denote each of the two bacterial species in each tube, and A(x+y) is a suspension mixture of 2 bacteria.

2.9. Cell surface hydrophobicity assay

The surface cell hydrophobicity was determined according to the method (Xing et al., 2017). LFB1295 or PP2397 cells without or encapsulated with CMFH from OPL were grown in MRS broth medium at 30°C for 18 hours and then centrifuged (10,000 rpm, for 5 minutes). The cell pellet was washed twice with urea magnesium phosphate (UMP) buffer (pH 7.1). Then, the washed pellet was resuspended in PUM buffer and adjusted to a similar cell density (about 10^8 CFU/mL). 3.0 mL of the cell suspension was measured, and then 1.0 mL of xylene was added and mixed thoroughly using a vortex and incubated at 30°C for 10 minutes. Subsequently, this mixture was thoroughly remixed using a vortex for 1 minute and then incubated at 30°C for 1 hour to allow phase separation. The water phase was carefully removed, and the absorbance was measured at 600 nm. The surface hydrophobicity (%) was calculated as the percentage decrease in the absorbance of the aqueous phase between the initial suspension and after mixing.

2.10. Data analysis

Data on safety evaluation were analyzed descriptively, and data on antioxidant activity, autoaggregation, coaggregation, and hydrophobicity were statistically analyzed using analysis of variance using IBM SPSS version 23 software. If Fcount ≥ Ftable, it will be continued with the Duncan multiple range test at 5%.

RESULTS AND DISCUSSION

A hemolytic activity test was conducted to ensure that LAB used as a probiotic candidate was safe. The discovery of a distinct zone of hydrolysis surrounding the colony, which suggested hemolytic activity, was a positive outcome. The results of the hemolytic activity test of LFB1295 and PP2397 cells without or encapsulated with CMFH of oil palm leaves can be seen in Table 1.

Table 1. Hemolytic activity, production of cytolysin, gelatinase, and biogenic amines by lactic acid bacteria cells encapsulated with or without CMFH from OPL

Treatments	Hemolytic activity	Production of cytolysin	Production of gelatinase	Production of biogenic amines
WPP	No	No	No	No
WLF	No	No	No	No
EPP	No	No	No	No
ELF	No	No	No	No

The absence of a clear zone surrounding the colony indicated that LFB1295 and PP2397 cells without or encapsulated with CMFH from OPL had a negative result, meaning that these two LABs did not have hemolytic activity and were, therefore, safe to use as probiotics (Table 1). A clear zone can be formed due to the lysis of blood cells by bacterial activity, which indicates that the bacteria are pathogenic (Alang *et al.*, 2020). This study's results align with Hawaz (2014), who conducted hemolytic activity tests on several Lactobacillus probiotic strains isolated from curd, including *L. fermentum*, *L. lactis,* and *L. brevis,* showing negative results of hemolytic activity. Additionally, 23 Lactobacillus strains, including *L. plantarum* and *L. paracasei* as probiotic possibilities isolated from raw sheep milk and cheese, were tested for hemolytic activity by Pisano *et al.* (2014), but no hemolytic activity was discovered. The *L. fermentum* TIU19 strain was also tested for hemolytic activity in later research by Das *et al.* (2022), but the results were negative. Hence, it was deemed safe for use as a probiotic. Numerous Pediococcus species identified from kombucha did not exhibit harmful hemolytic activity (Nit et al., 2020).

On the other hand, it was discovered that harmful bacteria like Salmonella enterica can create hemolysin, which can produce hemolytic activity. The hemolysin produced will cause the decomposition of proteins and lipids of red blood cells, which results in the release of hemoglobin, thereby damaging the cell membrane. The destruction of red blood cells can cause a decrease in hemoglobin levels or a lack of blood, known as anemia (Beshiru *et al.*, 2018). Non-hemolytic activity is considered a safety prerequisite for the selection of probiotic strains.

Cytolysin is a compound produced by microorganisms that can cause lysis in the cells of other organisms, such as humans. The clear zone around the colony shows a positive result, presumed that the probiotic produces cytolysin. The absence of clear zones around the colonies after 24 hours of incubation showed that the LFB1295 and PP2397 cells without or encapsulated with CMFH from OPL had negative findings in the cytolysin production test. The absence of a clear zone indicated that LAB does not produce cytolysin, which means it is safe to use as a probiotic. This study's results align with Tan *et al.* (2013), who evaluated the safety of probiotics, namely the cytolysin production test on *Enterococcus faecium* YF5 isolated from sourdough, which showed negative results. Another study by Santos *et al.* (2015) assessed the safety of probiotics and found that tests for the formation of cytolysin in different strains of Lactobacillus plantarum and Lactobacillus rhamnosus produced negative results. Cytolysin is a toxin compound produced by pathogenic bacteria, which can cause hemolysis. Cytolysin can lyse human, rabbit, and horse erythrocytes (Moraes *et al.*, 2012).

Tyne *et al.* (2013) stated that Enterococcus faecalis is a pathogenic bacterium capable of producing cytolysin. Cytolysin produced by E. faecalis is resistant to drugs and causes endophthalmitis infection in postoperative patients. Tan *et al.* (2013) also stated that cytolysin could significantly exacerbate the severity of endocarditis, which is inflammation of the heart's inner lining, and endophthalmitis, which is inflammation of the eyeball.

Gelatinase is a virulence factor for a microbe that can cause immunosuppression and immunoevasion, reducing the host's immune system (Chandra *et al*., 2014; Hussain & Khan, 2022). The clear zone around the colony shows a positive result, presumed that the probiotic produces gelatinase. Data Table 1 shows the production test of LAB gelatinase without encapsulation or encapsulation with CMFH of oil palm leaves. The results showed negative results marked by the absence of clear zones around the colonies, which meant that both LABs did not produce gelatinase, so they were safe to use as probiotics. Endocarditis can be brought on by gelatinase, which hydrolyzes gelatin, collagen, and specific bioactive peptides (Jaouani *et al.*, 2015). Tests for gelatinase production of new strains before application in food need to be carried out to determine the safety of these strains. Choudhary *et al*. (2019) conducted a gelatinase production test as a safety evaluation of probiotics. It showed that *L. pentosaceus* MMP4 did not produce gelatinase, so it was considered safe and could be used as a probiotic. In subsequent research, Zhou *et al*. (2021) also conducted a gelatinase production test as a safety evaluation for the probiotic Enterococcus durans A8-1 with negative results. Research by Beshiru *et al.* (2018) showed that the gelatinase enzyme can be produced by pathogenic bacteria such as Salmonella enterica, which can break down gelatin into amino acid, peptide, and polypeptide compounds so that they can pass through the host cell immunogenic response and continue their growth and transmission.

Biogenic amines are one of the dangerous metabolites produced by several bacteria, including probiotics, so determining the safety of probiotics before consumption is essential. The results of this study indicate that LFB1295 and PP2397 without or encapsulation with CMFH in oil palm leaves do not produce biogenic amines from several amino acids such as L-histidine, tyrosine, L-ornithine, and lysine. Thus, both LABs are used as probiotics. The findings of this study show that LFB1295 and PP2397 could not create BAs from the four different types of amino acids. Previous research also showed that some LABs, including *L. paracasei*, *L. plantarum*, and *L. brevis*, did not create BAs. (Barbieri et al., 2019). Tyramine is produced by several LABs, especially Enterococci, Carnobacteria, and Lactobacilli. The main producers of BAs such as cadaverine and putrescine are Enterobacteriaceae (Bover-cid & Holzapfel, 1999).

The ability to capture DPPH radicals was to determine the potential of LFB1295 and PP2397 without encapsulation or encapsulation with CMFH of oil palm leaves in producing antioxidant compounds. The antioxidant compounds produced by LAB will scavenge free electrons from DPPH radicals. The reduction of DPPH radicals resulted in a color change from purple to pale yellow, indicating the sample's antioxidant activity (Silalahi *et al.*, 2018). The ability to capture DPPH radicals is calculated as an inhibitory concentration (IC50), which means the concentration that can counteract 50% of DPPH free radicals. The smaller the IC50 value, the greater the ability to counteract DPPH free radicals. The analysis of variance showed that the treatment of encapsulated and non-encapsulated LABcells significantly influenced the scavenging ability of DPPH radicals (Table 2).

Table 2. The scavenging ability of DPPH radicals by lactic acid bacteria cells encapsulated with or without CMFH from OPL

Treatments	The scavenging ability of DPPH radicals (IC_{50})(ppm)*
WPP	99.31^{e}
WLF	58.65^{c}
EPP	73.89^{d}
ELF	51.38^{b}

Table 2 shows that encapsulated LAB cells show a lower IC50 value than non-encapsulated LAB cells. The higher the IC50 value, the higher the oxidant activity. This finding is because encapsulants can maintain high cell viability compared to cells without encapsulation. The results of this study showed that the results of antioxidant activity on D-0 were 64.13 ppm, lower than that of Shehata *et al*. (2019), who showed that the probiotic *L. plantarum* DMSZ20079 had robust antioxidant activity at DPPH free radical scavengers, namely 12.99 ppm. Tristantini *et al.* (2016) stated that a compound has a very strong antioxidant ability if the IC50 value is <50 ppm, 50-100 ppm strong, 100-150 ppm moderate, and 150-200 ppm weak. The IC50 value for all treatments ranged from 51.38 to 99.91 ppm, which was still relatively strong. The P. pentosaceus strain 2397 used in this study produced good antioxidants compared to some Pediococcus spp isolated from kombucha and, therefore, did not have antioxidant activity in the form of DPPH (Nit et al., 2020).

HRS activity tests the ability of encapsulated LAB to scavenge hydroxyl radicals using the Fenton reaction. The HRS activity of encapsulated LAB is calculated in % inhibition, which means the ability of an antioxidant to counteract free radicals. The higher the % inhibition value indicates the more robust the antioxidant ability of the compound. The results of variance showed that encapsulated or non-encapsulated cells significantly ($P<0.05$) affected HRS activity value (Table 3).

Table 3. HRS activity value by lactic acid bacteria cells encapsulated with or without HMFH from OPL

Treatments	Hydroxyl radical scavenging activity (%)
WPP	66.17^{a}
WLF	70.88^{ab}
EPP	65.58^{a}
ELF	75.29^{b}

Data from Table 3 shows that the hydroxyl radical scavenging activity of cells encapsulated with CMFH from OPL was higher than that of cells without encapsulation, with the highest activity obtained in LFB1295 cells, which were encapsulated at 75.29%. The results of this study are not much different from those of Duz *et al.* (2020), which showed a high HRS activity value in *L. plantarum* İH18L without storage treatment, namely 73.13% and *L. sake* İH23L, namely 73.53%. However, the results of this study have a better HRS activity value than the research of Zhang *et al.* (2013), who showed that *L. plantarum* C88 had a hydroxyl radical scavenging ability of only 50%. This finding shows that *L. fermentum* InaCC B1295 encapsulated in CMF hydrogel from oil palm leaves has a good antioxidant in counteracting hydroxyl radicals. Hence, it has the potential to be a probiotic.

Autoaggregation refers to the ability of microorganisms, for example, LAB, to interact and form their groups or aggregates. This is a phenomenon in which bacteria of the same species can interact and form larger clumps or groups. In some cases, LAB may use their autoaggregation capabilities to facilitate attachment to their hosts. This can help LAB in preventing the process of infection by pathogenic bacteria in the digestive tract. Table 4 shows that encapsulated or non-encapsulated cells significantly (P<0.05) affected autoaggregation value.

Table 4. Autoaggregation value by lactic acid bacteria cells encapsulated with or without CMFH from OPL

Treatments	Autoaggregation values (%)
WPP	29.88^{c}
WLF	24.29^{b}
EPP	12.65^{a}
ELF	35.41^{d}

The data in Table 4 shows that LFB1295 cells that were not encapsulated had the lowest autoaggregation value of 12.65%. Conversely, LFB1295 cells encapsulated with CMF hydrogel from oil palm leaves showed the highest autoaggregation value of 35.41%. LFB1295 and PP2397 cells in this investigation had a lower autoaggregation value than Pediococcus acidilactici NCDC 252, which had a value of 95.8% (Kumar et al., 2020).

The coaggregation test was performed to determine whether interactions between bacterial isolates in the GI could cohere and resist being easily washed out by feces. To build a powerful host defense against the introduction of pathogens from the outside, colony formation is crucial as probiotics (Peres et al., 2014). Table 5 shows that encapsulated or non-encapsulated cells significantly (P<0.05) influenced the coaggregation values.

Table 5. Coaggregation values by lactic acid bacteria cells encapsulated with or without HMF from OPL

Treatments	Coaggregation values (%)
WPP-LF	17.32[f]
WPP-EC	12.67[d]
WPP-SA	9.41[c]
WLF-PP	17.32[f]
WLF-EC	7.67[b]
WLF-SA	4.59[a]
EPP-LF	15.06[e]
EPP-EC	7.09[b]
EPP-SA	8.29[bc]
EFL-PP	17.37[f]
EFL-EC	7.54[b]
EFL-SA	5.31[a]

The results showed that there was coaggregation between LAB cells that were encapsulated or not encapsulated with fellow LAB or pathogenic bacteria with different percentage values from 4.59% in TEL-SA to 17.37% in EFL-PP. The coaggregation test of the same species but without encapsulation yields different values. The opinion of some researchers states that the coaggregation value is influenced by the specific strain, incubation time, and the presence of inhibitors (Bao et al., 2010).

Cell wall constituent components such as phospholipids and lipopolysaccharides play a role in the hydrophobic interactions of bacterial cells. Table 4 shows that encapsulated or non-encapsulated cells had a significant ($P<0.05$) effect on the hydrophobicity value.

Table 6. Hydrophobicity value by lactic acid bacteria cells encapsulated with or without HMF from OPL

Treatments	Hydrophobicity value **(%)**
WPP	12.93[b]
WLF	10.66[a]
EPP	18.08[c]
ELF	12.20[ab]

A high hydrophobicity value indicates the presence of hydrophobic molecules on the surface of the tested bacterial cells. High hydrophobicity bacteria may survive, reproduce, and invade tissues on the surface of the intestinal tract (Priadi et al., 2020). The hydrophobicity value resulted in the DEP treatment (non-encapsulated LFB1295 cells) of 18.08%. The hydrophobicity value of LAB in this study ranged from 10.66 to 18.08%, much lower than research (Ding et al., 2017), which tested several L. plantarum strains, and the highest hydrophobicity value was only 60.97%. According to Panjaitan et al. (2018), the hydrophobicity of microbes is influenced by bacterial strains, growth media, bacterial age, and bacterial surface structure. The diversity of these factors causes each species and strain to express different hydrophobicity values (Priadi et al., 2020).

CONCLUSIONS

Based on the research data, it can be concluded that LFB1295 and PP2397 without encapsulation or encapsulation with CMFH of oil palm leaves do not produce hemolysin activity and do not produce cytolysis, gelatinase, and biogenic amines, so these two LABs are safe to use as probiotics. LFB1295 and PP2397 cells without encapsulation or encapsulation with CMFH of oil palm leaves produced relatively strong DPPH activity. LFB1295 cells encapsulated with CMFH of OPL produced the most HRS activity. Compared to free cells and closed cells in these two LABs, LFB1295 and PP2397 showed higher levels of coaggregation with the harmful bacteria S. aureus and E. coli. Unencapsulated LFB1295 cells showed the highest hydrophobicity score.

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DEVELOPMENT OF PLANT SEED BASED BIODEGRADABLE AND EDIBLE PACKAGING MATERIAL

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ABSTRACT

Most food packaging is made from petroleum, a non-degradable material that can cause serious "white pollution" and release toxic chemicals, posing a serious risk to food and environmental safety. For this reason, studies on edible coatings with eco-friendly, sustainable and various functional properties have gained momentum in the food packaging industry. It is also known that edible packaging offers additional benefits, such as protection against mechanical and microbial damage, aesthetic appearance, and prevention of loss of desired flavour components.

Various seeds/kernels that are by-products in the food industry produce mucilages with different properties that can be used for this purpose. The mucilages obtained from different parts of plants have their own unique composition and properties. Their unique colloidal properties, low production cost, and ease of extraction make them attractive as potential packaging materials. They are often used to reduce moisture and flavor loss and have good gas and water vapor barrier properties. The use of mucilage, a plant hydrocolloid, in edible films can maintain good physicochemical and barrier properties by ensuring food stability. Edible packaging materials with superior functional properties can be obtained with additional components to the mucilage extracted from these seeds, which are used according to the zero-waste principle. These edible films interact positively with food and the environment, reducing the rate of spoilage reactions, extending food shelf life and meeting consumer demands.

Key words: Seed musilage, edible packaging, eco-friendly packaging

1. Introduction

Petroleum-based synthetic polymers are used as unavoidable packaging materials due to their easy availability and excellent mechanical and physical properties (Sukhija et al., 2016). The vast majority of existing food preservation materials are non-degradable plastics that pollute the environment with waste after use. They also threaten food safety and human health through the migration of plasticizing chemical components into food. These negative effects of plastics on the environment and human health necessitate alternative, biodegradable and edible packaging materials that can be consumed with food. The fact that these edible and biodegradable packaging materials protect and control food quality (Hira et al., 2022), as well as being an environmentally friendly technology, has brought them into the focus of research (Kumar et al., 2022).

Edible films are obtained by extracting the mucilages from plant seeds by passing them through various processing steps with or without the addition of bioactive components (Figure 1). They are applied by wrapping the film obtained by drying in the food or by dipping the food in the film solution. Cheese, meat, fruit, vegetables, chocolate, etc. foods can be packaged in edible films made from vegetable biopolymers. They are defined as a thin layer that prevents/reduces water loss, gas exchange and lipid oxidation of foods (Korkmaz 2017). Since edible films are consumed with food, there is no negative impact on the environment. Considering these characteristics, it can be concluded that edible films are suitable for less processed (peeling, cutting, etc.) fruits and vegetables, sliced cheese, meat and fish fillets, and meatballs (Ayana and Turhan 2010). With the increasing environmental awareness, the demand for food protection through natural processes has increased and the work in this direction has accelerated. In this context, the production of edible films with natural antimicrobial and antioxidant properties that resist microbial and chemical deterioration for a long time has gained importance (Seydim and Sarıkuş 2006; Duan et al., 2007; Di-Pierro et al., 2007; Di-Pierro et al., 2011).

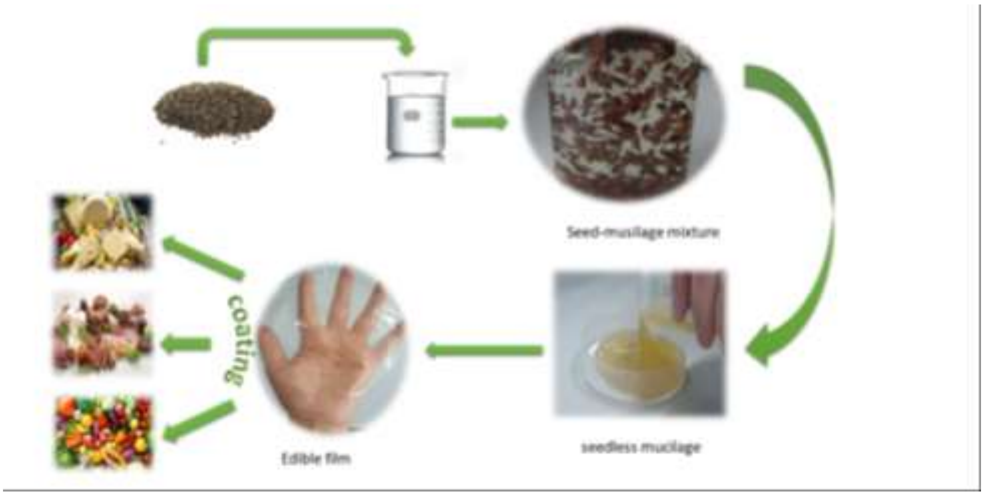

Fig. 1. Example schema of edible film production stages from plant seeds

Food production generates large amounts of solid and liquid waste through food preparation and consumption. Waste is a valuable product as biomass and food ingredient. As in the rest of the world, a large amount of waste is generated in our country when fruits and vegetables are processed into products. During the production process, wastes such as peels, seeds, and pulp with high polysaccharide, protein, and lipid content are generated. These wastes can be reused in different areas. For this reason, studies under the zero waste approach have focused on the use of organic wastes after food production for various purposes in food production, but also for use in various fields such as chemistry, medicine and cosmetics by subjecting them to various processes.

Food packaging plays an important protective role by extending the shelf life of the product and significantly reducing food waste. Most food packaging is made from petroleum, a non-degradable material that can cause serious "white pollution" and release toxic chemicals, posing a serious risk to food and environmental safety (Dong et al., 2022). Therefore, it is important for the food industry to develop environmentally friendly and sustainable packaging films. Recently, edible films have received more attention due to their low cost, abundant and renewable resources of natural biopolymers such as proteins, polysaccharides, lipids, etc., biodegradability, biocompatibility and ability to form films (Requena et al., 2018; Hadidi et al., 2022). The global transition to a bioeconomy is also largely focused on the sustainable use of renewable resources (mainly byproducts of agribusiness and the food industry). In this framework, the use of bioactive compounds from renewable sources represents a new perspective that contributes (among other things) to a food waste prevention strategy (Papadaki et al., 2022).

The increasing preference for a vegan diet is leading to an increased demand for plant-based alternatives due to changing dietary habits as well as the high content of essential amino acids, polyunsaturated fatty acids, and rich lipid profile and positive environmental impact. In this context, mucilages from plant seeds/seeds have recently come to the fore as ingredients with potential use in plant-based products. In fact, it acts as an innovative food ingredient by being used as gelling agents, texture modifiers, stabilizers and emulsifiers in foods. In addition, they can provide better technological, functional and sensory properties by enriching the nutritional content of foods (Lira et al., 2023). In addition to all these beneficial effects, their cheap and easy availability and excellent functional and technological properties make them promising candidates for use as edible plant-based film components.

2. Use of Plant Seed/Seed Mucilages as Edible Coatings in Various Foods

Recently, various biodegradable polymers, polysaccharides from different sources (Priyadarshi et al 2022) and proteins (Gonzalez et al., 2019; Hu et al., 2021; Wang et al., 2020) have been used to develop edible coatings to extend the shelf life of foods. The quality of edible film coatings is significantly influenced by the properties of the material. Therefore, a suitable edible biopolymer film should be selected taking into account the physical and chemical properties of its components, as well as the permeability and mechanical properties of the film (Marquez et al., 2017).

Polysaccharides, proteins and lipids are widely used for the synthesis and production of composite films (Wang et al., 2015). Various plant-based mucilages, which are a source of hydrocolloid, are considered as edible films due to their low production cost compared to many biopolymers, their ease of extraction and their remarkable nutritional value. Gums/mucilages, which are plant hydrocolloids, represent a subgroup of hydrocolloids that form a monosaccharide gel containing organic acids in the presence of water, as they are hydrophilic components (Beikzadeh et al., 2019). Numerous studies have been carried out on the preparation of edible films with the above-mentioned plant seed mucilages. Some important recent studies on this subject are summarized below.

The effects of edible films containing flaxseed mucilage and xanthan gum in different proportions were studied in Cheddar cheese during 90 days of ripening in cold storage at 8±2 °C. They found that sample pH, acidity, and oil content in the dry matter were significantly affected by treatment with xanthan gum and flaxseed gum. They found that edible coatings had no statistical effect on the growth of non-starter lactic acid bacteria and total mesophilic aerobic bacteria, while bacterial growth changed significantly under the influence of edible coating materials (Soleimani-Rambod et al., 2018).

There are several studies investigating the physical, thermal, structural, permeability, mechanical, and antioxidant properties of edible films prepared with quince seed mucilage. In these studies, in which the prepared films gave positive results in terms of most of the properties, it was found that quince seed mucilage has the potential to be used for the packaging of numerous food products (Jouki et al. 2013; Jouki et al. 2014).

Erkaya-Kotan et al. (2023), made an edible film from quince seed mucilage as an alternative to plastic vacuum packaging to solve the mold problem in Kashar cheese. They found that all film formulations with or without the addition of oregano oil significantly inhibited mould growth. In addition, the researchers found that quince seed mucilage contributes to healthy living and improves food shelf life by increasing ACE inhibitory activity in the coated samples compared to the uncoated samples.

According to Xiong et al. (2020), research was conducted on edible coating material with grape seed extract for pork, which is very sensitive to oxidation and microbiological degradation. As a result of the research, they found that the antioxidant activity against meat oxidation increased further by adding 0.5% grape seed extract to the film formulation. The researchers suggested that the film formulation works well in pork preservation and therefore could be developed as an innovative technology for fresh meat preservation.

In another study, UV-blocking and potentially biodegradable composite films were developed with the addition of flaxseed mucilage, pectin, and other active ingredients. According to the results of the analysis of the developed biodegradable composite films, it was found that they could potentially be used in food packaging as a UV-blocking layer with good moisture resistance if additional active ingredients were added to the plant seed mucilage (Akhila et al., 2023).

In a study on the synthesis of edible films from Balangu seed mucilage as a new carbohydrate source, the excellent mechanical and barrier properties of the film showed that it could be a potential material for the packaging industry (Sadeghi-Varkani et al., 2018). Using the same mucilage, Behbahani et al. (2020) aimed to produce an environmentally friendly edible coating by adding cumin essential oil as an active ingredient to the mucilage. The researchers found that coating with 2% cumin essential oil gave the beef better quality characteristics and extended its shelf life in the refrigerator. As a result, they found that the coating material derived from Balangu seed mucilage and enriched with cumin essential oil can be used as active packaging to improve the quality characteristics and microbial safety of beef and other fresh foods.

In the study in which two- and three-layer films were prepared based on basil (Ocimum basilicum L.) seed mucilage as another plant polysaccharide, the formulation was found to have good mechanical properties without cytotoxicity (Hosseini et al., 2022).

The researchers, noting that food packaging materials are one of the main contributors to environmental pollution, explained that there has been an increased interest in using biopolymers as an alternative packaging material. To this end, they set out to develop a film with a blend of Chia seed mucilage, which contains high levels of polysaccharides, and a polyol containing glycerol and sorbitol. They concluded that edible films, which have different superior properties depending on their glycerol and sorbitol content, can be successfully used to develop biofilms that have the potential to be used in food packaging (Urbizo-Reyes et al., 2020).

In another study examining the production of probiotic edible film based on Chia seed mucilage and sodium caseinate, it was observed that the survival rate of probiotic cells increased significantly in the presence of Chia mucilage. The study results support the feasibility of Chia mucilage as an edible preservative that could act as a suitable probiotic carrier for the safe delivery of probiotics for consumption. As a result, they state that plant-based natural preservatives can offer an alternative to synthetic compounds and chemicals (Semwal et al., 2022).

Jiang and Zheng, (2023) produced biodegradable active composite films composed of Chia seed mucilage, chitosan, and Xanthoceras sorbifolium Bunge (X. sorbifolium) leaf extract as the active ingredient, and investigated some of their properties. According to the research results, Chia seed mucilage/chitosan films with the addition of X. sorbifolium leaves can replace petroleum-based packaging materials as an environmentally friendly functional packaging material with the necessary improvements. They also pointed out that the study provides a new and effective way to recycle biowaste.

Mousavi et al. (2021) studied the effect of an edible coating of chia seed mucilage and bacterial cellulose nanofibers on bioactive compounds and antioxidant enzyme activity of strawberries. According to their results, it was found that the use of edible coatings preserved the phenolic, flavonoid, ascorbic acid and antioxidant activities of strawberries, and this effect was more pronounced in the samples covered with films containing both components. On the other hand, the study found that the activity of polyphenol oxidase and peroxidase enzymes, which are responsible for the degradation of phenolic compounds in the product and brown color, can be effectively controlled by the edible coating.

In a study from chia seed mucilage, its film-forming ability was investigated by obtaining mucilage at different temperatures and with levan biopolymers. According to the results of the study, it was found that the films obtained with Chia mucilage and levan retained their antibacterial properties but lost their antifungal properties. In addition, the study reported for the first time that chia seed mucilage obtained at 55 °C has both an anti-quorum sensing effect and an antibacterial effect. The researchers stated that this result is an important development that can prevent bacteria from forming biofilm, and explained that chia seed mucilage is not only environmentally friendly, but also important for improving the quality of composite materials by preventing the formation of biofilm by using it in various composite structures (Ağçeli, 2022).

In a study on the usability of black cumin mucilage in edible films, various quality parameters were investigated by adding xantham in different amounts to the film solution. According to the results, highly transparent and hydrophobic films with very low water vapor permeability, low water solubility and good mechanical properties were developed. They also found that the films had good antioxidant activity and antibacterial activity *against E. coli, S. aureus,* and *P. aeruginosa*. As a result, they found that biofilms applied to strawberries by dipping method have great potential for edible coating and packaging applications (Ashooriyan et al., 2023).

In another study, an edible film was prepared based on basil seed mucilage and alginate. It was found that the films obtained had good physical properties (thickness, tensile strength, crystallinity and thermal stability). As a result, it was found that the shelf life of these edible films developed from vegetable mucilage can be extended by using different foods and their moisture and oxygen barrier properties (Nazir, and Wani, 2022).

In the study investigating the use of Dracocephalum Moldavica seed mucilage as a new source of hydrocolloids, glycerol was added in varying amounts. The results show that the barrier, mechanical and some physical properties of the film samples increased significantly with increasing glycerol content, and all film samples exhibited significant antioxidant properties. As a result, they found that Dracocephalum Moldavica seed mucilage can be used as an innovative component in the production of edible antioxidant films (Beigomi et al., 2018).

In another study, the efficacy of an edible coating developed from Malva sylvestris (great mallow) seed mucilage and *Saccharomyces cerevisiae* var. *boulardii* ATCC MYA-796 derived postbiotics in preserving lamb was investigated. The results show that the edible composite coating effectively reduces microbial growth and prolongs meat shelf life, significantly inhibits lipid oxidation, and reduces the formation of primary and secondary oxidation intermediates (Abbasi et al., 2023).

CONCLUSION

Edible, biodegradable films are attracting the attention of the scientific world as a good alternative to plastics that both pollute the environment and endanger human health. The extraction of these edible films from vegetable waste is a very important detail. They have become the focus of interest both because of the reduction of waste and because of their economic benefits. In addition, various bioactive properties of mucilages extracted from plant seeds confer superiority to edible films. On the other hand, physical, mechanical, antioxidant, and antimicrobial properties can be improved by producing composite films with various bioactive components. Evaluating the current studies from a comprehensive perspective, the application of the obtained films in industry is promising in terms of completely eliminating plastic packaging from our lives.

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EFFECT OF GROWING CONDITIONS ON THE STRUCTURAL ELEMENTS AND YIELD OF WINTER BARLEY GENOTYPES

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Abstract

The aim of this study was to determine the impact of growing conditions on the structural elements and yield of winter barley genotypes. In the period 2019-2021, two varieties of two-row barley and two varieties of six-row barley were studied in a multifactorial field experiment with three sowing rates and four fertilization variants. The study was conducted at the experimental field of the Institute of Agriculture - Karnobat, Bulgaria. The results showed that higher yields were obtained by increasing the fertilization levels, and for the two-row varieties it was N16 fertilization, whereas for the multi-row varieties it was N8 fertilization. It was established that the year and fertilization factors played the greatest role in forming grain yield. The analysis of variance for the structural elements proved that year and genotype had the strongest impact on the studied indicators and determined their variation.

Key words: genotype, barley, conditions, structural elements, yield

INTRODUCTION

Depending on the growing conditions, deviations from the variety may occur in the seed production process, which are due to reasons such as unevenly sown crops, fertilization, soil fertility, low or high temperatures in critical phases for the crop, and others. According to Ivanova and Tsenov (2009), the meteorological conditions are the main agrotechnical factor for the formation of biological and economic traits in fourteen varieties of winter common wheat, whereas fertilization rate mainly affects grain yield. The weather conditions of the year have the greatest impact on yield and grain quality (Terziev, 2000). Yields, as well as their structural elements, are strongly affected by the year conditions and the variety plasticity (Georgieva et al., 2004).

In order to obtain optimal yields, it is necessary to introduce new high-yielding varieties and determine an appropriate varietal structure. In this regard, a more in-depth study of the relationship between variety and specific weather conditions of a given region is imperative (Penchev and Stoeva, 2004; Habibi et al., 2011; Kucek et al., 2019). The correct varietal structure, depending on the specific agro-ecological conditions of the area, can significantly increase yield and production quality (Ilieva, 2011).

The aim of this study was to determine the impact of growing conditions on the structural elements and yield of winter barley genotypes.

MATERIAL AND METHODS

In the period 2019-2021, the impact of growing conditions on the structural elements and yield of winter barley genotypes was studied at the Institute of Agriculture - Karnobat, Bulgaria. In a field multifactorial experiment with 4 fertilization variants (T0 – no fertilization; T1- fertilization with N8; T2- fertilization with N12; T3- fertilization with N16) and three sowing rates (P1 - 250, P2 - 350 and P3 - 450 germinable seeds) were grown winter barley varieties Kuber, Saira, Zemela and Bozhin. The varieties were sown on a harvest plot size of 10 m^2 in 4 replications. The grain yield of each plot was reported. When full maturity was reached, biometrics were taken from each plot for certain traits related to the structural elements and yield - plant height (cm), spike length (cm), grain number per spike, number of sterile spikelets, grain weight per spike (g), 1000-grain weight (g). The results were statistically processed by analysis of variance using the JMP and SPSS 19.0 software products.

The weather conditions during the three years of testing were contrasting (Figures 1 and 2), two of them being favorable for the development of the varieties and one was unfavorable. In the first year (2019), the average monthly temperatures during the growing season, except for December, were close to the mean multi-year values. From the beginning of the growing season until April, rainfall was significantly below normal. The rainfall during the period of grain filling and ripening had the most favorable effect.

The second year (2020) was the driest year in the region for the last 20 years, which adversely affected the development of the barley varieties. Plant growth occurred at higher average monthly temperatures than the multi-year values for the period. The amount of rainfall was 41.7% less than the multi-year values. As a result, yields were greatly reduced.

In the third year (2021), the weather conditions were also favorable for barley development. The cooler spring and the rainfall during the period of grain filling and ripening were the reason for the formation of higher yields.

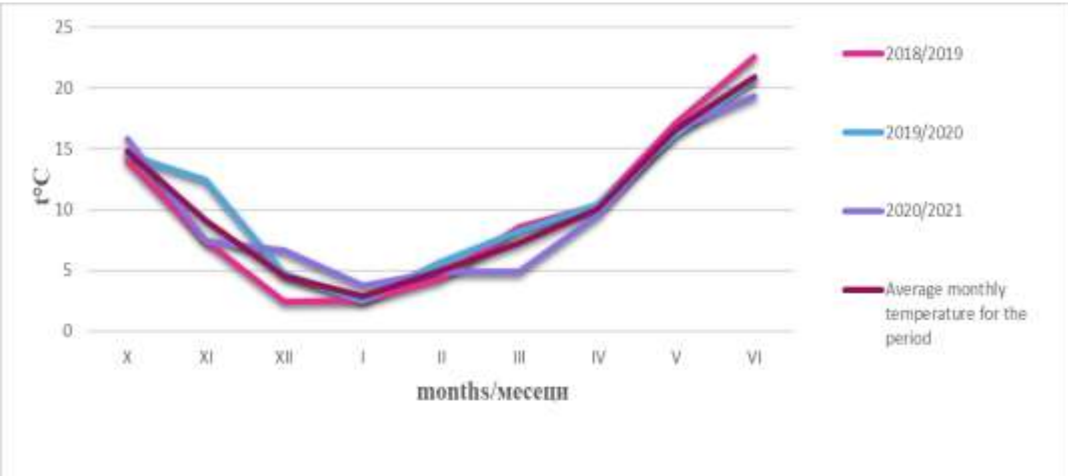

Figure 1. Average monthly air temperatures in the period 2019-2021

Figure 2. Average monthly rainfall in the period 2019-2021

RESULTS AND DISCUSSION

Table 1 presents data on the yield and structural elements of the two-row Kuber and Saira barley varieties by fertilization variants and sowing rates as mean for the period 2019-2021.

The lowest yield average for the period for both varieties was formed at 250 g.s. for the Saira variety (3.07 t/ha) and 350 g. seeds for the Kuber variety (3.22 t/ha) in the unfertilized variants (P1T0 and P2T0). The yield variation in these variants in both varieties was strong, from 16.04% for Kuber to 38.41% for Saira. The highest yields were in the variants with 350 g.s. and N16 fertilization for Kuber variety (P2T3) - 5.80 t/ha, and 450 g.s. and N8 fertilization for Saira (P3T1) - 5.66 t/ha. During the study period, variant yields varied from medium to high in both varieties except for the yields of variants P1T1 (VC%=9.45%) and P3T1 (VC%=7.77%) in Saira variety. Analyzing the obtained results by fertilization variants, it is noticeable that increasing the fertilization rate increases the yield. In the Kuber variety, the unfertilized variants yielded from 3.22 t/ha to 3.55 t/ha, and in the Saira variety, yield ranged from 3.07 t/ha to 3.66 t/ha. The yields were highest in the variant with N16 fertilization. For the Kuber variety they were from 5.00 t/ha to 5.80 t/ha, and for Saira variety – 5.30 t/ha to 5.59 t/ha (Table 1).

Spike length in barley is one of the relatively constant indicators. Regarding this indicator, genotype has a stronger impact (Bonchev, 2017). The spike length of the varieties studied during the study period varied from low to medium. The indicator varied the least in the variant with 250 g.s. in both varieties, and unfertilized (P1T0) for the Kuber variety, as well as when fertilized with N8 (P1T2) for the Saira variety. The shortest spikes were formed in both varieties in the unfertilized variants with 250 g.s. for Kuber variety (P1T0) and with 450 g. seeds (P3T0) for Saira variety. The varieties had the longest spikes at 250 g.s. with N12 fertilization for Kuber and N16 for Saira (Table 1).

The grain number per spike varied from low to medium by variant and by variety. For both varieties, the fewest grains per spike were formed at 450 g.s. and unfertilized (P3T0) – 22.29 grains for Kuber variety and 23.49 for Saira. Their number was the highest for the Kuber variety – 26.46 grains, and for the Saira variety – 28.04 grains at 250 g.s. and the high levels of fertilization (N12 and N16) (Table 1).

Grain weight is one of the indicators that varies greatly, as it is influenced by the conditions of the year (Dimova, 2015). In this study, grain weight per spike ranged from medium to strong, with VC% by variants and varieties ranging from 16.49% to 44.56%. The lowest values for the indicator in both varieties were found in the unfertilized variants at 250 and 350 g.s. for Kuber (0.99 g) and 450 g.s. for Saira (1.03 g). The highest weight per spike was in the P2T2 variant (1.16 g) for the Kuber variety and P2T2 and P1T3 (1.21 g) for Saira (Table 1).

The results for 1000-grain weight in Table 1 show low (VC%=9.26%) to medium (VC%=22.18%) variation by variant and variety. For the Kuber variety, the indicator had the lowest values in the P1T3 variants – 45.25 g, and for the Saira variety in P3T3 – 44.46 g, which showed that the grain was not large at high levels of fertilization. The highest values were formed for both varieties in the unfertilized variants at 450 g.s. for Kuber (47.94 g) and 350 g.s. for the Saira variety (48.97 g). Analyzing the data, it can be said that 1000-grain weight in the unfertilized variants was high where the least number of grains were formed and, conversely, in the variants with high levels of fertilization, the grain number was high and 1000-grain weight was low. This interrelationship between the indicators should be considered when

Table 1. Yield and structural elements of yield in two-row barley varieties average for the period 2019-2021

Variety	Variant	Grain yield (t/ha)				Spike length (cm)				Grain number per spike				Grain weight per spike (g)				1000-grain weight (g)			
		Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %
Kuber	P_1T_0/ SR_1F_0	3.26±0.79	2.60	4.14	24.27	6.99±0.59	6.62	7.67	8.44	22.56±0.67	21.88	23.21	2.95	0.99±0.21	0.77	1.18	20.87	47.62±6.59	40.23	52.88	13.84
	P_2T_0/ SR_2F_0	3.22±0.52	2.85	3.81	16.04	7.00±0.99	6.33	8.14	14.22	22.52±2.34	20.88	25.20	10.39	0.99±0.30	0.71	1.31	30.34	47.04±7.14	39.08	52.87	15.18
	P_3T_0/ SR_3F_0	3.55±0.82	3.01	4.50	23.16	7.02±0.97	6.24	8.11	13.83	22.29±2.00	20.70	24.54	9.00	1.01±0.23	0.83	1.27	22.84	47.94±7.00	40.55	54.48	14.61
	P_1T_1/ SR_1F_1	4.74±1.51	3.05	5.95	31.82	7.91±1.04	7.06	9.07	13.15	25.50±2.14	23.40	27.67	8.38	1.13±0.36	0.79	1.50	31.37	47.67±8.49	39.51	56.46	17.81
	P_2T_1/ SR_2F_1	5.02±0.64	4.30	5.50	12.71	7.86±0.94	7.08	8.91	12.02	25.10±1.88	23.22	26.97	7.47	1.12±0.31	0.83	1.45	27.72	47.07±7.29	40.76	55.05	15.48
	P_3T_1/ SR_3F_1	4.72±1.92	2.54	6.14	40.61	7.62±1.32	6.24	8.86	17.27	24.38±3.48	20.52	27.26	14.26	1.08±0.43	0.63	1.49	40.03	46.42±8.87	37.73	55.46	19.11
	P_1T_2/ SR_1F_2	5.66±1.50	4.02	6.96	26.46	8.17±1.14	7.08	9.35	13.91	26.46±2.57	23.59	28.55	9.72	1.15±0.39	0.77	1.54	33.60	46.70±7.95	39.61	55.29	17.02
	P_2T_2/ SR_2F_2	5.24±1.66	3.37	6.54	31.69	8.08±1.04	7.09	9.17	12.92	26.07±2.23	23.51	27.62	8.57	1.16±0.36	0.82	1.54	31.04	46.63±8.72	39.37	56.30	18.70
	P_3T_2/ SR_3F_2	4.99±1.99	2.76	6.58	39.86	7.85±1.37	6.84	9.41	17.42	25.15±3.24	22.03	28.50	12.89	1.12±0.45	0.70	1.60	40.63	47.07±10.44	37.31	58.08	22.18
	P_1T_3/ SR_1F_3	5.64±2.60	2.69	7.60	46.11	8.02±1.42	6.60	9.43	17.65	25.88±4.21	21.05	28.78	16.28	1.08±0.48	0.58	1.54	44.56	45.25±8.69	38.01	54.89	19.20
	P_2T_3/ SR_2F_3	5.80±1.45	4.16	6.94	25.09	7.81±1.51	6.19	9.17	19.30	25.05±5.02	19.27	28.30	20.03	1.12±0.45	0.65	1.54	39.99	46.02±9.55	37.26	56.21	20.76
	P_3T_3/ SR_3F_3	5.00±2.38	2.32	6.87	47.61	7.76±1.02	6.86	8.86	13.10	24.62±2.37	21.92	26.38	9.64	1.10±0.37	0.74	1.47	33.31	47.44±9.40	39.24	57.69	19.81
Saira	P_1T_0/ SR_1F_0	3.07±1.18	2.04	4.36	38.41	8.06±0.78	7.46	8.94	9.66	26.41±1.71	25.07	28.33	6.47	1.20±0.20	1.07	1.43	16.65	48.36±4.48	45.28	53.50	9.26
	P_2T_0/ SR_2F_0	3.65±1.43	2.63	5.28	39.17	7.63±1.40	6.19	8.98	18.32	24.80±3.26	21.58	28.09	13.13	1.15±0.28	0.89	1.44	24.14	48.97±4.59	46.10	54.27	9.38
	P_3T_0/ SR_3F_0	3.66±1.11	2.60	4.82	30.50	7.27±0.71	6.47	7.82	9.73	23.49±1.54	21.73	24.62	6.58	1.03±0.17	0.93	1.23	16.49	46.78±5.20	42.41	52.54	11.13
	P_1T_1/ SR_1F_1	4.69±0.44	4.22	5.10	9.45	8.41±0,73	7.90	9.25	8.69	27.59±0.40	27.20	27.99	1.43	1.20±0.22	1.07	1.46	18.48	46.23±6.54	41.36	53.66	14.15
	P_2T_1/ SR_2F_1	4.69±0.89	3.67	5.29	18.90	8.26±1,12	7.59	9.56	13.59	26.86±2.48	24.90	29.64	9.22	1.19±0.31	0.97	1.54	26.01	46.01±6.57	42.10	53.60	14.29
	P_3T_1/ SR_3F_1	5.66±0.44	5.22	6.10	7.77	7.99±1.03	7.39	9.18	12.86	26.08±2.07	24.30	28.35	7.94	1.13±0.30	0.91	1.47	26.43	45.64±5.94	42.20	52.50	13.02
	P_1T_2/ SR_1F_2	4.75±1.08	3.70	5.85	22.65	8.57±1.07	7.81	9.80	12.51	27.73±1.52	26.38	29.38	5.49	1.19±0.29	1.00	1.52	24.11	45.87±5.44	42.48	52.14	11.85
	P_2T_2/ SR_2F_2	5.30±0.92	4.24	5.89	17.36	8.57±1.24	7.71	10.00	14.52	27.64±2.04	26.40	30.00	7.39	1.21±0.31	1.03	1.57	25.46	45.75±6.96	40.81	53.71	15.21
	P_3T_2/ SR_3F_2	5.10±1.20	4.08	6.42	23.54	8.16±1.00	7.57	9.32	12.27	26.43±1.97	24.57	28.49	7.44	1.15±0.28	0.97	1.48	24.59	44.99±7.24	40.60	53.35	16.09
	P_1T_3/ SR_1F_3	5.59±0.95	4.62	6.53	17.09	8.60±1.03	7.96	9.79	12.03	28.04±1.33	26.98	29.53	4.74	1.21±0.27	1.02	1.51	21.96	45.69±7.10	40.10	53.68	15.55
	P_2T_3/ SR_2F_3	5.44±1.60	3.59	6.40	29.46	8.48±1.02	7.73	9.65	12.08	27.41±2.09	25.47	29.63	7.64	1.19±0.27	0.96	1.48	22.44	46.02±5.34	42.30	52.14	11.61
	P_3T_3/ SR_3F_3	5.30±0.93	4.75	6.38	17.58	8.20±1.05	7.51	9.41	12.86	26.71±2.35	24.75	29.31	8.78	1.13±0.29	0.92	1.46	25.35	44.46±6.96	38.51	52.11	15.65

determining appropriate fertilization levels and sowing rates. Similar correlations were reported by Popova (2019).

Table 2 presents data on the yield and structural elements of the multi-row barley varieties Zemela and Bozhin by fertilization variants and sowing rates, average for the period 2019-2021.

The lowest yield, average for the period, for both varieties was formed at 250 g.s. for the Bozhin variety (3.49 t/ha) and 350 g. seeds for the Zemela variety (3.36 t/ha) in the unfertilized variants (P1T0 and P2T0). The yield variation in these variants for both varieties was average from 10.61% for Bozhin to 18.52% for Zemela. The highest yields were in the variants with 350 g.s. for Zemela variety (P2T1) – 5.51 t/ha, and 450 g.s. for Bozhin variety (P3T1) – 6.55 t/ha and N8 fertilizing. During the study period, the yields by variants varied from medium to high in both varieties, with the exception of the yields of the P3T0 variant (VC%=7.66%) in the Bozhin variety (Table 2).

Spike length of the studied varieties during the study period varied from low to moderate (7.04% to 21.68%). The indicator had the weakest variation in the unfertilized variant with 450 g.s. in Bozhin variety (P3T0). The shortest spikes were formed for both varieties in the unfertilized variants with 450 g.s. (P3T0). The varieties formed the longest spikes at 250 g.s. at N12 fertilization for the Zemela variety and N8 for the Bozhin variety (Table 2).

Grain number per spike in both varieties ranged from low to medium, with VC% ranging from 7.91% to 26.14%. In both varieties, the fewest grains per spike were formed at 450 g.s. and unfertilized (P3T0) – 57.89 grains for the Zemela variety and 53.72 grains for the Bozhin variety. The largest number was for the Zemela variety – 75.76 grains at 250 g.s. and N12 fertilization, and for the Bozhin variety – 67.57 grains at 250 g.s. and N8 fertilization (Table 2).

In this study, grain weight per spike varied greatly, with VC% ranging from 22.13% to 50.97%. The lowest values of the indicator in both varieties were reported for the unfertilized variants at 450 g.s. (P3T0) – 1.66 g for the Zemela variety and 1.68 g for the Bozhin variety. The weight per spike was highest in the 250 g.s. variant fertilized with N16 (P1T3) – 2.32 g for the Zemela variety and 2.23 g for the Bozhin variety (Table 2).

The 1000-grain weight showed that VC% varied from low (VC%=6.07%) to moderate (VC%=18.62%) by variants and varieties. For the Zemela variety, the indicator had the lowest values in the variant at 450 g.s. and N16 fertilization – 33.31 g, and for the Bozhin variety at 350 g.s. and N16 fertilization – 33.58 g.

Table 2. Yield and structural elements of yield in multi-row barley varieties average for the period 2019-2021

Сорт	Variant	Grain yield (t/ha)				Spike length (cm)				Grain number per spike				Grain weight per spike (g)				1000-grain weight (g)			
		Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %	Mean ± STDEV	min	max	VC %
Zemela	P_1T_0/ SR_1F_0	3.63±0.72	3.06	4.44	19.79	4.91±0.80	3.98	5.41	16.38	67.73±11.36	55.25	77.48	16.78	2.11±0.80	1.23	2.79	37.87	38.31±4.50	33.16	41.48	11.75
	P_2T_0/ SR_2F_0	3.36±0.62	2.77	4.01	18.52	4.89±0.56	4.25	5.29	11.45	65.58±5.19	60.13	70.46	7.91	2.10±0.53	1.58	2.63	25.05	38.20±3.31	34.73	41.33	8.67
	P_3T_0/ SR_3F_0	3.48±0.55	3.09	4.10	15.68	4.40±0.46	3.96	4.87	10.34	57.89±8.04	50.45	66.42	13.89	1.66±0.72	0.96	2.39	43.00	36.87±4.13	32.40	40.53	11.19
	P_1T_1/ SR_1F_1	4.81±2.00	2.61	6.53	41.65	5.13±1.01	3.96	5.73	19.71	71.83±18.78	50.15	83.13	26.14	2.15±1.04	0.98	2.97	48.38	36.40±2.94	34.65	39.79	8.08
	P_2T_1/ SR_2F_1	5.51±1.52	3.78	6.65	27.63	5.12±0.73	4.28	5.64	14.31	73.79±12.73	59.10	81.55	17.25	2.29±0.79	1.39	2.86	34.48	36.80±2.23	34.38	38.78	6.07
	P_3T_1/ SR_3F_1	5.05±2.18	2.55	6.55	43.14	4.98±0.87	4.00	5.68	17.56	70.00±14.58	53.44	80.90	20.83	2.22±0.89	1.20	2.82	40.04	36.61±5.21	32.75	42.53	14.22
	P_1T_2/ SR_1F_2	5.09±2.17	2.94	7.29	42.74	5.29±1.14	3.98	5.97	21.49	73.92±17.57	53.65	84.81	23.77	2.21±1.05	1.03	3.04	47.50	34.82±3.35	32.25	38.61	9.63
	P_2T_2/ SR_2F_2	5.04±2.23	2.47	6.42	44.26	5.01±0.97	3.90	5.69	19.38	71.23±16.07	52.70	81.34	22.56	2.07±0.94	1.01	2.81	45.49	33.52±3.80	31.03	37.89	11.34
	P_3T_2/ SR_3F_2	5.42±2.28	2.85	7.20	42.06	5.02±1.05	3.80	5.65	21.01	70.99±17.58	50.70	81.60	24.76	2.11±0.94	1.04	2.79	44.42	34.47±2.35	33.03	37.18	6.82
	P_1T_3/ SR_1F_3	5.09±2.78	2.32	7.89	54.68	5.26±0.95	4.19	6.00	18.04	75.76±14.11	59.47	84.05	18.63	2.32±0.73	1.49	2.88	31.61	34.72±2.59	32.28	37.43	7.45
	P_2T_3/ SR_2F_3	5.41±2.17	2.91	6.78	40.10	4.96±0.90	3.93	5.58	18.14	70.11±16.73	50.84	80.95	23.87	2.01±0.90	1.00	2.71	44.60	33.96±2.42	31.21	35.78	7.13
	P_3T_3/ SR_3F_3	5.13±2.83	1.87	6.84	55.07	4.96±1.08	3.72	5.64	21.68	69.09±19.24	46.95	81.80	27.85	2.00±1.02	0.83	2.69	50.97	33.31±3.84	30.85	37.74	11.54
Bozhin	P_1T_0/ SR_1F_0	3.49±0.37	3.07	3.76	10.61	4.75±0.53	4.14	5.09	11.15	61.95±5.83	55.30	66.16	9.41	2.10±0.53	1.49	2.45	25.17	38.84±5.81	32.13	42.24	14.96
	P_2T_0/ SR_2F_0	4.38±0.89	3.52	5.30	20.33	4.51±0.34	4.24	4.89	7.55	57.75±4.60	55.00	63.06	7.96	1.88±0.42	1.49	2.32	22.13	38.02±6.33	30.85	42.86	16.66
	P_3T_0/ SR_3F_0	4.44±0.34	4.06	4.72	7.66	4.29±0.30	4.01	4.61	7.04	53.72±4.45	51.05	58.85	8.28	1.68±0.39	1.29	2.07	23.26	37.16±5.90	30.48	41.63	15.87
	P_1T_1/ SR_1F_1	4.56±0.87	3.82	5.52	19.10	5.29±0.97	4.24	6.15	18.32	67.57±13.19	52.55	77.25	19.52	2.16±0.79	1.26	2.71	36.38	35.61±6.63	28.26	41.14	18.62
	P_2T_1/ SR_2F_1	5.35±1.48	3.82	6.77	27.62	5.03±0.90	4.03	5.77	17.85	65.09±13.59	49.62	75.10	20.88	2.05±0.84	1.08	2.61	41.09	35.05±5.32	29.51	40.12	15.18
	P_3T_1/ SR_3F_1	6.55±1.23	5.25	7.71	18.87	5.02±0.76	4.17	5.61	15.05	63.65±11.65	50.30	71.75	18.30	2.00±0.68	1.23	2.49	33.80	34.76±5.70	28.50	39.64	16.39
	P_1T_2/ SR_1F_2	4.33±1.38	2.76	5.37	31.97	5.18±0.91	4.13	5.76	17.55	66.93±14.62	50.12	76.65	21.84	2.00±0.84	1.07	2.71	42.09	34.73±5.79	30.97	41.40	16.67
	P_2T_2/ SR_2F_2	5.89±134	4.79	7.38	22.72	5.19±0.91	4.15	5.88	17.64	65.70±12.00	51.85	72.90	18.26	2.06±0.67	1.31	2.62	32.77	34.29±5.96	29.28	40.88	17.38
	P_3T_2/ SR_3F_2	6.31±1.96	4.07	7.70	31.06	4.91±0.81	3.98	5.42	16.43	61.58±11.52	48.35	69.35	18.70	1.95±0.69	1.17	2.50	35.60	35.10±4.40	30.50	39.26	12.53
	P_1T_3/ SR_1F_3	5.12±1.58	3.32	6.27	30.86	5.28±0.99	4.14	5.78	18.79	67.31±15.42	49.55	77.20	22.90	2.23±0.89	1.25	2.98	39.80	35.17±2.56	33.31	38.09	7.28
	P_2T_3/ SR_2F_3	5.69±1.87	3.55	7.00	32.82	5.12±0.73	4.28	5.62	14.33	63.93±11.61	50.53	70.95	18.16	1.99±0.60	1.34	2.53	30.29	33.58±3.56	30.75	37.58	10.61
	P_3T_3/ SR_3F_3	6.23±1.33	4.87	7.53	21.36	5.01±0.73	4.22	5.67	14.64	63.89±11.73	50.90	73.70	18.36	1.99±0.50	1.41	2.31	25.20	33.72±4.28	30.16	38.47	12.69

The highest values for both varieties were formed in the unfertilized variants at 250 g.s. for Zemela variety (38.31 g) and for Bozhin variety (38.84 g). Here also, along with the two-row varieties, it can be said that 1000-grain weight in the unfertilized variants was high, where the grain number was small, and, conversely, in the variants with high levels of fertilization, the grain number was high, and the 1000-grain weight was low.

Table 3 presents the results from the analysis of variance performed on yield and some structural elements. The year conditions were of essential importance for yield variation, with factor strength of η=25.84%. Fertilization had an almost equal effect on yield formation (η=23.42%). The role of genotype and sowing rate was insignificant compared to the other two factors. The interaction between year conditions and fertilization also had high values of factor strength (η=24.75%).

Spike length is one of the parameters that are strongly genotypically predetermined (Ur et al., 2023). The strength of the genotype factor for it was 74.71%. Naturally, under the pressure of the contrasting year conditions, spike length varied, but the factor strength was significantly lower (η=14.38%). The genotype x year interaction of factors also contributed to the indicator variation, with strength of η=4.70% (Table 3).

Similar results were obtained for grain number per spike. Their variation was due to the genotype role, and the factor strength was 85.72%. The year conditions also contributed to this variation, although with a lower strength (η=6.55%). The two factors interacted and their strength was η=4.08%.

Regarding grain weight per spike, the year and genotype factors contributed with an almost equal effect, with a strength of η=37.48% and η=46.58%, respectively. The variation of the indicator also depended on the interaction of the year x genotype factors, with a strength of 10.33%.

The genotype role for 1000-grain weight was the greatest, with η=53.16%, followed by year with a factor strength of 33.96%. The year and genotype interaction further contributed to the variation in 1000-grain weight (η=6.26%). Fertilization was also important for the manifestation of the indicator, whose strength was 2.18%.

An analysis of the results led us to draw the conclusion that the year and fertilization factors, as well as their interaction, had a limiting effect on yield. In the studied varieties, the structural elements were genetically determined and their variation depended on the year conditions and the interaction of these factors.

Table 3. Analysis of variance of yield and some structural elements in barley varieties for the period 2019-2021

Factor	Grain yield		Spike length		Grain number per spike		Grain weight per spike		1000-grain weight	
	MS	ŋ	MS	ŋ	MS	ŋ	MS	ŋ	MS	ŋ
Year	172.825***	25.84	121.989***	14.38	9341.274***	6.55	50.617***	37.48	5555.002***	33.96
Genotype	7.356***	1.65	422.677***	74.71	81536.775***	85.72	41.934***	46.58	5796.907***	53.16
Sowing rate	10.079***	1.51	5.620***	0.66	535.510***	0.38	0.817***	0.61	16.029***	0.10
Fertilization	104.418***	23.42	14.827***	2.62	946.050***	0.99	0.656***	0.73	237.314***	2.18
Year x Genotype	18.971***	8.51	13.294***	4.70	1938.494***	4.08	4.652***	10.33	340.992***	6.26
Year x Sowing rate	1.321***	0.39	0.251***	0.06	30.724***	0.04	0.088***	0.13	8.234**	0.10
Year x Fertilization	55.178***	24.75	3.747***	1.32	429.361***	0.90	0.561***	1.25	92.128***	1.69
Genotype x Sowing rate	7.001***	3.14	0.243***	0.09	64.077***	0.13	0.120***	0.27	9.145***	0.17
Genotype x Fertilization	0.754***	0.51	0.593***	0.31	82.038***	0.26	0.068***	0.23	17.932***	0.49
Sowing rate x Fertilization	1.174***	0.53	0.091**	0.03	33.967***	0.07	0.140***	0.31	3.483**	0.06
Year x Genotype x Sowing rate	3.388***	3.04	0.200***	0.14	20.903***	0.09	0.059***	0.26	3.592**	0.13
Year x Genotype x Fertilization	1.347***	1.81	0.159***	0.17	69.916***	0.44	0.080***	0.53	12.511***	0.69
Year x Sowing rate x Fertilization	0.479***	0.43	0.265***	0.19	11.113***	0.05	0.034**	0.15	3.753**	0.14
Genotype x Sowing rate x Fertilization	1.215***	1.63	0.148***	0.16	19.649***	0.12	0.080***	0.53	4.045**	0.22
Year x Genotype x Sowing rate x Fertilization	1.056***	2.84	0.215***	0.46	14.127***	0.18	0.045***	0.60	5.891***	0.65

Conclusions:

1. Higher yields are obtained by increasing the fertilization levels, and for two-row varieties it is N16 fertilization, and for the multi-row varieties it is N8 fertilization.
2. Spike length increases at fertilization levels from N8 to N16.
3. The highest number of grains per spike is formed at low sowing rates (250 g.s.) and at fertilization rates from N8 to N16
4. Grain weight per spike has the highest values at sowing rates of 250 g.s. and 350 g.s. and N12 to N16 fertilization.
5. In two-row barley varieties, a high 1000-grain weight is formed at high sowing rates and no fertilization, and in multi-row varieties – at low sowing rates and no fertilization.
6. It has been established that year, fertilization and their interaction play the greatest role in forming grain yield.
7. The variation in the studied structural elements is influenced by year and genotype, as well as by their interaction.

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EFFECT OF GROWING CONDITIONS ON GRAIN YIELD AND SEED YIELD IN WINTER BARLEY GENOTYPES

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Abstract

The aim of this study was to determine the impact of growing conditions on the formation of grain yield and seed yield in barley genotypes. The research was conducted in field and laboratory experiments at the Institute of Agriculture - Karnobat, Bulgaria, in the period 2019-2021, as a multifactorial field experiment with four fertilization variants (N0:N8:N12:N16) and three sowing rates (250, 350 and 450 germinable seeds). It was established that there were demonstrable differences in the studied genotypes. The data showed that grain yield was highest in the variants with a seeding rate of 250 germinable seeds and N_{12} and N_{16} in the two-row Kuber and Saira varieties. With the six-row Zemela and Bozhin varieties, the highest grain yield was in the variants with a sowing rate of 350 and 450 germinable seeds and fertilization with N_8, N_{12} and N_{16}. Average for the years of the study, seed yield was highest in 2019 in the unfertilized and N8-fertilized variants at all sowing rates. It can be seen from the analysis of variance that year conditions and fertilization, as well as their interaction, had the greatest impact on grain yield and seed yield.
Keywords: genotype, barley, conditions, grain yield, seed yield

INTRODUCTION

Barley yield depends on the varietal genetic predisposition and growing conditions - the soil-climatic factors of the area and the applied agrotechnical measures. The agroecological and climatic conditions in different regions of the country affect barley development and productivity (Penchev, Gramatikov, 2001). Since these conditions are uncontrollable, the main factors for the full development of the potential varietal capabilities are the properly selected agrotechnical units (Arisnabarreta, Miralles, 2006; Ivanova et al., 2007; Tonev et al., 2008). Depending on their biological requirements and adaptive potential, crops respond with a corresponding increase in yield (Gastal & Lemaire, 2002; Hirel et al., 2007). The proper varietal structure, depending on the specific agro-ecological conditions of the area, can significantly increase the production yields and quality (Ilieva, 2011). In barley seed production, the varietal response to the growing conditions and utility coefficient of seed preparation, which shows what part of the grain yield is taken by seed yield, is of great importance (Bonchev, 2017).
The aim of this study was to establish the impact of growing conditions on grain yield and seed yield in winter barley genotypes.

MATERIAL AND METHODS

In the period 2019-2021, at the Institute of Agriculture - Karnobat, Bulgaria, was studied the impact of growing conditions on grain yield and seed yield in winter barley genotypes. In a field multifactorial experiment with 4 fertilization variants (T_0 – no fertilization; T_1 – fertilization with N_8; T_2 – fertilization with N_{12}; T_3 – fertilization with N_{16}) and three sowing rates (P_1 - 250, P_2 - 350 and P_3 - 450 germinable seeds) were grown winter barley varieties

Kuber, Saira, Zemela and Bozhin. The P3T1 variant was adopted as the optimal cultivation variant as determined in the barley cultivation technology in Bulgaria (Gramatikov et al., 2004). The varieties were sown on a harvest plot size of 10 m^2. The grain yield of each plot was reported as well as the seed yield. The results were statistically processed by analysis of variance using the JMP and SPSS 19.0 software products.
The weather conditions during the three years of testing were extremely contrasting (Figures 1 and 2). In the first (2019) and third (2021) years of the barley growing season, the weather conditions were beneficial for the development of the varieties and obtaining high yields from them. The rainfall during the filling and ripening phases of the grain had the most favorable effect. In the second year (2020), the weather conditions were unfavorable for the development of the varieties.

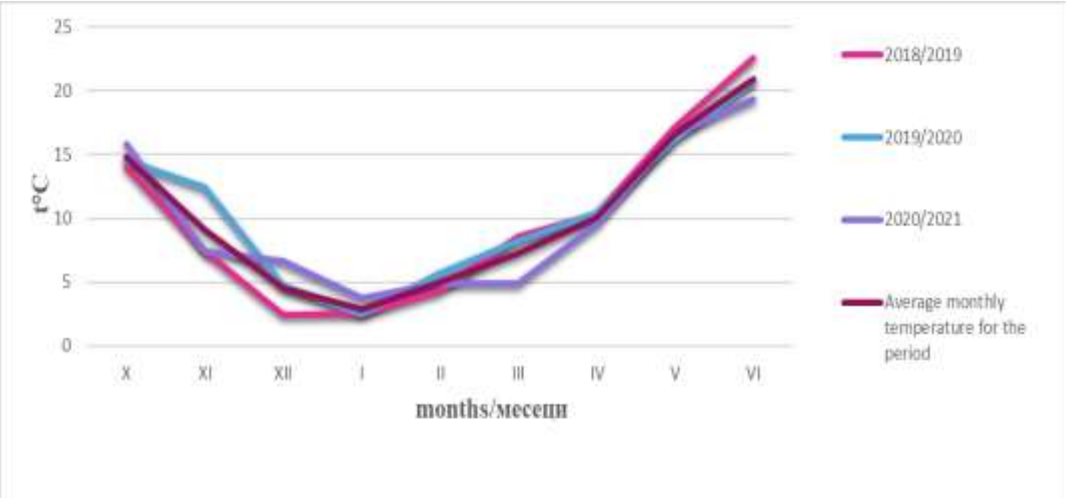

Figure 1. Average monthly air temperatures in the period 2019-2021

The plant growth in 2020 occurred at higher average monthly temperatures compared to the multi-year average values for the period. The rainfall amount was very small. It was the driest year in the region for the last 20 years.

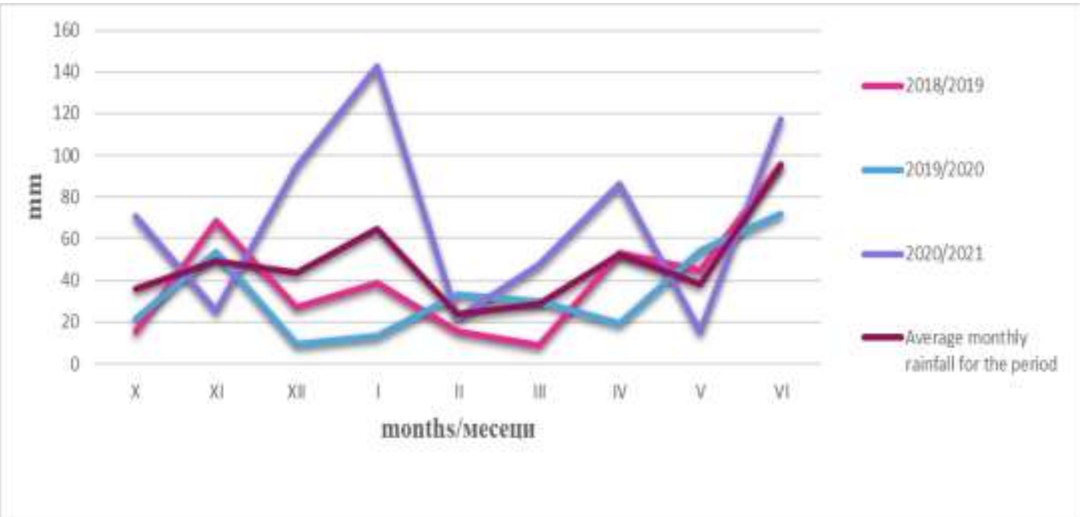

Figure 2. Average monthly rainfall in the period 2019-2021

RESULTS AND DISCUSSION

Table 1 presents data on the grain yield obtained from the four tested varieties by year and average for the period. In 2019 and 2021, average yields of 5.38 t/ha and 5.45 t/ha, respectively, were obtained from the studied varieties in all variants. The most unfavorable year was 2020 with an average grain yield of 3.76 t/ha from the studied varieties in all variants. The decrease in yield was 1.62 t/ha compared to the first year and 1.69 t/ha compared to the third. The great drought during the growing season of 2019/2020 played a negative part in the final value of the experiment - grain yield (Figure 2). Of the two-row varieties, the highest average grain yields in all variants were formed in 2019 in the amount of 5.40 t/ha for the Kuber variety and 5.28 t/ha for the Saira variety (Table 1). The yields in 2020 were lowest for the two-row varieties - 3.47 t/ha for the Kuber variety and 4.47 t/ha for the Saira variety, average for all variants. The multi-row varieties formed the highest yield in 2021 in the amount of 5.92 t/ha for the Zemela variety and 5.96 t/ha for the Bozhin variety, average for all variants. Both in the two-row varieties and in the multi-row varieties, the drought had a negative impact on the yield and it was the lowest in 2020 – 2.93 t/ha for the Zemela variety and 4.17 t/ha for the Bozhin variety, average for all variants.

The data shows that the highest average yield for the period at the different fertilization rates was obtained after fertilization with N16 - an average of 5.46 t/ha for the three years. In 2019 and 2021, the average grain yield was highest when fertilized with N16 – 6.48 t/ha and 6.47 t/ha, respectively. In 2020, the yield fertilized with N16 was the lowest (3.42 t/ha), and the highest values were obtained in the unfertilized variants (N0). This was due to the small amount of rainfall in the spring of 2020 and insufficient water reserves from the winter months. The

Table 1. Results of grain yield obtained from barley varieties at different fertilization and sowing rates in the period 2019-2021

Factors	Grain yield per ha																					
	Varieties																					
Fertilization	Kuber					Saira					Zemela					Bozhin						
Sowing rates	P1	P2	P3	Avg.	LSD for sowing rates	P1	P2	P3	Avg.	LSD for sowing rates	P1	P2	P3	Avg.	LSD for sowing rates	P1	P2	P3	Avg,	Avg. of the year varieties	LSD for sowing rates	LSD avg. for sowing rates
Year	**2019**																					
T0	2.60^{b}	2.85^{ab}	301^{a}	2.82^{b}	0.39	2.82^{a}	3.03^{a}	3.54^{a}	3.13^{ab}	0.84	3.06^{b}	4.01^{a}	4.10^{a}	3.72^{a}	0.75	3.07^{b}	3.52^{b}	4.06^{a}	3.55^{ab}	**3.31**	0.57	0.82
T1	5.95^{a}	5.50^{a}	548^{a}	5.64^{a}	0.48	5.10^{a}	5.29^{a}	6.10^{a}	5.50^{a}	1.38	5.30^{b}	6.09^{b}	6.55^{a}	5.98^{a}	1.01	4.34^{c}	5.47^{b}	6.70^{a}	5.50^{a}	**5.66**	0.86	1.38
T2	6.96^{a}	6.54^{ab}	564^{b}	6.38^{a}	1.11	5.85^{a}	5.89^{a}	6.42^{a}	6.05^{a}	1.29	5.04^{b}	5.73^{ab}	6.19^{a}	5.65^{a}	0.91	5.37^{b}	5.50^{b}	7.70^{a}	6.19^{a}	**6.07**	0.87	1.52
T3	7.60^{a}	6.94^{ab}	580^{b}	6.78^{a}	1.15	6.53^{a}	6.33^{a}	6.38^{a}	6.41^{a}	1.49	5.07^{b}	6.55^{a}	6.69^{a}	6.11^{a}	0.65	5.78^{b}	6.51^{b}	7.53^{a}	6.61^{a}	**6.48**	0.85	1.47
Average	5.78	5.46	498	**5.40^{a}**		5.08	5.14	5.61	**5.28^{a}**		4.62	5.60	5.89	**5.37^{a}**		4.64	5.25	6.50	**5.46^{a}**	**5.38**		**1.19**
LSD for fertilization	0.88	0.76	0.84			1.34	1.09.	1.25			0.56	0.98	0.84			0.87	0.57	0.83				
	2020																					
T0	4.14^{a}	3.81^{a}	4.50^{a}	4.15^{ab}	2.13	4.36^{a}	5.28^{a}	4.82^{a}	4.82^{a}	0.22	4.44^{a}	3.30^{a}	3.09^{a}	3.61^{b}	2.41	3.76^{a}	5.30^{a}	4.72^{a}	4.59^{ab}	**4.29**	2.63	1.14
T1	3.05^{a}	4.30^{a}	2.54^{a}	3.30^{ab}	2.00	4.75^{ab}	3.67^{b}	5.66^{a}	4.69^{a}	1.78	2.61^{a}	3.78^{a}	2.55^{a}	2.98^{b}	2.25	3.82^{a}	3.82^{a}	5.25^{a}	4.30^{ab}	**3.82**	2.33	1.62
T2	4.02^{a}	3.37^{a}	2.76^{a}	3.38^{ab}	2.92	3.70^{a}	4.24^{a}	4.08^{a}	4.01^{a}	2.03	2.94^{a}	2.47^{a}	2.85^{a}	2.75^{b}	1.42	2.76^{a}	4.79^{a}	4.07^{a}	3.87^{ab}	**3.50**	2.24	1.18
T3	2.69^{a}	4.16^{a}	2.32^{a}	3.05^{ab}	2.90	4.62^{a}	3.59^{a}	4.78^{a}	4.33^{a}	2.57	2.32^{a}	2.91^{a}	1.87^{a}	2.37^{b}	1.81	3.32^{a}	3.55^{a}	4.87^{a}	3.91^{a}	**3.42**	2.01	1.44
Average	3.48	3.91	3.03	**3.47^{bc}**		4.36	4.20	4.84	**4.47^{a}**		3.08	3.12	2.59	**2.93^{c}**		3.42	4.37	4.73	**4.17^{ab}**	**3.76**		**0.87**
LSD for fertilization	2.54	2.73	1.97			2.38	2.05	1.80			2.10	2.13	1.54			1.51	2.42	2.60				
	2021																					
T0	3.05^{a}	3.00^{a}	3.15^{a}	2.43^{c}	0.72	2.04^{a}	2.63^{a}	2.60^{a}	2.42^{c}	0.75	3.40^{a}	2.77^{a}	3.24^{a}	3.14^{b}	0.96	3.65^{a}	4.33^{a}	4.53^{a}	4.17^{a}	**3.04**	1.11	0.69
T1	5.22^{a}	5.28^{a}	6.14^{a}	5.55^{ab}	1.17	4.22^{a}	5.10^{a}	5.22^{a}	4.85^{b}	1.10	6.53^{a}	6.65^{a}	6.04^{a}	6.41^{a}	1.30	5.52^{b}	6.77^{ab}	7.71^{a}	6.67^{a}	**5.87**	1.70	1.29
T2	6.01^{a}	5.82^{a}	6.58^{a}	6.14^{ab}	1.48	4.70^{a}	5.77^{a}	4.79^{a}	5.09^{b}	1.18	7.29^{a}	6.42^{a}	7.20^{a}	6.97^{a}	1.38	4.87^{b}	7.38^{a}	7.17^{a}	6.47^{ab}	**6.17**	1.57	1.54
T3	6.64^{a}	6.29^{a}	6.87^{a}	6.60^{ab}	1.31	5.62^{ab}	6.40^{a}	4.75^{b}	5.59^{b}	1.11	7.89^{a}	6.78^{b}	6.84^{b}	7.17^{a}	0.74	6.27^{a}	7.00^{a}	6.29^{a}	6.52^{ab}	**6.47**	1.76	1.09
Average	5.23	5.10	5.69	**5.34^{ab}**		4.15	4.98	4.34	**4.49^{b}**		6.28	5.66	5.83	**5.92^{a}**		5.08	6.37	6.43	**5.96^{a}**	**5.45**		**0.93**
LSD for fertilization	1.36	0.81	1.24			1.20	0.82	0.98			1.36	1.01	0.80			1.41	1.14	1.87				
	Average for period																					
T0	3.26	3.22	3.55	3.34		3.07	3.65	3.66	3.46		3.63	3.36	3.48	3.49		3.49	4.38	4.44	4.10	**3.55**		
T1	4.74	5.02	4.72	4.83		4.69	4.69	5.66	5.01		4.81	5.51	5.05	5.12		4.56	5.35	6.55	5.49	**5.11**		
T2	5.66	5.24	4.99	5.30		4.75	5.30	5.10	5.05		5.09	5.04	5.42	5.18		4.33	5.89	6.31	5.51	**5.25**		
T3	5.64	5.80	5.00	5.48		5.58	5.44	5.30	5.44		5.09	5.41	5.13	5.21		5.12	5.69	6.23	5.68	**5.46**		
Average	4.83	4.82	4.57	**4.74**		4.52	4.77	4.93	**4.74**		4.66	4.83	4.77	**4.75**		4.38	5.33	5.88	**5.20**	**4.84**		

nitrogen feeding under the drought conditions of February 2020 led to absence of nitrogen uptake by the plants and even impediment to their development. The rainfall in May and June could not compensate for the delay in plant development and this had a negative impact on grain yield. Similar results were also reported by other authors (Valchev, Valcheva, 2019).

The sowing rate also affected yield formation. Analyzing the data, it can be seen that in 2019, in the Kuber variety, there was a demonstrably higher yield in the P1T3 variant (7.60 t/ha), while in the Saira variety, there were no demonstrable differences between the variants with different sowing rates. In the same year, the multi-row varieties formed the highest yield in variants P3T3 for Zemela (6.69 t/ha) and P3T2 for Bozhin (7.70 t/ha). In 2020, for Kuber, Zemela and Bozhin, there were no demonstrable differences in the yield from variants of different sowing rates. The Saira variety had the demonstrably highest yield in the P3T1 variant, which was considered in the study to be the optimal variant for barley cultivation. In 2021, there were no demonstrable differences in the yield of the Kuber variety in the variants of different sowing rates. With the Saira variety, the highest yield was demonstrated in the P2T3 variant in the amount of 6.40 t/ha. The yield of the Zemela variety was demonstrably highest in the P1T3 variant – 7.89 t/ha, while for the Bozhin variety, the highest values were in the P3T1 (7.71 t/ha) and P2T2 (7.38 t/ha) variants. The lack of a clearly expressed tendency by years and varieties to form high yields at certain sowing rates indicates that the impact of other environmental factors was stronger.

Table 2 presents data on the seed yield obtained from the four tested varieties by year and average for the period. In 2019 and 2021, average seed yields of 2.47 t/ha and 1.94 t/ha, respectively, were obtained from the studied varieties in all variants. It can be seen that both in terms of grain yield and seed yield, the most unfavorable year was 2020 - with an average yield of 1.84 t/ha from the studied varieties of all variants.

The highest average yield for the period at the different fertilization rates was obtained in the unfertilized variants (N0) - an average of 2.10 t/ha for the three years, followed by N8 - 2.09 t/ha. The seed yield was lowest when fertilized with N16 for the entire study period – 2.06 t/ha. The data show that higher yields in total for the period were obtained without fertilization (N0) and with N8 fertilization, which is also the optimal variant for barley cultivation. In the third year of the experiment, although there was a higher amount of rainfall in total for the growing season, February and May were very dry and this impeded nitrogen uptake and affected seed yield (Figure 2).

Regarding the sowing rate, it is clear that it did not affect the formation of seed yield. During the study period there were no demonstrable differences between variants of different seeding rates in all the varieties. In 2021, Zemela produced the highest seed yield in the P2T0 variant (1.97 t/ha), and the lowest in the P3T3 variant (1.83 t/ha). In the same year, Bozhin produced the highest seed yield in the variants without fertilization (T0) and at the three sowing rates (P1, P2 and P3) - 1.96 t/ha, and the lowest seed yield in the P3T3 variant - 1.75 t/ha.

Table 2. Results of seed yield obtained from barley varieties at different fertilization and sowing rates in the period 2019-2021

Factors	Seed yield per ha																					
	Varieties																					
Fertilization	Kuber					Saira					Zemela					Bozhin						
Sowing rates	P1	P2	P3	Avg.	LSD for sowing rates	P1	P2	P3	Avg.	LSD for sowing rates	P1	P2	P3	Avg.	LSD for sowing rates	P1	P2	P3	Avg,	Avg. of the year varieties	LSD for sowing rates	LSD avg. for sowing rates
Year	**2019**																					
T0	2.49[a]	2.49[a]	2.49[a]	2.49[a]	0.14	2.48[a]	2.48[a]	2.48[a]	2.48[b]	0.16	2.48[a]	2.47[a]	2.47[a]	2.47[c]	0.10	2.47[a]	2.47[a]	2.47[a]	2.47[c]	**2.48**	0.17	0.06
T1	2.49[a]	2.49[a]	2.49[a]	2.49[a]	0.17	2.48[a]	2.48[a]	2.48[a]	2.48[a]	0.13	2.47[a]	2.47[a]	2.46[a]	2.47[b]	0.27	2.46[a]	2.46[a]	2.47[a]	2.46[b]	**2.48**	0.23	0.09
T2	2.49[a]	2.49[a]	2.49[a]	2.49[a]	0.12	2.48[a]	2.48[a]	2.48[a]	2.48[a]	0.18	2.46[a]	2.44[a]	2.47[a]	2.46[b]	0.39	2.46[a]	2.45[ab]	2.44[b]	2.45[b]	**2.47**	0.18	0.17
T3	2.49[a]	2.49[a]	2.48[a]	2.48[a]	0.13	2.49[a]	2.48[a]	2.49[a]	2.49[a]	0.15	2.47[a]	2.47[a]	2.47[a]	2.47[b]	0.27	2.43[a]	2.43[a]	2.42[a]	2.43[c]	**2.47**	0.24	0.09
Average	2.49	2.49	2.49	**2.49[a]**		2.48	2.48	2.48	**2.48[b]**		2.47	2.46	2.47	**2.47[c]**		2.46	2.45	2.45	**2.45[d]**	**2.47**		0.08
LSD for fertilization	0.16	0.13	0.13			0.18	0.15	0.12			0.13	0.37	0.24			0.19	0.16	0.24				
	2020																					
T0	1.95[a]	1.95[a]	1.96[a]	1.95[a]	0.26	1.96[a]	1.96[a]	1.96[a]	1.96[a]	0.26	1.91[a]	1.89[a]	1.90[a]	1.90[a]	0.11	1.56[a]	1.65[a]	1.44[a]	1.55[b]	**1.84**	0.46	0.10
T1	1.93[a]	1.95[a]	1.94[a]	1.94[a]	0.49	1.96[ab]	1.94[b]	1.97[a]	1.96[a]	0.18	1.86[a]	1.87[a]	1.88[a]	1.87[a]	0.14	1.68[a]	1.49[a]	1.63[a]	1.60[b]	**1.84**	0.40	0.09
T2	1.93[a]	1.94[a]	1.95[a]	1.94[a]	0.39	1.94[a]	1.96[a]	1.95[a]	1.95[a]	0.28	1.85[a]	1.81[a]	1.83[a]	1.83[b]	0.13	1.67[a]	1.54[a]	1.52[a]	1.58[c]	**1.83**	0.29	0.08
T3	1.94[a]	1.93[a]	1.94[a]	1.94[a]	0.24	1.95[a]	1.94[a]	1.95[a]	1.95[a]	0.33	1.82[a]	1.87[a]	1.82[a]	1.84[b]	0.18	1.59[a]	1.57[a]	1.43[a]	1.53[c]	**1.82**	0.54	0.09
Average	1.94	1.94	1.95	**1.95[a]**		1.95	1.95	1.96	**1.96[a]**		1.86	1.86	1.86	**1.86[b]**		1.63	1.56	1.51	**1.57[c]**	**1.84**		0.06
LSD for fertilization	0.43	0.32	0.28			0.28	0.27	0.21			0.13	0.14	0.15			0.43	0.44	0.38				
	2021																					
T0	1.98[b]	1.98[b]	1.98[a]	1.98[a]	0.04	1.95[a]	1.97[a]	1.97[a]	1.96[b]	0.30	1.96[a]	1.97[a]	1.96[a]	1.96[b]	0.19	1.96[a]	1.96[a]	1.96[a]	1.96[b]	**1.97**	0.54	0.12
T1	1.97[a]	1.98[a]	1.98[a]	1.98[a]	0.10	1.96[b]	1.96[b]	1.97[a]	1.96[b]	0.07	1.94[a]	1.95[a]	1.96[a]	1.95[c]	0.24	1.92[a]	1.93[a]	1.93[a]	1.93[d]	**1.96**	0.28	0.13
T2	1.97[a]	1.98[a]	1.98[a]	1.98[a]	0.17	1.96[a]	1.95[a]	1.95[a]	1.95[a]	0.13	1.90[a]	1.84[a]	1.91[a]	1.88[b]	0.78	1.83[a]	1.83[a]	1.90[a]	1.85[b]	**1.92**	0,11	0.53
T3	1.97[a]	1.97[a]	1.97[a]	1.97[a]	0.23	1.96[a]	1.94[a]	1.94[a]	1.95[ab]	0.27	1.92[a]	1.89[ab]	1.83[b]	1.88[b]	0.68	1.87[a]	1.80[a]	1.75[a]	1.81[c]	**1.90**	0.16	0.72
Average	1.97	1.98	1.98	**1.98[a]**		1.96	1.96	1.96	**1.96[b]**		1.93	1.91	1.92	**1.92[c]**		1.90	1.88	1.89	**1.89[d]**	**1.94**		0.14
LSD for fertilization	0.16	0.16	0.08			0.29	0.16	0.15			0.33	0.65	0.54			0.50	1.23	1.02				
	Average for period																					
T0	2.14	2.14	2.14	2.14		2.13	2.14	2.14	2.14		2.12	2.11	2.11	2.11		2.00	2.03	1.96	2.00	**2.10**		
T1	2.13	2.14	2.13	2.13		2.13	2.13	2.14	2.13		2.09	2.10	2.10	2.10		2.02	1.96	2.01	2.00	**2.09**		
T2	2.13	2.14	2.14	2.14		2.13	2.13	2.13	2.13		2.07	2.03	2.07	2.06		1.99	1.94	1.95	1.96	**2.07**		
T3	2.13	2.13	2.13	2.13		2.13	2.12	2.13	2.13		2.07	2.08	2.04	2.06		1.96	1.93	1.87	1.92	**2.06**		
Average	2.13	2.14	2.14	**2.14**		2.13	2.13	2.14	**2.13**		2.09	2.08	2.08	**2.08**		1.99	1.97	1.95	**1.97**	**2.08**		

The year conditions are a factor with a large share of the total variation and largely determine the possibilities for realizing the barley productive potential (Dimova, 2015). Table 3 presents data on the strength of the factors under which the different variants were grown - year, genotype, fertilization, sowing rate. In this study, the analysis of variance showed that the year had the biggest role with a strength of 25.84% for grain yield and 88.82% for seed yield with very good demonstration. Many researchers highlight fertilization as a factor with a decisive role in yield formation (Savova, 2001; Dimitrova-Doneva, 2007). Fertilization had a major impact on high grain yields, with a strength of η=23.42 %. Unlike grain yield, seed yield was not affected by the fertilization levels, and the factor's influence was only 0.24%.

The genotype role in forming the seed yield was stronger (η=5.20 %). The share of interaction between year conditions and fertilization for grain yield (η=8.51 %) and seed yield (η=4.60 %) was demonstrably large.

The tested varieties responded almost identically to the growing conditions and the analysis results show that the genotype role was weak for grain yield (η=1.65 %) and seed yield (η=5.20 %). Under contrasting conditions of the year, the genotype role was more pronounced, with the interaction between the two factors being η=8.51% for grain yield and 4.60% for seed yield, followed by the genotype x sowing rate interaction – 3.14%. As an independent factor, the impact of sowing rate was weakest on grain yield (η=1.51 %) and seed yield (η=0.03 %); as well as fertilization on seed yield (η=0.24 %), and its interaction with the year conditions for grain yield η=0.39 % and for seed yield (η=0.03 %).

Table 3. Analysis of variance on grain and seed yields in barley varieties at different fertilization and sowing rates in the period 2019-2021

Factors	Grain yield		Seed yield	
	MS	ŋ	MS	ŋ
Year	172.825***	25.84	227907.848***	88.82
Genotype	7.356***	1.65	8894.813***	5.20
Sowing rate	10.079***	1.51	74.883*	0.03
Fertilization	104.418***	23.42	406.239**	0.24
Year x Genotype	18.971***	8.51	3938.441***	4.60
Year x Sowing rate	1.321***	0.39	34.066 n.s.	0.03
Year x Fertilization	55.178***	24.75	78.957*	0.09
Genotype x Sowing rate	7.001***	3.14	67.568*	0.08
Genotype x Fertilization	0.754***	0.51	104.369**	0.18
Sowing rate x Fertilization	1.174***	0.53	62.180 n.s.	0.07
Year x Genotype x Sowing rate	3.388***	3.04	44.291 n.s.	0.10
Year x Genotype x Fertilization	1.347***	1.81	37.049 n.s.	0.13
Year x Sowing rate x Fertilization	0.479***	0.43	37.055 n.s.	0.09
Genotype x Sowing rate x Fertilization	1.215***	1.63	37.601 n.s.	0.13
Year x Genotype x Sowing rate x Fertilization	1.056***	2.84	29.108 n.s.	0.21

Conclusions:

1. Of the two-row varieties, the highest average grain yields of all variants were formed in 2019 in the amount of 5.40 t/ha for the Kuber variety and 5.28 t/ha for the Saira variety.
2. The multi-row varieties formed the highest yield in 2021 in the amount of 5.92 t/ha for the Zemela variety and 5.96 t/ha for the Bozhin variety, average of all variants.
3. The highest average yield for the period at the different levels of fertilization was obtained with fertilization with N16 - an average of 5.46 t/ha for the three years.
4. The lack of a clearly expressed tendency by years and varieties to form high yields at certain seeding rates shows that the influence of other environmental factors is stronger.

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DETERMINATION OF SOME MORPHOLOGICAL CHARACTERISTICS OF HERBICIDE-RESISTANT M$_3$ MUTANT QUINOA (*Chenopodium quinoa* Willd) LINES

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ABSTRACT

Quinoa (*Chenopodium quinoa* Willd) is an important crop that is resistant to salinity, can adapt to harsh climatic conditions, is gluten-free and has seeds rich in nutrients. It has a high adaptability. It has been cultivated in South America for a very long time but has recently become widespread due to its designation as astronaut food by NASA. The plant contains high quality amino acids and protein. It can be used in human and animal nutrition. Since there is no selective herbicide for quinoa cultivation, it is necessary to develop herbicide-resistant quinoa lines. This study was carried out to determine some morphological characteristics of herbicide resistant mutant M$_3$ generation quinoa lines under greenhouse conditions. In the study, 4 mutant lines and control plants were planted in pots according to a completely randomized experimental design. When the plants matured, some morphological characteristics such as plant height, stem thickness and number of branches were determined. According to these results, the highest average plant height was 71 cm and the highest average stem thickness was 6.04 mm in the control plants and the highest number of leaf was 17 in the EM-6 line.

Keywords: Quinoa, mutation, improvement, line, plant characteristics

INTRODUCTION

In recent years, climatic conditions like extremely cold, hot and rains have negatively affects crop production because Differences have emerged in average precipitation, temperature and their distribution. In this case, plants that can adapt to climatic conditions are needed. Because of this situation alternative plants gaining imporant. Alternative plant must have a good adaptation ability as well a high value nutrition content. Quinoa can thinkable an alternative plant with its resistant to harsh climate conditions and salinity and heat and its seed have rich nutrient.

Quinoa is a annual plant, belong to C$_3$ plants groups. It have a branched root and its root can penetrable in soil 0.5-2.5 m (Tan and Temel, 2019). Plants can long 0.5-3.5 m it has woody stem and much branches and alternative sequence leaves. Some leaves have hairs while others have not. At begin its flowers is green but harvest time can yellow, red, pink purple. The flowers is in the form of a cluster and hermaphrodite and average 85-90% self pollination, seeds is 1,5-4 mm diameter and round shaped. Its thousand-seeds weight between 1.99-5.08 g. Seeds clour can pink, yellow, black, red, white (Tan and Yöndem, 2013). It has 250 varieties at the wordwide and it classifield according to seeds and plant color or plant morphology (Gordillo-Bastidas et al., 2016).

Its growth period is 90-200 days, it like good drenage, lite, middle and heavy soil although grown everybody soil conditions can grown, middle sensitive to salinity, it must between pH 6-8,5 (Türkmen 2019). It can join crops rotation with pea, potatoes, barley. When soil heat 7-10 ^{0}C seeds sow, because of seeds size soil so good must be processed. Seeds should be planted 1.5-2 cm deep. It is very important to weeds control due to plants slow-growing in the beginning. weeds fast develops and pressured to quinoa.

With the increasing population and urbanization, food consumption has also increased. However, diets previously high in complex carbohydrates, micronutrients, fiber, and phytochemicals have been replaced by diets high in animal fats and refined carbohydrates and fats (Gordillo-Bastidas et al., 2016). Because of the perception this situation led to some chronic diseases consumers has began searching healty food which is non alergical and non GM as well as nutritious like conventional food. Quinoa is best herbal protein source and its protein content more than creals like wheat, maize, rice. Proteins biological value is important and measured with body absorbed from a food. Moreover quinoa protein content high its changes between 12-16% and numerous essential amino acids like histidine, leucine, isoleucine, methionine, phenylalanine+tyrosine, threonine, tryptophan, valine this reason quinoa a good nutrition source for people. Quinoa is a good source of carbohydrates, its carbohydrate content is between 58.1% and 64.2% dry matter, 14% fat content and its the unsaturated fatty acid ratio of this content is 70-90%. Fiber content, averaging 10%. The glycemic index is very important for blood sugar level and is between 0-100 and it needs to be low for low blood sugar. Glycemic index of quinoa is between 30-50. Quinoa has satisfactory vitamins content which including thiamine, folic acid, C vitamine, E vitamine, B16 and pantothenic acid as well as has sufficient mineral quantities like calcium, magnesium and potassium (Gordillo-Bastidas et al., 2016).

Quinoa can use as a nutrition like rice, wheat, after its seed be removed coat. Its taste is not dominant and it suits the consumption habits of our people. In the USA, it is used to make pilaf like rice. Moreover quinoa use as soup, salad, its flour can use pasta, biscuit, bread, its leaf can use like spinach. Quinoa has rich nutrition content as well as glüten free this reason it is an important food for people with gluten sensitivity.

Quinoa is generally grown for its seeds, but it has enough straw for animal nutrition. Quinoa hay and straw have been used in animal nutrition for centuries in South America. With a good cultivation and selected suitable variety its forage yiels can increasing to 2400 kg/da (Tan and Temel, 2017 and 2018). Its forage hay yield rate is 26-28%, crude protein rate is 13-22% harvest time hay digestion rate is 63-69%. It must harvested during the three-leaf period because its protein content is high and its fiber content is low because It's forage when drying forage fiber content increase and don't like by animal. Plants fast grown and produced high yield, moreover suitable harsh cultivation conditions. Its use as plant silage has become widespread due to the decrease in digestive content as the forage matures. It used as a silage plants a lot conuntries. The dry matter content must be sufficient for fermentation. The dry matter ratio is suitable for silage when the plants start to set seeds. For a good fermentation, if the dry matter content of the plants is insufficient, dry matter should be added and the plants should be chopped into pieces smaller than 1 cm (De Braeckelaer, 1993). Silage is excelled a food for dairy cattle It is recommended to use quinoa silage at a rate of 20% of the dairy cattle ration (Zom et al., 2002). Quinoa is a valuable forage plant with its rich protein, mineral, vitamins content and it can an alternative to grain (Gül and Tekce, 2016). It seeds can add to cattle and sheeps ration for increasing protein content. But its seeds husk has sapponins and this matter negative effects on the animals this reason seeds husk to must separared (Tan and Temel, 2019).

Plant seeds are used as flour in human food production. In countries where quinoa is grown intensively, food industry has developed. Plants seed hull has a lot saponins and this matter use to make soap, detergent, shampoo, fire extinguisher, medicine and cosmetics. In South America, indigenous people has been used laundry detergent and antiseptic. Its use paper production due to its cellulose content. There are 66 color tone of quinoa seeds it used fabric painting (Tan and Temel, 2019).

MATERIALS AND METHODS

Study uses four mutant lines (M_3) and one control of Titicaca variety. Mutant lines were previously obtained in a study conducted at Atatürk University (Eğritaş, 2022). In this doctoral study, the chemical mutation agent sodium azide was applied to quinoa seeds, then the seeds were planted in the field. Seeds were produced from the planted seeds in the first year, the seeds were replanted in the field in the second year, and when the plants had three leaves, total herbicide containing imidazoline was applied and seeds were taken from resistant plants (M_2) (Egritas et al., 2022).

M_2 seeds were used in our study, and some characteristics of mutant lines planted in pods and control plants were compared. Seeds were planted in pods in 10 replicates. Measured plant height, stem thickness and leaf number values were compared with control plants.

Plant Heigth (cm): When plants matured, plant height was determined by measuring the plants from the soil to the top point.

Plant Stem thickness (mm): When plants matured, plant stem thickness was determined by measuring the plants from the above of the second node by help composing stick.

Leaf Number: When plants mature, leaf number was determined by to count leaf of plants.

The obtained data were analyzed with the help of the MSTAT-C package program and the significant differences were grouped with the LSD multiple comparison test.

RESULTS

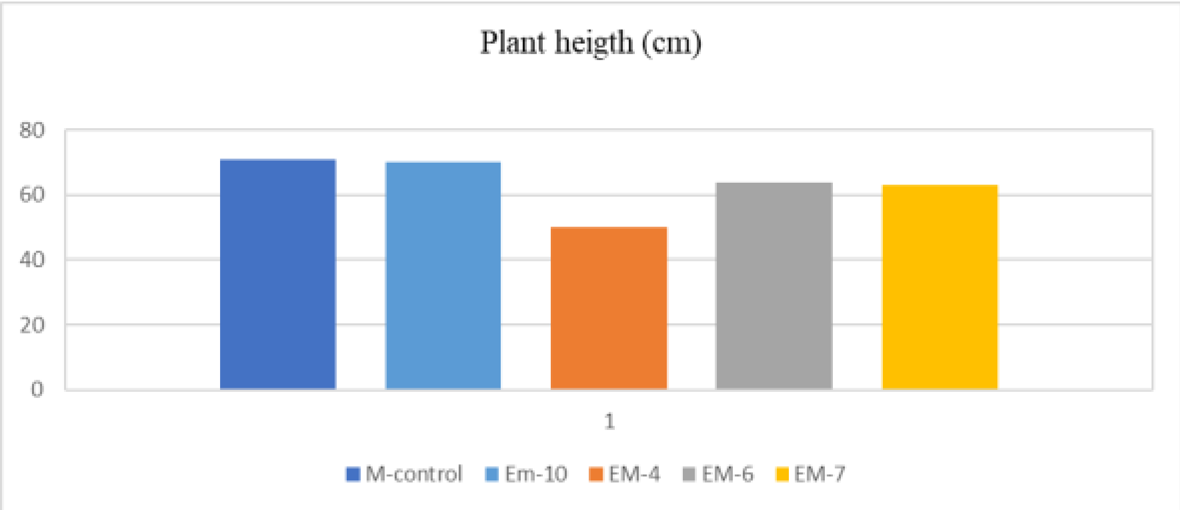
The data obtained in the study regarding plant height, stem thickness and number of leaf are given in Table 1. According to these results, plant heights and leaf numbers of the lines showed statistically significant differences; stem thickness was found to be insignificant.

Table 1. Some characteristics of control and mutant quinoa genotypes

Line Number	Plant heigth (cm)	Plant Stem thickness (mm)	Leaf Number
Em-10	70.33 a	4.44	16.00 a
Em-4	50.33 c	5.24	8.33 c
Em-6	64.67 b	3.78	17.00 a
Em-7	63.33 b	4.38	15.00 ab
Control	71.00 a	6.04	13.67 b
Variety	0.05	ns	0.05
Cv	21.42	23.57	28.31
LSD	4.2	-	2.2

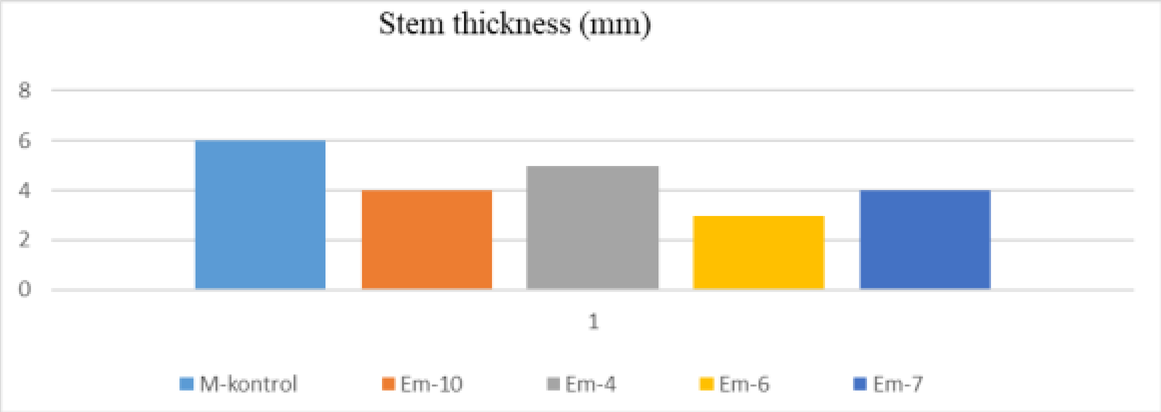
ns: Non-significant statistically

Plant heigth (cm): According to statistical analyse at the M_3 generation difference between mutant plants and control plants are signifcant at the 5% level. Highest plants height measured from control plants 71.00 cm, lowest plants height measured from Em-4 lines 50.33 cm.



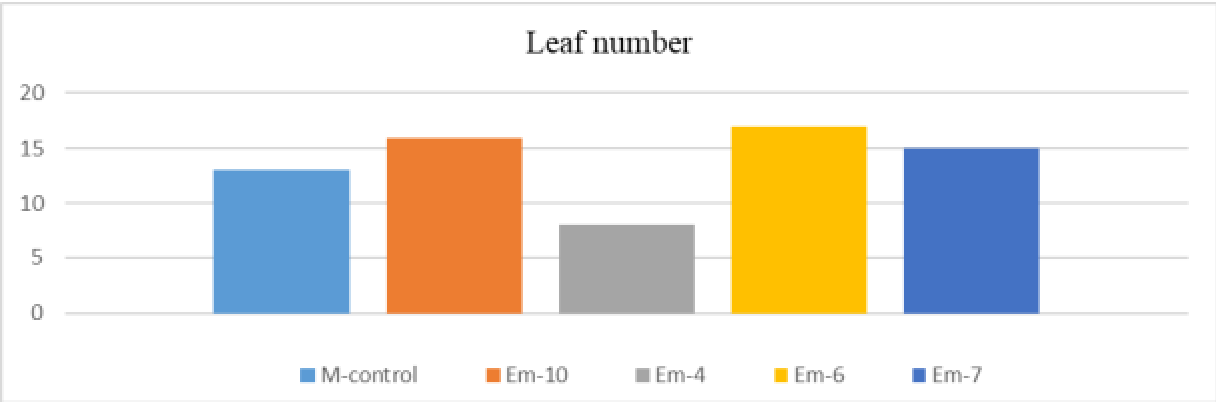
There are also different studies this issue; In the study conducted on to determined of the impact of EMS-induced Mutations on the growth and morpho-phenological Traits in pea (*Pisum sativum* L), mutation application negative effected on plant height and mutant plants height lower compared control plants (Savant et al., 2016). Another the study conducted on to determined the effect of mutation application in the M_1 generation on growth and flowering in chrysanthemum varieties, mutation negative effected on plant height and a decrease in mutant plant height (Ghormade, 2020). Another the study conducted on to determined Assessment of the effects of gamma radiations on various morphological and agronomic traits of common wheat (*Triticum aestivum* L.) var. WH-147. According to this study conclusion mutation negative effected on plant height and a decrease in mutant plant height (Kahah et al,. 2015). At the another study conducted on to determined mutagenic effects of MH and MMS on induction of variability in broad bean (*Vicia faba* L.) According to this study conclusion mutation negative effected on plant height and a decrease in mutant plant height (Laskar and Khan 2014).

Plant stem thickness (mm): According to statistical analyse at the M_3 generation difference between mutant plants and control plants Stem thickness are nonsignifcant. It is highest stem thickness measured from control plants 6.04 mm, lowest plants stem thickness measured from Em-6 lines 3.78 mm.



There are also different studies this issue; in the study conducted on to determined the effect mutagenic effect of sodium azide (NaN_3) on M_2 generation of *Brassica napus* L. (variety Dunkled), highest plant stem thickness measured from control plants 3.84 cm, lowest plants Stem thickness is 3.49 cm and measured from T4-0.8% line (Hussain et al., 2017). At the another study conducted on to determination of some plant properties in herbicide resistant mutant quinoa (*Chenopodium quinoa* Willd.) lines highest stem thickness measured from control plants 4.03 mm, lowest stem thickness measured from Et-7 lines is 2.20 mm (Egritas et al., 2020).

Leaf Number: According to statistical analyse at the M_3 generation difference between mutant plants and control plants leaf number are significant at the 5% level. Highest leaf number is Em-6 plants, it is 17 piece, lowest leaf number measured from Em-4 lines 8.33 piece.



According to this results, mutant lines, except the Em-4 line, have higher leaf numbers than control plants. There are also different studies this issue; According to a study to determined Morphological characterization and assessment of genetic variability, character association, and divergence in soybean mutants, there are variations amongs mutant and control plants branch number (Malek et al., 2014). A study conducted for determination of some vegetative characteristics in herbicide-resistant mutant quinoa (*Chenopodium quinoa* Willd.) lines, was determined variation amongs mutant and control plants branch number according to this study some mutant lines have hight branch number than control while some mutants (Egritas et al., 2020). Another the study on determining the morphological effects of sodium azide on tomato (*Lycopersicon esculentum* Mill). Mutant lines have been reported to have lower leaf numbers than control plants. (Adamu and Aliyu, 2007). Another study conducted on to determination effect of physical and chemical mutagens on morphological behavior of tomato (*Solanum lycopersicum*) under heat stress conditions. According to this study conclusion mutant lines have low leaf number from control plants and mutation doses negative effect on leaf number (Akhtar, 2014). According to the results of the study conducted to determine the effect of sodium azide on the agro-morphological characteristics of four types of kenaf (*Hibiscus cannabinus*), the highest number of leaves was measured from control plants. It has been reported that mutation application has negative effects on the number of leaves in black rice, tomato, *Amaranthus caudatus* and *Helianthus annus* plants (Ehoniyotan and Aiyenigba, 2019).

CONCLUSIONS

Quinoa is an important plant with nutrition value and useful area as well as its adaptation ability harsh climate and soil conditional. Especially wheat, corn plants cannot grow at the regions it can an alternative nutrition source for people and animal feed. The plant has been grown for years in a small area in South America for family needs. However, there are some problems in its cultivation, such as weed control. Quinoa is morphologically similar to lamb's quarters (*Chenopodium album*) and red-root amaranth (*Amaranthus retroflexus*). This situation causes problems in cultivation because the plant seeds are mixed and the value of quinoa seeds decreases. Moreover competition with weeds decreasing yield and quality. There aren’t selective hercibide for quinoa. All this reasons it necessary improvement herbicide resistant quinoa variety. At the our study was compared control and mutant lines which resistant to IMI group herbicides some morphological properties using M_2 seeds. Statistically analyzed plant height, stem thickness and number of leaves were reduced in the mutant lines. This situation can be predicted because mutation applications have some negative affects on mutant plants. According to the results obtained, the average of the mutant lines is fluctuating and it can be considered that the mutant lines are not genetically pure. In the next stage, control plants and herbicide-resistant lines should be observed in the same way and studied in terms of yield and quality. With use herbicide resistant quinoa lines marginal fields will use agriculture production, herbal production will increase, changed climate conditions will compatible. Quinoa can be rotated with other herbicide-resistant plants. Moreover fallow land will decrease as well as erosion decrease.

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THE IMPACT OF GEOTHERMAL ON LAND PRICES IN THE REGION

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ABSTRACT

Geothermal energy resources have positive effects on land purchase and sale criteria and agricultural land prices. The aim of this research is to investigate the effects of geothermal energy on land prices in Germencik county of Aydin province. The data of the research consist of face-to-face survey data obtained from producers who continue their agricultural activities in the region subject to the study. Descriptive statistical analyses were used to analyses the data obtained in accordance with the purpose of the study. According to the findings obtained as a result of the analysis of the data, more than 50% of the farmers stated that they have agricultural lands close to geothermal resources and that geothermal resources positively affect the economy in the region. 85% of the farmers stated that the economic value of the lands close to geothermal energy sources is high and that these agricultural lands are very valuable. The majority of the producers in the region subject to the research stated that geothermal energy plants affect the prices of fruit, vegetable, field and vineyard lands, facilitate irrigation in agriculture and increase productivity in agriculture. As a result; it was determined that geothermal energy resources have positive effects on agricultural land prices. While the decrease in energy costs increases the profitability of agricultural activities, it increases the potential of agriculture with special applications.

Keywords: Geothermals, Land Prices, Agriculture

INTRODUTION

Geothermal energy, which is one of the renewable energy sources, means the energy provided by hot water and its vapour coming out of the underground. The positive or negative effects of renewable energy sources on the environment have been the subject of many discussions (Külekçi 2009). Geothermal energy, which is one of the renewable energy sources, is a clean energy source. Compared to conventional energy sources, it is cheaper and ready to use. However, the minerals in the geothermal fluid cause water and soil pollution (Ataman 2007).

Geothermal Power Plants can cause serious environmental problems such as air and noise pollution, safety and land use. Recently, the effects of renewable energy sources on the environment continue to be a subject of debate. One of these debates is the impact of geothermal renewable energy sources on land pricing (Akova 2008). Land value is calculated not only by evaluating the land itself, but also by adding yield, assets, buildings if available, whether there are resources such as water, electricity, natural gas, topographical condition of the land and transaction costs (Töremen 2018). Researchers conducted before the establishment of geothermal power plants are very important in terms of the land where the power plant will be established, the structure of the land, its importance in terms of agriculture, which products are produced around the power plant, and how the power plant will benefit or harm them. In this study, the effects of geothermal power plants, one of the renewable energy sources, on land prices have been investigated by taking these situations into consideration. Determining the effects of GPP's on land prices may be important for policy makers both in expropriation processes and in determining the real estate declaration value. Geothermal energy is important in agriculture and many other factors on land. The aim of this study is to investigate the effects of geothermal power plants on land prices in the region.

MATERIAL AND METHOD

The main material of this study is the data obtained from the enterprises that carry out farming activities in the Germencik county of Aydin province. The main reason why the farmers in Germencik county are the main material is the intense geothermal energy resources in the region. These data were obtained by using a questionnaire prepared in accordance with the objectives. In this study, the sample size was determined randomly due to the Covid-19 pandemic process in Turkey and in the world in determining the number of farmers that could reflect the region. During the period when the pandemic measures were reduced by the state (April 2021), 20 farmers were interviewed randomly. Based on the data obtained from the producers, simple statistical analyzes were made in accordance with the purpose of the research.

FINDINGS

According to the findings obtained from the survey study of this research, it was determined that 40% of the farmers were primary school graduates, 10% secondary school graduates, 20% high school graduates and 30% university graduates. The average age of the producers participating in the research is 52 years.

In Table 1, while 30% of the farmers stated that they did not have sufficient knowledge about geothermal resources, 40% of the farmers stated that they had sufficient knowledge about geothermal resources.

Table 1. Knowledge Level of Farmers About Geothermal Resources

I have sufficient knowledge about geothermal resources		Frequency	%	Cumulative %
	I don not agree	6	30,0	30,0
	I am undecided	6	30,0	60,0
	I agree	7	35,0	95,0
	Absolutely I agree	1	5,0	100,0
	Total	20	100,0	

While 30% of the farmers participating in this study stated that Geothermal Power Plants did not affect the economic vitality, 50% of the farmers stated that they did (Table 2).

Table 2. The Impact of GPPs on Economic Vitality

GPPs have an impact on economic viability		Frequency	%	Cumulative %
	I strongly disagree	2	10,0	10,0
	I don not agree	4	20,0	30,0
	I am undecided	4	20,0	50,0
	I agree	8	40,0	90,0
	Absolutely I agree	2	10,0	100,0
	Total	20	100,0	

In Table 3, when these living participating households were asked whether they exceeded the house prices around the GPPs, 40% of the farmers did not agree with this statement, while 30% stated that they agreed with this statement. In Table 4, 70% of the farmers stated that the leakage of GPP pipes from their lands did not increase the land prices in the region, while 30% stated that they increased the prices.

Table 3. The Effect of Geothermal Power Plants in the Research Area on Housing Prices

GPPs increase house prices in the area		Frequency	%	Cumulative %
	I don not agree	2	10,0	10,0
	I am undecided	6	30,0	40,0
	I agree	6	30,0	70,0
	Absolutely I agree	6	30,0	100,0
	Total	20	100,0	

Table 4. Effect of GPP Pipes on Land Pricing

The passing of the GPP pipe in the land affects the land price		Frequency	%	Cumulative %
	I never agree	1	5,0	5,0
	I agree	13	65,0	70,0
	Absolutely I agree	6	30,0	100,0
	Total	20	100,0	

85% of the farmers participating in this research stated that the proximity of Geothermal Power Plants to agricultural lands increases the land prices (Table 5).

Table 5. Price Levels of Lands Near GPPs

Proximity to GPPs affects land price		Frequency	%	Cumulative %
	I never agree	1	5,0	5,0
	I don not agree	1	5,0	10,0
	I am undecided	1	5,0	15,0
	I agree	12	60,0	75,0
	Absolutely I agree	5	25,0	100,0
	Total	20	100,0	

In Table 6, 35% of the farmers participating in the research stated that the presence of Geothermal Power Plant wells on the land would not affect the land pricing positively, while 50% of the stated that it would positively affect them.

Table 6. Effect of Geothermal Power Plant Well on Land Prices

The presence of the GPP well on the land positively affects the price of the land		Frequency	%	Cumulative %
	I never agree	3	15,0	15,0
	I don not agree	4	20,0	35,0
	I am undecided	3	15,0	50,0
	I agree	6	30,0	80,0
	Absolutely I agree	4	20,0	100,0
	Total	20	100,0	

In this research, 20% of the farmers stated that Geothermal Power Plants do not affect the prices of agricultural lands in the region and 60% of the producers stated that Geothermal Power Plants have an impact on the prices of agricultural lands (Table 7).

Table 7. Impact of GPPs on the Prices of Orchards, Field Lands and Vineyards in the Region

GPPs have an impact on the prices of orchards, farmland and vineyard lands in the region		Frequency	%	Cumulative %
	I never agree	1	5,0	5,0
	I don not agree	3	15,0	20,0
	I am undecided	4	20,0	40,0
	I agree	9	45,0	85,0
	Absolutely I agree	3	15,0	100,0
	Total	20	100,0	

In Table 8, 65% of the farmers stated that Geothermal Power Plants did not increase the productivity of agricultural lands, while 20% stated that Geothermal Power Plants increased the yield of agricultural lands.

Table 8. Effect of Geothermal Power Plants on Efficiency

Yield increases in the land where GPPs are located		Frequency	%	Cumulative %
	I never agree	4	20,0	20,0
	I don not agree	9	45,0	65,0
	I am undecided	3	15,0	80,0
	I agree	2	10,0	90,0
	Absolutely I agree	2	10,0	100,0
	Total	20	100,0	

65% of the farmers participating in this research stated that Geothermal Power Plants reduced their olive and olive exports (Table 9). Most of the farmers who produce in the region stated that geothermals disrupt the ecological balance and negatively affect health.

Table 9. Effect of Geothermal Power Plants on Olive and Fig Exports

GPPs reduce olive and fig exports		Frequency	%	Cumulative %
	I never agree	2	10,0	10,0
	I don not agree	5	25,0	35,0
	I am undecided	10	50,0	85,0
	I agree	3	15,0	100,0
	Absolutely I agree	20	100,0	

SONUÇ

In the region subject to this research, figs, olives, cotton and many other agricultural products are cultivated along with the intensive geothermal power plants. It is important for sustainable life and sustainable development to objectively investigate the positive and negative effects on the regions where geothermal power plants are established. The scope of this study is limited to agricultural lands and producers in Germencik county of Aydın province. The degree of impact of geothermals on land prices may differ in other regions of Turkey and the world. Therefore, developing similar studies, sharing the results with the stakeholders of agricultural valuation activities and ensuring that it functions as a public system may provide solutions to the problems experienced in valuation activities. In this study, the effects of geothermal geothermal on land prices in Germencik county were investigated. According to the data and information obtained from farmers carrying out agricultural activities, it is stated that the economic value of the lands close to geothermal power plants is high. However, it can be said that geothermal power plants located in agricultural lands and the pipes passing through these lands limit the use of agricultural lands. In addition, it was stated that the yield of crops such as olives and figs decreased due to the pollution caused by geothermal power plants. As a result, farmers stated that even if the economic value of the lands close to geothermal power plants increases, geothermals harm people, plants, animals and the environment. For a sustainable life and sustainable development, healthy renewable energy sources that do not harm the ecology can be used.

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THE EFFECT OF GEOTHERMALS ON FARMERS PREFERENCE FOR ALTERNATIVE CROPS

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ABSTRACT

The use of geothermal energy sources in agricultural production areas, in addition to providing the temperature required by the plant, allows production to be carried out uninterruptedly throughout the year, except for extremely hot periods. Geothermal resources are important in terms of agricultural production as well as other areas of use and some of the benefits they provide. The use of geothermal energy in agricultural production and greenhouse heating allows producers to produce continuously. The aim of this study is to investigate the effect of geothermal energy resources on farmers' alternative crop preference in Aydin province. The data of the study consist of survey data obtained from the owners of agricultural enterprises in Germencik county of Aydin province. Statistical analyses were used to determine the effect of geothermal in the region on farmers' alternative product preference. According to the data obtained as a result of statistical analysis, the majority of the producers in the region stated that they want to produce alternative products with high added value, but they do not want to utilize geothermal energy resources too much for agricultural production. The reason why the majority of producers do not want to utilize geothermal energy sources is that geothermal energy sources dry the trees of certain crops such as figs and olives and pollute the environment. Approximately 60% of the producers stated that the establishment of geothermal power plants in the region directed producers to alternative products. 76.7% of the producers stated that they would like to receive support from the government or municipality to produce alternative products. Supporting and encouraging producers to produce alternative products with natural and clean energy sources by policy makers, sectoral organizations and universities can make positive contributions to sustainable agriculture.

Keywords: Energy of Geothermal, Alternative Product, Agricultural Production, Aydin Province

INTRODUCTION

Geothermal energy is a renewable energy source that uses heat from the Earth's core to generate electricity and provide heating and cooling for a variety of applications. It can be said that this energy is considered as a sustainable and alternative energy source compared to fossil fuels. Rapid depletion of energy resources, unconscious use of non-renewable resources such as oil, coal, nuclear energy, factors such as the pollution these sources give to the environment and the atmosphere have led people to use renewable energy sources (Elveren, 2013). Geothermal energy, which is one of the renewable energy sources, is used in many fields such as electricity generation, medicine, tourism, agriculture and industry. In order to meet the world's ever-increasing energy needs, scientists have carried out many studies to increase the use of renewable energy sources in every field and have developed projects such as the use of solar cells to utilize the sun and wind mills to utilize the wind. (Hergül, 2009; Ocak Gemici and Önder, 2018). Geothermal resources are directly utilized in important areas such as heating and cooling, aquaculture, industrial processes and agriculture.

Although geothermal energy resources have many benefits, the main ones are that they are renewable as mentioned before, that is, they are a type of energy that is difficult to be exhausted with proper use, they are easy to detect and produce, their cost is low, the investment made returns in a very short time, and they are also environmentally friendly compared to other resources. The use of geothermal energy in agricultural production areas, in addition to providing the warmth required by the plant, allows production to be carried out at all times of the year without interruption, except for extremely hot periods. For this reason, geothermal resources are of great importance in terms of agricultural production in addition to other areas of use and benefits (Hergül, 2009; Ataseven et al., 2014). Intensive and diverse production in greenhouses, which is an important branch of agriculture, factors such as heat, temperature, humidity, air and irrigation are mandatory needs of plants. Meeting such mandatory needs in greenhouses through geothermal energy resources will increase the development of greenhouse cultivation and the income and investments of producers (Cebeli and Kendirli, 2011). Use of geothermal energy in agricultural and greenhouse heating; Although geothermal resources provide continuity in the cultivation of producers, sometimes they can have negative effects on the environment, human and plant health. In regions with geothermal energy sources, there are problems in the cultivation of some agricultural products and these products are damaged, so alternative products should be grown instead of damaged products. In the face of the changing global world, it can be said that the producers prefer alternative products and/or increase the cultivation of alternative products, increase the sufficient income of the farmer and it will be important for sustainable agriculture. The aim of this study is to investigate the effect of geothermal energy resources on the alternative product preference of farmers in the Germencik county of Aydın province.

MATERIAL AND METHOD

The main material of this study consists of face-to-face survey data obtained from farmers who carry out farming activities in the Germencik county of Aydın province. The main reason why the farmers in Germencik county are the main material is the intense geothermal energy resources in the region. In addition, previous articles, theses, books and research data on the subject are among the other secondary sources used in this study. In this study, the sample size was determined randomly due to the Covid-19 pandemic process in Turkey and in the world in determining the number of farmers that could reflect the region. During the period when the pandemic measures were reduced by the state (April 2021), 30 farmers were interviewed randomly. The data obtained from the producers were analyzed in accordance with the purpose of the research.

FINDINGS

Some Sociodemographic Characteristics of Producers

Produers participating in the survey; the minimum age was 33, the maximum age was 68, and the mean age was 49.23. The education level of the producers; primary school (26,7%), secondary school (20%), high school (26,7%) and university (26,7%). Produers stated that 50% of the producers have non-agricultural income (Table 1).

Table 1. Some Features of Producers

		Number	Minimum	Maximum	Average
Age		30	33	68	49,23
Educational Status		Frequency	%	Cumulative %	
	Primary school	8	26,7	26,7	
	Middle school	6	20	46,7	
	High school	8	26,7	73,3	
	University	8	26,7	100	
	Total	30	100		
Do you have non-agriulture income?		Frequency	%	Cumulative %	
	No answer	2	6,7	6,7	
	Yes	15	50	56,7	
	No	13	43,3	100	
	Total	30	100		

Producers and Alternative Product Relationships

When the producers were asked whether they would like to receive support from the state and municipality for alternative crops, 76,7% of them stated that they would like to receive support and 23,3% stated that they did not receive support (Table 2). If producers receive support from these organisations, it may reduce producer costs.

Table 2. Ideas of Producers to Get Support for Alternative Products

Would you like to receive support from the state or municipality for alternative products?		Frequency	%	Cumulative %
	Yes	23	76,7	76,7
	No	7	23,3	100,0
	Total	30	100,0	

Among the producers who participated in the research; 56,7% stated that alternative products increase my income, 16,6% stated that alternative products do not increase my income and 26,6% stated that they were undecided about such a statement (Table 3).

Table 3. The Producers' Participation in the Idea of Alternative Products to Increase Income

I think alternative products increase my income		Frequency	%	Cumulative %
	I'm Never Agree	1	3,3	3,3
	I don't Agree	4	13,3	16,7
	I'm undecided	8	26,7	43,3
	I'm Agree	12	40,0	83,3
	Absolutaly I Agree	5	16,7	100,0
	Total	30	100,0	

When the producers participating in this research were asked whether they think that they contribute to the national economy with alternative crop production, 50% of them stated that they agreed with this statement. (Table 4).

Table 4. Ideas of Produers to Contribute to the National Economy with Alternative Products

I think that I contribute to the country's economy with the production of alternative products.		Frequency	%	Cumulative %
	I'm Never Agree	1	3,3	3,3
	I don't Agree	4	13,3	16,7
	I'm undecided	10	33,3	50,0
	I'm Agree	12	40,0	90,0
	Absolutaly I Agree	3	10,0	100,0
	Total	30	100,0	

According to Table 5, 36,7% of the producers have increased their orientation towards alternative crops due to geothermals, 40% disagreed with such a statement and 23,3% were undecided. According to Table 6, 53,4% of the respondents stated that they changed the crop pattern on the land due to environmental conditions.

Table 5. Orientation of Proucers to Alternative Products

Increased our orientation towards geothermal alternative products		Frequency	%	Cumulative %
	I'm Never Agree	3	10,0	10,0
	I don't Agree	9	30,0	40,0
	I'm undecided	7	23,3	63,3
	I'm Agree	9	30,0	93,3
	Absolutaly I Agree	2	6,7	100,0
	Total	30	100,0	

Table 6. Crop Change Status of the Producers

Environmental conditions changed my crop pattern in the field		Frequency	%	Cumulative %
	I'm Never Agree	2	6,7	6,7
	I don't Agree	9	30,0	36,7
	I'm undecided	3	10,0	46,7
	I'm Agree	11	36,7	83,3
	Absolutaly I Agree	5	16,7	100,0
	Total	30	100,0	

When the producers were asked whether the trees of specific crops such as figs and olives dry out after geothermal power plants, 86,6% of the producers agreed with this question (Table 7).

Table 7. Damage to Trees Caused by geothermal

Can fig and olive trees dry up after geothermal resources?		Frequency	%	Cumulative %
	I'm Never Agree	1	3,3	3,3
	I'm undecided	3	10,0	13,3
	I'm Agree	7	23,3	36,7
	Absolutaly I Agree	19	63,3	100,0
	Total	30	100,0	

Produccers and Alternative Product Preferences

According to Table 8, producers stated that the most productive product group is industrial crops (36,7%), fodder crops (26,7%), cereals (13,3%) fruits (13,3%) and horticultural crops (10%).

Table 8. The Most Productive Agricultural Crop in the Region Preferring Alternative Crops

The most efficient product considering the region where I prefer alternative products I think;		Frequency	%	Cumulative %
	Cereals	4	13,3	13,3
	Industrial Plants	11	36,7	50,0
	Forage Crops	8	26,7	76,7
	Fruits	4	13,3	90,0
	Horticulture Plants	3	10,0	100,0
	Total	30	100,0	

When the producers participating in this research were asked which alternative crop is the most profitable, 33,3% of the producers said strawberry, 23,3% said cotton, 13,3% said tomato (Table 9).

Table 9. Most Profitable Alternative Product Preferences for Producers

What do you think is the most profitable alternative product?		Frequency	%	Cumulative %
	No Answer	5	16,7	16,7
	Strawberry	10	33,3	50,0
	Tomato	4	13,3	63,3
	Cotton	7	23,3	86,7
	Medicinal Plants	1	3,3	90,0
	Bait	3	10,0	100,0
	Total	30	100,0	

According to Table 10, the reasons why the producers started alternative production due to geothermal power plants were factors such as the disappearance of the products I used to produce (16,7%), decrease in production area (10%), deterioration of soil structure (23,3%) and pollution of water (46,7%). In Table 11, when the producers were asked which alternative agricultural products the increase in geothermal resources caused you to turn to in terms of agriculture, 50% of the producers answered strawberry.

Table 10. Reasons for Producers to Start Alternative Production

The reason why I started alternative production due to geothermal energy;		Frequency	%	Cumulative %
	Disappearance of the Products I Produced	5	16,7	17,2
	Reduction of My Production Area	3	10,0	27,6
	Deterioration of Soil Structure	7	23,3	51,7
	Pollution of Water	14	46,7	100,0
	Total	29	96,7	
	No answer	1	3,3	
	Total	30	100,0	

Table 11. Preferences of Produers for Alternative Products Originated from Geothermal

Which alternative products did the increase in geothermal resources cause you to turn to in terms of agriculture?		Frequency	%	Cumulative %
	Oregano	4	13,3	13,8
	Canola	4	13,3	27,6
	Dragon Fruit	2	6,7	34,5
	Strawberry	15	50,0	86,2
	Other	4	13,3	100,0
	Total	29	96,7	
	No Answer	1	3,3	
	Total	30	100,0	

In Table 12, when the producers were asked whether their trees dried up after geothermal power plants, 70% of the producers answered yes and 30% answered no. In Table 13, 46.7% of the producers stated that their plant pattern changed after geothermal power plants, while 53.3% stated that their plant pattern did not change after geothermal power plants.

Tablw 12. Are Trees Affected by Geothermal Energy Sources

Are Trees Affected by Geothermal Energy Sources?		Frequency	%	Cumulative %
	Yes	21	70,0	70,0
	No	9	30,0	100,0
	Total	30	100,0	

Table 13. The Situation of Plant Patterns of Producers Originating from Geothermal

Has there been any change in your plant pattern after the geothermal power plants?		Frequency	%	Cumulative %
	Yes	14	46,7	46,7
	No	16	53,3	100,0
	Total	30	100,0	

In Table 14, the preferences of the producers for the products produced in the past and alternative products produced now are given. 33.3% of the producers have started to grow products such as strawberries, cotton and thyme as alternative products instead of the products they used to grow in the past.

Table 14. Past and Present Alternative Product Preferences of Produers

Yes, if yes, which is the product produced in the past and which is now produced alternatively		Frequency	%	Cumulative %
	No Answer	20	66,7	66,7
	Fig - Strawberry	1	3,3	70,0
	Fig - Oregano	1	3,3	73,3
	İFig - Cotton	2	6,7	80,0
	From Cotton and Corn I Turned to Horticulture	1	3,3	83,3
	Vegetable	2	6,7	90,0
	Olive - Strawberry	1	3,3	93,3
	Olive - Oregano	1	3,3	96,7
	Olive - Cotton	1	3,3	100,0
	Total	30	100,0	

CONCLUSION

It is important for a sustainable life to objectively investigate the positive and negative effects of geothermal energy sources in the regions where geothermal power plants are located. Since the Geothermal Power Plants are dense in the Germencik county, which is the subject of this research, the statements of the producers who continue their agricultural activities in this region have been taken into account in order to reveal the effect of geothermals. According to the information and data obtained from the producers, when geothermals are intense in the region; figs, olives and other crops are damaged and the ecological balance is also damaged. In this study, the effect of geothermals on the alternative product preference of farmers was determined. The producers stated that they have turned to alternative products when their incomes are insufficient and because geothermal power plants damage the agricultural products they grow. It can be said that producing alternative products will both increase farm incomes and contribute to the national economy. Strawberry is the most preferred alternative product due to its low cost, high yield and profit. As a result, due to the intense use of geothermal in Germencik county, producers stated that there is a change in alternative product preferences in the past and today. In order for farmers to sustain production, efforts should be made to increase their economic income and welfare levels. In addition, healthy renewable energy sources should be preferred for the sustainability of ecological balance and a quality environment.

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