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Effects of Adding Fennel (*Foeniculum vulgare*) In Rations on Fattening Performance and Blood Oxidant-Antioxidant Balance in Rams

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Abstract

This study aimed to determine the effects of adding fennel seeds (*Foeniculum vulgare*) to the diet on the biochemical parameters and live weight gains in rams. Thirty male rams of the Eşme breed, with an average live weight of 39 kg, were used, divided into two groups, each containing 15 rams. The control group received a ration without any additional supplementation, while the treatment group had fennel seeds added to their rations at a rate of 1.5 % per animal per day for 60 days. At the end of the experiment, blood samples were collected from the sheep to analyze malondialdehyde (MDA), protein carbonyl concentrations (PCO), superoxide dismutase (SOD), and catalase (CAT) enzyme activities. The study found a statistically significant difference between the groups in terms of daily live weight gain, with the control group showing 0.300 kg and the fennel group showing 0.330 kg. The MDA, SOD, protein, and catalase values were examined in the collected blood samples, revealing significant differences in MDA, SOD, and catalase values, while no significant difference was observed in PCO values. The addition of fennel seeds showed an increase in antioxidant levels and a decrease in lipid peroxidation, which could effectively protect rams from oxidative stress that reduces their productivity and resilience. Moreover, the study concluded that the use of fennel seeds in ram rations did not have any harmful effects.

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fattening performance

1. Introduction

The general aim of livestock enterprises is for the producer to obtain the highest level of efficiency from the animals he/she raises and reproduces in his/her enterprise. Genetic and environmental factors significantly affect the productivity of animals. Nutrition, in other words, feed, has an important place among environmental factors. Roughages are of great importance in the feeding of ruminant animals in terms of both feeding physiology and business economics. However, as the vegetation period and duration of each region are different as a natural result of different climatic conditions, animals can naturally meet some of their green fodder needs only during certain periods of the year. Especially due to limiting factors in the winter season, the use of roughage comes to the fore. The use of these roughages causes significant economic losses. For this reason, productivity differences can be seen depending on the season. These undesirable productivity losses in livestock enterprises can be prevented by using silages obtained from different sources (Kurtoğlu, 1998; Çiftçi et al., 2005). Due to the geographical features of Turkey, many medicinal and aromatic plants can be grown and they are collected from nature, as in many parts of the world. While some of the medicinal and aromatic plants in Turkey (laurel, sage, linden blossom, licorice root, rosemary, juniper bark, etc.) are collected from nature, some of them (coriander, thyme, cumin, anise, fenugreek, mint, fennel) are cultivated (Özhatay and Koyuncu, 1998). Fennel is one of the 300 genera in the Apiaceae family (Davis, 1978). It is grown mostly in European countries, India, Egypt, Turkey, China, Argentina, Indonesia and Pakistan in the world. It is known to be grown in the provinces of Istanbul, Zonguldak, Kastamonu, Artvin, Bursa, Çanakkale, Kırklareli, Kocaeli, Samsun, Sinop and Trabzon in the north of Turkey and in the region where Amanos Mountains in Hatay province are located in the south (Koru, 2019). Fennel is cultivated in the provinces of Antalya (150 kg da⁻¹), Burdur (120 kg da⁻¹) and Konya (150 kg da⁻¹) (Anonymus, 2019). The chemical composition

of fennel includes essential oil, fatty acids, phenylpropanoids, monoterpenes, sesquiterpenes and coumarin. It also contains triterpene, tannin, flavonoid, cardiac glycosides and saponin. Fennel contains approximately 20 % fatty acids. Petroselinic acid is a characteristic fatty acid of fennel oil. Petroselinic acid level can be between 70 % and 80 % (He and Huang, 2011). Fennel has been reported to contain 6.3 % moisture, 9.5 % protein, 10 % fat, 13.4 % minerals, 18.5 % fibre and 42.3 % carbohydrates. Minerals and vitamins found in Fennel are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C (Rather et al., 2016). This study was conducted to determine the effects of adding the antioxidant fennel plant (*Foeniculum vulgare*) to the diets of sheep of the Eşme breed on live weight gain and biochemical parameters.

2. Materials and Methods

The study was conducted in a private enterprise in Eşme district of Uşak province, following the approval of the Uşak University Animal Experiments Local Ethics Committee (USAKHADYK 2023/01). The animal material of the study consisted of 30 Eşme breed sheep, approximately 7-8 months old, with an average live weight of 39 kg. The animals were divided into two groups, one control and one experimental, with 15 sheep in each group. Care was taken to ensure that the animals in the groups were with similar birth dates and weights, and the groups were placed in separate pens so that they could not come into contact with each other. The animals were fed with feed prepared to meet the daily dry matter and nutrient needs reported by NRC (2007) for sheep. In the study, the animals were placed in the pens in their usual conditions in such a way as to prevent them from reaching each other's feed. In the control group, no changes were made in the feeding and water consumption patterns, and only 1.5% fennel seed per day was added to the rations of the animals in the experimental group. Following a seven-day eating acclimatization period, they were fed with the food allocated to them for a total of 60 days of the main study period.

Feeding was done in two meals a day, morning and evening, and roughage and mixed feed were given together in the same meal. At the end of the study, blood samples were taken before the morning feeding. Adequate blood samples were taken from the jugular vein of the sheep into anticoagulant tubes and brought to the Uşak University Central Research Laboratory for biochemical parameters. Plasma and serum samples and erythrocyte packets obtained by centrifugation (Nüve, NF 1000R) (1500 g, 4 °C, 15 min) were placed in eppendorf tubes and stored in the freezer at -20 °C until analysis. Preparation of erythrocytes was carried out according to the method reported by Witterbourn et al. (1975). MDA (Malondialdehyde) levels in the blood samples were determined according to the method reported by Draper and Hardley (1990), PCO

levels were determined according to the method reported by Levine et al. (1990), and SOD and CAT enzyme activities in erythrocyte packages were determined according to the methods reported by Sun et al. (1998) and Luck (1995) using a spectrophotometer device (Shimadzu UV 1601). Independent samples t test was used for statistical calculations on the data of the groups obtained in the study and to determine the significance of the differences between the mean values of the groups. The level of significance was taken as $p < 0.05$. All the analyses were conducted in SPSS 23.0 statistical program package. In the study, the sheep were fed with diets containing 14.4 % crude protein and a metabolic energy of 2760 kcal kg⁻¹. The raw material and calculated nutrient compositions of the treatment rations are given in Table 1.

Table 1. Raw material and nutrient compositions of intensive feed mix

Raw materials	Quantity, kg	Nutrients	Quantity, %
Barley	400.0	Dry Matter	88.7
Wheat	300.0	Crude Protein	14.4
Bran	84.6	Raw Fat	3.7
Cotton Seed Meal	92.6	Crude Cellulose	7.0
Full Fat Soy	83.4	Ash	4.9
Vegetable Oil	17.0	Calcium	0.7
Marble Dust	13.4	Phosphorus	0.5
Salt	7.0	Nitrogen-Free Extracts	58.6
Vit.-Min. premix*	1.0	Metabolic Energy kcal/kg	2760.0

The roughage and concentrate feeds used in the ration were mixed with each other and given to the sheep as complete rations. Live weights of the sheep were determined by weighing them on an empty stomach at the beginning of fattening and at the end of the 60th day of fattening. Feed and water were

given ad libitum throughout the treatment period. In this study, which lasted a total of 60 days and investigated the effects of fennel supplementation on live weight gain and blood oxidant-antioxidant balance in sheep, data on live weight gains are given in Table 2.

Table 2. Mean live weight increases as a result of fennel supplementation

	Control		Fennel		t	F	P
	X	Sx	X	Sx			
Mean Live Weight per livestock, kg.	39.8640	1.2851	39.3993	1.3960	0.948	0.012	P>0.05
Mean Live Weight at the end of fattening, kg.	57.8987	1.2439	59.2247	1.8019	-2.345	2.274	P>0.05
Total Live Weight increase	18.0347	0.5320	19.8253	0.6997	0.027	1.797	P<0.05
Daily Live Weight increase, kg	0.3000	0.084	0.3307	0.116	0.889	0.72	0.05

At the end of the study, as a result of the addition of 1.5 % fennel in the treatment group,

the daily live weight gain was found to be 0.330 ± 0.01 kg, and in the control group it was

found 0.300 ± 0.08 kg. Total live weight gain was measured as 19.82 ± 0.6 kg in the fennel supplemented group and 18.03 ± 0.5 kg in the control group. A statistical difference was

detected between the groups ($p < 0.05$). The effects on MDA (Malondialdehyde), protein levels, SOD (Superoxide dismutase) and CAT (Catalase) activities are given in Table 3.

Table 3. Effects of fennel supplementation on MDA, protein, SOD and CAT levels in lambs.

	Control		Fennel				
	Average	Standard deviation	Average	Standard deviation	t	F	P
MDA mg/dl	39.8640	1.2851	39.3993	1.3960	0.948	0.012	$P > 0.05$
SOD mg/dl	57.8987	1.2439	59.2247	1.8019	-2.345	2.274	$P > 0.05$
Protein mg/dl	18.0347	0.5320	19.8253	0.6997	0.027	1.797	$P < 0.05$
CAT mg/dl	0.3000	0.084	0.3307	0.0116	-7.889	2.072	$P < 0.05$

Some biochemical parameters were examined in blood samples taken from the jugular vein at the beginning and end of the treatment. While a statistical difference ($p < 0.05$) was detected between the groups in terms of MDA, SOD and CAT activities, which reveal the antioxidant-oxidant status of the biochemical values measured, it was found that the difference between the protein carbonyl levels of the control and 1.5% fennel group is not significant.

3. Discussion

In this study, which was carried out to determine whether fennel, which is among the feed additives used to increase the utilization of the feed given to animals, increase the quality of the products obtained, ensure that the animals are raised in a healthy way and reduce the cost of the obtained product, can be an alternative, the sheep used as animal material were fed with two different rations, the control and the treatment group with 1.5 % fennel added to the feed, during the study, which lasted a total of 2 months. Fennel seed addition to the feed was performed by selecting amounts close to the level used in previous studies (Hajalizadeh et al., 2019). In order for natural herbal products to be used as feed additives, they must not adversely affect both the productivity and health of the animal (Rochfort et al., 2008). In the current study, total live weight gains and daily live weight gains were found to be high in the group which were feed with feed supplemented with fennel seeds, which is consistent with the study

conducted by Hajalizadeh et al. (2019). In the study of Hassan and Hassan (2009), in which they added black cumin, rosemary and probiotics to the rations of Karadi sheep, the increase in live weight gain is compatible with the study. Shahsavari et al. (2021) found that total live weight gain and daily live weight gain were significant ($p < 0.05$) in Kermani lambs supplemented with fennel in their rations, which is consistent with the current study. Liu et al. (2021), in their study investigating the effect of fennel seed powder supplementation on feed intake and carcass in Cobb chickens, determined that fennel supports the growth and development of chickens, but found that there was no significant difference ($P > 0.05$) in the growth performance of broiler chickens. The results they obtained differ from the current study in terms of fattening performance. Recent research on antioxidants has focused on naturally occurring molecules to reduce consumer concerns about the safety and toxicity of their synthetic counterparts (Gharaghani et al., 2015). It is recommended to use dietary antioxidants in animal feed to reduce lipid peroxidation and protect the quality of animal products (Wood and Enser 1997). It is known that fennel seed is a potential source of natural antioxidants (Oktay et al., 2003). Free radicals produced during the function of body cells cause oxidative stress and damage cells. Cells have enzymatic and non-enzymatic antioxidant defence systems to protect themselves from the damage of free radicals (İnce et al., 2010). In the current study, a statistically significant difference ($p < 0.05$)

was determined between the groups in terms of the lipid peroxidation product MDA levels, which are indicators of oxidative stress, and SOD, an enzymatic antioxidant, and CAT enzyme activity levels (Table 3). This finding shows the effect of fennel, which has antioxidant properties (Marino et al., 2007; Parejo et al., 2004; Ruberto et al., 2000; Shahat et al., 2011), on blood oxidant- antioxidant balance when added at the rate of 1.5% to the feed of sheep. SOD enzyme reduces superoxide levels within the cell by converting superoxide radical into hydrogen peroxide and molecular oxygen, thus it shows antioxidant properties. SOD is one of the most important antioxidant systems in the organism, together with catalase and glutathione redox system (Repine et al., 1997). SOD measurement can give an idea about the antioxidant capacity of the organism. Aydin et al. (2020) studied the effect of using thyme oil (*Origanum vulgare*) on fattening performance and blood oxidant-antioxidant balance in weaned Tuj breed lambs, and the effect of adding thyme oil to the ration was found to be insignificant on fattening performance, which is different from the current study, but its effect on SOD and GPx values, two of the blood oxidant-antioxidant parameters, was found to be significant ($p < 0.05$). Studies on fennel have reported that the presence of trans-anethole as an active ingredient at levels of 38-82 % gives fennel antioxidant properties (Gulfraz et al., 2008; Shahat et al., 2011, Zoubiri et al., 2014). However, Cengiz (2018) suggested that the addition of rosemary and fennel essential oil mixture to the quail diet reduced the MDA level in breast meat stored at +4 °C under refrigerator conditions. They also found that the addition of fennel to the feed of quail was found to affect liver MDA, GSH, Vit C levels and GPx and CAT activities. In the current study, MDA and CAT activities were also found to be significant. The current study is consistent with the study (Sheweita et al. 2016), in which they stated that administering fennel essential oils to mice significantly increased CAT activities, one of the endogenous antioxidant enzymes, and did not induce lipid peroxidation. Choi and Hwang

(2004) reported that oral administration of fennel fruit methanolic extract had antioxidant effects by reducing plasma MDA level and increasing CAT activity in an experimental model of acute and subacute inflammatory diseases and type IV allergic reaction in rats. Koppula and Kumar (2013) studied the lipid peroxidation of fennel in liver and brain tissues against stress in rats, and found that the fennel group had lower liver MDA levels compared to the control group. Kazak et al. (2020) determined that the addition of 1% ground fennel seed in quail diets reduced liver MDA level and GPx activity and increased Vit C levels ($p < 0.05$). The significant decrease in MDA level is compatible with the current study. Ghiasvand et al. (2021) studied the effects of adding fennel essential oil to the diet on growth performance and serum biochemistry in broiler chickens and found that it had no effect on serum MDA concentrations, but these results are different from the results of the current study. PCO (Protein carbonyl) products are formed as a result of damage to many amino acid residues and peptide backbones as a result of the interaction of ROS (reactive oxygen species) with proteins. The most commonly measured product of protein oxidation is protein carbonyls. Detection of PCO levels is a sensitive method for determining oxidative protein damage (Evans et al. 1999). No literature data could be found regarding the examination of PCO levels in studies conducted with the addition of fennel, and this study is the first in this regard. It has been found that adding fennel, which has antioxidant properties, to the feed of sheep at a level of up to 1.5% has no effect on PCO levels.

4. Conclusion

Along with the increasing demands for livestock, the increasing presence of foodborne diseases reveals the necessity of ensuring food safety and developing new strategies to raise healthy farm animals. In recent years, as consumers have turned to healthy and safe food consumption, the use of medicinal and aromatic plants, especially as productivity enhancers, in the production of animal

products has come to the fore. When fennel seeds are added to feed, they make the aroma of the feed more delicious and cause sheep to consume it more willingly. In the current study, fennel supplementation increased feed consumption in sheep. The mean weight gain achieved at the end of 60 days and the higher daily live weight gain have revealed that fennel will provide more economic profit for livestock enterprises, considering its low cost. The fact that the addition of fennel to the diet increases blood antioxidant levels and decreases lipid peroxidation may be effective in protecting sheep from oxidative stress, which reduces productivity and resistance in sheep. With this study, it can be concluded that the use of fennel seed in the diets of sheep does not have a harmful effect. It was concluded that fennel can be used as an alternative feed additive to synthetic products to meet consumer demand in animal products, but this potential should be confirmed with different ruminant species and long-term studies. If the data obtained as a result of such studies support the findings of the current study, it will be possible for fennel to be used especially by organizations that produce feed additives and animal breeders, and thus the use of fennel in ruminant animals will become widespread.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Investigation of Dry Herbage Yield and Some Quality Characteristics of Sorghum x Sudan Grass Hybrid Varieties Grown Under Semi-Humid Ecological Conditions

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Abstract

This research was conducted in the 2019 and 2020 growing seasons to determine the most suitable sorghum x sudan grass hybrid variety or varieties in terms of dry herbage yield and quality characteristics in Bingöl, which has a semi-humid climate. Dry herbage yield (DHY), crude protein ratio (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV) were determined for ten sorghum x sudan grass hybrid varieties grown in Bingöl University Faculty of Agriculture, Agricultural Application and Research Center according to coincidence blocks experimental design with 3 replications. In the varieties examined in the research, DHY varied between 12216-16397 kg ha⁻¹, CP 5.15-8.15 %, ADF 39.43-45.85 %, NDF 51.45-62.89 %, ADL 9.20-13.15 %, DMD 53.18-58.18 %, DMI 1.91-2.33 % and RFV 78.68-104.30. According to the two-year combined averages, the Master BMR variety produced a higher dry herbage yield than the other varieties. Among the varieties, the highest CP, DMI, RFV, and the lowest NDF were obtained in the Master BMR variety. It was concluded that the Master BMR variety gave high values in terms of dry herbage yield and quality characteristics in Bingöl province's ecological conditions.

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1. Introduction

Türkiye ranks first in the world in terms of the number of animals, it has not reached the desired level in the yield, production, and quality of animal products. It should be in an important place due to its location, potential, and natural ecology. However, feed, which constitutes 60-75 % of the majority of production inputs, cannot be obtained easily and cheaply. The solution to livestock feed problems is not only concentrated feed resources. In addition, the production of quality forage resources should be increased. Forage crops, which have a very important share in agricultural production, are the most important way of roughage production (Açıkgöz et al., 2005). One of the main reasons for the high forage deficit in Türkiye is the insufficiency of forage crop cultivation areas in arable agriculture. In Türkiye, 55.4 million tons of quality roughage, including grass and silage corn, is produced on 2.1 million hectares annually. The production of forage crops is approximately 13.1 million tons and 10.3 million tons from meadow-pasture areas, while the total amount of quality dry herbage produced is 23.4 million tons per year (Tuik, 2022).

The low cost of roughages such as dry grass, green feeds, and silo feeds reduces production inputs (Bilgen et al., 1996). For this reason, to meet the quality roughage requirement of animal husbandry, in addition to the improvement of meadow pastures, it is necessary to expand forage plant agriculture and increase the production of cheap and alternative roughage resources (Serin and Tan, 2001; Yolcu and Tan, 2008). Sorghum x sudangrass hybrid, which is the result of sorghum crossing with sudangrass, is a plant species with high biomass yield, wide adaptability, drought tolerant, close to maize in terms of nutritional value, and more resistant to diseases and pests (Bean et al., 2002). Sorghum x sudan grass hybrid can be grown in marginal areas due to its low water and fertilizer demand. Therefore, it is seen that it can be a very important source in closing the roughage deficit. It is one of the alternative

plants that can be cultivated as a second crop. It can also be used to reduce diseases and pests in areas where sugar beet is grown (Akdoğan 2004; Avcıoğlu et al., 2009; Erdurmus et al., 2018).

Many studies have been carried out on dry herbage yield and quality characteristics of sorghum x sudangrass hybrids cultivated for energy and forage crops under different ecological conditions (Kara et al., 2019; Ateş and Atalay, 2020; Bilen and Türk, 2021; Kardeş et al., 2023). However, studies on dry herbage yield and quality characteristics of sorghum x sudan grass hybrid varieties under Bingöl ecological conditions are insufficient. Therefore, this study aimed to determine the dry herbage yield and quality characteristics of ten different sorghum x sudan grass hybrid varieties under the ecological conditions of Bingöl province.

2. Materials and Methods

The research was conducted in Bingöl, which has a semi-humid climate, in the Bingöl University Agricultural Application and Research Centre experiment area for two years in 2019 and 2020. The climatic data of Bingöl province, where the research was carried out, for the long years and the years in which the experiment was carried out were given in Table 1 (GDM, 2021). In 2019 and 2020, the average temperature of June-September, which is the vegetation period, was 24.9 °C in both years and the long-term average temperature was 24.0 °C. An increase of approximately 1 °C was observed between the years in which the experiment was carried out and the long-term average temperature. Total precipitation during the vegetation period was 8.7 mm in 2019, 17.5 mm in 2020, and 46.9 mm for long years. It is seen that the rainfall was very low in the periods when the experiment was carried out compared to long years, especially in 2019. The average relative humidity is 33.6 % in 2019, 34.5 % in 2020, and 39.6 % for long years. There was no significant difference in relative humidity between the period in which the experiment was conducted and long years (Table 1).

Table 1. Climatic data of the research area for long years (1961-2018), 2019 and 2020

Years/Months	June	July	August	September	Total/Average
Average Temperature Values ($^{\circ}\text{C}$)					
Long Years (1961-2018)	21.9	26.6	26.4	21.2	24.0
2019	24.6	26.2	27.3	21.6	24.9
2020	22.4	27.0	26.5	24.0	24.9
Total Precipitation (mm)					
Long Years (1961-2018)	22.1	7.5	4.7	12.6	46.9
2019	3.6	0.5	4.6	0.0	8.7
2020	10.0	5.7	0.6	1.2	17.5
Average Relative Humidity (%)					
Long Years (1961-2018)	44.3	36.4	36.0	41.7	39.6
2019	37.8	31.8	30.5	34.3	33.6
2020	40.6	35.3	29.9	32.3	34.5

With the irregular distribution of rainfall throughout the year, the decrease in the amount of precipitation during vegetation periods, and

the increase in temperature, the symptoms of drought began to be seen.

Table 2. Soil properties of the study area

Soil Texture	Water Saturation (%)	EC (%)	pH	Lime (%)	Organic Matter (%)	P ₂ O ₅ (kg da ⁻¹)	K ₂ O (kg da ⁻¹)
Clayey-loamy	54	0.02	6.1	-	1.89	3.60	37.0

As a result of the analyses performed on the soil samples taken from 0-30 cm depth from the research area, the soil has a clayey-loamy texture. The soil was determined as slightly acidic, salt-free, and lime-free. It was found to be 'low' in terms of organic matter and poor in terms of phosphorus (P) and potassium (K) content (Table 2).

In the study, 10 sorghum x sudan grass hybrid varieties (Aneto, Greengo, Jumbo, Master BMR, Nutri Honey, Nutrima, Sugar Graze II, Supergraze 1000, Super Su 22 and Tonka) obtained from private sector companies were used. The study was established according to the randomized block design with three replications. The plot area was determined as $5 \times 1.8 = 9 \text{ m}^2$ and four rows were placed at 45 cm row spacing. Sowing was done by hand and the seed rate was set at 4 kg da^{-1} . Fertilization was done with 10 kg da^{-1} NPK compound fertilizer at sowing and 22 kg da^{-1} Urea (46 % N) as the upper fertilizer when the plants were 40-50 cm. For aphids, 50 g l^{-1} Lambda-cyhalothrin insecticide was used

once. Irrigation was carried out by sprinkler system until the emergence of the plants and then by drip irrigation. Hand hoeing was done once to control weeds. Harvesting was carried out at the dough stage of the grains.

Fresh herbage samples taken from the harvested plants were dried at 70°C , weighed and dry herbage yield was determined. The plant material was then ground in a 0.5-1 mm sieve and prepared for analysis. Nitrogen ratios determined by the Kjeldahl device (Kacar and Inal, 2008) were multiplied by a coefficient of 6.25 to determine the crude protein ratio. Acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) ratios were determined according to the method described by Van Soest (1963) and, Van Soest and Wine (1967). DMD, DMI, and RFV are determined by the following equations (Morrison, 2003).

DMD (%): $88.9 - (0.779 * \% \text{ ADF})$

DMI (%): $120 / (\% \text{ NDF})$

RFV: $(\text{DMD} * \text{DMI}) / 1.29$

The data obtained from the experiment were analyzed by using JMP statistical software according to the random blocks experimental design. According to the results of variance analysis, the differences between the means were determined by the LSD multiple comparison test.

3. Results and Discussion

The mean values of DHY, CP, ADF, NDF, ADL, DMD, DMI, and RFV of sorghum x sudangrass hybrid varieties were given in Table 3,4,5,6.

Table 3. Mean values of DHY and CP parameters of sorghum x sudangrass hybrid varieties

	DHY (kg ha ⁻¹)			CP (%)		
	2019	2020	Mean	2019	2020	Mean
Aneto	14071	15971	15021 b	6.93	7.57	7.25 de
Greengo	14691	16108	15399 b	7.47	6.52	7.00 e
Jumbo	14847	16247	15547 b	8.23	7.40	7.82 bc
Master BMR	15896	16898	16397 a	8.43	7.87	8.15 a
Nutri Honey	12762	11706	12234 e	8.17	7.37	7.77 bc
Nutrima	14172	16039	15106 b	8.33	7.95	8.14 a
Sugar Graze II	13869	12810	13339 d	5.76	4.53	5.15 f
Supergraze 1000	13424	12700	13062 d	7.50	8.33	7.92 ab
Super Su 22	11519	12914	12216 e	7.33	7.97	7.65 bc
Tonka	13882	14542	14212 c	7.57	7.47	7.52 cd
Mean	13913 b	14593 a		7.57 a	7.29 b	
CV (%)		3.89			3.46	

a, b, c: Differences between means indicated by different letters in the same row ; **:p< 0.01 significant. DHY: dry herbage yield, CP: crude protein

It was determined that there was significant ($p<0.01$) variation in terms of dry herbage yield in terms of years, varieties, and year x variety interaction. The average dry herbage yield was 13913 kg ha⁻¹ in the first year and 14593 kg ha⁻¹ in the second year. Dry herbage yield among the varieties varied between 12216-16397 kg ha⁻¹, the lowest yield was obtained from Super Su 22 (12216 kg ha⁻¹) and Nutri Honey (12234 kg ha⁻¹), which were statistically in the same group, and the highest yield was obtained from Master BMR variety with 16397 kg ha⁻¹. In terms of dry herbage yield of sorghum x sudangrass hybrid varieties, Master BMR variety stood out. This shows that the variety is better adapted to environmental conditions. In many studies conducted with sorghum x sudangrass hybrid varieties, significant differences were recorded between dry herbage yields. For example, dry herbage yield was determined as Çoban and Acar 2018 (1794-2581 kg da⁻¹), Kara et al., 2019 (1003-1701 kg da⁻¹), Ateş and Atalay 2020 (528-4542 kg da⁻¹), Bilen and Türk 2021 (1177-1314 kg da⁻¹) ve Erecek et al., 2023 (1814-2004 kg da⁻¹). The genotype differences, location, slope, orientation, altitude, climatic factors during the vegetation period, and soil structure affect the

yield and some quality characteristics of the plant. It was found that the variations were significant ($p<0.01$) among years, varieties, and year x variety interactions in terms of crude protein ratio. The average CP rate was 7.57 % in the first year and 7.29 % in the second year. The CP ratio between the varieties varied between 5.15-8.15 %. In terms of this parameter, Sugar Graze II (5.25 %) had the lowest CP rate, Master BMR (8.15 %), and Nutrima (8.14 %), which were statistically in the same group, had the highest CP rate. According to Adesogan (2006), the crude protein content of sorghum should be more than 8 %. In this case, Master BMR and Nutrima varieties had a rate higher than 8 %. In previous studies, some researchers, Nazlı et al., 2013, Karadağ and Özkurt 2014, Dursun Sahan 2017, Canyigit and Okant 2018, Budak and Kır 2019, Yıldırım and Bengisu 2019 determined the crude protein ratio as 10.0-10.5 %, 9.5-11.0 %, 7.8-10.5 %, 9.1-13.1 %, 12.0-12.9 %, 14.1-16.2 % respectively. The difference in crude protein ratio may be because the ecologies in which the trials were conducted, the genotypes used and the plant growth stages in the harvest periods were not the same.

Table 4. Mean values of ADF and NDF parameters of sorghum x sudan grass hybrid varieties

	ADF (%)			NDF (%)		
	2019	2020	Mean	2019	2020	Mean
Aneto	40.63	39.43	40.03 def	56.77	55.80	56.28 ef
Greengo	42.26	38.77	40.52 cd	59.47	58.25	58.86 c
Jumbo	45.26	43.24	44.25 b	60.40	59.39	59.90 b
Master BMR	40.35	39.83	40.09 def	51.90	51.00	51.45 g
Nutri Honey	42.70	39.31	41.01 c	57.70	54.13	55.92 f
Nutrima	42.72	38.17	40.45 cd	58.97	56.40	57.69 d
Sugar Graze II	44.90	46.80	45.85 a	62.36	63.42	62.89 a
Supergraze 1000	40.13	38.77	39.45 ef	58.69	55.40	57.05 de
Super Su 22	40.57	39.83	40.20 de	59.60	55.50	57.55 d
Tonka	40.13	38.73	39.43 f	57.38	55.10	56.24 ef
Mean	41.96 a	40.28 b		58.32 a	56.43 b	
CV (%)		1.56			1.35	

a, b, c: Differences between means indicated by different letters in the same row ; **:p< 0.01 significant. ADF: acid detergent fiber, NDF: neutral detergent fiber

The ADF ratio was found to be statistically significant at the level of $p<0.01$ for years, varieties, and year x variety interactions. In this respect, ADF, which was 41.96 % in the first year of the experiment and 40.28 % in the second year, varied between varieties as 39.43-45.85 %. The lowest ADF rate was obtained from Tonka (39.43 %) and the highest was obtained from Sugar Graze II (45.85 %). As a result of the analysis of variance, it was found that there were significant differences between years, varieties, and year x variety interactions in terms of NDF ratio ($p<0.01$). The mean values were 58.32 % in the first year and 56.43 % in the second year of the study, and this trait

varied between 51.45-62.89 % among the varieties. The lowest NDF rate was determined in the Master BMR (51.45 %) variety, while the highest NDF rate was determined in the Sugar Graze II (62.89 %) variety. ADF value gives an idea about the digestibility of feed. NDF ratio, which is composed of cell wall components, is also desired to be low as in ADF. The ADF, NDF and ADL ratios obtained in the studies conducted by some researchers and the data obtained in this study are compatible with each other (Güven, 2017; Kır and Dursun Sahan, 2019; Bilen and Türk, 2021; Erecek et al., 2023).

Table 5. Mean values of ADL and DMD parameters of sorghum x sudangrass hybrid varieties

	ADL (%)			DMD (%)		
	2019	2020	Mean	2019	2020	Mean
Aneto	9.83	8.56	9.20 e	57.25	58.18	57.72 abc
Greengo	10.60	8.43	9.52 de	55.98	58.70	57.34 cd
Jumbo	12.10	11.78	11.94 b	53.65	55.21	54.43 e
Master BMR	9.87	8.73	9.30 e	57.47	57.87	57.67 abc
Nutri Honey	11.17	10.07	10.62 c	55.63	58.28	56.96 d
Nutrima	11.43	10.57	11.00 bc	55.62	59.17	57.39 cd
Sugar Graze II	12.63	13.67	13.15 a	53.92	52.44	53.18 f
Supergraze 1000	10.63	10.25	10.44 cd	57.64	58.70	58.17 ab
Super Su 22	11.63	11.11	11.37 bc	57.30	57.87	57.58 bc
Tonka	11.66	10.36	11.01 bc	57.64	58.73	58.18 a
Mean	11.15 a	10.35 b		56.20 b	57.51 a	
CV (%)		8.16			0.88	

a, b, c: Differences between means indicated by different letters in the same row ; **:p< 0.01 significant. ADL: acid detergent lignin, DMD: dry matter digestibility

Significant differences were found between years and varieties in terms of ADL ratio ($p<0.01$), while year x variety interaction was not significant. The average ADL was 11.15% in the first year and 10.35 % in the second year; this value varied between varieties as 9.20-

13.15 %. The lowest ADL rate was obtained from Aneto (9.20 %) and Master BMR (9.30 %) varieties and the highest ADL rate was obtained from Sugar Graze II (13.15 %) varieties. As a result of statistical analysis, significant differences were found between

years, varieties, and year x variety interactions at $p < 0.01$ level for DMD. DMD average was 56.20 % in the first year and 57.51 % in the second year. DMD rate varied between

varieties as 53.18-58.18 %. The lowest DMD rate was found in Sugar Graze II (53.18 %) and the highest DMD rate was found in Tonka (58.18 %).

Table 6. Mean values of DMI and RFV parameters of sorghum x sudangrass hybrid varieties

	DMI (%)			RFV		
	**			**		
	2019	2020	Mean	2019	2020	Mean
Aneto	2.11	2.15	2.13 bc	93.82	97.02	95.42 b
Greengo	2.02	2.06	2.04 e	87.59	93.74	90.67 e
Jumbo	1.99	2.02	2.00 f	82.64	86.48	84.56 f
Master BMR	2.31	2.35	2.33 a	103.03	105.57	104.30 a
Nutri Honey	2.08	2.22	2.15 b	89.70	100.15	94.93 bc
Nutrima	2.03	2.13	2.08 d	87.74	97.59	92.66 d
Sugar Graze II	1.92	1.89	1.91 g	80.44	76.92	78.68 g
Supergraze 1000	2.05	2.17	2.11 cd	91.38	98.57	94.98 bc
Super Su 22	2.01	2.16	2.09 d	89.47	97.00	93.24 cd
Tonka	2.09	2.18	2.13 bc	93.44	99.16	96.30 b
Mean	2.06 b	2.13 a		89.92 b	95.22 a	
CV (%)		1.34			1.61	

a, b, c. Differences between means indicated by different letters in the same row; **: $p < 0.01$ significant. DMI: dry matter intake, RFV: relative feed value

Statistically years, varieties, and year x variety interactions were found statistically significant at $p < 0.01$ level in terms of DMI ratio. The average DMI ratio was 2.06 % in the first year and 2.13 % in the second year. This value was determined as 1.91-2.33 % among the varieties. The highest DMI rate was obtained from Master BMR (2.33 %) and the lowest DMI rate was obtained from Sugar Graze II (1.91 %). Significant differences ($p < 0.01$) were found in terms of RFV according to years, varieties, and year x variety interactions. The average RFV was 89.92 in the first year and 95.22 in the second year. RFV between varieties was determined as 78.68-104.30. The highest RFV was found in Master BMR (104.30) and the lowest RFV was found in Sugar Graze II (78.68). According to the classification of Linn and Martin (1989), sorghum x sudangrass hybrid varieties were classified as medium quality in terms of DMD, DMI ratios, and RFV. The values obtained in the studies conducted by Özmen (2017) and, Bilen and Türk (2021) are similar to the values obtained in this study.

4. Conclusion

Dry herbage yield and quality characteristics of ten sorghum x sudan grass hybrid varieties were determined under ecological conditions of Bingöl Province. It

was observed that there were statistically significant differences between the varieties in terms of dry herbage yield and all quality parameters (CP, ADF, NDF, ADL, DMD, DMI, and RFV). The highest dry herbage yield, CP, DMI ratio, and RFV were obtained from the Master BMR variety. The lowest ADF rate was obtained from the Tonka variety and the lowest NDF rate was obtained from the Master BMR variety. Therefore, it was concluded that the Master BMR variety gave the highest values in terms of dry herbage yield and quality characteristics of sorghum x sudan grass hybrid varieties examined in Bingöl province ecological conditions. It is recommended that this variety should be prioritized in terms of cultivation in Bingöl and regions with similar ecological conditions.

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Trend Analysis of Some Crop Yields and Agro-Meteorologic Drought in the Trakya Region

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Abstract

Drought is one of the most dangerous-meteorologic events affecting agriculture. Drought can be determined by indexes. In this study, the relationship between the Standard Precipitation Index (SPI) results and trend analysis results was examined. Thus, the consistency of the drought in Trakya with the results of the trend analysis was investigated. For this purpose, using the precipitation data of Tekirdag, Kırklareli, and Edirne Meteorological Observation Stations (MOS), drought analysis was done with SPI, trend analysis was done with the help of Mann-Kendall, Sen and Linear Regression methods. In addition, the deviation value of crop yield value from the mean were produced and compared with the drought index result. As a result, the crop most incompatible with the drought results in Trakya is the wheat. In addition, agriculture is not expected to be affected by droughts in Trakya where meteorological drought is not dominant. Edirne is prone to drought while Kırklareli is expected to be prone to humidity, relatively. Nevertheless, there is no significant trend in Trakya in general.

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1. Introduction

Drought is a natural disaster that starts with a decrease in precipitation and negatively affects the hydrological balance and water budget. Mainly drought types are meteorological, hydrological and agricultural drought (Okkan and Altun, 2019). Classification of the drought severity overlaps with the philosophy of methods such as trend analysis and fuzzy logic. In drought studies in the literature, the duration, severity, trend, temporal and spatial effects of drought have been investigated (Dabanli, 2019). The main tool used to determine drought severity is drought indices. For meteorological drought, Palmer Drought Severity Index, Reconnaissance Drought Index and Standard Precipitation Index (SPI) can be used (Gumus, 2017). In addition, remote sensing and GIS are important tool for drought studies (Ozsoy, 2021).

Climate series such as precipitation and runoff fit the Gamma distribution compared to the normal distribution. The first step of the SPI is to fit the data to the Gamma probability density function. 1-3-month periods of SPI indicate meteorological drought, 6-9-month periods indicate agricultural drought, and 12-24-month periods indicate hydrological drought (Oguz et al., 2021).

Climate change and drought are expected to have different effects on many different regions and crops. According to climate models and scenarios, it is expected that the temperature in Trakya might increase by 5 °C by 2100 (40 % of water resources might be lost) and 30 % of water resources might be lost by 2050. The main plants that might be affected by drought in Trakya was estimated. It is predicted that these are wheat, rice and sunflower. According to future climate conditions in Trakya, wheat yield is expected to increase by 60 % and sunflower yield is expected to decrease by 20 % (Alkan, 2021; Alkan and Konukcu, 2022).

Various studies have been carried out related to drought in the world. Gocic and Trajkovic (2013) analyzed the trend of SPI by using Mann–Kendall tests at the 5 %

significance level in Serbia. They said that year 2000 was extremely dry for all stations and there were increasing trends in winter and fall precipitation in the seasonal series.

Vido et al. (2019) determined decreasing trend of drought during the winter months and determined increasing trend during the summer months with the help of Standardized Precipitation Evapotranspiration Index (SPEI) and Mann-Kendall (M-K) test in Slovakia. As a result, the trend analyses revealed no significant increase in drought impacts on agriculture in the studied period. Ouatiki et al. (2019) determined the annual rainfall deficit with the help of SPI and then analyzed trends with the help of Mann–Kendall test for 1970–2010 period. As a result, the researchers found that the OER River basin in Morocco tends to have drier conditions.

Naz et al. (2020) determined drought trend at the 3-month in Balochistan-Pakistan with the help of Mann-Kendall test. They found the decreasing precipitation trend in four stations. Alsafadi et al. (2020) used SPI, SPEI and M-K methods. The M-K method showed a significant negative trend (SPI-12) in 11.5 % over the western Hungary.

Various studies have been carried out related to drought in Turkey. Bacanlı (2017) analyzed the trends (with Mann-Kendall) of monthly SPI results for Aegean drought between 1960-2013. It is understood that Mann-Kendall and Spearman rho methods give similar results. After all, It was found a negative trend in Aydın, Denizli, Kutahya, Manisa and Mugla stations.

Oguz et al. (2021) examined the trend and drought in Mugla for the period 1960-2018. Mann-Kendall and SPI were applied to precipitation data. After all, in Mugla, the extreme dry period has been experienced more than the extreme wet period. According to SPI, the most severe droughts were between December 2006 and September 2007, and the longest droughts were between June 2000 and May 2001.

Kankal and Akcay (2019) determined the trend analysis of Trabzon precipitation using

Mann-Kendall and Şen methods. In both stations, increasing trends dominate in fall and spring. While no trend was observed in Trabzon in summer, a decreasing trend was observed in Akçaabat.

Nouri and Homaei (2019) applied gamma and log-logistic distribution in their study for SPI and SPEI, respectively. They also used the Mann-Kendall trend test for results of SPI and SPEI. After all, they determined that SPI do not correlate well with SPEI in arid regions.

Vaheddost (2020) determined the trend using monthly precipitation between 2005-2018 in Counties of Bursa, with the help of linear trend, Mann-Kendall, Spearman, Sen and SPI methods.

Dabanli (2019) calculated the Drought Disaster Indicator and Drought Sensitivity Indicator. The main goal of his work was to produce drought risk maps with precipitation data and fuzzy logic from 250 stations (1971-2010) in Turkey. After all, he determined that 5 out of 81 Provinces in Turkey have low drought risk, 61 provinces are at medium drought risk and 14 provinces have high drought risk.

Demir et al. (2017) investigated the effects of precipitation trends on regional agriculture in Bingöl. As a result, they determined that annual and seasonal precipitation (except in April, not statistically significant) decreased in Bingöl. It has been determined that this decrease will not affect the agriculture of Bingöl in the short term.

In a study similar to our study, Eroglu (2021) analyzed the seasonal trend of precipitation in the Meriç River Basin using the Mann-Kendall and Sen method. For this purpose, the positive-negative trends of 50-year precipitation data of 10 stations in Bulgaria and Turkey were periodically examined. As a result, a significant negative trend was determined in the annual precipitation in the 1965-1990 period in Ipsala and a significant positive trend in Kirklareli in the 1991-2015 period. Besides, in the 1965-2015 period, no statistically significant trend could be detected at any station.

Also, there is a significant decrease trend in annual precipitation in Luleburgaz considering the rainfall data of the Marmara between 1930 and 1993. Except for Luleburgaz, no significant trend could be determined at the level of 95 % at any of the stations (Eroglu 2021).

Considering the scarcity of studies on meteorological drought in Trakya, which is one of the arid regions in Turkey, this study, which aims to investigate the effect of drought on agriculture in Trakya, is really important. Using meteorological data (precipitation) with the study, comments will be made on all types of drought. For this purpose, Since the 6-9-month SPI values represent agricultural drought (Oguz et al., 2021), results related to agricultural drought will be produced.

In this study, the success of drought in Trakya in representing the trend was investigated by examining the relationship between the results of the drought index and the results of the trend analysis. For this purpose, using the precipitation data of Tekirdag, Kirklareli and Edirne Meteorological Observation Stations (MOS), drought analysis was done with SPI, trend analysis was done with the help of Mann-Kendall, Sen and Linear Regression methods. Besides, the deviation value of crop yield value from the mean were obtained and compared with the SPI result. Thus, the drought effect on crop yield was determined.

2. Materials and Methods

2.1. Materials

2.1.1. Study area: the Trakya region (Tekirdag, Kirklareli, Edirne)

In Turkey, 1600 m³ water falls per person while 500 m³ water falls in Trakya (Bagdatli and Belliturk, 2016; Alkan, 2021). Considering that the agricultural sector in the region consumes excessive water, the future water shortage will become more evident. In terms of agricultural drought in Turkey, Trakya is one of the driest regions (Alkan, 2021; Balkan et al., 2023). Especially the Trakya side of the Marmara Region is more prone to drought (60 % of Trakya is

agricultural land). For example, in 1971, while the north of Trakya was humid, drought was seen in Biga. In 2008, droughts were observed in Trakya in winter and in Kırklareli in fall (Alkan, 2021).

Main agriculture provinces in Trakya are Tekirdag, Edirne and Kırklareli (Figure 1). The years joint to all 3 stations (MOS no: 17056 Tekirdag, 17052 Kırklareli, 17050 Edirne) were chosen as the observation period (Between 1941 and 2020 years).

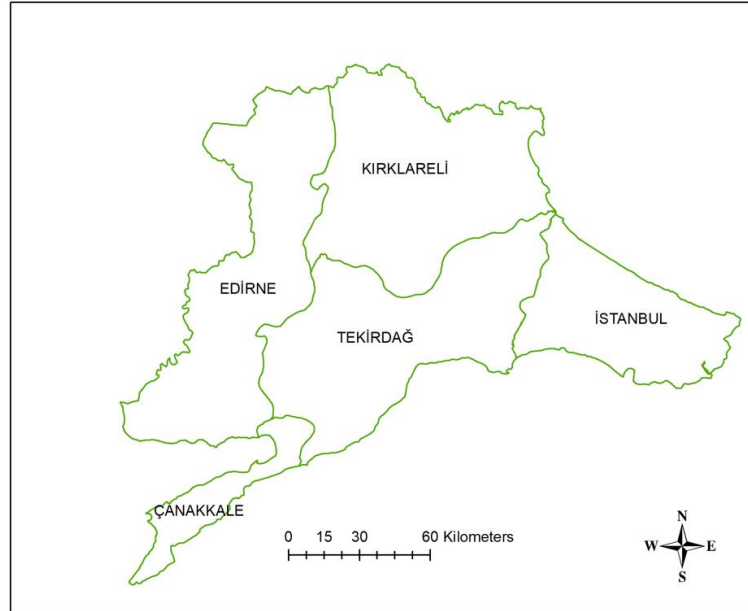


Figure 1. Trakya map

2.1.2. Agricultural production data in the Trakya region

The cities where wheat yields are from high to low are Tekirdag, Edirne and Kırklareli, respectively. The cities where sunflower yields

are from high to low are Edirne, Kırklareli and Tekirdag, respectively. The cities where Barley yields are from high to low are Tekirdag, Kırklareli and Edirne, respectively (Table 1).

Table 1. Some crops yields in Trakya (kg da^{-1}) (TSI, 2023)

Year	Wheat			Sunflower			Barley		
	Edirne	Kırklareli	Tekirdag	Edirne	Kırklareli	Tekirdag	Edirne	Kırklareli	Tekirdag
2004	321	326	428	171	165	184	374	425	477
2005	352	333	405	179	164	193	377	444	435
2006	325	306	340	202	184	215	347	367	411
2007	420	383	436	164	181	121	462	482	542
2008	422	390	465	167	174	188	502	466	541
2009	343	308	372	181	179	183	339	361	398
2010	399	283	322	289	180	190	347	318	347
2011	303	324	355	215	178	196	298	351	387
2012	455	402	489	197	228	176	409	471	478
2013	365	378	378	227	247	229	406	424	441
2014	424	407	430	290	258	230	495	443	477
2015	356	346	407	230	258	208	431	394	441
2016	362	381	429	225	218	200	418	355	444
2017	362	437	459	243	248	235	476	477	548
2018	346	390	340	248	258	235	417	392	339
2019	381	393	451	263	285	251	412	393	487
2020	358	390	392	264	291	248	347	361	400
2021	474	460	534	266	248	240	468	430	563

2.2. Methods

Precipitation data and analysis scale were determined, then drought severity was

calculated and compared with trend analysis (Figure 2).

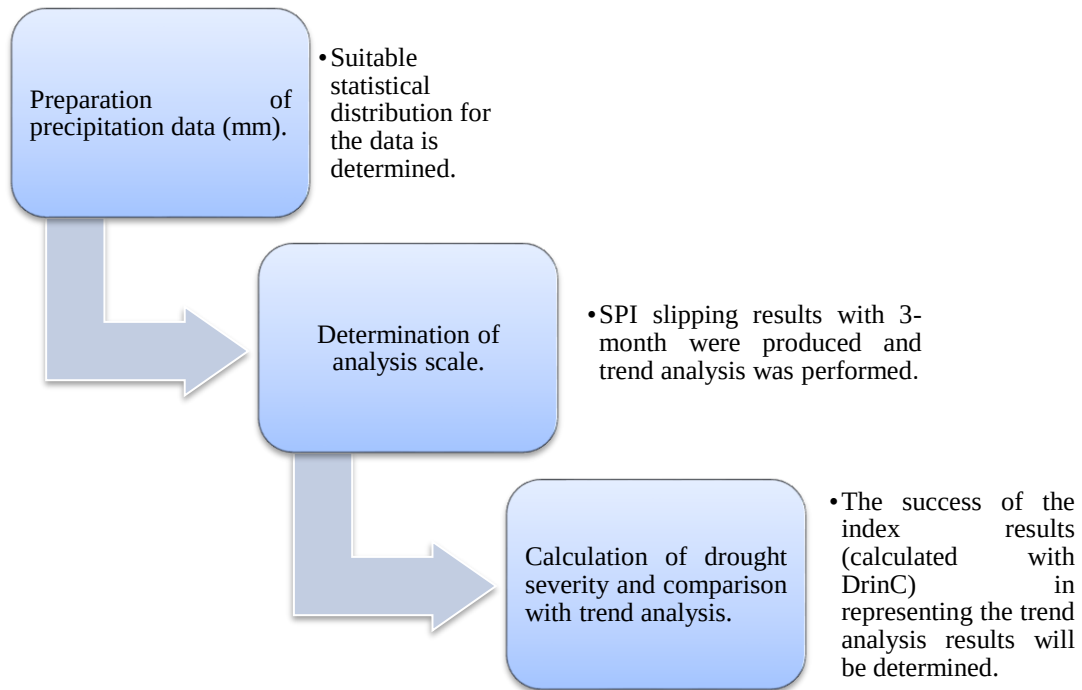


Figure 2. Flow chart of the study

2.2.1. Standard Precipitation Index

SPI, which was developed by McKee et al. in 1993, is an index that determines the meteorological drought. SPI drought classes are derived from standard normal distributed of precipitation sequences. However, the probability distribution function of precipitation sequences does not generally fit the normal distribution. Precipitation sequences generally conform to the gamma distribution. For this reason, standard precipitation sequences must be obtained in order to use precipitation data in SPI. The simplicity of the application of the method causes it to be preferred (Akbas, 2013).

Equation of Standard Precipitation Index: For the standard precipitation index, monthly precipitation data are needed. In equation 1, X_i is the amount of precipitation (mm), while X_i^{ort} is the mean amount of precipitation (mm) and σ is the standard deviation. A distribution function is fitted to the rainfall data. The gamma distribution is the most suitable for rainfall data. Cumulative probabilities are calculated by fitted distribution. Then, cumulative probabilities are converted to SPI values, which has a standard deviation of 1 and a mean of 0. The SPI results are available in some classes in Table 2 (McKee et al., 1993).

$$SPI = \frac{X_i - X_i^{ort}}{\sigma} \quad (1)$$

Table 2. Drought classes of standard precipitation index (McKee et al., 1993)

SPI Result	Drought Class
≥ 2	Extreme wet
1.50 ~ 1.99	Very wet
1.00 ~ 1.49	Mid wet
0.99 ~ 0	Slight wet
0 ~ -0.99	Slight drought
-1.00 ~ -1.49	Mid drought
-1.50 ~ -1.99	Very drought
≤ -2	Extreme drought

2.2.2. Trend analysis methods (mann-kendall, sen, linear regression)

Mann-Kendall Method: It is a non-parametric method that is not affected by missing data in a data series. In this method,

$$t = \sum_{i=1}^n n_i \quad (2)$$

The mean of the t test value is E(t) and its variance is var(t).

$$E(t) = \mu = \frac{n(n-1)}{4} \quad (3)$$

$$\text{var}(t) = \mu = \frac{n(n-1)(2n+5)}{72} \quad (4)$$

The Mann-Kendall test value is u(t).

$$u(t) = (t - E(t)) / \sqrt{\text{var}(t)} \quad (5)$$

The fact that u(t) is close to zero indicates that there is no change in time, while large values of u(t) indicate that there is a change. The first point where u(t) and u'(t) intersect is where the change begins. If u(t) and u'(t) are parallel to each other, it is understood that there is no trend (Oguz et al., 2021).

Sen's slope Method: A slope estimator is regarded as median for all data sets for different variation to identify the trend existing in data sets of a time series. The positive value of m indicates an upward trend while negative value of m indicates a downward trend. The slope is determined using the following equation (Agarwal et al., 2021).

$$m_i = \frac{T_J - t_i}{J - 1} \quad (6)$$

Where, T_J and t_i are the time values at time J and i.

The Sen's estimator slope is the median of these N counts. The Sen's slope is determined using the following equation (Agarwal et al., 2021).

$$m = m \left(\frac{N+1}{2} \right) \quad (7)$$

$$m = \frac{1}{2} \left(\frac{N}{2} + \frac{N+1}{2} \right) \quad (8)$$

As a result, m is calculated using two tailed test at 100 (1- α) % confidence level, then right slope is given by the nonparametric test.

$$x_t = a.t + b \quad (9)$$

Where coefficient a and b are determined based on the relationship between two variables, t and x_t . Later, the significance of coefficient a is tested using a t value at a significance (generally at 0.05 significance). In this case, the assumption of normal distribution is essential, while the results are sensitive to the outliers (Vaheddoost, 2020).

3. Results and Discussion

The results of the M-K trend and the SPI results are in parallel. If it is accepted that drought might decrease the sunflower yield, a parallelism is determined between the SPI results and the crop yield trend. Even in the prone to drought climate in Tekirdag, wheat and barley yields were very high. In this case, it was concluded that wheat and barley yields were not adversely affected by drought in Tekirdag conditions. Moreover, the crop most

Linear Regression Method: Linear regression is determined using,

coherent with the drought results in Trakya is the sunflower. The trend analysis method that best represents the result of the SPI is M-K (Kendall's tau). Nouri and Homaei (2019) determined that the SPI do not correlate well with the SPEI in arid regions. Namely, the correlation of SPI results with other method results is quite variable. For this reason, the drought indices to be used should be carefully selected according to the region and the type of drought. The graphs (Figure 3-5) of wheat and barley yields in the grain group were very similar to each other in terms of both shape and count (Table 3).

Similar to Kendall's tau results, according to Sen's slope results, a relative increase was detected in Edirne and Kırklareli and a relative decrease in Tekirdag although there is no significant trend (Table 3).

Table 3. Drought-trend and (crop yield) comparison in Trakya (Between 2004 and 2021)

Province	Drought (SPI), 2004-2021	Crop yield (kg da ⁻¹), 2004-2021			Trend of the drought (SPI) (Mann-Kendall and Sen, 2004-2021)		
		Wheat	Sunflower	Barley	Kendall's tau	p-value	Sen's slope
Edirne	0.39 (normal)	376	223	407	0,072 No trend (Increasing)	0.705	0.006
Kırklareli	0.47 (normal)	369	219	409	0,098 No trend (Increasing)	0.596	0.029
Tekirdag	-0.03 (normal)	413	207	453	-0,150 No trend (Decreasing)	0.405	-0.049

Naz et al. (2020) determined drought trend in Pakistan. They found the decreasing precipitation trend in half of the stations. Alsafadi et al. (2020) found a significant negative trend in 11.5% over the western Hungary. Ouatiki et al. (2019) determined the meteorological drought for 1970–2010 period. As a result, it was found that Morocco tends to have drier conditions. These results show that drought tendency is increasing in the World, especially in the Mediterranean watershed.

Dabanli (2019) produced drought risk maps at 250 stations in Turkey for the period 1971–2010. After all, the researcher determined that 5 out of 81 provinces in Turkey have low drought risk, 61 provinces are at moderate drought risk, and 14 provinces have high drought risk. Bacanlı (2017) detected a negative trend in half of the Aegean Region between 1960 and 2013. Oguz et al. (2021) investigated the drought trend in Mugla for the period 1960–2018. After all, the extreme dry

period was experienced more often than the extremely humid period. Moreover, the most severe droughts occurred in 2007, and the longest in 2001. Kankal and Akcay (2019) determined the trend of precipitation in Trabzon. Increasing trends dominate the city in autumn and spring. While no trend was observed in Trabzon in summer, a decreasing trend was observed in Akçaabat. These results showed that the cities of Turkey, which has a semi-arid climate, especially close to the Aegean and Mediterranean Seas, were more affected by drought. It was understood that

humidity was observed in places where the Black Sea climate was dominant. Therefore, Edirne, close to the Aegean, is prone to drought and Kırklareli, close to the Black Sea, is expected to be prone to humidity. In this study, compared to Kırklareli, Edirne is more prone to drought. In Trakya, while no trend was observed in the SPI values between 2004 and 2021 years, only in Tekirdag, there was a significant increase trend in SPI 6, 9 and 12 values. This situation shows that Tekirdag Province is relatively more humid than Edirne and Kırklareli (Table 4).

Table 4. Trend analysis of the SPI in Trakya

		P values ($\alpha=0.05$)	Kendall's tau	Sen's slope
Edirne	SPI 1	0.940	-0.002 (↓)	0
	SPI 3	0.459	0.017 (↑)	0
	SPI 6	0.447	0.018 (↑)	0
	SPI 9	0.532	0.015 (↑)	0
	SPI 12	0.453	0.018 (↑)	0
Kırklareli	SPI 1	0.822	0.006 (↑)	0
	SPI 3	0.173	0.034 (↑)	0
	SPI 6	0.330	0.024 (↑)	0
	SPI 9	0.436	0.019 (↑)	0
	SPI 12	0.245	0.029 (↑)	0
Tekirdag	SPI 1	0.322	0.021 (↑)	0
	SPI 3	0.066	0.040 (↑)	0
	SPI 6	0.039 (Significant)	0.045 (↑)	0
	SPI 9	0.034 (Significant)	0.046 (↑)	0
	SPI 12	0.022 (Significant)	0.050 (↑)	0

Demir et al. (2017) investigated the effects of precipitation trends on agriculture in Bingöl. As a result, it is determined that the decrease in precipitation will not affect the agriculture of Bingöl in the short term. Even if there is a decrease in precipitation, agriculture will not be affected in the short term. Therefore, agriculture is not expected to be affected by droughts because meteorological humidity is dominant in Trakya. The presence of various climate types and maritime effect in Trakya can be expressed as a factor that reduces meteorological factor. Eroglu (2021) analyzed the precipitation data of 10 stations for 50 years in Bulgaria and Turkey in order to determine the trend of precipitation in the Meric (Maritza) River Basin. As a result, a significant negative trend was determined in İpsala during the 1965-1990 period and a significant positive trend in Kırklareli during the 1991-2015 period. The researcher, in the 1965-2015

period, no statistically significant trend could be detected at any station. Moreover, there is a significant decrease trend in annual precipitation in Luleburgaz between 1930 and 1993, when the Marmara region is examined. Except for Luleburgaz, no significant trend could be detected at any of the stations. This result in the literature shows parallelism with the results of this study. It is also supported by the literature that Edirne is prone to drought, Kırklareli to humidity, and that there is no significant trend in Trakya in general. The data period between 2004 and 2021 was used to compare with crop yields and drought index results. The strongest crop yield-drought relationship was in the sunflower yield in Edirne (R^2 : 0.122 in Figure 4) and the weakest of the relationship was in the sunflower yield in Kırklareli (R^2 : 0.0002 in Figure 4). Generally, in Tekirdag conditions, there were stronger relationships between the effects of

drought and crop yields (R^2 : 0.042-0.11 in Figure 3-5). The precipitation data between January and December (not the October-September water year) were used to calculate the SPI index (Figure 3, 4, 5 and 6). In Edirne, Kirkclareli and Tekirdag, regression graph

between drought and wheat yield deviation were produced for 18 years (2004-2021). The cities, which has correlation from strong to weak between drought and wheat yield deviation are Tekirdag, Kirkclareli and Edirne, respectively (Figure 3).

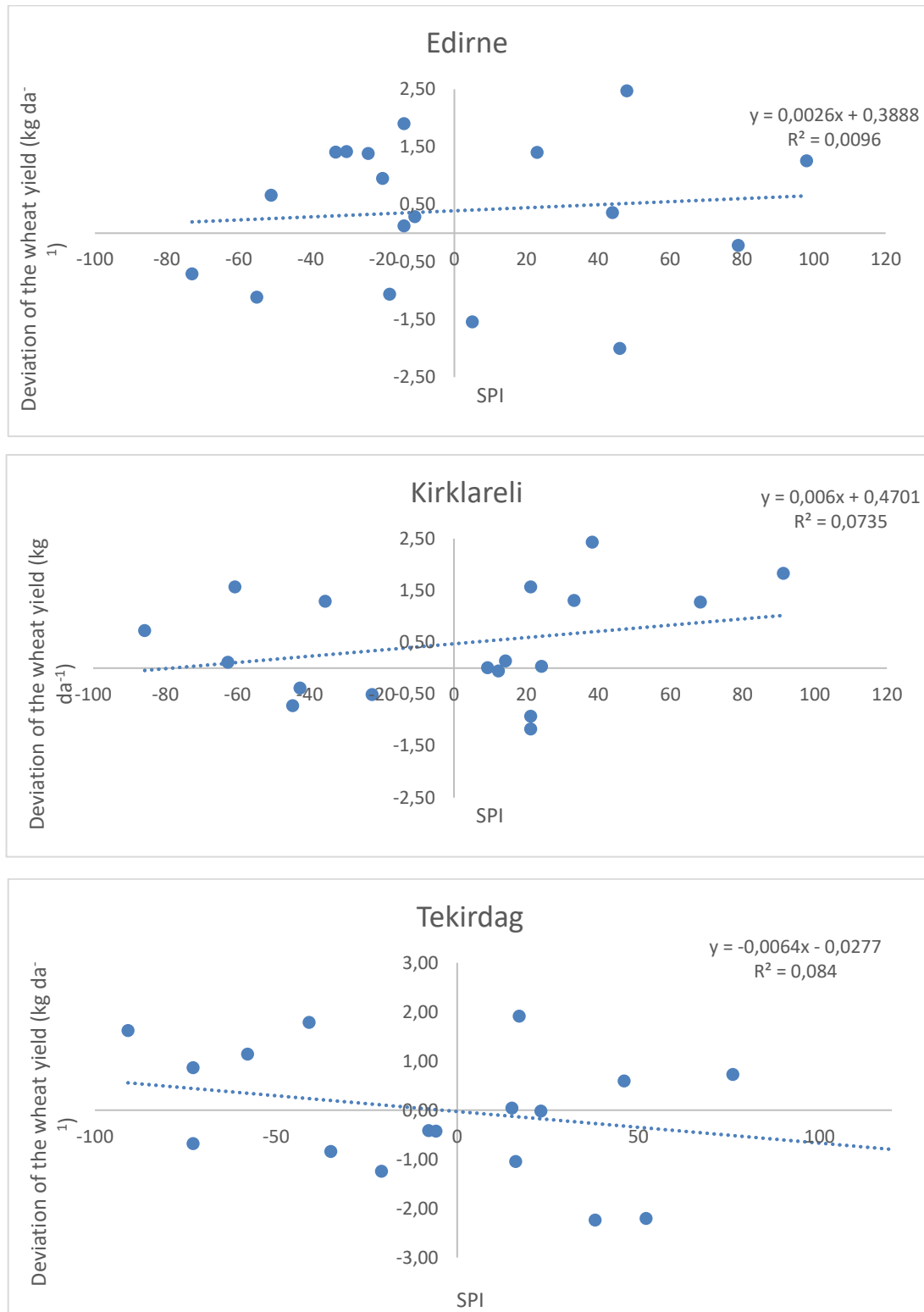


Figure 3. Regression graph of Trakya wheat yields and drought results

R^2 is 0.0096 in the regression graph for wheat in Edirne. It was understood that non-climatic factors were more effective on wheat yield for Edirne. This situation is thought to have occurred due to non-climatic condition for wheat in Edirne. In Edirne, Kırklareli and Tekirdağ, regression graph between drought

and sunflower yield deviation were produced for 18 years (2004-2021). The cities, which has correlation from strong to weak between drought and sunflower yield deviation are Edirne, Tekirdağ and Kırklareli, respectively (Figure 4).

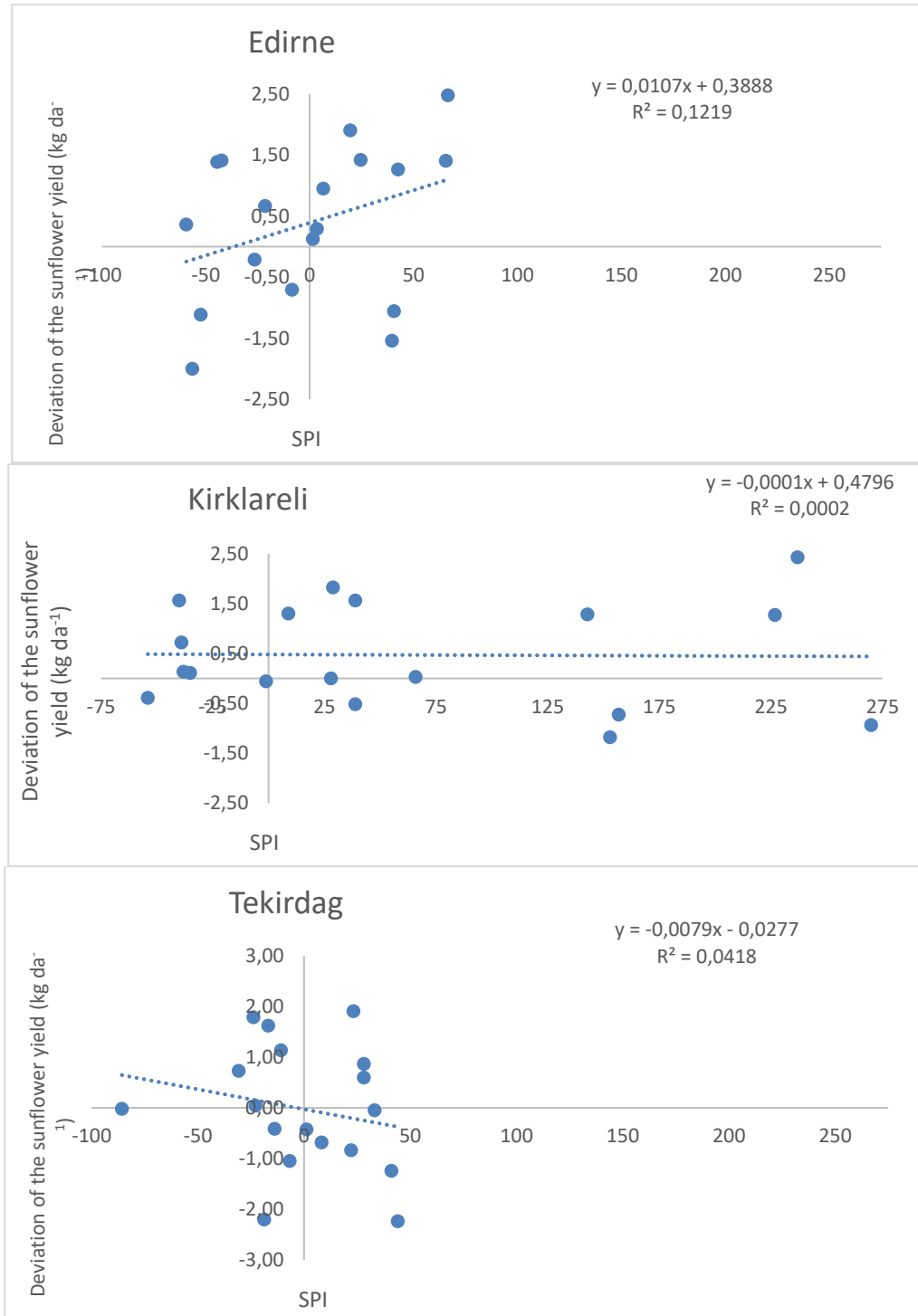


Figure 4. Regression graph of Trakya sunflower yields and drought results

R^2 is 0.0002 in the regression graph for sunflower in Kırklareli. It was understood that non-climatic factors were more effective on sunflower yield for Kırklareli. This situation is thought to have occurred due to non-climatic condition for sunflower in Kırklareli. In Edirne, Kırklareli and Tekirdağ, regression

graph between drought and barley yield deviation were produced for 18 years (2004-2021). The cities, which has correlation from strong to weak between drought and barley yield deviation are Tekirdağ, Kırklareli and Edirne, respectively (Figure 5).

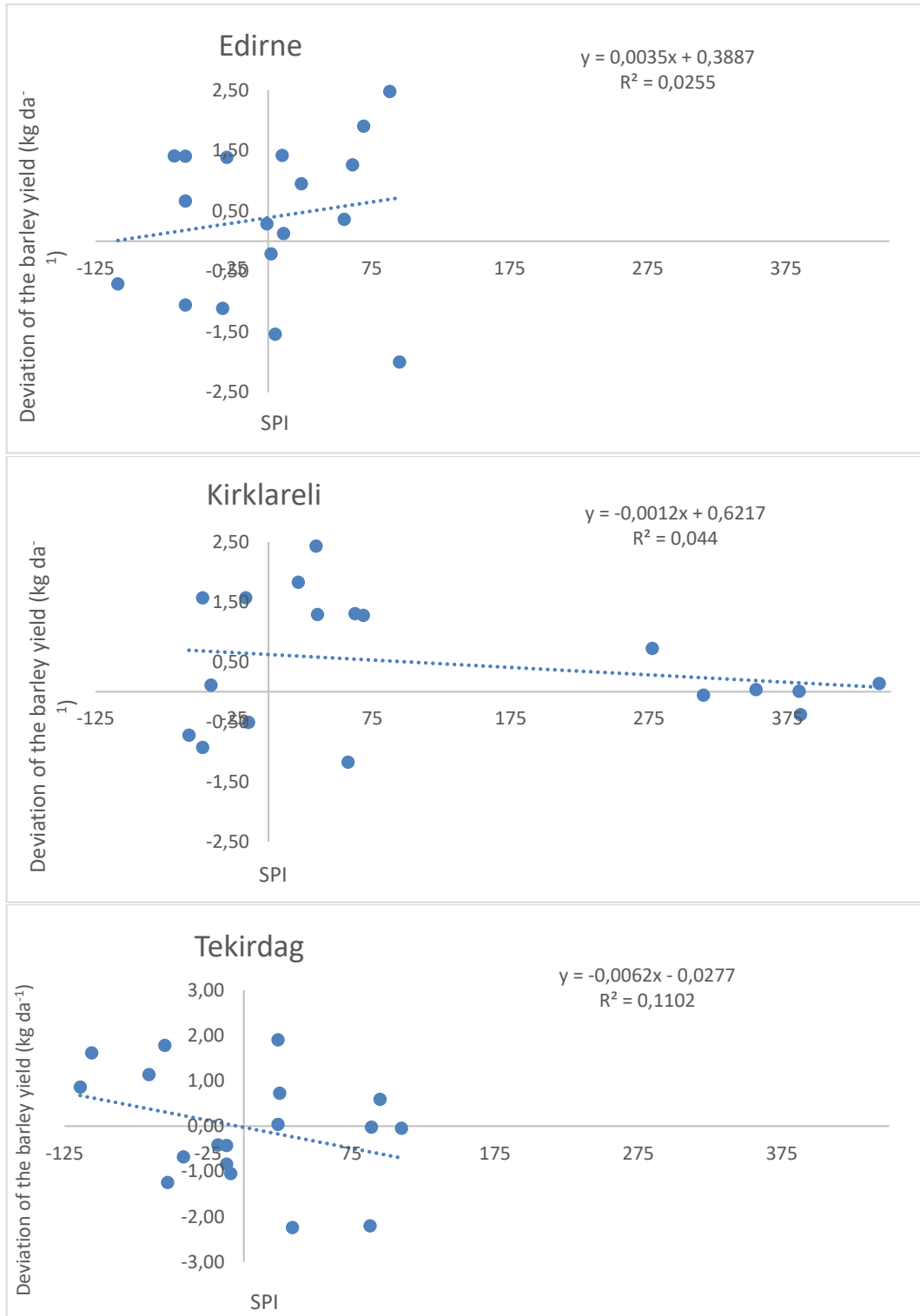


Figure 5. Regression graph of Trakya barley yields and drought results

R^2 is 0.0255 in the regression graph for barley in Edirne. It was understood that non-climatic factors were more effective on barley yield in Edirne. This situation is thought to have occurred due to non-climatic condition for barley in Edirne.

4. Conclusion

Drought tendency is increasing in the World, especially in the Mediterranean watershed. Therefore, the cities of Turkey, which has a semi-arid climate, especially close to the Aegean and Mediterranean Seas, were more affected by drought. It was understood that humidity was observed in places where the Black Sea climate was dominant. Therefore, Edirne, close to the Aegean, is prone to drought and Kirklareli, close to the Black Sea, is expected to be prone to humidity. In this study, compared to Kirklareli, Edirne is more prone to drought. An agriculturally wet situation prevails in only Tekirdag. Wheat and barley yields were not adversely affected by drought in Tekirdag conditions. Moreover, the crop most incompatible with the drought results for Trakya region was the wheat crop, when compared R^2 to each other in figure 3-5. Even if there is a relative decrease in precipitation, agriculture will not be affected in the short term. Inasmuch as meteorological drought is not dominant in Trakya, agriculture is not expected to be affected by droughts. The presence of various climate types and maritime effect in Trakya can be expressed as a factor that softens the climate of the Trakya. As a result, Edirne is prone to drought, Kirklareli to humidity, relatively. Besides, there is no significant trend in Trakya in general.

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The Effect of Different Planting Times on the Agricultural Characters of Some Safflower Cultivars Planted in Elazig Ecological Conditions

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Abstract

This study was carried out in 2021-2022 and 2022-2023 growing seasons to determine the yield and some yield characteristics of different safflower cultivars at different planting times in Elazig ecological conditions. The planting dates were 25 October, 10 November and 25 November in both years and Dincer, Linas, Olas and Zirkon cultivars were used. Among the agricultural traits examined in this study, winter resistance rate ranged from 4.58-100 %, flower yield ranged from 143.55-508.25 mg plant⁻¹, plant height ranged from 51.90-82.04 cm, number of lateral branches ranged from 1.71-5.50 pcs plant⁻¹, number of heads ranged from 3.20-10.01 pcs plant⁻¹, head diameter ranged from 15.91-24.43 mm, thousand seed weight ranged from 33.73-46.17 g, and seed yield ranged from 23.74-160.96 kg da⁻¹. In Elazig ecological conditions, it was determined that safflower, which is generally planted as summer safflower, can also be grown as winter safflower, although early sowing causes too much plant loss, planting can be done between 10 November and 30 November, and Dincer and Zirkon varieties came to the fore in this study.

Research Article

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1. Introduction

Fats, carbohydrates and proteins are the most important organic molecules in living organisms. In living organisms, fats serve many functions, such as providing more energy than carbohydrates and proteins, ensuring the absorption of vitamins A, D, E and K, and protecting internal organs. Fats of vegetable and animal origin are categorised into two groups, and their importance for human health has increased in terms of human health, as vegetable origin fats are more unsaturated. Oil is found in the seeds or fruits of many plants, but oil ratios are greater in certain plant groups. In general, vegetable oils are obtained from sunflower, canola, soya, safflower, peanut, sesame, olive, palm, etc., plants (Arioğlu et al., 2010).

In the last 10 years, vegetable oil production and consumption have increased worldwide. Vegetable oil production increased from 172 million tonnes to 218 million tonnes, and vegetable oil consumption increased from 167 million tonnes to 213 million tonnes (Anonymous, 2023b). Looking at the oilseed production in Turkey, oilseed production increased from 2.7 million tonnes to 4.2 million tonnes in the 10-year period from 2013-2014 to 2022-2023, while vegetable oil consumption increased from 991 thousand tonnes to 1.9 million tonnes (Anonymous, 2024). According to these figures, it is predicted that vegetable oil consumption will increase in parallel with population growth in the future.

Most vegetable oils are used for food purposes, and some are utilised in industry. Vegetable oils are used in industry in areas such as paint, varnish, and biodiesel (Kılı and Beycioğlu, 2019). Today, oils from plants such as rape and safflower are utilised in biodiesel. However, the spread of electric vehicles worldwide, reservations in ensuring food safety, and the negative effects of biodiesel on diesel engines and

automotive manufacturers, which reduce the production of diesel vehicles due to emission problems, suggest that biodiesel production will decrease in the future (Yusuf et al., 2011; Cunanan et al., 2021).

The continuous production of vegetable oils worldwide is important for food security. In particular, global climate change, the COVID-19 pandemic, and the Russia-Ukraine War negatively affected food security. Due to these problems of global origin, increases and volatilities were observed in vegetable oil prices in Türkiye, which is a net importer in terms of vegetable oils. To overcome such problems in Türkiye, it is imperative to increase the production of oil crops and to develop appropriate policies (Ortaş, 2022; Kılavuz and Yücer, 2023).

Drought caused by climate change in our country in recent years has negatively affected vegetable oil production. The safflower plant, which has the potential to close vegetable oil deficit and is resistant to drought, has potential for use in this area. When evaluated in terms of cultivation, safflower plants are easy to produce. Since safflower plants can be cultivated in summer and winter, it is important to utilise fallow areas effectively. The presence of oleic-type cultivars in safflower may enable the product to find buyers at higher prices. Input costs are low because there are few diseases and pests, and the need for fertiliser is low. However, bird damage in sunflower is not observed in safflower. In addition, seed casting problems observed in plants such as rapeseed and sesame in the later stages of maturation do not exist in safflower. The safflower plant is very suitable for mechanised use in cereals in agriculture, and it can be easily cultivated without any equipment changes (Yılmaz et al., 2015; Taşlıgil and Şahin, 2016).

Not only oil is produced from safflower seeds. After the oil is extracted from safflower seeds, the remaining cake is used

for animal nutrition. The breeding of varieties with thin seed coats has potential for the use of seeds as nuts. As safflower flowers can colour food and fabrics such as saffron flowers, safflower plants are also known as false saffrons (Taşıgil and Şahin, 2016). Due to the high cost of saffron flowers, the use of safflower flowers has increased in recent years (Andırman and Karaaslan, 2021).

The safflower production in Türkiye and in Elazığ Province over the last 10 years fluctuated with the safflower cultivation area and production in Türkiye, but this fluctuation was less in Elazığ Province. The

highest safflower cultivation area and production in our country in the last 10-year period were observed in 2014 and 2015. In Türkiye, in 2014, 62 000 tons were produced on an area of 443 000 da, and in 2015, 70 000 tons were produced on an area of 431 000 da. In Elazığ Province, while production was carried out in the 220 da area in 2014, production started in the 1000 da area, with a five fold increase in 2015. In the last 10 years, the highest production in Elazığ was obtained in 2018, with an area of 3993 da and 596 tons of production. By 2022, 353 tons of safflower were produced from the 2670 da area (Anonymous, 2023a).

Table 1. Safflower cultivation area (da) and production amount (tons) in the last ten years in Türkiye

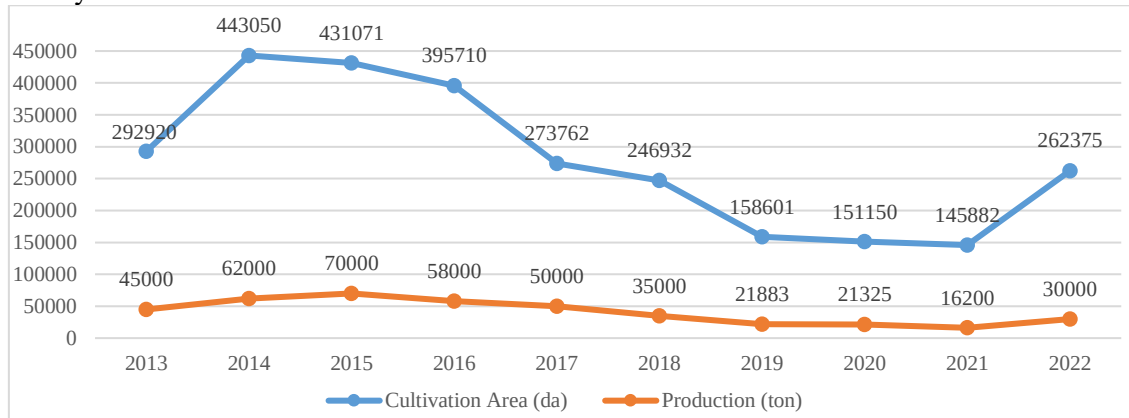
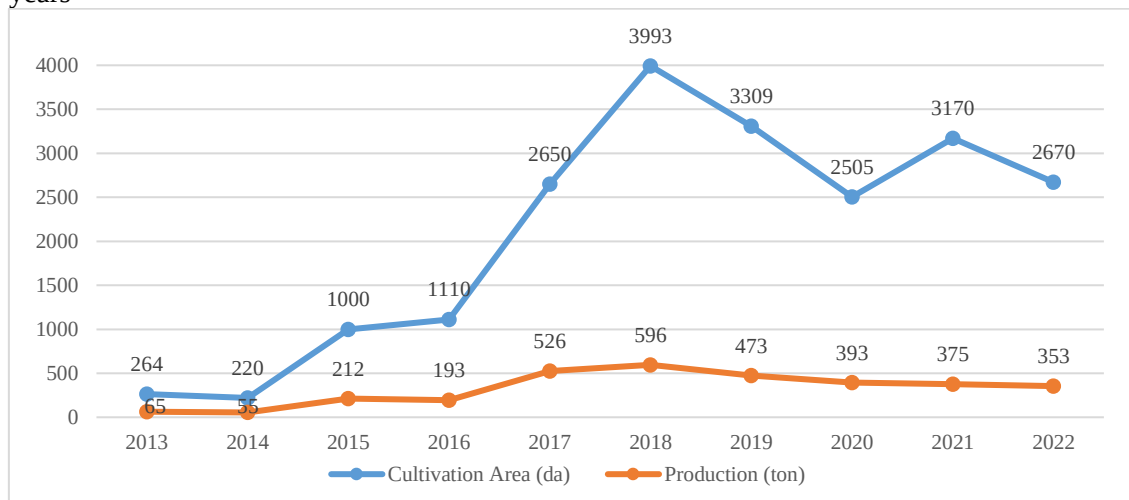


Table 2. Safflower cultivation area (da) and production amount (tons) in Elazığ over the last ten years



In safflower plants, issues such as planting time, row spacing, cultivar and fertilisation have important effects on agricultural characters and are important in terms of cultivation practices (Oruç and Yılmaz, 2019; Andırman and Karaaslan, 2021; Arslan and Güler, 2022). According to this perspective, there are few scientific studies on safflower and no studies on its cultivation in Elazığ Province. The aim of this study was to determine the agronomic characteristics of different safflower cultivars sown at different planting times for winter in Elazığ Province. It is thought that further studies on cultivation will contribute both to the literature and to safflower cultivation practices in Elazığ Province.

2. Materials and Methods

The trial was conducted during the 2021-2022 and 2022-2023 growing seasons at the Agriculture and Livestock Research and Application Centre of Firat University. In the trial, four different cultivars were used at three different planting times. The experiment was carried out according to the split-split design in randomised blocks with 4 replications, with the main plots were designed as planting times and the subplots were designed as cultivars. In this study, the length and width of each sub-plot were determined as 6 m and 1.2 m, respectively, and consisted of 6 rows. The seeds were sown between 20 cm row spacing with 5 kg per decare and no thinning was done. The plantings were made on 25 October, 10 November, 25 November in two growing years. The cultivars used were Dincer, Linas, Olas and Zirkon. The cultivars were obtained from Isparta University of Applied Sciences Faculty of Agriculture (Zirkon), Dicle University Faculty of Agriculture (Dincer) and Trakya Agricultural Research Institute (Linas and Olas). In the trial, 5 kg da⁻¹ of pure nitrogen and 5 kg da⁻¹ of pure phosphorus were applied to the planted winter plants, and 5 kg da⁻¹ of pure nitrogen

was applied as top fertiliser in March (Katar et al., 2012). Safflower fly (*Acanthiophilus helianthi*) and seedhead weevil (*Bangasternus planifrons*) were observed in the trials, and were treated with *imidacloprid*. Outlet irrigation was used in cases where rainfall was insufficient, especially at the first planting times. Since all plants matured at the same time in the experiment, harvesting was carried out on 19 August 2022 in the first year and 14 August 2023 in the second year. To determine whether the error variances of the trials of both years were homogeneous, the error variances of both years of the trial were subjected to Bartlett's homogeneity test. According to the results of this test, since the error variances were homogeneous, the trials of the two years were combined and analysed for variance. Duncan's multiple comparison test was used to compare the statistically significant means (Açıkgöz, 1993). Winter resistance rate, flower yield, plant height, number of lateral branches, number of heads, head diameter, thousand seed weight and yield per decare were analysed.

The Climatic data for the experimental site were obtained from the 13th Elazığ Regional Directorate. According to the average temperatures in Table 3, the coldest month was January, with a temperature of -1.6 °C in the 2021-2022 growing years; -1.1 °C in February in the 2022-2023 growing years; -0.8 °C in February. The hottest month was August in both growing years and long years, and these values were higher in both growing years than in long years.

When the monthly maximum temperatures were analysed, the lowest monthly maximum temperature occurred in January in both growing years and long years. The highest monthly maximum temperature occurred in August in both growing years and in July in the long years. The analysis of the monthly minimum

temperatures showed that the monthly minimum temperature decreased to -24.6 °C in December and January, while it decreased to -11.2 °C in January in the first growing year and to -9.5 °C in February in the second growing year. In August, which

is one of the hottest months of the year, a decrease of up to 10.2 °C was observed, while a decrease of up to 18.6 °C was observed in the first growing year, and a decrease of 14.9 °C was observed in the second growing year (Table 3).

Table 3. Climatic data of the experimental site

Months	Average temperature			Monthly maximum temperature (°C)			Monthly minimum temperature (°C)			Monthly total precipitation (mm)		
	2021 2022	2022 2023	Long Years	2021 2022	2022 2023	Long Years	2021 2022	2022 2023	Long Years	2021 2022	2022 2023	Long Years
October	14.8	16.1	15.00	25.8	28.5	32.40	3.2	5.1	-2.20	37.40	13.60	39.50
November	9.5	8.8	7.50	18.4	18.3	24.30	1.9	2.4	-15.20	23.50	73.70	48.90
December	1.9	4.7	1.80	10.6	11.7	19.60	-9.4	-1.6	-22.60	20.10	13.90	45.70
January	-1.6	0.1	-0.80	7.2	6.5	13.00	-11.2	-8.7	-22.60	27.20	16.10	42.00
February	2.8	-1.1	0.70	12.4	13.6	18.60	-5.4	-9.5	-21.40	44.50	30.30	42.60
March	1.8	8.1	5.60	16	16	26.40	-7.9	-3.5	-17.00	78.90	217.50	56.10
April	13.9	10.5	12.00	25.3	21.3	32.20	-2.7	-2.6	-7.00	6.00	84.90	62.90
May	14.6	15	17.20	30.9	24.6	36.60	4.5	4.2	0.00	49.9	84.40	53.10
June	22.5	21	22.80	33.7	32	38.60	11.5	12	4.00	45.00	4.90	12.40
July	25.8	25.7	27.20	36.2	36.2	42.40	13.5	12.8	6.70	0.00	0.60	3.30
August	28.5	28.4	27.00	36.8	38.3	42.20	18.6	14.9	10.20	0.50	4.30	1.80
Total										333.00	544.20	408.30

When the total monthly precipitation amounts were analysed, it was found that while 408.3 mm of precipitation occurred in the long years, 333.0 mm of precipitation was received in the first growing year, and 554.2 mm of precipitation was received in

the second growing year. Accordingly, the amount of precipitation received in the first growing year was lower than in the long years, while the amount of precipitation received in the second growing year was greater than that in the long years (Table 3).

Table 4. Soil analysis results of the experimental site

Name of Analysis	Results	Rating
Saturation (%)	60.5	Clay - Loamy
pH	8.03	Slightly Alkaline
Total Salt (%)	0.02	Unsalted
Lime (%)	26.04	Too much lime
Organic Matter (%)	2.59	Medium
Available Phosphorus (P ₂ O ₅ - kg da ⁻¹)	4.29	Low
Available Potassium (K ₂ O- kg da ⁻¹)	63.23	High

Soil analyses of the experimental area were carried out at the Soil Analysis Laboratory of the Elazığ Provincial Directorate of Agriculture and Forestry. According to the data obtained, Table 4 shows that the soil texture is clayey-loamy, the soil pH is slightly alkaline at 8.03, the total salt content is salt-free at 0.02 %, it contains high lime content at 26.04 %, the organic matter content in the soil is moderate at 2.59 %, the available

phosphorus is low at 4.29 kg per decare, but the available potassium is high at 63.23 kg per decare.

3. Results and Discussion

3.1. Winter resistance rate

When the years were analysed separately, the lowest winter resistance rate was obtained for the Dincer cultivar, with 4.58 % at the 1st planting time in 2021-2022.

In the following year, it was determined that Dincer cultivar was found to be the most sensitive cultivar to winter under Elazığ ecological conditions, with 5.90 % sensitivity at 1st planting time (Table 8). Koç (2019), in his study conducted under Konya ecological conditions, determined that the most sensitive cultivar to winter was Dincer, followed by Linas cultivar. However, winter resistance rates were lower in the study conducted by Koç (2019). This is because the minimum

temperature values in the years in which the trial was conducted were lower than the minimum temperature values in this trial (Table 3). Koç (2019) and Johnson et al. (2016) determined a resistance between 80 % and 100 % in the Chinese origin BJ-27 line, and these values were found to be low compared to this study, and the reason for this situation can be said that this Chinese origin line used in these trials has a genetically higher cold resistance.

Table 5. Variance analysis results of the trial

VK	DF	Winter resistance rate (%)	Flower yield (mg plant ⁻¹)	Plant height (cm)	Number of lateral branches (pcs plant ⁻¹)	Number of heads (pcs head ⁻¹)	Head diameter (mm)	Thousand seed weight (g)	Yield per decare (kg da ⁻¹)
B	3	**	NS	NS	NS	NS	NS	NS	NS
Y	1	**	**	NS	**	*	**	**	*
PT	2	**	**	**	**	**	NS	NS	**
Y x PT	2	**	**	**	**	**	**	NS	**
C	3	**	**	**	**	**	NS	**	**
Y x C	3	**	**	NS	**	**	NS	**	**
PT x C	6	**	**	**	NS	*	**	NS	**
Y x PT x C	6	**	**	NS	NS	NS	**	*	**
CV (%)		9.23	13.79	6.32	15.62	15.52	4.59	4.39	14.48

**: Statistically significant at the 1% level, * Statistically significant at the 5% level, NS: Not significant, B: Block, Y: Year, PT: Planting time, C: Cultivar, CV: Coefficient of variation, DF: Degree of freedom.

3.2. Flower yield

Since the safflower flowers are used in different fields and have a positive relationship with seed yield, it is important to investigate this character (Singh et al. 2008). Table 5 shows that the effects of year, planting time, cultivar, year × cultivar, year × planting time, planting time × cultivar, and year × planting time × cultivar on flower yield were statistically significant at the 1 % level. The effects of different years, planting times and cultivars alone and together caused differences in flower yield. Table 6 shows that flower yield was higher in the first year of the experiment than in the second year. When the development of the plants was examined, the flowering period occurred in June. An analysis of the climatic data (Table 3) revealed that the amount of rainfall in the first year was significantly higher than that

in the second year in June during the first and second years of the experiment. The flower yield in the second year decreased due to the effects of heat and drought. The highest flower yield per plant was obtained from 508.25 mg of Olas and 494.50 mg of Zirkon at 1st planting time in the 2021–2022 growing period (Table 8). The obtained data were lower than those in the study of Uysal et al. (2006), and it was determined that this difference was not due to a lack of rainfall during the years when the experiment was carried out; rather, the plant density affected flower yield, possibly due to low plant density. It was determined that the flower yield was higher than that in the study conducted by Koç and Güneş (2021) in summer. The rainfall in June, especially in the first year of the experiment, and the cultivar used and other ecological factors affected this situation.

3.3. Plant height

Plant height is an important agricultural character of safflower, as is it for many crops. According to the results of the variance analysis, year, year $\times$ cultivar interaction and year $\times$ planting time $\times$ cultivar interaction had no significant effect on plant height. The effects of planting time, cultivar and year $\times$ planting time, and planting time $\times$ cultivar interactions on plant height were found to be statistically significant at the 1 % level (Table 5). When the effect of planting time was analysed, it was observed that plant height increased gradually from 1st planting time to 3rd planting time (Table 6). This can be explained by the fact that 1st planting time was exposed to more cold and drought than was 3rd planting time. The longer this period is, the more the meristem tissues that support plant growth will be negatively affected. The lowest and highest plant heights were obtained from the first year of the experiment. The lowest plant height was obtained from Dincer cultivar at 51.90 cm at 1st planting time, while the highest plant height was obtained from Linas cultivar at 82.04 cm at 3rd planting time (Table 8). In a study conducted by El Bey et al. (2021), Linas cultivar was found to have higher plant height than Olas cultivar, which was similar to the findings of this study. However, when the general plant height values of the trials were examined, the values were lower than those reported by El Bey et al. (2021), Paşa (2008), and Hatipoğlu et al. (2012) and were somewhat similar to those reported by Oruç and Yılmaz (2019). This may be related to the response of the ecological conditions in which the trials were carried out, especially whether the winter conditions were harsh.

3.4. Number of lateral branches

The number of lateral branches is an important character in safflower plants, and a head is usually formed at the end of each lateral branch. In this study, the differences between the averages of the number of lateral branches, year, planting time, year $\times$ planting time, cultivar and year $\times$ cultivar were statistically significant at the 1% level, while the differences between the interactions of planting time $\times$ cultivar and year $\times$ planting time $\times$ cultivar were not statistically significant (Table 5). The number of lateral branches varied between 2.71 and 5.50. The lowest number of lateral branches was obtained from Zirkon cultivar at 3rd planting time in the 2022-2023 growing season, while the greatest number of lateral branches was obtained from Olas cultivar at 3rd planting time in the 2021-2022 growing season (Table 8). Kızıl (2002) and Hatipoğlu et al. (2012), who studied winter planting times, reported that the number of lateral branches in safflower decreased with increasing planting time increased, and in this study, when the effect of planting time alone on the number of heads was examined, it was determined that there was a decrease from 1st planting time to 2nd planting time and an increase in 3rd planting time (Table 6). This can be explained by the fact that the number of plants per m² at 1st and 3rd planting times was less than that at 2nd planting time. This result is in partial agreement with the study conducted by Baran and Andırman (2019). Number of lateral branches was lower than the values obtained by Kızıl (2002), Hatipoğlu et al. (2012), and Baran and Andırman (2019). This may be due to the ecological conditions under which the studies were conducted.

Table 6. Data from the experiment for year, planting time, cultivar, and year $\times$ planting time interaction

Table 6. Data from the experiment for year, planting time, cultivar, and year x planting time interaction																	
		WRR		FY		PH		NLB		NH		HD		TSW		Y	
		Year															
2021		52.95	A	423.29	A	67.02		3.76	A	6.25	A	22.67	A	25.76	A	105.88	A
2022																	
2022		40.56	B	270.42	B	67.85		2.86	B	5.17	B	16.92	B	22.11	B	73.88	B
2023																	
		Planting Time															
1. PT		18.02	C	377.15	A	65.03	B	3.44	A	6.07	A	19.60		39.76		64.25	B
2. PT		41.52	B	352.99	A	66.98	AB	2.87	B	4.76	B	19.66		39.90		106.63	A
3. PT		80.73	A	310.42	B	70.30	A	3.61	A	6.30	A	20.12		40.04		98.74	A
		Cultivar															
Dincer		42.14	C	355.57	A	67.57	B	3.13	B	6.60	A	19.84		41.64	A	87.62	BC
Linas		45.75	B	290.69	B	74.45	A	3.33	B	5.10	B	19.94		40.09	B	92.03	AB
Olas		46.80	B	366.51	A	66.24	B	3.78	A	6.08	A	19.85		38.21	C	80.61	C
Zirkon		52.35	A	374.65	A	61.48	C	3.00	B	5.06	B	19.55		39.65	B	99.26	A
		Year x Planting Time Intraction															
2021	1. PT	19.65	D	427.25	AB	57.22	D	3.11	BC	4.74	B	21.93	B	41.93		69.72	C
	2. PT	39.20	C	450.13	A	70.30	AB	3.18	BC	5.19	B	22.66	AB	43.34		142.78	A
	3. PT	100.00	A	392.50	B	73.53	A	4.98	A	8.81	A	23.41	A	43.53		105.13	B
2022	1. PT	16.39	D	327.05	C	72.83	A	3.77	B	7.39	A	17.28	C	37.58		58.78	C
	2. PT	43.84	C	255.86	D	63.66	C	2.57	CD	4.34	B	16.66	C	36.45		70.49	C
	3. PT	61.46	B	228.34	D	67.06	BC	2.24	D	3.78	B	16.82	C	36.54		92.35	B

WRR: Winter resistance rate, FY: Flower Yield, PH: Plant Height, NLB: Number of Lateral Branches, NH: Number of Heads, HD: Head Diameter, TSW: Thousand Seed Weight, Y: Yield, PT: Planting Time.

3.5. Number of heads

The effects of planting time, year $\times$ planting time, cultivar, year $\times$ cultivar were statistically significant at the 1 % level; year and planting time $\times$ cultivar interaction were statistically significant at the 5 % level; and year $\times$ planting time $\times$ cultivar interaction was not significant (Table 5). The number of heads varied between 3.20 and 10.01. The lowest number of heads was obtained from Zirkon cultivar at 3rd planting time and from Linas cultivar at 2nd planting time in the growing years 2022-2023, while the greatest number of heads was obtained from Olas cultivar at 1st planting time in the same growing year (Table 8). When the single effect of number of heads was analysed, similar to number of lateral branches, there was a decrease in number of heads from 1st planting time to 2nd planting time and then an increase from 2nd planting time to 3rd planting time (Table 6). This situation can be explained by plant density per m². Plant density per m² at 2nd planting time was higher than that at 1st and 3rd planting times. Therefore, fewer heads were obtained during 2nd planting time. While the

data on number of heads were consistent with those of El Bey et al. (2021) and Aslantaş and Akınerdem (2020), they were lower than those of Baran and Andırman (2019), Öz (2016) and Samancı et al. (2001).

3.6. Head diameter

The effects of year, year $\times$ planting time, planting time $\times$ cultivar and year $\times$ planting time $\times$ cultivar were statistically significant at the 1 % level, while the effects of planting time, cultivar and year $\times$ cultivar were not statistically significant. Head diameter varied between 15.91 and 23.43 mm (Table 5). The smallest head diameter was obtained for the Olas cultivar at 3rd planting time in the 2022-2023 growing season, while largest head diameter was obtained for Olas cultivar at 3rd planting time in the 2021-2022 growing season (Table 8). In the first year of the trial, the number of plants per m² at 3rd planting time was low at 3rd planting time, and in second year, it was high at 2nd planting time, so head diameter remained low due to the increase in density. Head diameter was similar to that reported by Hatipoğlu et al. (2012) and lower than

that reported by Aslantaş and Akınerdem (2020).

3.7. Thousand seed weight

According to the analysis of variance, the effects of year, year $\times$ planting time, planting time $\times$ cultivar and year $\times$ planting time $\times$ cultivar were statistically significant at the 1 % level; the effect of year was statistically significant at the 5 % level; and the effects of planting time, cultivar and year $\times$ cultivar were statistically nonsignificant on the difference between the average thousand seed weights (Table 5). The thousand seed weight varied between 33.73 and 46.17 g. The lowest thousand seed weight was obtained from Linas cultivar at 3rd planting time of the 2022-2023 growing season, and the highest thousand seed weight was obtained from Linas cultivar at 2nd planting time of the 2021-2022 growing season (Table 8). Considering these values, Linas cultivar showed wide variation in terms of thousand-seed weight. In general, it was found to be higher than that of Öztürk (2019) and Aslantaş and Akınerdem (2020) and lower than that of El Bey et al. (2021),

Hatipoğlu et al. (2012) and Karaaslan et al. (2011). One of the factors affecting thousand seed weight in safflower is related to seed filling after the flowering period. In particular, in this study, poor seed formation was observed due to the drought experienced after the flowering period, and it was observed that some seeds were not filled.

3.8. Seed yield

According to the results of variance analysis, the effects of planting time, cultivar, year $\times$ planting time, year $\times$ cultivar, planting time $\times$ cultivar, year $\times$ planting time $\times$ cultivar and year $\times$ planting time $\times$ cultivar were statistically significant at the 1 % level, and the effect of year at the 5 % level on the difference between the mean seed yields (Table 5). The lowest seed yield was obtained from Dincer cultivar at 1st planting time in the 2022-2023 growing season (23.74 kg da⁻¹), while the highest seed yield was obtained from Dincer cultivar at 2nd planting time in the 2021-2022 growing season (160.96 kg da⁻¹), (Table 8).

Table 7. Data of the trial for year $\times$ cultivar and planting time $\times$ cultivar interactions.

		WRR		FY		PH		NLB		NH		HD		TSW		Y	
Year x Cultivar Intraction																	
2021- 2022	Dincer	47.09	CD	391.92	B	66.75		2.80		6.26	AB	22.71		27.00	A	106.04	A
	Linas	54.09	B	376.75	B	73.32		3.03		5.64	BC	22.42		26.70	A	109.96	A
	Olas	50.98	BC	464.17	A	65.96		3.18		6.95	A	23.06		24.42	B	88.66	B
	Zirkon	59.64	A	460.33	A	62.04		2.44		6.15	ABC	22.49		24.92	B	118.85	A
2022- 2023	Dincer	37.18	E	319.22	C	68.40		3.13	B	6.93	A	16.98		22.96	C	69.19	C
	Linas	37.40	E	204.63	D	75.57		3.33	B	4.56	DE	17.46		21.40	D	74.09	BC
	Olas	42.62	D	268.86	C	66.53		3.78	A	5.21	CD	16.64		21.42	D	72.55	C
	Zirkon	45.06	D	288.97	C	60.92		3.00	B	3.98	E	16.61		22.67	C	79.67	BC
Planting Time x Cultivar Intraction																	
1. PT	Dincer	5.24	F	402.53	A	59.09	E	3.26		7.66	A	19.40	AB	41.00		28.64	E
	Linas	18.70	E	303.28	BCD	72.18	B	3.45		5.42	BCD	19.77	AB	40.96		74.64	D
	Olas	25.48	D	405.40	A	65.19	CDE	3.93		6.36	B	19.11	AB	37.35		75.85	D
	Zirkon	22.68	DE	397.40	A	63.65	DE	3.14		4.83	CDE	20.13	A	39.71		77.88	D
2. PT	Dincer	39.56	C	385.53	A	71.73	B	2.84		5.64	BC	19.95	AB	41.73		123.50	A
	Linas	39.83	C	303.78	BCD	70.80	BC	2.59		3.80	E	19.78	AB	40.28		97.82	BC
	Olas	36.50	C	338.65	ABC	66.09	BCD	3.45		5.36	BCD	20.27	A	38.30		89.77	CD
	Zirkon	50.21	B	384.03	A	59.30	E	2.62		4.27	DE	18.66	B	39.27		115.45	AB
3. PT	Dincer	81.61	A	278.65	CD	71.90	B	3.28		6.50	AB	20.17	A	42.18		110.71	AB
	Linas	78.71	A	265.03	D	80.36	A	3.94		6.09	BC	20.27	A	39.03		103.61	BC
	Olas	78.42	A	355.48	AB	67.45	BCD	3.96		6.51	AB	20.17	A	38.96		76.20	D
	Zirkon	84.17	A	342.53	ABC	61.49	DE	3.25		6.09	BC	19.86	AB	39.98		104.45	BC

WRR: Winter Resistance Rate, FY: Flower Yield, PH: Plant Height, NLB: Number of Lateral Branches, NH: Number of Heads, HD: Head Diameter, TSW: Thousand Seed Weight, Y: Yield, PT: Planting Time.

Dincer cultivar showed a wide variation seed yield per decare. In the first planting of both years, it was determined that seed yield

per decare was low in Dincer cultivar. It is understood from the winter resistance rate that Dincer cultivar is highly affected by

cold, especially during early planting, such as in winter. In a winter cold resistance study conducted by Koç (2019) with different safflower cultivars and lines, Dincer cultivar was among the most sensitive cultivars to winter cold. Considering 2nd planting times of both years, the highest seed yields were obtained from Dincer cultivar compared to the other cultivars (2021-2022: 160.96 kg da⁻¹, 2022-2023: 86.04 kg da⁻¹). When 3rd planting times were also evaluated, although it followed Linas cultivar in the first year of the experiment, it had almost the same seed yield (Linas: 124.75 kg da⁻¹, Dincer: 123.62 kg da⁻¹) and followed Zirkon cultivar in 2nd year of the experiment (Zirkon: 100.51 kg da⁻¹, Dincer: 97.79 kg da⁻¹). When the general situation of the trial was evaluated, it was determined that the seed yield of the first year was higher than that of the second year due to precipitation, and in terms of planting times, the highest seed yield was obtained from 2nd planting time, but the same statistical group was shared with 3rd planting time.

The highest seed yield was obtained from Zirkon cultivar (99.26 kg da⁻¹), and it

was determined that this cultivar had a more stable yield than the other cultivars. According to the 2-year average of the experiment, while the lowest seed yield was obtained from Dincer cultivar at 1st planting time, the highest seed yield was obtained from Zirkon cultivar, and it shared the same statistical group with the other cultivars except Dincer. At 2nd planting time, the greatest seed yield was obtained from Dincer cultivar followed by Zirkon cultivar and the lowest seed yield was obtained from Olas cultivar. At 3rd planting time, the highest seed yield was obtained from Dincer cultivar, followed by Zirkon cultivar. At the same time, Zirkon and Linas shared the same statistical group. The lowest seed yield was obtained from Olas cultivar at the same planting time (Table 7). In this study, seed yield was higher than that obtained by Aslantaş and Akınerdem (2020) and lower than that obtained by Culpun (2023), Hatipoğlu et al. (2012), and Karaaslan et al. (2011). These differences are due to the cultivars used, the different ecological conditions in which the trials were conducted and some differences in the conduct of the trials.

Table 8. Data of the trial for year × planting time × cultivar intraction

		WRR		FY		PH	NLB	NH	HD	TSW		Y			
2021-2022	1. PT	Dincer	4.58	K	383.50	BCDE	51.90	3.20	5.31	21.93	BC	44.03	ABCD	33.55	KL
		Linas	22.34	IJ	322.75	DEF	62.58	2.75	4.18	21.53	BC	43.01	BCDE	73.96	GHIJ
		Olas	21.60	IJ	508.25	A	56.73	3.60	5.05	21.57	BC	39.54	FGHI	73.77	GHIJ
		Zirkon	30.09	HI	494.50	A	57.67	2.90	4.43	22.69	ABC	41.16	EFG	97.62	EFG
	2. PT	Dincer	36.70	FGH	438.50	ABC	71.98	2.70	4.85	22.82	ABC	45.51	AB	160.96	A
		Linas	39.92	FG	421.00	ABCD	75.35	2.95	4.40	23.31	ABC	46.17	A	131.17	BC
		Olas	31.35	GH	463.25	AB	70.81	4.04	6.49	23.18	ABC	40.23	EFGH	128.45	BC
		Zirkon	48.84	DE	477.75	AB	63.07	3.02	5.03	21.34	C	41.46	DEFG	150.53	AB
	3. PT	Dincer	100.00	A	353.75	CDEF	76.37	4.45	8.63	23.37	AB	45.48	AB	123.62	CDE
		Linas	100.00	A	386.50	BCDE	82.04	5.19	8.35	22.42	BC	44.34	ABC	124.75	BCD
		Olas	100.00	A	421.00	ABCD	70.36	5.50	9.30	24.43	A	42.34	CDE	63.76	HIJ
		Zirkon	100.00	A	408.75	ABCD	65.37	4.80	8.98	23.43	AB	41.96	CDEF	108.40	CDEF
2022-2023	1. PT	Dincer	5.90	K	421.55	ABCD	66.28	3.32	10.01	16.88	DE	37.98	HIJKL	23.74	L
		Linas	15.05	J	283.80	EFGH	81.78	4.15	6.65	18.01	D	38.91	GHIJ	75.32	GHIJ
		Olas	29.35	HI	302.55	EFG	73.65	4.25	7.68	16.65	DE	35.17	LMN	77.94	GHIJ
		Zirkon	15.27	J	300.30	EFG	69.62	3.37	5.23	17.57	DE	38.26	HIJK	58.13	IJK
	2. PT	Dincer	42.41	EF	332.55	DEF	71.48	2.98	6.43	17.07	DE	37.96	HIJKL	86.04	FGHI
		Linas	39.75	FG	186.55	HI	66.25	2.23	3.20	16.25	DE	34.39	MN	64.48	HIJ
		Olas	41.64	EF	214.05	GHI	61.38	2.85	4.23	17.37	DE	36.38	JKLMN	51.09	JK
		Zirkon	51.57	D	290.30	EFG	55.53	2.23	3.50	15.97	E	37.08	IJKL	80.37	GHI
	3. PT	Dincer	63.23	BC	203.55	GHI	67.43	2.11	4.37	16.97	DE	38.88	GHIJ	97.79	EFG
		Linas	57.41	CD	143.55	I	78.68	2.70	3.83	18.12	D	33.73	N	82.47	FGHI
		Olas	56.85	CD	289.97	EFG	64.55	2.43	3.73	15.91	E	35.58	KLMN	88.64	FGH
		Zirkon	68.34	B	276.30	FGH	57.60	1.71	3.20	16.30	DE	37.99	HIJKL	100.51	DEFG
Ort.		46.76		346.85		67.44	3.31	5.71	19.79		39.90		89.88		

WRR: Winter Resistance Rate, FY: Flower Yield, PH: Plant Height, NLB: Number of Lateral Branches, NH: Number of Heads, HD: Head Diameter, TSW: Thousand Seed Weight, Y: Yield, PT: Planting Time.

4. Conclusions

An evaluation of the results obtained from this trial over two years revealed that safflower, which is generally grown as a summer crop under Elazığ ecological conditions, can also be grown as a winter crop. Planting time should be taken into consideration as an important factor in winter cultivation. Early planting had a particularly negative effect on seed yield. For high yields, winter planting should be started on 10-15 November and completed on 25-30 November. These dates are generally a period of increased rainfall, and there is limited time to reach the land for planting. Considering the 2-year averages of the cultivars in terms of yield, Dincer cultivar was more prominent than the other cultivars in terms of yield at 2nd and 3rd planting times, but Zirkon cultivar should be considered separately in terms of cultivar preference since it has the greatest single effect in terms of yield and the highest winter resistance rate.

Authors' Declaration of Contribution

The authors declare that they have contributed equally to the manuscript and have read and approved the final version of the manuscript for publication.

Conflict of Interest Declaration

All authors declare that they have no conflict of interest for this study.

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Andırın-Çiğsar Bölgesi Kiraz Bahçesi Topraklarının Bazı Fiziko-Kimyasal Özelliklerinin Değerlendirilmesi

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Özet

Tarımsal üretimin sürdürülebilirliği, bitkilerin dengeli beslemesi ile mümkündür. Bitkilerin dengeli beslenebilmesi için toprak özelliklerinin bilinmesi gerekmektedir. Bu bağlamda, Kahramanmaraş-Andırın ilçesi Çiğsar bölgesinde kiraz bahçelerinde, 24 farklı noktadan 0-30 ve 30-60 cm derinlikten tesadüfî örnekleme yöntemine göre toprak örnekleri alınmıştır. Alınan toprak örneklerinde tekstür, hidrolik iletkenlik (Hİ), tarla kapasitesi (TK), solma noktası (DSN), saturasyon yüzdesi (SY) yarayışlı su miktarı (YS), pH, kireç, tuz, organik madde (OM), alınabilir kalsiyum (Ca), magnezyum (Mg), fosfor (P₂O₅), potasyum (K₂O), demir (Fe), bakır (Cu), çinko (Zn) ve mangan (Mn) analizleri yapılmıştır. Toprakların 0-30 ve 30-60 cm toprak derinliğinde ortalama pH, kireç, OM, TK, DSN, YS, Hİ ve SY sırası ile; 8.01-8.05, %24.6-24.5, % 3.39-3.21, %34.9-34.6, %22.6-22.1, % 12.2-12.5, 0.67-0.73 cm/saat, %47.5-47.3 olarak tespit edilmiştir. Toprakların (0-30 ve 30-60 cm) ortalama makro (Ca, Mg, K₂O ve P₂O₅) ve mikro (Fe, Zn, Cu, Mn) element içerikleri genel olarak yeterli ya da fazla sınıfta yer almıştır. Toprakların üst ve alt derinlikleri için yapılan bağımlı t testine göre, toprak özelliklerinden pH, K₂O, Cu ve Mn değişkenleri istatistiksel olarak (p<0.05) birbirinden farklı bulunmuştur. Sonuç olarak bölge toprakları genel olarak değerlendirildiğinde, kiraz beslenmesini, verim ve kaliteyi olumsuz yönde etkileyen faktörlerin yüksek pH, kireç ve kil içeriği olduğu söylenebilir. Bu nedenle, yüksek kil, pH ve kirecin olduğu alanlarda organik madde (hayvan gübresi, kompost, leonardit), humik asit ve kükürt gibi materyallerin uygulanması önerilebilir.

Assessment of Some Physico-Chemical Properties of Cherry Orchard Soils of Andırın-Çiğsar Region

Abstract

The sustainability of agricultural production is achievable through balanced plant nutrition. Understanding soil properties is essential for plants to be adequately nourished. In this context, soil samples were collected from 24 different points in the orchards of cherry in Çiğsar region of Kahramanmaraş, Andırın district, at depths of 0-30 cm and 30-60 cm using random sampling. Physical (texture, hydraulic conductivity (HI), field capacity (TK), wilting point (DSN), saturation percentage (S), and available water amount (YS)) and chemical (pH, lime, salt, organic matter (OM), calcium (Ca), magnesium (Mg), phosphorus (P₂O₅), potassium (K₂O), iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn)) analyses were conducted on the soil samples. Average pH, lime, OM, TK, DSN, YS, HI and SY of the soils at 0-30 and 30-60 cm soil depth, respectively; It was determined as 8.01-8.05, 24.6-24.5 %, 3.39-3.21 %, 34.9-34.6 %, 22.6-22.1 %, 12.2-12.5 %, 0.67-0.73 cm/hour, 47.5-47.3 %. The average macro (Ca, Mg, K₂O and P₂O₅) and micro (Fe, Zn, Cu, Mn) element contents of the soils (0-30 and 30-60 cm) were generally in the sufficient or excess class. According to the dependent t test for the upper and lower depths of the soil, pH, K₂O, Cu and Mn variables were found to be statistically different from each other (p<0.05). As a result, when the soils of the region are evaluated in general, it can be said that the factors that negatively affect cherry nutrition, yield and quality are high pH, lime and clay content. Therefore, in areas with high clay, pH and lime, it may be recommended to apply materials such as organic matter (animal manure, compost, leonardite), humic acid and sulfur.

Araştırma Makalesi

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1.Giriş

Tarımsal üretimde verimin artırılması, kaliteli ve standart ürünlerin alınabilmesi ve sürdürülebilir kılınması bitkilerin dengeli beslemesi ile mümkündür (Çokkızgın ve ark., 2022; Karadağ ve ark., 2022). Bitkilerin dengeli beslenebilmesi için toprak özelliklerinin ve toprakta mevcut olan bitki besin madde miktarının bilinmesi gereklidir. Toprak analiz sonuçlarını dikkate almadan tarım alanlarına yapılan kontrolsüz uygulamalar, toprakların fiziksel ve kimyasal özelliklerini olumsuz yönde etkilemektedir.

Toprak yapısına bağlı olarak bitkide beslenme ve besin elementi eksiklikleri olabileceğinden kloroz, fotosentez oranında düşüş, meyve kalitesinde ve veriminde düşüş gibi çeşitli problemler ortaya çıkmaktadır. Bununla birlikte aşırı besin maddesi kullanımı toprak ve su kalitesi üzerinde de olumsuz etki oluşturur. Bu nedenle, toprak analizleri sonuçlarına göre bitki besin elementlerinin uygulanması ve tarım topraklarına verilecek gübre çeşit ve miktarlarının buna göre yapılması tarımsal ve ekolojik açıdan önemlidir (Parlak, 2008). Toprakların olumlu ve olumsuz fiziksel ve kimyasal özellikleri, bitki besin elementlerinin azlığı veya çokluğu tarım ürünlerinin verim ve kalitesini etkileyen önemli etkenlerdendir (Boydak, 2010). Meyve ağaçlarında verim ve kaliteyi etkileyen etmenler içerisinde beslenme ile ilgili sorunlar önemli bir yer tutar. Bu sorunların ortaya çıkarılmasında ve giderilmesinde bitki ve toprak analizleri sonuçlarına göre bitki besleme uygulamalarının yapılması önem arz etmektedir (Uysal ve Katkat, 2005).

Türkiye farklı coğrafik ve ekolojik özelliklere sahip olması nedeniyle, meyve üretiminde dünyada önemli potansiyele sahiptir. Dünyada söz sahibi olduğumuz meyvelerden birisi de kirazdır. Türkiye sahip olduğu zengin ekolojik koşullar nedeni ile erken, orta ve geç dönemde kiraz yetiştirebilen nadir ülkelerden birisidir (Özbiçerler, 2006). Türkiye'nin yıllık kiraz üretimi 662.224 ton olup bunun yaklaşık 89.000 tonu ihraç edilmektedir (Tüik, 2020). Kahramanmaraş ili

kiraz üretimi yıllık 7.000-8.000 ton civarında olup, genellikle temmuz ve ağustos ayında (10 Temmuz-10 Ağustos) hasat edilmektedir. Kahramanmaraş'ın en önemli kiraz üretim alanı Andırın ilçesi Çokak ve Çiğşar bölgesidir.

Ülkemizde kiraz hasadı genel olarak haziran ayında yapılmakta olup arz yüksekliği nedeniyle haziran ayında kiraz fiyatları düşmektedir. Ekonomik bakımdan en karlı kiraz üretimi erken ve geç hasat edilen kirazdır (Özyalın, 2023). Andırın Çiğşar bölgesinde kiraz hasadı 10-15 Temmuz'da başlamakta olup Ağustos ayının ilk haftasına kadar sürmektedir. Andırın Çiğşar bölgesi, ülkemizde geç hasat edilen kiraz bölgelerinden birisidir. Bu nedenle üretilen kirazlar genellikle ihraç edilmektedir. Son yıllarda kiraz ihraç eden firmaların, ihraç için daha iri taneli, standart ve kaliteli kiraz talebi etmektedir. Teknik olarak bölgede kalibresi yüksek, daha iri taneli, kaliteli ve standart kiraz üretiminin, toprak ve bitki analizlerine dayalı bitki besleme ve gübreleme programları ile başarılabileceği düşünülmektedir.

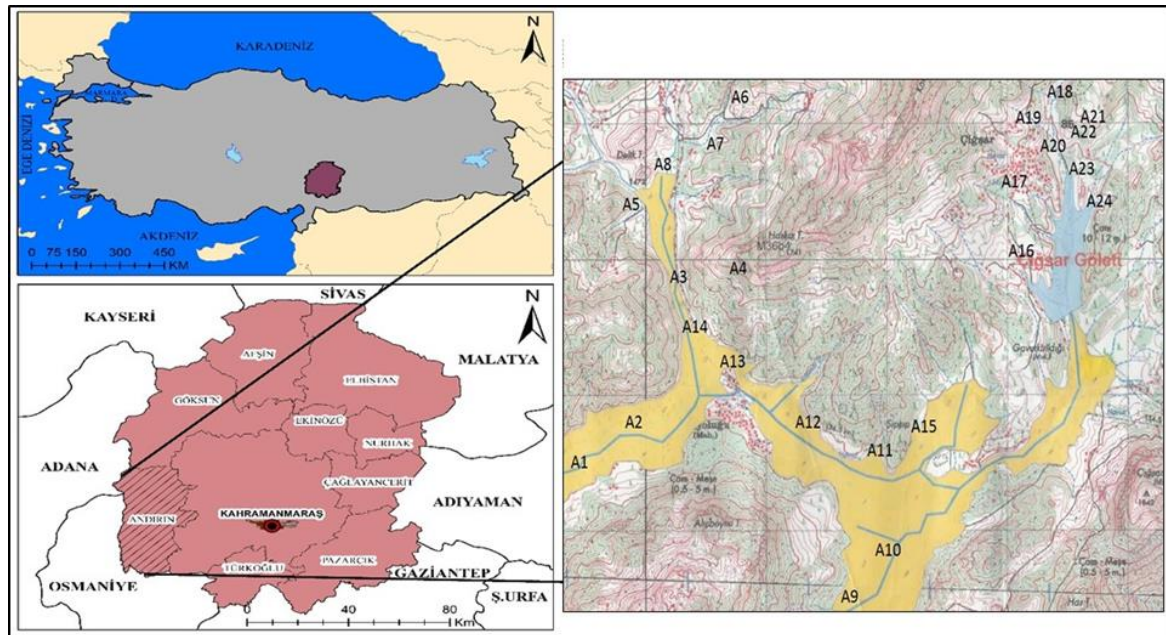
Bu çalışmanın amacı; Kahramanmaraş-Andırın ilçesi Çiğşar Kiraz Bölgesi topraklarının bazı fiziksel ve kimyasal özelliklerini belirlemek ve elde edilen verileri yorumlayarak kaliteli kiraz üretimi için toprak ve bitki besleme konusunda öneriler sunmaktır.

2.Materyal ve Yöntem

Kahramanmaraş'ın Andırın ilçesine bağlı Çiğşar köyü ve çevresinde yer alan kiraz bahçeleri çalışma alanı olarak belirlendi. Kiraz bahçelerinden üst (0-30 cm) ve alt (30-60 cm) toprak derinliklerden toplam 48 adet toprak örneği tesadüfî örnekleme yöntemine göre alındı. Alınan toprak örneklerinin koordinatları Tablo 1'de ve haritada gösterimi ise Şekil 1'de verilmiştir. Araştırma alanının jeolojisine bakıldığında genel olarak çakıl taşı, kum taşı, kil taşından oluşan gölsel-akarsu çökelleri oluşturmaktadır. Ayrıca, araştırma alanının batı yönünde gnays, amfibolit şist ve kalkerli şist formasyonlarına ve bunlarla birlikte peridotit, diabaz, spilit, serpantin, kireçtaşı, kumtaşı ve şeyl'den oluşan blok ve dilimlere rastlanmaktadır (MTA, 2008).

Tablo 1. Alınan toprak örneklerinin koordinat noktaları (Datum: WGS-84, Türü: UTM)

Örnek No	X	Y	Örnek No	X	Y
1	521571,012	4178371,793	13	524821,058	4181328,413
2	523026,420	4179966,219	14	524491,147	4182801,172
3	524417,212	4182976,693	15	526719,927	4180723,885
4	525230,880	4182578,334	16	527489,796	4181814,770
5	523949,518	4183077,064	17	527203,968	4182396,539
6	536732,572	4183077,064	18	527574,492	4183566,290
7	524763,876	4184880,038	19	527399,358	4183251,222
8	524645,580	4183532,341	20	527738,097	4182940,956
9	526028,298	4179599,407	21	528708,177	4183354,341
10	526144,860	4179898,840	22	528547,358	4183156,459
11	526316,908	4180451,271	23	528418,566	4182184,816
12	525385,250	4180926,238	24	528298,866	4181407,447



Şekil 1. Meyve bahçelerinden alınan toprak örneklerinin topografik haritada gösterimi

2.1. Yöntem

2.1.1. Fiziksel analizler

Toprakların tekstür analizi hidrometre yöntemi ile yapılmıştır (Bouyoucos, 1951). Darcy yasası uyarınca laboratuvar koşullarında doymuş hale getirilen topraklarda hidrolik iletkenlik ölçülmüştür (Özdemir, 1998). Toprakların tarla kapasitesi ve daimi solma noktası Klute (1986) yöntemi esas alınarak belirlenmiştir. Toprakların saturasyon (doymunluk) yüzdesi ise Demiralay (1993) tarafından önerilen yöntemle göre tespit edilmiştir.

2.1.2. Kimyasal analizler

Toprakların pH ve yüzde tuz içeriği Richards (1954) metodu esas alınarak

belirlenmiştir. Toprakların organik madde içeriği Walkley-Black yöntemi ile (Nelson ve Sommers, 1996), toplam kireç içeriği ise Scheibler kalsimetre cihazı kullanarak belirlenmiştir (Allison ve Moodie, 1965). Toprakların ekstrakte edilebilir K, Mg ve Ca içeriği amonyum asetat yöntemine göre (Helmke ve Sparks, 1996), alınabilir fosfor içeriği ise Olsen ve arkadaşları (1954) tarafından geliştirilen yöntemlere göre tespit edilmiştir. Toprakların mikro besin elementleri (Fe, Zn, Cu ve Mn) DTPA yöntemi esas alınarak belirlenmiştir (Lindsay ve Norvell, 1978).

2.1.3. İstatistiksel değerlendirme

Elde edilen verilerin korelasyon analizi ve tanımlayıcı istatistiği yapılmıştır. Ayrıca

toprakların alt ve üst derinliğini kıyaslamak amacı ile bağımlı t testi yapılmıştır.

3.Bulgular ve Tartışma

Meyve bahçelerinden alınan toprakların tanımlayıcı istatistiği Tablo 2’de verilmiştir.

Buna göre toprakların ortalama bünyesi killi tın sınıfında yer almıştır. Ancak, alt ve üst katmanlarda 1, 2, 4, 5, 6, 12, 13, 15, 16, 17 ve 21 nolu alanlar killi tekstüre sahiptir. Toprak özellikleri arasındaki ilişkiler Tablo 3’de verilmiştir.

Tablo 2. Üst ve alt derinlik toprakların tanımlayıcı istatistik sonucu

	0-30 cm derinlik			30-60 cm derinlik		
	En Düşük	En Yüksek	Ortalama	En düşük	En Yüksek	Ortalama
pH	7.30	8.20	8.01	7.40	8.30	8.05
Kil (%)	16.00	55.00	36.00	10.00	50.00	34.88
Silt (%)	13.00	46.00	23.96	12.00	29.00	23.33
Kum (%)	8.00	71.00	40.04	27.00	78.00	41.79
Tuz (%)	0.01	0.07	0.04	0.01	0.06	0.04
Kireç (%)	1.00	70.00	24.63	2.00	55.00	24.50
OM (%)	0.8	8.00	3.39	1.1	5.8	3.21
TK (%)	20.40	42.80	34.91	16.60	41.80	34.65
DSN (%)	11.90	32.60	22.65	7.90	30.00	22.09
YS (%)	8.50	15.80	12.26	8.70	18.00	12.56
SY (%)	42.60	56.30	47.53	40.80	52.10	47.36
Hİ (cm saat ⁻¹)	0.085	3.35	0.675	0.134	3.614	0.732
K ₂ O (kg da ⁻¹)	30.00	668.00	122.88	25.00	537.00	74.38
Ca (kg da ⁻¹)	940.00	2269.00	1556.17	999.00	2886.00	1604.33
Mg (kg da ⁻¹)	58.00	396.00	191.17	72.00	514.00	240.13
P ₂ O ₅ (kg da ⁻¹)	2.00	15.80	6.90	1.50	15.50	6.52
Fe (ppm)	10.10	18.70	14.10	7.80	19.20	13.65
Cu (ppm)	2.00	7.90	4.65	0.80	8.30	4.00
Zn (ppm)	0.70	2.70	1.40	0.20	6.10	1.16
Mn (ppm)	10.60	47.90	24.13	10.60	37.60	20.34

*OM: Organik madde, TK: Tarla kapasitesi, DSN: Daimi solma noktası, YS: Yarıyışlı su, SY: saturasyon yüzdesi, Hİ: Hidrolik iletkenlik, K₂O: Potasyum, Ca: Kalsiyum, Mg: Magnezyum, P₂O₅: Fosfor, Cu: Bakır, Zn: Çinko, Mn: Mangan

Tablo 3. Üst ve alt derinlik toprakların korelasyon analiz sonucu

	pH	Kil	Silt	Kum	Tuz	Kireç	OM	TK	DSN	YS	SY	Hİ	K ₂ O	Ca	Mg	P ₂ O ₅	Fe	Cu	Zn	Mn		
30-60 cm derinlik toprakları	pH	1	0,375	0,205	-,408*	,563**	,487*	0,067	0,396	0,355	0,240	0,213	-,0297	0,184	0,092	-,0225	-,0394	-,0249	0,341	-,541**	-,0402	pH
	Kil	0,257	1	0,062	-,791**	,683**	0,286	0,358	,964**	,992**	0,103	,577**	-,757**	0,147	0,401	-,477*	-,0143	0,284	0,394	-,0252	-,0022	Kil
	Silt	-,0190	0,218	1	-,659**	0,219	0,105	-,0008	0,200	0,016	,741**	0,359	-,0110	0,178	0,127	-,0145	-,0165	-,0108	0,057	0,050	0,187	Silt
	Kum	-,0166	-,950**	-,510*	1	-,648**	-,0280	-,0264	-,849**	-,757**	-,655**	,637**	-,0220	-,0379	,448*	0,209	-,0147	-,0332	0,159	-,0098		Kum
	Tuz	,552**	,570**	0,000	-,502*	1	0,402	0,091	,660**	,651**	0,181	0,331	-,486*	0,200	,450*	-,0394	-,0230	-,0045	,445*	-,0166	-,0254	Tuz
	Kireç	,545**	0,351	0,324	-,412*	0,273	1	0,180	0,354	0,303	0,269	0,210	-,0308	0,170	-,0008	-,0039	-,0309	-,0149	0,383	-,0255	-,0171	Kireç
	OM	0,190	0,381	-,0297	-,0241	,418*	-,0054	1	0,392	,442*	-,0105	,843**	0,029	,471*	-,0044	0,046	0,054	0,377	0,029	-,0169	0,009	OM
	TK	0,337	,958**	0,123	-,883**	,605**	0,311	,456*	1	,969**	0,337	,635**	-,820**	0,283	0,379	-,497*	-,0147	0,203	,440*	-,0170	0,115	TK
	DSN	0,271	,993**	0,197	-,938**	,603**	0,336	,470*	,969**	1	0,093	,626**	-,742**	0,205	0,382	-,461*	-,0122	0,292	0,395	-,0237	0,008	DSN
	YS	0,261	-,0153	-,0302	0,231	0,001	-,0104	-,0059	0,112	-,0137	1	0,171	-,476*	0,361	0,071	-,0247	-,0129	-,0295	0,265	0,219	,430*	YS
	SY	0,371	,619**	-,0060	-,526**	,693**	0,170	,821**	,733**	,693**	0,153	1	-,0154	,434*	0,180	-,0219	0,000	0,317	0,181	-,0181	0,016	SY
	Hİ	-,0214	-,884**	-,0273	,866**	-,0335	-,0331	-,0182	-,896**	-,869**	-,0096	-,406*	1	-,0097	-,0347	,441*	0,240	-,0010	-,0397	-,0070	-,0260	Hİ
	K ₂ O	0,041	0,146	-,0201	-,0065	0,096	-,0068	,456*	0,176	0,179	-,0013	0,267	-,0114	1	-,0147	-,0228	0,308	-,0090	0,149	-,0123	0,303	K ₂ O
	Ca	-,0307	0,055	-,0102	-,0016	-,0159	-,0173	-,0026	0,163	0,060	,410*	0,116	-,0166	-,0355	1	-,0061	-,0173	-,0240	-,0093	0,390	0,042	Ca
	Mg	-,0053	-,540**	-,0114	,512*	-,0390	-,0338	-,0264	-,493*	-,542**	0,204	-,425*	0,337	-,0216	-,0010	1	0,031	-,0226	-,492*	0,157	-,0048	Mg
	P ₂ O ₅	-,0361	-,0063	0,202	-,0008	0,022	-,0118	-,0113	-,0203	-,0087	-,467*	-,0213	0,206	0,359	-,0345	-,0278	1	0,208	0,032	0,080	0,071	P ₂ O ₅
	Fe	0,033	0,200	-,0090	-,0147	0,067	0,235	,423*	0,142	0,244	-,409*	0,281	-,0065	,521**	-,0381	-,0368	0,224	1	0,112	-,0136	-,0099	Fe
	Cu	-,0078	,452*	0,118	-,435*	0,247	0,266	0,362	,443*	,469*	-,0110	0,366	-,414*	,516**	-,0105	-,520**	0,325	,440*	1	-,0004	-,0096	Cu
	Zn	-,0022	0,130	-,0168	-,0061	0,063	-,0010	,459*	0,177	0,167	0,037	0,286	-,0114	,910**	-,0149	-,0195	0,239	,409*	,527**	1	0,394	Zn
	Mn	-,0269	0,129	0,070	-,0136	-,0047	-,0193	0,361	0,188	0,172	0,064	0,344	-,0102	0,393	0,153	-,0261	0,000	0,216	0,228	,552**	1	Mn
0-30 cm derinlik toprakları	pH	1	0,375	0,205	-,408*	,563**	,487*	0,067	0,396	0,355	0,240	0,213	-,0297	0,184	0,092	-,0225	-,0394	-,0249	0,341	-,541**	-,0402	pH
	Kil	0,257	1	0,062	-,791**	,683**	0,286	0,358	,964**	,992**	0,103	,577**	-,757**	0,147	0,401	-,477*	-,0143	0,284	0,394	-,0252	-,0022	Kil
	Silt	-,0190	0,218	1	-,659**	0,219	0,105	-,0008	0,200	0,016	,741**	0,359	-,0110	0,178	0,127	-,0145	-,0165	-,0108	0,057	0,050	0,187	Silt
	Kum	-,0166	-,950**	-,510*	1	-,648**	-,0280	-,0264	-,849**	-,757**	-,655**	,637**	-,0220	-,0379	,448*	0,209	-,0147	-,0332	0,159	-,0098		Kum
	Tuz	,552**	,570**	0,000	-,502*	1	0,402	0,091	,660**	,651**	0,181	0,331	-,486*	0,200	,450*	-,0394	-,0230	-,0045	,445*	-,0166	-,0254	Tuz
	Kireç	,545**	0,351	0,324	-,412*	0,273	1	0,180	0,354	0,303	0,269	0,210	-,0308	0,170	-,0008	-,0039	-,0309	-,0149	0,383	-,0255	-,0171	Kireç
	OM	0,190	0,381	-,0297	-,0241	,418*	-,0054	1	0,392	,442*	-,0105	,843**	0,029	,471*	-,0044	0,046	0,054	0,377	0,029	-,0169	0,009	OM
	TK	0,337	,958**	0,123	-,883**	,605**	0,311	,456*	1	,969**	0,337	,635**	-,820**	0,283	0,379	-,497*	-,0147	0,203	,440*	-,0170	0,115	TK
	DSN	0,271	,993**	0,197	-,938**	,603**	0,336	,470*	,969**	1	0,093	,626**	-,742**	0,205	0,382	-,461*	-,0122	0,292	0,395	-,0237	0,008	DSN
	YS	0,261	-,0153	-,0302	0,231	0,001	-,0104	-,0059	0,112	-,0137	1	0,171	-,476*	0,361	0,071	-,0247	-,0129	-,0295	0,265	0,219	,430*	YS
	SY	0,371	,619**	-,0060	-,526**	,693**	0,170	,821**	,733**	,693**	0,153	1	-,0154	,434*	0,180	-,0219	0,000	0,317	0,181	-,0181	0,016	SY
	Hİ	-,0214	-,884**	-,0273	,866**	-,0335	-,0331	-,0182	-,896**	-,869**	-,0096	-,406*	1	-,0097	-,0347	,441*	0,240	-,0010	-,0397	-,0070	-,0260	Hİ
	K ₂ O	0,041	0,146	-,0201	-,0065	0,096	-,0068	,456*	0,176	0,179	-,0013	0,267	-,0114	1	-,0147	-,0228	0,308	-,0090	0,149	-,0123	0,303	K ₂ O
	Ca	-,0307	0,055	-,0102	-,0016	-,0159	-,0173	-,0026	0,163	0,060	,410*	0,116	-,0166	-,0355	1	-,0061	-,0173	-,0240	-,0093	0,390	0,042	Ca
	Mg	-,0053	-,540**	-,0114	,512*	-,0390	-,0338	-,0264	-,493*	-,542**	0,204	-,425*	0,337	-,0216	-,0010	1	0,031	-,0226	-,492*	0,157	-,0048	Mg
	P ₂ O ₅	-,0361	-,0063	0,202	-,0008	0,022	-,0118	-,0113	-,0203	-,0087	-,467*	-,0213	0,206	0,359	-,0345	-,0278	1	0,208	0,032	0,080	0,071	P ₂ O ₅
	Fe	0,033	0,200	-,0090	-,0147	0,067	0,235	,423*	0,142	0,244	-,409*	0,281	-,0065	,521**	-,0381	-,0368	0,224	1	0,112	-,0136	-,0099	Fe
	Cu	-,0078	,452*	0,118	-,435*	0,247	0,266	0,362	,443*	,469*	-,0110	0,366	-,414*	,516**	-,0105	-,520**	0,325	,440*	1	-,0004	-,0096	Cu
	Zn	-,0022	0,130	-,0168	-,0061	0,063	-,0010	,459*	0,177	0,167	0,037	0,286	-,0114	,910**	-,0149	-,0195	0,239	,409*	,527**	1	0,394	Zn
	Mn	-,0269	0,129	0,070	-,0136	-,0047	-,0193	0,361	0,188	0,172	0,064	0,344	-,0102	0,393	0,153	-,0261	0,000	0,216	0,228	,552**	1	Mn

*OM: Organik madde, TK: Tarla kapasitesi, DSN: Daimi solma noktası, YS: Yarıyışlı su, S: saturasyon yüzdesi, Hİ: Hidrolik iletkenlik, K₂O: Potasyum, Ca: Kalsiyum, Mg: Magnezyum, P₂O₅: Fosfor, Cu: Bakır, Zn: Çinko, Mn: Mangan

Toprakların kil içeriği ile TK ve DSN arasında önemli pozitif ($p<0.01$) ilişki elde edilirken, kil içeriği ile Hİ arasında ise önemli negatif ilişki ($p<0.05$) elde edilmiştir. Toprakların kil miktarı arttıkça toprak nem sabitelerinin (TK, DSN ve SY) arttığı ve Hİ ise azaldığı birçok çalışmada belirtilmiştir (Gülser ve ark., 2007; Özdemir ve ark., 2018; Yakupoğlu ve ark., 2013). Tezcan (2006), Kemalpaşa kiraz bölgesinde yapılan bir çalışmada, kiraz için en iyi yetiştirme ortamının toprakları derin, iyi havalandan, drenaj sorunu olmayan, tınlı topraklar olduğunu bildirmiştir. Çiğşar kiraz bahçelerinde, kil bünyeli toprakların olduğu alanlarda iyi yanmış hayvan gübresi, kompost, leonardit ve gıda gibi materyallerin uygulanması topraklarda uygun hava su dengesini sağlanması, kil kaynaklı sorunların giderilmesi ve toprakların fiziksel özelliklerinin iyileşmesine katkı sağlayabilir.

Toprakların % SY ile Kil ve OM arasında önemli pozitif ($p<0.101$) ilişki elde edilmiştir. Bu durum toprakların kil içeriği ile % OM içeriğinin toprakların su tutma kapasitesini artırdığı şeklinde yorumlanabilir (Kara ve ark., 2022; Kara ve Yakupoğlu, 2023; Saltalı ve ark., 2023).

Toprakların üst katmanında (0-30 cm) organik madde içeriği ortalama % 3.39 bulunmuştur. Ortalama değerlere bakıldığında alt ve üst toprakların organik madde içeriği iyi sınıfında yer almaktadır (Güçdemir, 2006). Ancak 10, 13, 17, 18 ve 22 nolu alanlarda 0-30 cm'de OM içeriği % 2'nin altındadır. Toprakların üst ve alt derinliğinde % OM ile K_2O arasında önemli ($p<0.05$) pozitif ilişki elde edilmiştir. Benzer pozitif ilişkiler 30-60 cm OM ile alınabilir Fe ve Zn arasında da elde edilmiştir (Tablo 3). Bu durum OM'nin topraklarda bitki besin maddesi üzerine olumlu etkisini yansıtmaktadır. Bu nedenle kiraz yetiştiriciliğinde ve özellikle OM içeriği % 2'nin altında olan alanlarda mutlaka organik gübre uygulamaları yapılmalıdır.

Toprakların üst ve alt katmanda pH değeri ortalama 8.01 ve 8.05 bulunmuştur. Sağlam (2008) tarafından önerilen sınıflandırma sistemine göre toprakların ortalama pH'sı orta alkalin (7.9-8.4) sınıfında yer almaktadır.

Kiraz üretimi için ideal toprak pH'sı 6.5 ile 7.0 arasındadır (Eryüce, 2010). Toprakların her iki derinliğinde pH değeri ile % kireç arasında önemli pozitif ilişki elde edilirken, alınabilir Zn arasında 0-30 cm toprak derinliğinde önemli ($p<0.05$) negatif ilişki elde edilmiştir. Bu durum topraklarda pH yükseldikçe alınabilir Zn içeriğini olumsuz etkilediğini göstermektedir. Benzer bulgular farklı araştırmalarda da vurgulanmıştır (Lindsay ve Norvell, 1978; Velmurugan ve Swarnam, 2017).

Araştırma alan toprakların 0-30 ve 30-60 cm derinliğinde toplam kireç içeriği çok değişkenlik göstermekte olup ortalama 24.6 ve 24.5 bulunmuştur (Tablo 2). Toprakların ortalama kireç içeriği değerlendirildiğinde 3, 5, 7, 9, 13, 17, 18, 19, 20, 23 nolu alanlar hariç orta ve fazla kireçli sınıfında yer almaktadır (Eyüpoğlu, 1999). Araştırma bölgesi topraklarının kireç içeriğinin ana materyal kaynaklı olduğu düşünülmektedir. Toprakların yüksek pH ve fazla kireç içermesi kiraz yetiştiriciliği, beslenmesi ve kalitesi açısından sorun yaratabilir. Yüksek pH ve kireç içeriği yüksek olan toprakların kiraz yetiştiriciliğine uygun olmadığını, yüksek kireç nedeniyle, kirazın makro ve mikro elementler ile beslenmesi bakımından sorunlar yaşadığı rapor edilmiştir (Öz, 1988). Yapılan diğer çalışmalarda topraklardaki yüksek kireç içeriğinin bitkilerin çinko (Zn) ve fosfor (P) alımını olumsuz yönde etkilediği rapor edilmiştir (Kacar ve ark., 1998; Mengel ve Kirkby, 1987; Udo ve ark., 1970). Bölgede fazla kireçli alanlarda, kirecin olumsuz etkilerini azaltabilmek için elementel kükürt uygulaması önerilebilir. Ancak, kireç içeriği % 1-5 arasında olan toprakların oranı %31'dir. Bu tür alanlarda, diğer organik materyallere göre daha ucuz olan ve % 40-50 OM ve %30-40 kireç içeren gıda materyali de kullanılabilir (Saltalı ve Yıldırım, 2016).

Toprakların üst katmanda tarla kapasitesi (TK), daimi solma noktası (DSN), saturasyon yüzdesi (SY) ve yarayılı su içeriği (YS) sırası ile ortalama % 34.91, % 22.65, % 47.53 ve % 12.26 bulunmuştur. Alt derinlikte ise ortalama TK (% 34.65), DSN (% 22.09), SY (% 47.53)

ve YS (% 12.56) bulunmuş olup üst toprak ile benzer sonuçlar vermiştir (Tablo 2). Her iki toprak derinliğinde kil ile TK ve DSN arasında önemli ($p < 0.01$) pozitif ilişki elde edilmiştir (Tablo 3). Bu durum toprakların kil içeriğinin TK ve DSN artırdığı şeklinde değerlendirilebilir (Şenol ve ark., 2018; Tunçay ve ark., 2018).

Üst toprakların hidrolik iletkenliği $0.085 - 3.35 \text{ cm saat}^{-1}$ arasında değişirken ortalama değeri 0.675 cm/saat bulunmuştur. Toprakların alt derinliklerinde hidrolik iletkenlik ($0.134 - 3.61 \text{ cm saat}^{-1}$) ortalama $0.732 \text{ cm saat}^{-1}$ 'tir (Tablo 2). Araştırma bölgesinde 20 ve 29 nolu örnek alanları hariç Hİ düşük bulunmuştur. Black (1965) tarafından bildirilen sınıflandırmaya göre toprakların ortalama

hidrolik iletkenliği oldukça yavaş ($0.5-2 \text{ cm/saat}$) sınıfında yer almıştır. Toprakların oldukça yavaş geçirgen özelliği toprakların kil içeri ile ilişkilendirilebilir. Hİ değeri düşük olan topraklar genellikle kil tekstüre sahip olan topraklardır. Bölge topraklarında iyi bir hava-su dengesi için, kil içeriği yüksek olan alanlarda iyi yanmış hayvan gübresi ve leonardit gibi organik materyallerin kullanılması önerilebilir.

Toprakların K_2O içeriği alt katmana göre üst katmanda daha fazla iken, Ca ve Mg içeriği ise üst derinliğe göre alt derinlikte daha fazla bulunmuştur (Tablo 2). Ancak üst ve alt derinliğin Ca ve Mg içeriği arasında istatistiksel olarak önemli bir fark yoktur (Tablo 4).

Tablo 4. Üst ve alt derinlik toprakların bağımlı t testi analiz sonucu

Değişkenler	pH	Kil	Silt	Kum	Tuz	Kireç	OM	TK	DSN	YS	S	Hİ	K_2O	Ca	Mg	P_2O_5	Fe	Cu	Zn	Mn
t	-2.846	1.051	0.358	-0.856	.401	.077	.461	.441	.956	-.822	.329	-.502	3.606	-.568	-1.822	1.509	.817	2.164	.993	2.136
df	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
p	0.009	0.304	0.724	0.401	0.692	0.939	0.649	0.663	0.349	0.419	0.745	0.621	0.001	0.575	0.081	0.145	0.422	0.041	0.331	0.044

** t: Test Değeri; df: Serbestlik Derecesi; p: Anlamlılık Derecesi

Toprakların üst katmanında K_2O içeriği daha fazla olup, alt ve üst derinliklerin K_2O içeriği istatistiksel olarak önemli ($P < 0.01$) derecede farklı bulunmuştur. FAO (1990) tarafından önerilen sınıflandırma sistemine göre makro besin elementleri yeterli ya da fazla sınıftadır. Bağcı, (2013) tarafından da vurgulandığı gibi kiraz bahçesi topraklarının ana materyali bazik, ultrabazik, kireçtaşı, dolomit ve marn gibi materyallerden oluşması doğal olarak toprakların alınabilir Ca ve Mg içeriğinin yüksek çıkmasına neden olabilir. Bitki besleme açısından toprakların Ca içeriklerinin fazla olması K, P, Mg ve mikro bitki besin elementlerinin alımını olumsuz yönde etkileyebilir (Kılıç ve ark., 1999). Bu olumsuz etkileri azaltabilmek için toprak kalitesini artırıcı organik madde uygulaması önerilebilir. Topraklarda mikro elementler ve fosfor adsorpsiyonu konusunda yapılan araştırmalarda, topraklarda organik maddenin ayrışması sırasında açığa çıkan humik

maddelerin, toprakta mikro element ve fosforu sorbe edici aktif yüzeyleri (kireç, kil, oksit bileşikleri, Ca vb) maskeleyiği ve topraklarda alınabilir mikro element ve fosfor içeriğini artırdığı rapor edilmiştir (Agbenin ve Igbokwe, 2006; Saltalı ve Nedirli, 2021; Tani ve ark., 2010; Yu ve ark., 2013).

Toprakların ortalama alınabilir Fe, Cu ve Mn içeriği yeterli sınıfta yer almaktadır (FAO, 1990; Lindsay ve Norvell, 1978). Toprakların 0-30 cm derinliğinde alınabilir Zn içeriği 0.7 mg kg^{-1} 'in üzerinde yani yerli sınıftadır. Ancak, toprakların 30-60 cm derinliğinde 5, 7, 13, 20 ve 22 nolu toprakların alındığı bahçelerde alınabilir Zn içeriği yeterlilik sınır değeri olan 0.7 mgkg^{-1} 'in altındadır. Kiraz köklerinin 30 cm den daha derine gittiği düşünüldüğünde, Zn noksanlığı görülen alanlarda Zn içeren gübrelerin beslenme programına alınmasında fayda vardır. Toprakların 0-30 cm derinliğinde alınabilir Cu içeriği üst katmanda daha fazla

bulunmuş olup, farklı toprak derinliklerinin alınabilir Cu içeriği istatistiksel olarak birbirinde farklı ($p<0.05$) bulunmuştur (Tablo 4). Üst katmanda alınabilir Cu içeriğinin yüksek bulunması, çiftçilerin bitkilerde görülen mantari hastalıklara karşı Cu içerikli preparatların uygulanmasına bağlanabilir. Bitki gövde ve dallara uygulama sırasında topraklara Cu içerikli preparatların topraklara kontamine olması, gövde ve dal kabuklara uygulanan Cu içerikli preparatların zamanla topraklara düşmesi ile toprakların alınabilir Cu içeriğinin arttığı düşünülmektedir.

4. Sonuçlar ve Öneriler

Bu çalışmada, Kahramanmaraş-Andırın ilçesi Çiğşar Kiraz bölgesi topraklarının bazı fiziksel ve kimyasal özellikleri belirlenmiştir. Analiz sonuçlarına göre; toprakların hidrolik iletkenliği yüksek kil içeriğine sahip alanlarda düşük bulunmuştur. Toprak yapısı genellikle killi ve killi tınlı bünyeye sahiptir. Toprakların pH değerleri bakımından % 87.5'i orta derecede alkalın özelliktedir. Çalışma alanında toprakların % 16.7'sinde alınabilir K_2O içeriği az'dır. Topraklarda K içeriği düşük alanlarda mutlaka K içeren gübreleri kullanılmalıdır. Araştırma alanı topraklarında alınabilir Fe ve Cu elementlerinde noksanlık sorunu yoktur, ancak toprakların % 19'unda Zn noksanlığı belirlenmiştir. Topraklarda Zn noksanlığı görülen alanlarda Zn içeren gübrele beslenme programına alınmalıdır. Araştırma bölgesinde yüksek pH, kireç ve kil bünyeli topraklarda, makro ve mikro besin elementleri açısından beslenme sorunları ortaya çıkabilir. Çiğşar kiraz bahçelerinde topraklara kireçli ve fazla kireçli sınıfta yer almıştır. Ancak düşük kireç içerikli bahçeler de bulunmaktadır. Bölgede yüksek pH, kireç ve kil içeriğine sahip topraklara asit karakterli kimyasal gübreler (amonyum sülfat), organik materyaller (hayvan gübresi, leonardit vb), ve elementel kükürt uygulaması önerilebilir. Kireç içeriği düşük olan alanlarda ise hem kireç hem de organik madde içeren gıda kullanımı önerilebilir.

Yazarların Katkı Beyanı

Yazarlar makaleye eşit katkıda bulunduklarını, makalenin yayına hazır son halini gördüklerini/okuduklarını ve onayladıklarını beyan ederler.

Çıkar Çatışması Beyanı

Tüm yazarlar, bu çalışma için herhangi bir çıkar çatışması olmadığını beyan etmektedir.

Açıklama

Bu çalışma; KSÜ Fen Bilimleri Enstitüsü, Toprak Bilimi ve Bitki Besleme ABD'da "K.Maraş-Andırın ilçesi Çiğşar kiraz bölgesi topraklarının fiziksel ve kimyasal özelliklerinin değerlendirilmesi" başlıklı tez verilerine ilave analizler yapılarak hazırlanmıştır. Destekleri için KSÜ Ziraat Fakültesine ve Çiğşar Köy Muhtarlığına teşekkür ederiz.

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Isparta İli Geleneksel Soğuk Hava Depolarında Oda Atmosferindeki Fungusların ve Elma Meyve Bulaşıklığının Belirlenmesi

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Özet

Bu çalışmada, Isparta iline bağlı Eğirdir, Gelendost ve Senirkent ilçelerindeki iki farklı soğuk hava deposunun iki farklı oda atmosferindeki fungal mikrofloranın ve elma çürüklük oranlarının belirlenmesi amaçlanmıştır. Bu sebeple, depo oda atmosferindeki funguslar tuzak besiyeri yöntemiyle yakalanmış ve cins düzeyinde koloni sayıları belirlenmiştir. Her ilçedeki depo odalarında, kasalardaki Golden Delicious çeşidi elma meyvelerindeki çürüklük sayıları ve çürüklük oranları (%) belirlenmiştir. Ayrıca, morfolojik özelliklerine göre *Penicillium*, *Botrytis*, *Alternaria*, *Aspergillus*, *Geotrichum* cinslerine ait funguslar ve bazı maya türleri tanımlanmıştır. Elma depo atmosferindeki fungus cinslerinin oranları ilçeler ve depolar arasında değişmiştir. Depo odalarında en yaygın izole edilen fungus *Penicillium* spp. olmuştur. Buna bağlı olarak depoya konan elma meyvelerindeki çürüklük oranları da değişkenlik göstermiştir. Çalışma sonucunda bu değişimin sebeplerinin depoya konan elma çeşidi ve depolarda sezon başı ve sonunda yapılan hijyen ve sanitasyon önlemlerine bağlı olduğu sonucuna varılmıştır.

Araştırma Makalesi

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Anahtar Kelimeler

Penicillium spp.

elma

fungal mikroflora

soğuk hava deposu

Determination of Fungi and Apple Fruit Contamination in the Room Atmosphere in Traditional Cold Storages in Isparta Province

Abstract

In this study, it was aimed to determine the fungal microflora and apple rot rates in two different room atmospheres of two different cold storages in Eğirdir, Gelendost and Senirkent districts of Isparta province. For this reason, fungi in the storage room atmosphere were captured by the trap medium method and colony numbers were determined at the genus level. The number of rots and rot rates (%) in variety of Golden Delicious fruits in crates in the storage rooms were determined for each district. The fungal genera were identified as *Penicillium*, *Botrytis*, *Alternaria*, *Aspergillus*, *Geotrichum* and some types of yeast according to the morphological characteristics. The rates of fungal species in the apple warehouse atmosphere varied between districts and warehouses. The most commonly isolated fungus in the storage rooms was *Penicillium* spp. Accordingly, the rot rates in apple fruits placed in storage also varied. As a result of the study, it was concluded that the reasons for this change vary depending on the apple variety placed in the warehouse and the hygiene and sanitation measures taken at the beginning and end of the season in the warehouses.

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cold storage

1.Giriş

Elma (*Malus domestica* Borkh.) önemli tarımsal ürünlerden birisi olup Rosaceae familyası, Pomoideae alt familyası *Malus* cinsinde yer alan ve farklı ekolojilerde üretimi yapılan yumuşak çekirdekli meyve türüdür. Ülkemizin bir çok bölgesi uygun ekolojik şartlara sahip olması nedeniyle elma yetiştiriciliği önemli bir konumdadır. Ülkemiz Isparta ili başta olmak üzere önemli gen merkezlerinden birisidir (Oğuz ve Karaçayır, 2009). Türkiye dünya elma üretiminde önemli bir yere sahiptir. Türkiye’de toplam 1 688 000 da alanda ve 4 493 000 ton elma üretilmektedir. Isparta ili 2022 yılı verilerine göre 214 803 da alanda 6 069 368 adet meyve veren ağaç ve 1 milyon 230 bin 580 ton elma üretimi ile Türkiye’de ilk sırada yer almaktadır (Tüik, 2022). Elma hasat edildikten sonra yaklaşık 8 ay süreyle depolanabilen bir meyvedir. Sağlıklı bir elma depolaması için vejetasyon süresince uygun yetiştiricilik koşullarında yetiştirilmesi de önemli bir konudur (Aydemir ve Kara, 2023). Bunun yanı sıra depolama süresince geleneksel soğuk hava depolarında muhafaza koşullarının iyi sağlanması gereklidir. Sağlıklı bir depolama süreci için de meyvelerin çeşide bağlı olarak belli hasat olgunluğunda hasat edilmesi gereklidir. Erken hasat edilen meyvede tat ve aromanın gelişmemesi nedeniyle kalite düşük olacağı gibi geç hasatta depolama ömrünün kısılmasına ve meyvelerde hasat sonrası patolojik bozulmaların daha erken dönemde ortaya çıkmasına neden olmaktadır. Bununla birlikte depolamadan önce elma meyvelerinin renk, irilik ve olgunluk düzeylerine göre sınıflandırılarak, zararlanmış ve hastalıklı meyvelerin ayrılması depo ömrünün uzatılmasında önemli bir etken olacaktır. Türkiye’nin soğuk hava depo kapasitesinin yaklaşık % 28.8’i Isparta ilinde bulunmaktadır (Kart Örmeci ve Demircan, 2013). Buna bağlı olarak elma üretiminin artmasından dolayı depolama teknolojisi ve kapasitesi bakımından da önemli bir potansiyele sahiptir. Özellikle ürün kayıplarını en aza indirebilmesi ve yıl boyunca ürün kalitesini koruması bakımından modern depoculuk önem arz etmektedir. Geleneksel depolama, hava bileşimine

müdahale edilmeden ortamın sadece sıcaklık ve oransal neminin kontrol edilebildiği depolama biçimidir. Bu depolar belirli ölçülerde dış koşulların etkilerine açıktır. Ancak, modern yani kontrollü atmosfer depolamasında sıcaklık ve nispi nem yanında ortamdaki oksijen ve karbondioksit gibi gazların oranı kontrol edilmektedir (Kart Örmeci ve Demircan, 2013). Her iki soğuk hava depolama yöntemi kıyaslandığında meyveler açısından kontrollü atmosfer depolamasında daha izole ve daha uzun süre sağlıklı bir depolama süreci oluşmaktadır. Tam aksine geleneksel soğuk hava depolamasında zaman zaman depo kapılarının açılıp kapatılarak yapılan havalandırma sebebiyle depo atmosferi dış koşullara daha açık hale gelmekte ve mikroorganizmalar açısından daha zenginleşmektedir (Saraçoğlu ve ark., 2011). Hasat sonrası taze meyve ve sebzelerde meydana gelen kayıpların oranı, gelişmiş ve gelişmekte olan ülkeler sırasıyla % 25 ve %50’ye kadar çıkabilmektedir (Rosenberger, 1997). Dolayısıyla meyve ve sebzelerde kalite ve depo ömrünün uzatılması önemli bir araştırma konusudur (Düzgün ve Çavuşoğlu, 2024).

Ürünlerin depolanması sürecinde meyve dokularında meydana gelen hassiyet nedeniyle patojenlere karşı direnç azaldığı için çürüklükler ortaya çıkmaktadır. Hasat sonrası kayıplara neden olan patojenler genellikle yara patojenleri olduğundan meyveye bu yolla girebilmektedir. Meyvelere bahçe döneminde bulaşarak depolama sürecinde herhangi bir mekanik yaralanma meydana geldiğinde gelişebilen en önemli fungal etmenler arasında; *Penicillium* spp., *Botrytis* spp., *Alternaria* spp., *Aspergillus* spp., *Geotrichum* spp. ve *Rhizopus* spp. sayılabilir. Zayıflık patojenleri depolama sürecinde kasalarda meyve bozulmalarına sebep olurken temas halinde olan meyvelere de bulaşma potansiyelindedir (Benli, 2000; Faten, 2005; Torres ve ark., 2006; Özgönen Özkaya ve Kılıç, 2013; Grohovac ve ark., 2011; Lore ve ark., 2011; Kalın, 2011; Rivera ve ark., 2012, Yıldız, 2015).

Her ortamda olduğu gibi soğuk hava depo atmosferi pek çok mikroorganizmaya ev sahipliği etmektedir. Mikroorganizmalar çok düşük sıcaklık ve nemde bile gelişme yeteneğine sahiptir. Dolayısıyla depo duvarları, depo atmosferi ve depolanmış meyveler üzerinde latent haldeki fungus sporları sürekli olarak bulunma potansiyelindedir. Geleneksel depolarda zaman zaman yapılan havalandırmaya bağlı olarak dışardan içeriye hava akımları ile fungus sporları gelebilir. Ayrıca meyveler üzerinde gelişen küflenme ile birlikte farklı cins funguslar depo atmosferine yerleşebilirler (Özgönen ve Çulal, 2013). Literatür incelendiğinde daha önce elma soğuk hava depo atmosferindeki fungusların ve etkilerinin belirlenmesine yönelik bir çalışma yapılmadığı belirlenmiştir. Bu çalışmada, Isparta ili geleneksel elma depolarında depo atmosferlerinin patojenik özellikte olabilecek mikroorganizma varlığının ve popülasyonlarının tespit edilmesi ve meyve bulaşıklığının belirlenmesi amaçlanmıştır.

2. Materyal ve Yöntem

2.1. Materyal

Çalışma, depo atmosferindeki mikroorganizma varlığının belirlenmesi amacıyla en yüksek depolama kapasitesinin bulunduğu Eğirdir, Gelendost ve Senirkent İlçelerinde pilot olarak seçilen 2 farklı ticari geleneksel soğuk hava deposunun ikişer odasında yürütülmüştür. Çalışmanın yürütüldüğü odalarda (3 adet) meyve bulaşıklığının belirlenmesi amacıyla kasalarda yer alan Golden Delicious elma çeşidi meyve materyali olarak kullanılmıştır. Laboratuvar çalışmaları ise ISUBU Ziraat Fakültesi Bitki Koruma Bölümü Mikoloji Laboratuvarında yürütülmüştür.

2.2. Depo atmosferinde tuzak besiyeri kullanılarak fungusların saptanması

Depo atmosferindeki fungal mikrofloranın izolasyonu için Patates Dekstroza Agar (PDA) kullanılmıştır. Besiyeri hazırlığı için hazır PDA (Merck, 39 g/L) kullanılmıştır. PDA saf su içerisine karıştırılarak 121°C ve 1 atm basınçta 20 dk. süreyle otoklav edilmiş ve daha

sonra 90 mm çaplı steril plastik Petri kaplarına yeteri miktarda dökülmüştür. Besiyerleri soğuk hava depo atmosferinde kullanılmak üzere polietilen poşetlere 30'lu paketler halinde hazırlanarak etiket yerleştirilmiştir. Her bir örnekleme odasında 30 adet 90 mm çaplı PDA içeren Petri kapları kapağı açık şekilde 5 dakika süreyle bekletilmiş ve daha sonra kapakları kapatılmıştır. Bu Petri kapları laboratuvara getirildikten sonra 25±2°C'de 3-7 gün süreyle inkübe edilmiştir. Bu sürenin sonunda besiyerinde gelişen fungus kolonilerinin cins düzeyinde sayımı yapılarak not edilmiştir (İnan ve Erkilic, 2018). Bu işlem yukarıda belirtilen toplam 6 adet depoda bir depolama sezonu boyunca aylık periyodik olarak yürütülmüştür. Çalışma 2020-2021 hasat döneminde Eylül ayından bir sonraki yılın Haziran ayına kadar sürdürülmüştür.

2.3. Depoda meyve sayımı

Atmosfer koşullarından örnekleme yapılan depo odalarında 3'er tekerrür olacak şekilde içerisinde 60'ar adet Golden Delicious çeşidinin yer aldığı elma kasaları yerleştirilmiştir. Elma meyveleri kasalara yerleştirilmeden önce % 1'lik sodyum hipoklorit solüsyonu ile yüzey dezenfeksiyonu yapılmış ve 1 kez saf su ile yıkanmıştır. Meyveler kurutma kağıdı ile fazla nemi alındıktan sonra kasalara yerleştirilmiş ve aylık olarak çürük meyve sayımı gerçekleştirilmiştir.

2.4. Değerlendirme

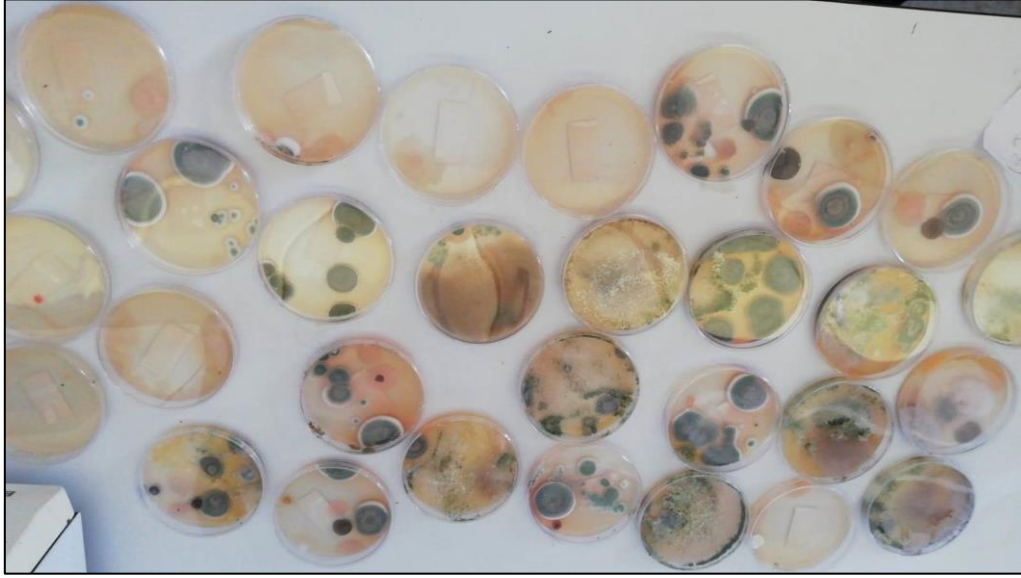
Elde edilen sonuçlar varyans analizine tabi tutulmuştur. Depolara göre fungus sayımları arasındaki farklılıklar LSD testine ($P<0.05$) ve çürük meyve oranları ise Tukey testine ($P<0.05$) tabi tutularak ortalamaların birbirinden farklılıkları ortaya konulmuştur. Depo atmosferinden elde edilen koloni sayımları üzerinden aylık değerlendirmeler yapılmış; fungusların toplam elde edilme miktarları (koloni/Petri), *Penicillium* türlerinin elde edilme miktarları (koloni/Petri), tüm depolardaki fungusların izole edilme oranları (%) ve kasalardaki meyvelerde çürüklük oranları (%) hesaplanmıştır (Karman, 1971).

3.Bulgular ve Tartışma

3.1.Depo atmosferindeki fungus yoğunluğunun belirlenmesi

Bu çalışmada, Eğirdir, Gelendost ve Senirkent ilçelerinde geleneksel depolama

koşullarında tuzak Petri kapları kullanılarak depo atmosferindeki fungal mikroflora belirlenmiştir. Petrilerin inkübasyon süresi sonunda gelişen fungusların cins düzeyinde koloni sayıları belirlenmiştir (Şekil 1).



Şekil 1. Petri kaplarında gelişen fungus kolonileri

Petri kaplarında sayımı yapılan fungus cinsleri sırasıyla *Penicillium* spp., *Botrytis* spp., *Alternaria* spp., *Aspergillus* spp., *Geotrichum* sp., bazı maya türleri, ve diğerleri olarak gruplandırılmıştır. Diğerleri kapsamına minör önemde sayılabilecek *Mucor* spp., *Rhizopus stolonifer* ve bakteri kolonileri dahil edilmiştir (Şekil 2). Fungusların cins

düzeyinde koloni sayımları yapılarak kaydedilmiş; fungusların total sayıları üzerinden varyans analizi yapılarak depo atmosferindeki yoğunlukları ve izole edilme oranları hesaplanmıştır. Ayrıca en yoğun izole edilen *Penicillium* türlerinin depo atmosferinden izole edilme oranları kıyaslanmıştır.



Şekil 2. Petri kaplarında gelişen bazı funguslar (*Penicillium* spp., *Alternaria* spp. ve *Aspergillus* spp.)

Depo atmosferinde Eylül-Haziran ayları arasında periyodik olarak örnek alınmış; depo odalarında bekletilen Petri kaplarında gelişen fungus kolonilerinin toplam miktarları

üzerinden depolar arasındaki toplam fungus kolonileri açısından birbirinden farklılıkları belirlenmiştir (Tablo 1 ve Şekil 3).

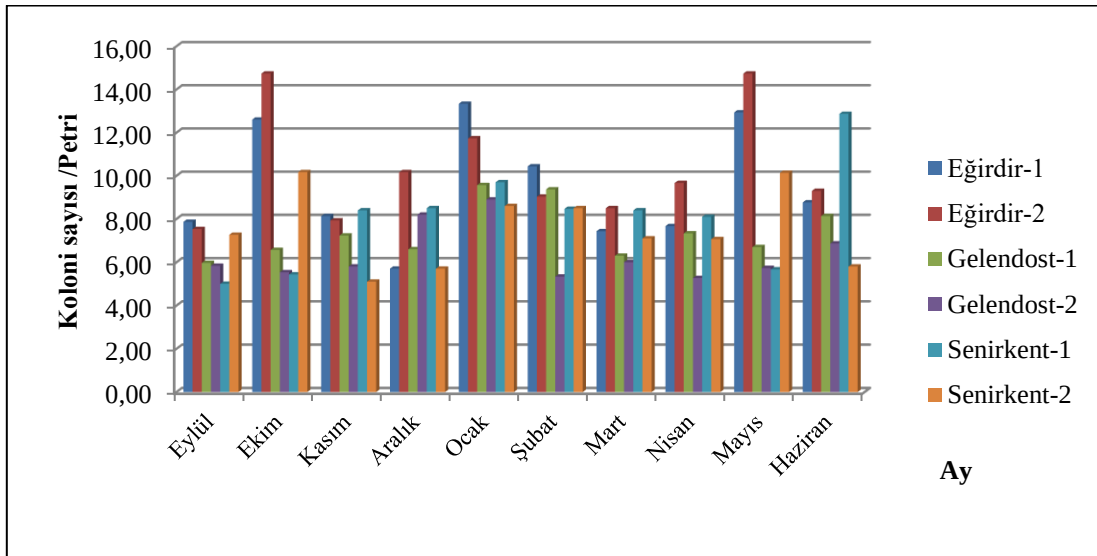
Tablo 1. İlçelerdeki soğuk hava depolarında toplam fungusların elde edilme miktarları (Koloni/Petri)

Aylar	Eğirdir-1	Eğirdir-2	Gelendost-1	Gelendost-2	Senirkent-1	Senirkent-2
Eylül	7.87 c	7.53 e	5.97 c	5.83 bc	5.00 c	7.27 bcd
Ekim	12.60 a	14.73 a	6.57 bc	5.53 bc	5.43 c	10.17 a
Kasım	8.13 c	7.93 de	7.23 bc	5.80 bc	8.40 b	5.10 f
Aralık	5.70 d	10.17 bc	6.60 bc	8.20 a	8.50 b	5.70 ef
Ocak	13.33 a	11.73 b	9.57 a	8.90 a	9.70 b	8.60 b
Şubat	10.43 b	9.03 cde	9.37 a	5.33 c	8.47 b	8.50 bc
Mart	7.43 cd	8.50 cde	6.30 c	6.00 bc	8.40 b	7.10 bcde
Nisan	7.67 cd	9.67 cd	7.33 bc	5.27 c	8.10 b	7.07 cdef
Mayıs	12.93 a	14.73 a	6.70 bc	5.73 bc	5.67 c	10.13 a
Haziran	8.77 bc	9.30 cde	8.13 ab	6.87 b	12.87 a	5.80 def

*Aynı sütun içerisinde farklı harf içeren ortalamalar LSD testine göre ($P<0.05$) birbirinden farklıdır.

Eğirdir, Gelendost ve Senirkent ilçelerindeki depo atmosferinden elde edilen toplam fungus sayıları arasında farklılıklar ve aylara göre dalgalanmalar gözlenmiştir (Tablo 1). Tablo 1 incelendiğinde, tüm depolarda ilk sayımın yapıldığı Eylül ayında toplam koloni sayıları düşük olduğu gözlemlenmiştir. Eğirdir'deki her iki depoda özellikle Ekim,

Ocak ve Mayıs aylarında koloni sayılarında artış görülmüştür. Gelendost'ta inceleme yapılan her iki depoda Petri başına koloni sayıları 10.0 adedin altında seyretmiştir. Benzer şekilde her iki depoda da Petri başına koloni sayıları aylık değişimle birlikte 10.0 ve 10.0 adedin altında seyretmiştir.



Şekil 3. İlçelerdeki soğuk hava depolarında toplam fungusların elde edilme miktarları (Koloni/Petri)

İlçelerdeki tüm depolardan baskın olarak izole edilen *Penicillium* türleri olduğu için soğuk hava depolarından elde edilme

miktarları ayrı olarak analiz edilmiş ve sonuçlar Tablo 2'de verilmiştir.

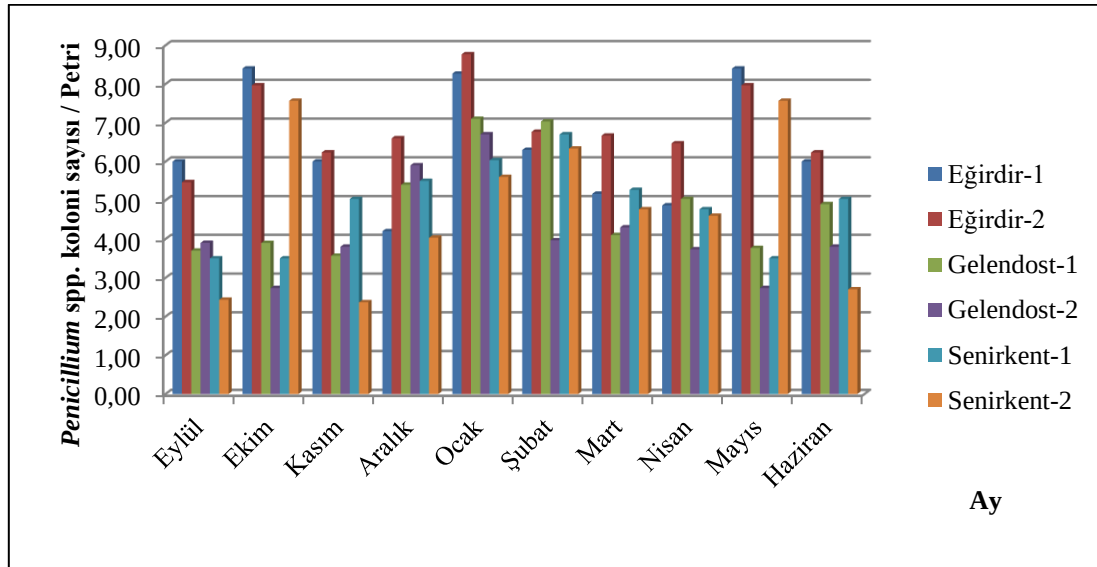
Tablo 2. İlçelerdeki soğuk hava depolarında *Penicillium* türlerinin elde edilme miktarları (Koloni/Petri)

Aylar	Eğirdir-1	Eğirdir-2	Gelendost-1	Gelendost-2	Senirkent-1	Senirkent-2
Eylül	6.00 b	5.47 c	3.70 e	3.90 b	3.50 d	2.43 e
Ekim	8.40 a	7.97 ab	3.90 de	2.73 c	3.50 d	7.57 a
Kasım	6.00 b	6.23 c	3.57 e	3.80 bc	5.03 bc	2.37 e
Aralık	4.20 c	6.60 bc	5.40 b	5.90 a	5.50 abc	4.03 d
Ocak	8.27 a	8.77 a	7.10 a	6.70 a	6.03 ab	5.60 bc
Şubat	6.30 b	6.77 bc	7.03 a	3.97 b	6.70 a	6.33 b
Mart	5.17 bc	6.67 bc	4.10 cde	4.30 b	5.27 bc	4.77 cd
Nisan	4.87 bc	6.47 bc	5.03 bc	3.73 bc	4.77 c	4.60 cd
Mayıs	8.40 a	7.97 ab	3.77 de	2.73 c	3.50 d	7.57 a
Haziran	6.00 b	6.23 c	4.90 bcd	3.80 bc	5.03 bc	2.70 e

*Aynı sütun içerisinde farklı harf içeren ortalamalar LSD testine göre ($P<0.05$) birbirinden farklıdır.

Penicillium türlerinin Petri başına koloni sayıları ilçelerdeki depolar ve aylara göre farklılık göstermiştir (Şekil 4). Eğirdir ilçesindeki depolarda yapılan sayımlar sonucunda Ekim, Ocak ve Mayıs aylarında en yüksek olmuştur. Gelendost'taki her iki depoda *Penicillium*'un koloni sayısı Ocak ayında en yüksek olmuş diğer aylarda daha

düşük oranda seyretmiştir. Senirkent'te ise *Penicillium* koloni sayıları açısından her iki depoda farklılıklar gözlenmiş; birinci depoda Ocak ve Şubat'ta en yüksek değerlere ulaşırken ikinci depoda Ekim, Şubat ve Mayıs aylarında en yüksek seviyeye ulaşmıştır (Tablo 2).

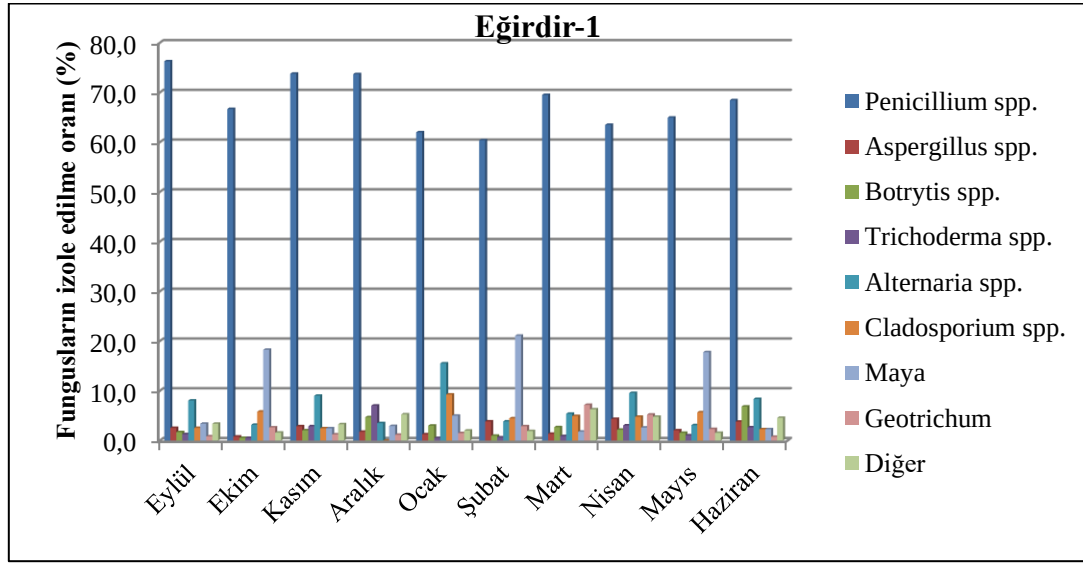
**Şekil 4.** İlçelerdeki soğuk hava depolarında *Penicillium* türlerinin elde edilme miktarları (Koloni/Petri)

Tüm ilçelerde depolardaki fungusların izole edilme oranları (%) hesaplanarak aylara göre oransal olarak dağılım Eğirdir için Tablo 3 ve Tablo 4; Gelendost için Tablo 5 ve Tablo 6; Senirkent için Tablo 7 ve Tablo 8'de verilmiştir. Elde edilen sonuçlar değerlendirildiğinde tüm ilçelere ait depolarda *Penicillium* türlerinin izole edilme oranlarının en yüksek olduğu dikkat çekmiş ve aylara göre dağılım farklı oranlarda görülmüştür. Eğirdir

ilçesindeki birinci soğuk hava deposunda Eylül-Haziran ayları arasında *Penicillium* türlerinin izole edilme oranları %60.4-76.3 arasında değişmiş ve en yüksek oran depolamanın ilk örneklemenin yapıldığı Eylül ayında belirlenmiştir (Tablo 3 ve Şekil 5). *Penicillium* türlerinin ardından *Alternaria*, *Cladosporium* ve maya türlerinin oranı önemli olmuştur.

Tablo 3. Eğirdir-1 soğuk hava deposunda fungusların izole edilme oranları (%)

Fungus	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran
<i>Penicillium</i> spp.	76.3	66.7	73.8	73.7	62.0	60.4	69.5	63.5	64.9	68.4
<i>Aspergillus</i> spp.	2.5	0.8	2.9	1.8	1.3	3.8	1.3	4.3	2.1	3.8
<i>Botrytis</i> spp.	1.7	0.5	2.0	4.7	3.0	1.0	2.7	2.2	1.5	6.8
<i>Trichoderma</i> spp.	1.3	0.5	2.9	7.0	0.5	0.6	0.9	3.0	1.0	2.7
<i>Alternaria</i> spp.	8.1	3.2	9.0	3.5	15.5	3.8	5.4	9.6	3.1	8.4
<i>Cladosporium</i> spp.	2.5	5.8	2.5	0.0	9.3	4.5	4.9	4.8	5.7	2.3
Maya	3.4	18.3	2.5	2.9	5.0	21.1	1.8	2.6	17.8	2.3
<i>Geotrichum</i>	0.8	2.6	1.2	1.2	1.5	2.9	7.2	5.2	2.3	0.8
Diğer	3.4	1.6	3.3	5.3	2.0	1.9	6.3	4.8	1.5	4.6

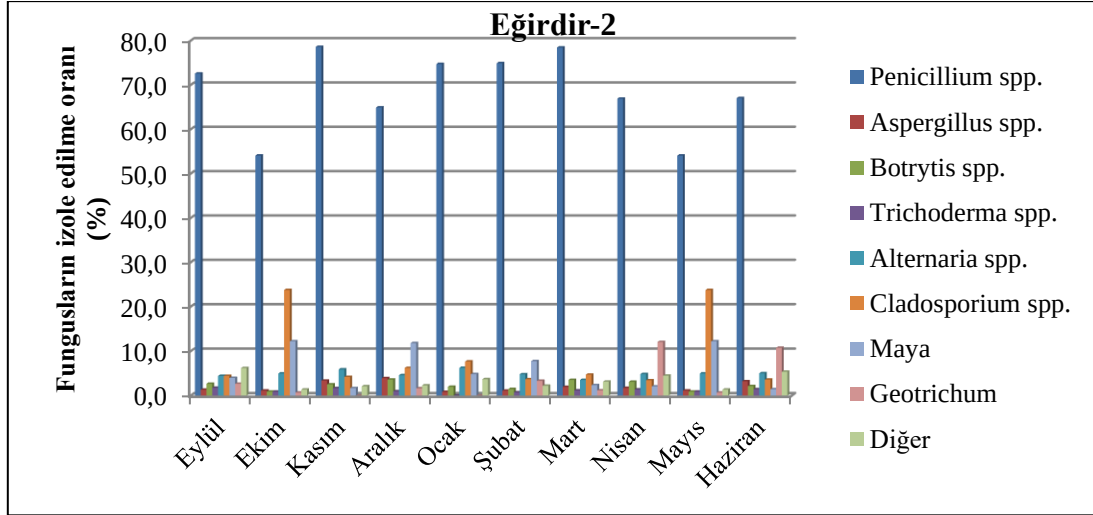
**Şekil 5.** Eğirdir-1 soğuk hava deposunda fungusların izole edilme oranları (%)

Eğirdir ilçesindeki ikinci soğuk hava deposunda Eylül-Haziran ayları arasında *Penicillium* türlerinin izole edilme oranları %54.1-78.6 arasında değişmiş ve en yüksek oran Kasım ayında belirlenmiştir (Tablo 4 ve

Şekil 6). Eğirdir'deki birinci depo sonuçlarına benzer şekilde ikinci depoda da *Alternaria*, *Cladosporium* ve maya türlerinin oranı ikincil önemde olmuştur.

Tablo 4. Eğirdir-2 soğuk hava deposunda fungusların izole edilme oranları (%)

Fungus	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran
<i>Penicillium</i> spp.	72.6	54.1	78.6	64.9	74.7	74.9	78.4	66.9	54.1	67.0
<i>Aspergillus</i> spp.	1.3	1.1	3.4	3.9	0.9	1.1	2.0	1.7	1.1	3.2
<i>Botrytis</i> spp.	2.7	0.9	2.5	3.6	2.0	1.5	3.5	3.1	0.9	2.2
<i>Trichoderma</i> spp.	1.8	0.9	1.7	1.0	0.0	0.7	1.2	1.4	0.9	1.4
<i>Alternaria</i> spp.	4.4	5.0	5.9	4.6	6.3	4.8	3.5	4.8	5.0	5.0
<i>Cladosporium</i> spp.	4.4	23.8	4.2	6.2	7.7	3.7	4.7	3.4	23.8	3.6
Maya	4.0	12.2	1.7	11.8	4.8	7.7	2.4	2.1	12.2	1.4
<i>Geotrichum</i>	2.7	0.7	0.0	1.6	0.0	3.3	1.2	12.1	0.7	10.8
Diğer	6.2	1.4	2.1	2.3	3.7	2.2	3.1	4.5	1.4	5.4



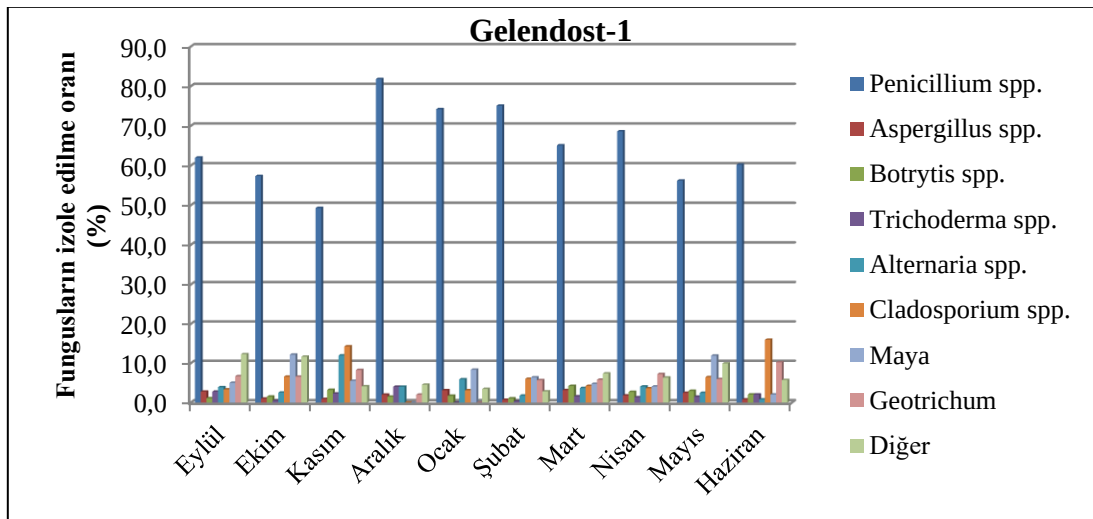
Şekil 6. Eğirdir-2 soğuk hava deposunda fungusların izole edilme oranları (%)

Gelendost ilçesindeki birinci soğuk hava deposunda Eylül-Haziran ayları arasında *Penicillium* türlerinin izole edilme oranları

%49.3-81.8 arasında değişmiş ve en yüksek oran Aralık ayında belirlenmiştir (Tablo 5 ve Şekil 7).

Tablo.5. Gelendost-1 soğuk hava deposunda fungusların izole edilme oranları (%)

Fungus	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran
<i>Penicillium</i> spp.	62.0	57.4	49.3	81.8	74.2	75.1	65.1	68.6	56.2	60.2
<i>Aspergillus</i> spp.	2.8	1.0	0.9	2.0	3.1	0.7	3.2	1.8	2.5	0.8
<i>Botrytis</i> spp.	1.1	1.5	3.2	1.5	1.7	1.1	4.2	2.7	3.0	2.0
<i>Trichoderma</i> spp.	2.8	0.5	2.3	4.0	0.0	0.4	1.6	1.4	1.5	2.0
<i>Alternaria</i> spp.	3.9	2.5	12.0	4.0	5.9	1.8	3.7	4.1	2.5	0.8
<i>Cladosporium</i> spp.	3.4	6.6	14.3	0.0	3.1	6.0	4.2	3.6	6.5	16.0
Maya	5.0	12.2	5.5	0.0	8.4	6.4	4.8	4.1	11.9	2.0
<i>Geotrichum</i>	6.7	6.6	8.3	2.0	0.0	5.7	5.8	7.3	6.0	10.2
Diğler	12.3	11.7	4.1	4.5	3.5	2.8	7.4	6.4	10.0	5.7



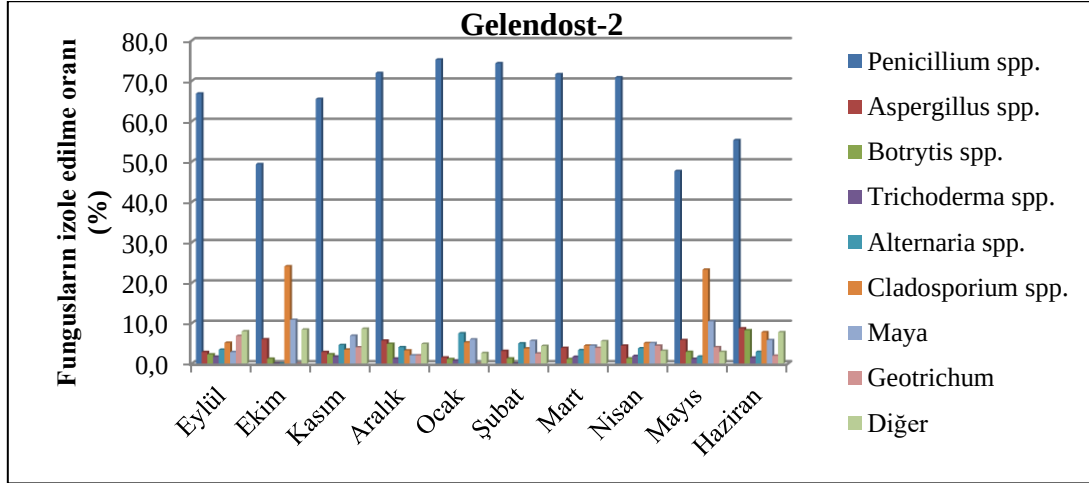
Şekil 7. Gelendost-1 soğuk hava deposunda fungusların izole edilme oranları (%)

Gelendost ilçesindeki ikinci soğuk hava deposunda Eylül-Haziran ayları arasında *Penicillium* türlerinin izole edilme oranları %

47.7-75.3 arasında değişmiş ve en yüksek oran Ocak ayında belirlenmiştir (Tablo 6 ve Şekil 8).

Tablo 6. Gelendost-2 soğuk hava deposunda fungusların izole edilme oranları (%)

Fungus	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran
<i>Penicillium</i> spp.	66.9	49.4	65.5	72.0	75.3	74.4	71.7	70.9	47.7	55.3
<i>Aspergillus</i> spp.	2.9	6.0	2.9	5.7	1.5	3.1	3.9	4.4	5.8	8.7
<i>Botrytis</i> spp.	2.3	1.2	2.3	4.9	1.1	1.3	1.1	1.3	2.9	8.3
<i>Trichoderma</i> spp.	1.7	0.0	1.7	1.2	0.7	0.0	1.7	1.9	1.2	1.5
<i>Alternaria</i> spp.	3.4	0.0	4.6	4.1	7.5	5.0	3.3	3.8	1.7	2.9
<i>Cladosporium</i> spp.	5.1	24.1	3.4	3.3	5.2	3.8	4.4	5.1	23.3	7.8
Maya	2.9	10.8	6.9	2.0	6.0	5.6	4.4	5.1	10.5	5.8
<i>Geotrichum</i>	6.9	0.0	4.0	2.0	0.0	2.5	3.9	4.4	4.1	1.9
Diğer	8.0	8.4	8.6	4.9	2.6	4.4	5.6	3.2	2.9	7.8



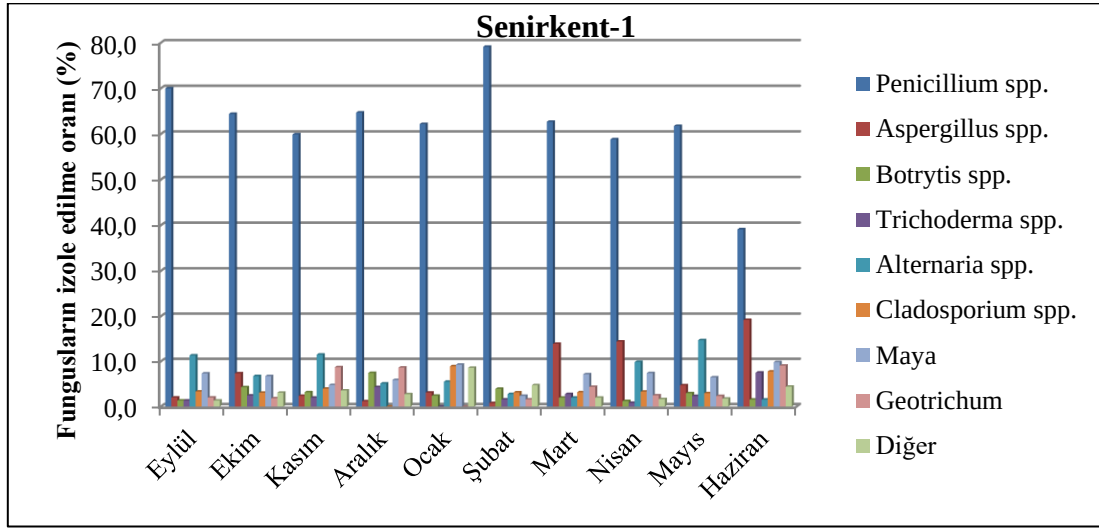
Şekil 8. Gelendost-2 soğuk hava deposunda fungusların izole edilme oranları (%)

Senirkent ilçesindeki birinci soğuk hava deposunda Eylül-Haziran ayları arasında *Penicillium* türlerinin izole edilme oranları

%39.1-79.1 arasında değişmiş ve en yüksek oran Şubat ayında belirlenmiştir (Tablo 7 ve Şekil 9).

Tablo 7. Senirkent-1 soğuk hava deposunda fungusların izole edilme oranları (%)

Fungus	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran
<i>Penicillium</i> spp.	70.0	64.4	59.9	64.7	62.2	79.1	62.7	58.8	61.8	39.1
<i>Aspergillus</i> spp.	2.0	7.4	2.4	1.2	3.1	0.8	13.9	14.4	4.7	19.2
<i>Botrytis</i> spp.	1.3	4.3	3.2	7.5	2.4	3.9	2.0	1.2	2.9	1.6
<i>Trichoderma</i> spp.	1.3	2.5	2.0	4.3	0.0	1.6	2.8	0.8	2.4	7.5
<i>Alternaria</i> spp.	11.3	6.7	11.5	5.1	5.5	2.8	2.0	9.9	14.7	1.6
<i>Cladosporium</i> spp.	3.3	3.1	4.0	0.0	8.9	3.1	3.2	3.3	2.9	7.8
Maya	7.3	6.7	4.8	5.9	9.3	2.4	7.1	7.4	6.5	9.8
<i>Geotrichum</i>	2.0	1.8	8.7	8.6	0.0	1.6	4.4	2.5	2.4	9.1
Diğer	1.3	3.1	3.6	2.7	8.6	4.7	2.0	1.6	1.8	4.4



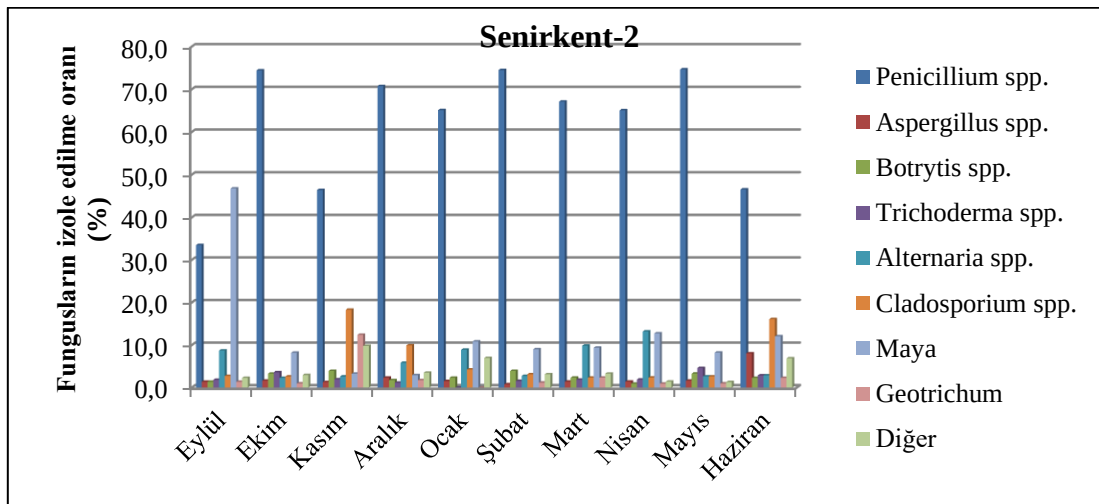
Şekil 9. Senirkent-1 soğuk hava deposunda fungusların izole edilme oranları (%)

Senirkent ilçesindeki ikinci soğuk hava deposunda Eylül-Haziran ayları arasında *Penicillium* türlerinin izole edilme oranları

%33.5-74.7 arasında değişmiş ve en yüksek oran Mayıs ayında belirlenmiştir (Tablo 8 ve Şekil 10).

Tablo 8. Senirkent-2 soğuk hava deposunda fungusların izole edilme oranları (%)

Fungus	Eylül	Ekim	Kasım	Aralık	Ocak	Şubat	Mart	Nisan	Mayıs	Haziran
<i>Penicillium</i> spp.	33.5	74.4	46.4	70.8	65.1	74.5	67.1	65.1	74.7	46.6
<i>Aspergillus</i> spp.	1.4	1.6	1.3	2.3	1.6	0.8	1.4	1.4	1.6	8.0
<i>Botrytis</i> spp.	1.4	3.3	3.9	1.8	2.3	3.9	2.3	0.9	3.3	2.3
<i>Trichoderma</i> spp.	1.8	3.6	2.0	1.2	0.0	1.6	1.9	1.9	4.6	2.9
<i>Alternaria</i> spp.	8.7	2.3	2.6	5.8	8.9	2.7	9.9	13.2	2.6	2.9
<i>Cladosporium</i> spp.	2.8	2.6	18.3	9.9	4.3	3.1	2.3	2.4	2.6	16.1
Maya	46.8	8.2	3.3	2.9	10.9	9.0	9.4	12.7	8.2	12.1
<i>Geotrichum</i>	1.4	1.0	12.4	1.8	0.0	1.2	2.3	0.9	1.0	2.3
Diğer	2.3	3.0	9.8	3.5	7.0	3.1	3.3	1.4	1.3	6.9



Şekil 10. Senirkent-2 soğuk hava deposunda fungusların izole edilme oranları (%)

Çalışmamızda Eğirdir, Gelendost ve Senirkent ilçelerindeki ikişer adet soğuk hava deposunda Eylül-Haziran ayları arasında tuzak besiyeri yöntemi ile elde edilen fungusların koloni sayımları yapılarak toplam fungus ve *Penicillium* türlerinin koloni sayıları üzerinden değerlendirmeler yapılmış ve her bir fungus cinsi için ilçelerdeki depolarda aylara göre fungusların izole edilme oranları hesaplanmıştır. Toplam fungus koloni sayıları üzerinden yapılan analizlerde aylara göre depolarda değişimler gözlenmiştir. *Penicillium* türleri koloni sayıları bakımından en fazla izole edilen baskın tür olarak belirlenmiş; Petri başına koloni sayıları ilçelerdeki depolar ve aylara göre farklılık göstermiştir. İlçeler bazında da *Penicillium* türlerinin en fazla izole edilen tür olduğu ve izolasyon oranlarının en yüksek olduğu gözlemlenmiştir. Çalışmamızda seçilmiş olan soğuk hava depoları konvansiyonel depolar olup hava kökenli fungal mikroflora belirlenmiştir. Aylık alınan değerler örnekleme yapıldığı gün, depo şartları, depo içerisinde bulunan meyvelerdeki bulaşmalar vb. gibi faktörlere bağlı olarak değişebilmekte ve bu bağlamda standart bir değişim gözlenmemektedir. Dolayısıyla bu değerler farklı zamanlara göre değişim gösterebilecektir. Özellikle geleneksel depolamada, meyvelerdeki birincil bozulma sebebinin meyveler üzerindeki latent sporlardan kaynaklı olduğu bilinmektedir. Diğer yandan kasa içerisinde depolanan meyvelerde bulaşmalar varsa bunlar temasla mekanik olarak zararlanmış diğer meyvelere bulaşabilmektedir. Bunun yanı sıra depolama süresince oda atmosferinde bulunan funguslar da meyvelerde bozulmalara sebep olabilir. İzole edilen fungus cinsleri açısından bir değerlendirme yapıldığında başta *Penicillium* türleri olmak üzere birçoğu potansiyel olarak meyvelerde bozulma sebepleri arasında sayılabilir. İnan ve Erkiş (2018) tarafından Mersin'deki turuncgil paketleme evinde yürütülen bir çalışmada, paketleme evlerinin farklı noktalarındaki atmosferden yoğunlukla *Penicillium* türleri izole edildiği bildirilmiştir. Paketleme evindeki en yüksek *Penicillium* popülasyonu ise paketleme evinin girişinden

elde edilmiştir. Paketleme evindeki en yaygın tür *P. digitatum* olarak belirlenmiştir. Lahlali ve ark. (2018) Fas'ta depo atmosferindeki fungus yoğunluklarını belirledikleri çalışmalarında fungal sporların en yüksek yoğunlukta Midelt'deki soğuk hava deposunda belirlenmiş ve yoğunluğu 464 spor/m³ olarak hesaplamışlardır. Konvansiyonel depolamada depo atmosferinde yapılan dezenfeksiyon işlemlerinin oldukça faydalı olduğu ifade edilmiştir. Nitekim soğuk hava depolamasında hava kökenli mikrobiyolojik analizlerin yapıldığı aynı çalışmada toplam mikroorganizma miktarı belirlenmiş (maya ve küfler) ve sonuçlar değerlendirildiğinde depo atmosferinin dezenfeksiyonla birlikte daha düşük oranda mikroorganizma içerdiği ortaya konulmuştur. Depolama süresince meyve kalitesinin depo çevre ve atmosferi ve meyvelerdeki mikroorganizma varlığına bağlıdır ve bunların aktivitesine bağlı olarak meyve çürüklükleri meydana gelmektedir. Kontrollü depolama koşullarında gaz karışımlarının kompozisyonunun depolama sürecini uzattığını ve mikroorganizmaların gelişimini azaltarak güvenli meyve depolaması yapılabileceğini bildirilmiştir (Juhvenica ve ark., 2011).

3.2. İlçelerdeki soğuk hava depolarındaki çürük meyve oranları

Atmosfer koşullarından örnekleme yapılan Eğirdir, Gelendost ve Senirkent ilçelerindeki 2'er soğuk odada içerisinde Golden Delicious çeşidinin yer aldığı elma kasaları yerleştirilmiş ve çürük meyve sayı ve oranları belirlenmiştir (Tablo 9). Depolara yerleştirilen kasalardaki çürüklük oranları ilçe ve depolara göre farklılıklar göstermiştir. İlçelerdeki depolarda çürüklük oranları % 13.3-37.8 arasında değişmiştir. En düşük çürüklük oranı Eğirdir'de örnekleme yapılan 2. depoda gözlemlenirken en yüksek çürüklük oranları Senirkent'teki depolarda gözlemlenmiştir (Tablo 9). Kasalarda çürük meyve sayıları belirlenirken çürüklüğe neden olan fungal etmenler dikkate alınmaksızın sayımlar yapılmıştır.

Tablo 9. Depo odalarına konan kasalardaki ortalama çürük meyve sayıları ve çürük meyve oranları (%)

İlçe	Toplam meyve sayısı (adet)	Ortalama çürük meyve sayısı (adet)	Çürük meyve oranı (%)
Eğirdir-1	180	18.0	30.0 c*
Eğirdir-2	180	8.0	13.3 a
Gelendost-1	180	13.0	21.7 b
Gelendost-1-2	180	14.7	24.4 b
Senirkent-1	180	22.7	37.8 d
Senirkent-2	180	21.0	35.0 d

*Aynı sütun içerisinde farklı harf içeren ortalamalar Tukey testine göre ($P<0.05$) birbirinden farklıdır.

Örnekleme yapılan depolarda (her ilçe için ikişer adet depo) çürük meyve sayılarının belirlendiği 60'şar meyve bulanan ve 3 tekerrürlü yürütülen deneme hâlihazırda küçük çaplı bir çalışma niteliğindedir. Meyvelere yüzey dezenfeksiyon işlemi dışında herhangi bir uygulama yapılmamıştır. Depolama sırasında meyveler doğal sürecine bırakılmış ve sayımlar yapılmıştır. Elma soğuk hava depolarında deponun özelliği, depolama şartları, elma çeşidine bağlı olarak yıldan yıla değişebilmektedir. Kontrollü atmosfer depolamasında muhafaza şartları optimum yapıldığı için kayıplar çok düşük oranlarda olmakla birlikte konvansiyonel depolarda kayıplar daha yüksek oranda olabilmektedir. Ülkemizde elmanın da dâhil olduğu bahçe ürünlerinde verim kayıplarının üretimden tüketiciye kadar olan süreçte % 10-30 oranında olduğu bildirilmiştir (Özdemir ve ark., 2009). Patolojik bozulmalar nedeniyle gerçekleşen kayıpların oranı depolanan ürün çeşidi, depolama koşulları ve meyvelerde yaralanmalara bağlı olarak % 20-50 oranındadır (Temur, 2012; Uslu, 2012). Lahlali ve ark. (2018) hasat sonu fungal patojenlerin 23 ayrı depo odasında yaptıkları surveylerde 138 hasta meyve üzerinde yaptıkları analizlerde dominant olarak *Penicillium expansum* (% 62) türünü tespit etmişlerdir. Bunun ardından en yaygın diğer türler *Alternaria tenuisima* (% 33) ve *Alternaria chartarum* (% 5) olmuştur.

4.Sonuçlar

Bu çalışmada Isparta iline bağlı Eğirdir, Gelendost ve Senirkent ilçelerindeki iki farklı deponun iki farklı oda atmosferindeki fungal mikrofloranın ve elma çürüklük oranlarının belirlenmesi amaçlanmıştır. Çalışmamızda

depo oda atmosferindeki funguslar tuzak besiyeri yöntemiyle yakalanmış ve cins düzeyinde koloni sayıları belirlenmiştir. Petri kaplarında sayımı yapılan fungus cinsleri sırasıyla *Penicillium* spp., *Botrytis* spp., *Alternaria* spp., *Aspergillus* spp., *Geotrichum* sp., bazı maya türleri ve diğerleri olarak gruplandırılmıştır. Diğerleri kapsamına minör önemde sayılabilecek *Mucor* spp., *Rhizopus stolonifer* ve bakteri kolonileri yer almıştır. Dolayısıyla elma depo atmosferinde hava kökenli fungusların geniş bir yelpaze ve çeşitlilikte olduğu dikkat çekmiştir. Eğirdir, Gelendost ve Senirkent ilçelerindeki depo atmosferinden elde edilen toplam fungus sayıları arasında farklılıklar ve aylara göre dalgalanmalar gözlenmiştir. Elma depo atmosferindeki fungus cinslerinin oranları ilçeler ve depolar arasında değişmiştir. Depo hava atmosferinde, hava kökenli *Penicillium* türleri en fazla sayımı yapılan fungus olmuştur. Elma depolamasında hasat sırasında meyve üzerine yerleşen latent fungus sporları, meyvede mekanik zararlanma meydana gelmesi halinde birincil bozulma kaynaklarıdır. Ancak, hava kökenli fungusların da elma depolarında en önemli bulaşma kaynakları olduğu düşünülmektedir. Depolama atmosferinde bu fungusların varlığı elmalarındaki depolama periyodunu da kısaltabilecek potansiyelde olabilmektedir. Elma hasadını takiben konvansiyonel depolamada depo atmosferindeki fungal çeşitliliğin zengin olma konusunun depocular tarafından iyi yönetilmesi gerekmektedir. Depolama şartlarının iyileştirilmesi ve hasat sonu teknolojilerinin geliştirilmesi elmada hasat sonu kayıplarının azaltılmasında önemli bir konudur. Nitekim kontrollü atmosfer depolaması 1 yıla yakın süre meyve kalitesi

bozulmadan depolama şansı sağlayan bir teknoloji olduğu bilinmektedir. Farklı odalarda kasalara konan Golden Delicious çeşidi meyvelerindeki çürüklük sayıları ve oransal olarak çürümenin de belirlendiği bu çalışmamızda depoya konan elma meyvelerindeki çürüklük oranları da depolar arasında değişkenlik göstermiştir. Elma meyvelerinin yetiştiricilik şartları, hasat sırasında yapılan uygulamalar ve hasat sonrası depolama koşullarının bu sonuçları etkileyebileceği düşünülmektedir. Tüm çalışma sonuçları bir arada değerlendirildiğinde bu farklılıkların sebeplerinin depoya konan elma çeşitleri ve depolarda sezon başı ve sonunda yapılan hijyen ve sanitasyon önlemlerine bağlı olarak değişkenlik gösterebileceği sonucuna varılmıştır. Depo atmosferindeki fungusların çeşitliliği ve yoğunluğu temizlik, hijyen ve sanitasyonun önemini ortaya koymuştur. Temizlik ve sanitasyon farklı uygulamalardır. Temizlik deterjan vb. ürünler kullanılarak yapılan işlemlerdir. Hijyen ve sanitasyon ise bazı kimyasallar kullanılarak mikroorganizmalardan tamamen arındırma işlemidir. Depo koşullarının en iyi standartlarda olmasının yanı sıra elma meyvelerinin depoya konulmadan öncesinde sezon başında ve depolama süreci sona erdiğinde iyi bir temizlik yapılması tavsiye edilebilir. Ardından önerilebilecek bazı kimyasallar ile depo boşken depo atmosferine uygulamalar yapılarak mikroorganizma yükü azaltılabilir. Uzun süreli depolamanın başarısı tüm bunlar dikkate alındığında daha da arttırılabilecektir.

Yazarların Katkı Beyanı

Yazarlar makaleye eşit katkıda bulunduklarını, makalenin yayına hazır son halini gördüklerini/okuduklarını ve onayladıklarını beyan ederler.

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Determination of The Effect of Nitrogenous Fertilization on Grain Quality Characteristics in Wheat

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Abstract

This research was carried out in the Tevekli Neighborhood rural area of Van Tusba District in the summer growing season of 2020 to determine the effects of four nitrogenous fertilizer doses (0 kg da⁻¹ N, (N0: control), 8 kg da⁻¹ N (N8), 12 kg da⁻¹ N (N12) and 16 kg da⁻¹ N (N16)) on some physiological properties (ash, moisture, starch, protein, cellulose and oil) of local (Bahare and Kose) and commercial (Seri-82 and Cemre) bread wheat varieties. The research was designed according to the split-plot design in randomized blocks with three replications, with N doses in the main plots and varieties in the subplots. During the heading period, live plant leaves were measured with the Normalised Difference Vegetation Index (NDVI) sensor, and leaf observations were made. According to the research results; the differences between the other characteristics examined except for grain moisture data were statistically significant (P<0.05). According to the interactions, the highest values were obtained from N12 x Cemre (0.320) and N16 x Kose in the same group in NDVI data, N0 x Bahre (1.036 %) and N12 x Bahare in the same group in ash ratios, N12 x Seri (44.704 %) and N12 x Cemre and N16 x Cemre in the same group in starch ratios, N12 x Bahare (15.901 %) in protein ratios, N16 x Kose (4.019 %) in cellulose ratios and N0 x Cemre (2.592 %) in oil ratios and five interactions in the same group. No statistically significant effect of N doses was observed on other examined traits except grain moisture and protein ratios. The grain moisture ratio was obtained from the highest N0 dose (8.788 %), and the protein ratio was obtained from other doses except N0. The effect of varieties on other properties except ash and oil contents in grain was found to be statistically insignificant (P>0.05). Bahare local variety had high values in ash ratio and commercial varieties had high values in oil ratio. It is thought that low rainfall and high temperatures during the season effectively obtained low results.

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1. Introduction

Due to its high adaptability, a very large area worldwide is used in wheat farming. Due to this feature, it is evaluated as the raw material of foods used as staple foods in many countries. 20 % of the world's food and 53 % of Türkiye's food is produced from wheat (Anonymous, 2017). Developing countries, in particular, meet 21% of their daily calorie needs and 20 % of their protein needs from wheat (Jones et al., 2020; Karaman et al., 2023; Balkan et al., 2023). Local varieties are considered to be very valuable genetic material used in breeding studies. Local varieties that continue to be cultivated in rural areas of the Van Lake Basin have the opportunity to be evaluated as a very valuable gene pool (Altuner et al., 2020).

While characteristics such as thousand-grain weight, hectoliter weight, grain color, and hardness in wheat are used to determine physical quality, the amounts of protein, dry matter, ash, oil, cellulose, and starch in the grain are also used as indicators of chemical quality (Erken, 2022). In particular, protein ratio is considered bread wheat's most important quality indicator. According to the Turkish Food Codex, the lowest protein ratio in bread wheat has been determined as 10.5 % (Menderis, 2006). However, high moisture in the grain reduces the commercial value of wheat, makes storage difficult and promotes germination. For this reason, a 14.6 % moisture level in wheat grain is considered critical (Unal, 2002). Wheat endosperm consists of 28.4 % cellulose and 23.7 % flour (Gartaula et al., 2018).

Physiological properties significantly affect yield and quality in wheat. The inclusion of these properties in breeding studies has become widespread in recent years. In this respect, some devices have been developed to measure physiological properties without damaging the structure of plants. SPAD meter, thermal camera and Normalized Difference Vegetation Index device (NDVI) are some of them (Bayhan et al., 2021). NDVI is one of the spectral index devices widely used to measure grain yield in living plants without the need for

cutting (Cabrera-Bosquet et al., 2011). NDVI measurements are based on the principle of measuring the difference in the absorption response of near-infrared reflected and red light in the parts with high leaf density in the chlorophyll. NDVI data taken especially in the flowering and grain filling stages show a significant correlation with grain yield (Mkhabela et al., 2011).

This study aimed to determine the NDVI values and some physiological quality traits such as protein, ash, cellulose, moisture, and starch in the grains of four bread wheat varieties, two of which were local, grown with four different nitrogen fertilization doses in the 2020 summer growing season.

2. Materials and Methods

2.1. Herbal material

Four types of summer-natured bread wheat seeds were used in the experiment. Seri-82 from commercial varieties was provided by the Eastern Mediterranean Agricultural Research Institute and Cemre from the GAP International Agricultural Research Institute. Bahare from local varieties was provided from the Bitlis rural area and Kose from the Mus rural area.

2.2. Climate and soil characteristics of the research area

The research was carried out in the rural area of the Tevekli Neighborhood of Tusba District, Van during the 2020 summer growing season. The precipitation, temperature and relative humidity values of the season and the long-term average (LTA) are given in Figure 1. Accordingly, it is seen that the highest precipitation was received in December, January, and April during the growing season and the season total was 230.3 mm, while the LTA precipitation total was 374.2 mm and the season total was 140 mm lower than this value. It is understood that the average temperature during the season was 9.7 °C, UYO was 8 °C and a growing season with an average temperature 1.7 °C higher than LTA was. It is understood that there was no significant difference between the relative humidity values (Anonymous, 2020).

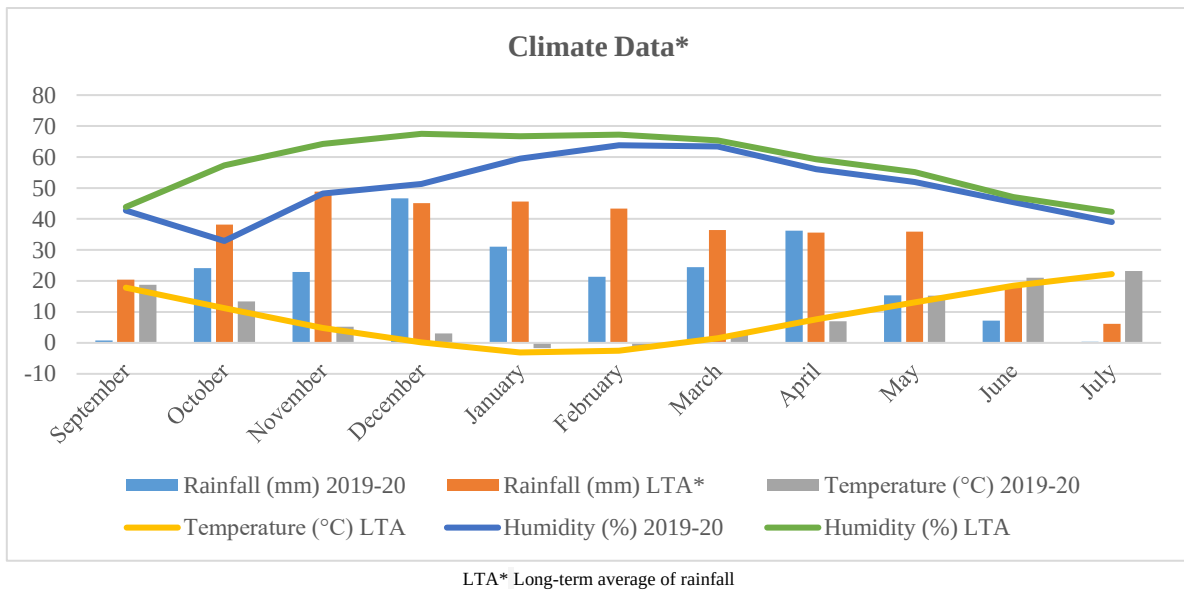


Figure 1. Experimental area research season and long-term average climate values (Anonymous, 2020)

Data on soil properties of the research area are given in Table 1. According to the analysis results of soil samples taken from 0-20 cm and 20-40 cm depths, it is understood that the trial

soils have a loamy texture, a pH around 7-90-7.96 and neutral, and a structure weak in terms of lime and organic matter.

Table 1. Some physical and chemical properties of the research area soils*.

Depth (cm)	Texture	pH	Clay (%)	Electrical conductivity/salt (dS m ⁻¹)	Organic matter (%)
0 - 20	Loamy	7.90	1.29	0.16	0.44
20 - 40	Loamy	7.96	1.25	0.17	0.42

*Soil analyses results of the Van YYYU Agriculture Faculty Labratuary.

2.3. Conducting the research and sample analyses

The research was established in the summer season of 2020 and the plot dimensions were determined as $5 \times 1.2 = 6\text{m}^2$. Nitrogenous fertilizer (N) was planted in the main plots and varieties in the sub-plots; with 450 seeds m^{-2} calculation were sown. Nitrogenous fertilizer was applied to the main plots with four fertilizer doses of $0 \text{ kg da}^{-1} \text{ N}$ (control), $8 \text{ kg da}^{-1} \text{ N}$, $12 \text{ kg da}^{-1} \text{ N}$, and $16 \text{ kg da}^{-1} \text{ N}$ in the form of NH_4SO_4 with 21% N content, 4 kg da^{-1} together with planting. Phosphorus (P_2O_5) was given in the form of 7 kg da^{-1} TSP at the same time as sowing. The remaining N fertilizer was applied as 46 % urea ($\text{CO}(\text{NH}_2)_2$) (Gucdemir, 2006). In the pre-tillering, stemming, and heading periods, limited

irrigation was applied three times (Akdogan and Soyulu, 2018). The trial was established on 48 plots with three replications according to the randomized block split-plot design. In the afternoon, measurements were made with the Normalized Difference Vegetation Index (NDVI) sensor without damaging the leaves of the plants during the heading period (Kilicaslan et al., 2020; Bayhan et al., 2021). In the Dicle University Faculty of Agriculture Laboratory, to determine the grain physiological properties such as protein ratio, starch ratio, moisture ratio, oil ratio, cellulose ratio and ash ratio as a percentage, the grains were determined without being subjected to grinding process with the FOSS brand XDS model Near Infrared Reflection (NIR) device (FOSS, Eden Prairie, North America) according to the AOAC 992-23 method

(Kilicaslan et al., 2020; Anonymous, 1990). Statistical analyzes of the data were performed using the CoSTAT (ver. 6.303) computer analysis program and the multiple comparison of the difference between the means was carried out according to the LSD (0.05 %) test.

3. Results and Discussion

The results obtained regarding the features examined in the research are shown in Table 2.

Table 2. Effects of nitrogen fertilization on NDVI and quality traits in wheat varieties*

N** Dose	Varieties	NDVI		Ash (%)		Moisture (%)		Starch (%)		Protein (%)		Cellulose (%)		Oil (%)	
N0	Bahare	0.210	ab	1.036	a	8.519		42.251	ab	11.798	b	3.716	b	1.549	ab
	Cemre	0.227	ab	0.383	b-d	8.822		43.221	ab	13.526	ab	3.220	c	2.592	a
	Kose	0.263	ab	0.551	a-d	8.724		43.653	ab	13.673	ab	3.046	cd	1.554	ab
	Seri-82	0.297	ab	0.517	a-d	9.082		43.655	ab	13.667	ab	2.920	d	2.546	a
N8	Bahare	0.213	ab	0.848	ab	8.485		41.740	ab	14.731	ab	3.242	c	1.218	ab
	Cemre	0.260	ab	0.328	cd	7.862		43.152	ab	15.014	ab	3.071	cd	1.948	ab
	Kose	0.263	ab	0.782	ab	8.280		40.152	b	15.383	ab	3.124	cd	1.506	ab
	Seri-82	0.227	ab	0.657	a-c	7.857		41.722	ab	13.762	ab	3.534	bc	2.132	a
N12	Bahare	0.260	ab	0.849	a	8.334		41.735	ab	15.901	a	3.314	bc	1.018	b
	Cemre	0.320	a	0.248	d	8.555		44.343	a	15.591	ab	4.037	a	2.533	a
	Kose	0.200	b	0.647	a-c	8.293		41.902	ab	15.541	ab	3.190	cd	1.392	ab
	Seri-82	0.230	ab	0.396	b-d	8.638		44.704	a	14.868	ab	2.937	d	2.461	a
N16	Bahare	0.220	ab	0.730	ab	8.268		42.059	ab	15.431	ab	3.441	bc	1.247	ab
	Cemre	0.260	ab	0.435	b-d	8.323		44.225	a	15.566	ab	2.978	c	1.653	ab
	Kose	0.303	a	0.571	a-c	8.534		42.107	ab	14.181	ab	4.019	a	1.897	ab
	Seri-82	0.177	b	0.480	b-d	8.060		43.050	ab	14.280	ab	3.100		2.100	a
C.V. (%)		26.009		32.857		5.821		4.996		10.989		19.595		31.989	
N Average	N0	0.249		0.621		8.788		A		13.166		B		2.059	
	N4	0.240		0.653		8.121		C		14.723		A		1.702	
	N8	0.252		0.535		8.456		B		15.477		A		1.850	
	N16	0.240		0.555		8.298		BC		14.864		A		1.724	
	LSD (0.05)	0.054		0.193		0.220				1.389		0.609		0.583	
Variety Average	Bahare	0.225		0.865		A		8.402		41.946		14.467		3.429	
	Cemre	0.266		0.347		C		8.393		43.735		14.923		3.326	
	Kose	0.257		0.638		B		8.458		41.954		14.695		3.346	
	Seri-82	0.232		0.514		B		8.409		43.283		14.145		3.124	
	LSD (0.05)	0.061		0.163				0.413		1.799		1.348		0.546	

*No statistically significant difference exists between the data shown with the same letter in the same column. There is a statistically significant ($P<0.05$) difference between the data shown with different lower and upper case letters in the same column. **N0: Control (0 kg da⁻¹ N), N8: 8 kg da⁻¹ N, N12: 12 kg da⁻¹ N, N16: 16 kg da⁻¹ N

3.1. NDVI

The NDVI measurement results of the varieties, N doses, and interactions used in the study are shown in Table 2. Accordingly, based on N dose averages, fertilizer doses did not have a statistically significant ($P>0.05$) effect on NDVI results. NDVI values varied between 0.240-0.252 according to fertilizer doses. There was also no statistically significant difference between NDVI data according to varieties. NDVI values of

varieties were determined between 0.225-0.266. The effect of N doses and variety interactions (N x V) on NDVI data was statistically significant ($P<0.05$). Accordingly, NDVI values varied between 0.177-0.320. According to the interactions, the highest NDVI value was taken from N16 x Kose interactions in the same group as N12 x Cemre and the lowest from N12 x Kose interactions in the same group as N16 x Seri-82. High NDVI data is considered an indicator of healthy plant

development (Kizilgeci et al., 2019). In a study conducted with some bread and advanced wheat genotypes and measurements taken during the heading period (Bayhan et al., 2021), it was determined that NDVI values varied between 0.53-0.67 and the trial average was 0.61. In a study conducted to determine the effects of some physiological traits on quality and yield traits in some triticale genotypes (Kizilgeci et al., 2017), it was determined that the NDVI values taken during the heading period were between 0.72-0.77 in the Mardin location and 0.77-0.81 in the Diyarbakir location. In another study (Kizilgeci and Yildirim, 2019), it was determined that the NDVI data taken during the heading period varied between 0.42-0.85. The data obtained in these studies are higher than our results. NDVI values are affected by many factors such as measurement phase and range, sensor height, environment, and growing conditions (Kizilgeci et al., 2017). The desired high chlorophyll content in plants is a trait that has a positive effect on yield in breeding programs (Bayhan et al., 2019; Albayrak et al., 2020).

3.2. Ash

The ash amounts determined in the grain of the varieties used in the study, N doses, and interactions are shown in Table 2. According to the table, no statistically significant difference was observed between the ash amounts in the grain-based on the N fertilizer averages ($P>0.05$). Accordingly, the ash amounts in the grain varied between 0.535-0.653 %. The differences between the ash amount in the grain according to the average of the varieties were found to be statistically significant ($P<0.05$). Accordingly, the ash amounts in the grain varied between 0.347-0.865 %. According to the variety averages, the highest ash amounts in the grain were determined in the Bahare local variety and the lowest in the Cemre variety. The differences between the ash amount in the grain according to the N fertilizer doses and variety interactions were found to be statistically significant ($P<0.05$). According to the interactions, the ash amounts in the grain were between 0.248-1.036 %. Accordingly, the highest ash ratios in

the grain were obtained from the N0, N12 x Bahare local variety and other interactions in the same group, and the lowest from the N12 x Cemre interaction. In a study conducted for three years in Yozgat ecological conditions (Mut et al., 2017), the ash ratios of 14 bread wheat varieties were determined to be 1.59 % in the first year, 1.68 % in the second year, and 1.87 % in the third year, varying according to the climate effect. In another study conducted on 11 barley varieties in Gumushane conditions (Sirat and Bahar, 2020), the ash ratio was determined between 2.00-2.31% according to the two-year average results. In Edirne conditions, the raw ash average of 6 bread wheat was determined between 1.14-1.62% (Erken, 2022). The findings obtained in our study are lower than these results. Water loss due to high temperature is also effective in the increase in the ash ratio in the grain (Egesel et al., 2009). Grain ash content varies depending on genotype, environment, and maintenance conditions (Anjum et al., 2014; Mahla et al., 2015).

3.3. Moisture

Data on grain moisture content are shown in Table 2. Accordingly, the effect of N doses on grain moisture content was statistically significant ($P<0.05$). According to N doses, grain moisture content varied between 8.121-8.788 %. Grain moisture content was taken from the highest N0 (control) dose and the lowest N8 dose. The effect of varieties on grain moisture content was statistically insignificant ($P>0.05$). According to varieties, moisture content varied between 8.393-8.458 %. The effect of interactions on grain moisture content was statistically insignificant ($P>0.05$). According to interactions, grain moisture content varied between 7.857-9.082 %. In a two-year study conducted in Gumushane conditions (Sirat and Bahar, 2020), the two-year average moisture content of 11 barley varieties was found to be between 9.21 % and 9.88 %. In another study conducted in Edirne conditions, the moisture content of 6 bread wheat was determined to be between 10.20 % and 15.30 % (Erken, 2022). The findings obtained in our study are lower than these

results. Low or ideal moisture content is accepted as one of the indicators of quality in barley. While very low grain moisture in cereals causes increased transportation losses, the commercial value and storage life of those with ideal moisture increases. The moisture content of cereals varies depending on climatic conditions, ripening period, storage type, and conditions (Kun, 1996; Sencar et al., 1997). 14.6 % moisture content is seen as the upper limit for Turkish wheat (Unal, 2002).

3.4. Starch

Data on starch content in grains of the varieties, N doses, and interactions used in the study are shown in Table 2. Accordingly, the effect of N doses and varieties on starch content in grains was statistically insignificant ($P>0.05$). Starch content was determined between 41.691-42.860 % according to N doses and 41.946-43.735 % according to varieties. Differences between starch content in grains according to interactions were found to be statistically significant ($P<0.05$). Starch content in grains of interactions occurred between 40.152-44.225 %. Accordingly, the highest starch content was obtained from N12 x Seri-82, N12 x Cemre, and N16 x Cemre from the same group, and the lowest from N8 x Kose interactions. In a study conducted in Diyarbakir conditions using 4 commercial varieties and 16 advanced bread wheat lines, starch ratios were determined between 61.36 % and 63.73 % and it was stated that there was a negative relationship between starch ratio and protein ratio in the grain (Bayhan et al., 2021). In a similar study (Mut et al., 2015), starch ratios in the grain were determined between 61.6 % and 65.0 %. In another study, the average starch ratios of 11 barley varieties were found between 52.61 % and 58.60 % (Sirat and Bahar, 2020). The findings obtained in our study are lower than these data. The reason for this is shown to be the change in starch ratio in the grain under the influence of genotype and ecological factors (Mahla et al. 2015).

3.5. Protein

Data on protein content in the grain of varieties, N doses, and interactions are shown

in Table 2. Accordingly, the effect of N doses on protein content in grain was found to be statistically significant ($P<0.05$). Protein ratios varied between 13.166-15.477 % according to N doses. Protein contents were obtained at the lowest N0 (control) dose and the highest N12 dose. The remaining N doses were also in the highest group. The differences between protein amounts in grain according to varieties were found to be statistically insignificant ($P>0.05$). Accordingly, protein contents were determined between 14.145-14.923 %. The differences between protein amounts in grain according to interactions were statistically significant ($P<0.05$). Accordingly, protein ratios in grain were determined between 11.798-15.901 %. The highest protein ratios were obtained from N12 x Bahare and the lowest from N0 x Bahare interactions. In a study conducted with 4 commercial and 16 advanced wheat varieties in Diyarbakır conditions (Bayhan et al., 2021), protein ratios were determined between 13.73-18.43 %. In another study conducted in Diyarbakır conditions (Kizilgeci et al., 2019), protein ratios varied between 14.36-16.48 %. In a study conducted with 14 bread wheat in Yozgat conditions, the protein ratio was 12.8 % according to the three-year average. The findings obtained in our study are similar to these results. It has been stated that the protein ratio in the grain varies under the influence of genotype, ecology, and maintenance conditions. It has also been reported that high protein increases grain hardness, the ratio in the grain varies between 8-18 % (Christen, 2009) and sometimes up to 25 % (Gooding and Davis, 1997) and positively affects other quality characteristics in the grain.

3.5. Cellulose

The cellulose contents of varieties, N doses, and interactions in grain are shown in Table 2. Accordingly, N doses and varieties did not have a statistically significant effect on cellulose contents in grain ($P>0.05$). Cellulose rates in grain varied between 3.228-3.385 % according to N doses and 3.124-3.429 % according to varieties. The differences between cellulose amounts in grain according to interactions were found to be statistically

significant ($P < 0.05$). Accordingly, cellulose contents in grain varied between 2.920-4.037 %. The highest cellulose rates in grain were detected in N16 x Kose interactions in the same group with N12 x Cemre and the lowest in N12 x Seri-82 interactions in the same group with N0 x Seri-82. In Edirne conditions, the crude cellulose ratios of 6 bread wheat were determined between 1.75-3.01 % (Erken, 2022). The results obtained in our research are higher than these data. On the other hand, in a study conducted in Harran Plain conditions (Yildiz and Tari, 2018), the cellulose amounts in barley were found between 6.1-7.2 %. The findings obtained in our research are lower than those of the data.

3.6. Oil

Data on the oil contents of varieties, N doses, and interactions in grain are shown in Table 2. Accordingly, the effect of N doses on oil amounts was found to be statistically insignificant ($P > 0.05$). Oil contents varied between 1.702-2.059 % according to N doses. The effects of varieties on oil contents were determined to be statistically significant ($P < 0.05$). The oil contents of varieties varied between 1.258-2.311 %. The highest oil content was determined in Seri-82, which is in the same group as Cemre, and the lowest in Kose, which is in the same group as Bahare. The effect of the interactions on oil content in the grain was determined to be statistically significant ($P < 0.05$). According to the interactions, oil contents varied between 1.018-2.592 %. The highest oil content in the grain was determined in other interactions in the same group as N0 x Cemre and the lowest in the N12 x Bahare interaction. In a study conducted in Yozgat conditions (Mut et al., 2017), the three-year oil content average of 14 varieties was found to be 1.61 %. In Edirne conditions, the crude oil ratio was found to be between 1.08-2.39 % according to the averages of 6 bread wheat (Erken, 2022). In Gumushane conditions, the two-year average of 11 barley varieties was found to be between 1.36-2.01 % (Sirat and Bahar, 2020). The findings obtained in our research are similar to these. It has been reported that the oil ratio is affected by

genotype, environment, and maintenance conditions (Ereifej et al., 2007; Barteczko et al., 2009).

4. Conclusion

According to the research examining the effects of nitrogenous fertilizer doses on NDVI and grain quality traits of local and commercial wheat varieties during the 2020 summer growing season: Nitrogenous fertilization significantly influenced the protein ratio in grain but had a limited effect on other quality traits. Commercial varieties demonstrated superior performance in grain oil content, while the Bahare variety showed high ash, starch, and protein ratios at a fertilization dose of 12 kg da⁻¹. Given the critical role of protein ratio in determining grain quality, our findings suggest that an 8 kg da⁻¹ nitrogenous fertilization dose is the most suitable dose for the trial conditions to obtain the highest protein ratio economically.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Antalya İlinde Marul Üretim Alanlarında Domates Lekeli Solgunluk Virüsü'nün (TSWV) Serolojik ve Moleküler Yöntemlerle Tanılanması

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Özet

Bu çalışma Antalya ilinde yetiştirilen marul üretim alanlarında domates lekeli solgunluk virüsü (tomato spotted wilt virus, TSWV)'nün tespit edilmesi amacıyla 2023 yılında yürütülmüştür. Son yıllarda bölge üreticilerinden marul yetiştiriciliği yapılan alanlarda TSWV benzeri belirtiler ile ilgili şikayetlerin gelmesi sonucunda bu çalışma planlanmıştır. TSWV'nün varlığı serolojik ve moleküler yöntemlerle belirlenmiştir. Araziden toplanan ve virüslü olduğundan şüphelenilen bitkiler, öncelikle DAS-ELISA (Double-antibody sandwich enzyme linked immunosorbent assay) yöntemi ile testlenmiştir. Testlenen toplam 92 örneğin 39 adedinin TSWV ile enfekteli olduğu saptanmıştır. Testlenen örneklerdeki hastalık oranı ise % 42.39 olarak belirlenmiştir. Toplam RNA ekstraksiyonu için bölgeleri temsil eden 5 bitki örneği seçilerek, RT-PCR (Ters transkripsiyon polimeraz zincir reaksiyonu) metodunda kullanılmışlardır. Agaroz jel elektroforezde 4 örnekte beklenen seviyede TSWV'ne spesifik 276 bp'lik bant oluşumu gözlenmiştir. Bir izolatta (Aksu izolatu) ve negatif kontrolde ise herhangi bir bant oluşumu gerçekleşmemiştir.

Araştırma Makalesi

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Anahtar Kelimeler

Marul
TSWV
DAS-ELISA
RT-PCR

Detection of Tomato Spotted Wilt Virus (TSWV) in Antalya Province Lettuce Production Areas by Serological and Molecular Methods

Abstract

This study was conducted to determine of Tomato spotted wilt virus (TSWV) in lettuce production areas in Antalya province in 2023 year. This study was planned as a result of receiving complaints from regional producers about TSWV-like symptoms in lettuce production areas in recent years. The presence of MWMV was determined by serological and molecular methods. Collected samples suspected to be TSWV were firstly tested with DAS-ELISA (Double-antibody sandwich enzyme linked immunosorbent assay) test. ELISA tests showed that among 92 samples, 39 were infected with TSWV and infection ratio for TSWV was determined as 42.39% of the field samples. In the study, 5 plant samples representing the regions were used for total RNA extraction and tested by RT-PCR (Reverse transcription polimerase chain reaction) for TSWV. The respected size of band with 276 bp in 4 samples was observed on agarose gel electrophoresis for TSWV. No band formation was observed in one isolate (Aksu isolate) and negative control.

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1.Giriş

Dünya nüfusunda oluşan artışla beraber, tarım ürünlerine olan ihtiyaçta gün geçtikte artmaktadır (Öncel ve ark., 2023). Beslenmemizde önemli bir yeri olan sebzeler vitamin ve mineraller bakımından oldukça zengindirler (Abak ve ark., 2010). Sebzeler işlenerek ya da taze olarak tüketilebilmektedirler (Erkan ve ark., 2013). Ülkemiz sebze üretiminde Çin, Hindistan ve ABD'den sonra 4. sırada yer almaktadır (Sırrı ve Özasan, 2020). Meyvesi yenilen sebzeler, toplam sebze üretiminin yaklaşık %83.2'sini oluştururken, % 10.6'sını kök ve yumrusu yenilen sebzeler izlemektedir. Marul, ıspanak, lahana ve brokoli gibi sebzeler ise %6.2'lik kısmı oluşturmaktadır. Marul, yaprağı yenilen bir bitki olup, Asteraceae familyası içerisinde yer almaktadır. A, C ve mineral madde bakımından oldukça zengindir. Ülkemizde marul açıkta ve örtü altında yıl boyunca yetiştirilebilmektedir (Balkaya ve ark., 2018).

Antalya ili coğrafi konumu, toprak ve iklim özelliklerinden dolayı marul yetiştiriciliğinde önemli bir yere sahiptir. Marul üretiminde Ankara ve Adana illerinden sonra Antalya 3. sırada yer almaktadır (Kandemir ve Balkaya, 2022).

Marul üretimini sınırlayan çok sayıda abiyotik ve biyotik hastalık etmenleri bulunmaktadır. Dünyada ve ülkemizde marul üretim alanlarında bazı virüs hastalıkları belirlenmiştir (Alan, 2012; Moreno ve Fereres, 2012; Sertkaya, 2015; Zelyüt, 2016).

İlk olarak 1915 yılında tanımlanan TSWV, Bunyaviridae familyasının Orthotospovirus cinsine aittir. Küresel partiküllere sahip olan TSWV, başta *Frankliniella occidentalis* Pergande ve *Thrips tabaci* Lindeman olmak üzere dokuz thrips türü ile taşınmaktadır (Adkins, 2000). Virüs ayrıca mekanik yolla mekanik yolla da taşınabilmektedir (Sherwood ve ark., 2009).

Sertkaya (2012) yaptığı çalışmada TSWV'nün enfeksiyon oranını % 8.2 olarak belirtirken; Wilson (1998) marulda bu virüsün % 5-60 oranında verim kaybına neden olduğunu bildirmiştir. Rosello ve ark. (1996)

ise TSWV'nün bitkilerde % 42.1-100 arasında kayıpların görüldüğünü ifade etmiştir. TSWV bitkilerde gelişme geriliği, cüceleşme, sararma, solgunluk, nekrotik ve klorotik lokal lezyonlar ve yapraklarda halkalı leke gibi belirtilere neden olmaktadır (Yardımcı ve Çulal-Kılıç, 2009).

Virüslerin neden olduğu kayıpların en düşük seviyeye indirilmesi ve yayılmasının engellenmesi için; öncelikle virüsün tanısının yapılması, yayılma yollarının ortaya konulması ve izlenecek yolun belirlenmesi büyük önem taşımaktadır.

Ülkemizde, marulda virüs hastalıklarının belirlenmesi ile ilgili farklı bölgelerde yapılan çalışmalar bulunmaktadır (Döken ve ark., 1993; Özdemir ve Erilmez, 2007; Yardımcı ve Çulal-Kılıç, 2009; Kamberoğlu ve Alan, 2011; Sertkaya, 2012; Sertkaya, 2015; Zelyüt, 2016; Sağlam ve Kamberoğlu, 2019; Ertunç ve Randa-Zelyüt, 2019; Akbaş ve ark., 2021; Yeşil ve Ağca, 2021).

Antalya ilinde 2009 yılında domates, biber ve marulda TSWV'nün varlığının belirlenmesi için Bozdoğan, (2009) tarafından yürütülen bir çalışmada; 58 marul örneğinden 54'ünün TSWV ile enfekteli olduğu bildirilmiştir.

Son yıllarda bölge üreticilerinden marul üretim alanlarında TSWV benzeri belirtiler ile ilgili şikayetlerin gelmesi sonucunda bu çalışma planlanmıştır. Bu çalışma ile marul yetiştiriciliğinin yapıldığı Antalya ilinde TSWV etmeninin varlığı serolojik yöntem DAS-ELISA ve moleküler yöntem RT-PCR yöntemi ile ortaya konulmuştur.

2.Materyal ve Yöntem

2.1. Arazi çalışmaları

2023 yılı, Temmuz-Ağustos-Eylül aylarında Antalya ili marul yetiştiriciliğinin yapıldığı Finike, Serik, Aksu, Kaş ve Muratpaşa üretim alanlarında arazi çalışması yapılarak marul yaprak örnekleri toplanmıştır.

2.2. Enfekteli bitki materyalinin temini

Arazi çalışmaları boyunca marullarda mozaik, kloroz, nekroz, şekil bozukluğu ve bodurluk gibi belirtiler gösteren bitkilerinden

örnekler alınmıştır. Toplanan örnekler -20 °C’de muhafaza edilmiştir.

2.3. Das-elisa

Çalışmada kullanılan ELISA kitleri (antiserumlar, pozitif ve negatif kontroller) BIOREBA-AG firmasından temin edilmiştir. Test sonuçlarının incelenmesinde ELISA okuyucusundan yararlanılmıştır. Böylece arazi çıkışlarında simptomatolojik olarak şüphe duyulan bitki örneklerinde TSWV etmeninin tanısının yapılması için DAS-ELISA yöntemi uygulanmıştır. Yöntem, firmanın yönergeleri doğrultusunda yapılmıştır.

2.4. Toplam nükleik asit izolasyonu

Bitkiden toplam nükleik asitler, RNA izolasyon kiti (Takara) kullanılarak izole edilmiştir. Sürvey yapılan alanlardan Finike, Serik, Muratpaşa ve Kaş’tan DAS-ELISA’da pozitif çıkan marul örneklerinden birer tane, Aksu’dan yine bir örnek seçilerek (toplam 5 örnek) toplam nükleik asit izolasyonu ve RT-PCR çalışmalarında kullanılmıştır.

2.5. RT-PCR çalışmaları

Ekstraksiyon aşamasından sonra, elde edilen total RNA’lar TSWV’ne spesifik primer kullanılarak (F-5-AATTGCCTTGCAACCAATTC-3; R-5-ATCAGTCGAAATGGTCGGCA-3) RdRp

geninin yaklaşık 276 bp’lik bir kısmı çoğaltılmıştır (Perez ve ark., 2014). RT-PCR çalışmaları tek aşamalı olarak gerçekleştirilmiştir. (Primescript One step RT-PCR kit; Takara Bio Inc). Tek bir reaksiyon karışımında; 1 µl RNA, 1’er µl virüs spesifik primer çifti (her biri 20 µM), 25 µl 2x1 step buffer (Reaksiyon buffer, dNTP mix), 2 µl 1x step enzim mix (Reverstranscriptase, Taq Polimeraz, Rnase inhibitör) ile hazırlanmıştır.

TSWV için PCR koşulları; 50 °C’de 30 dakika, 94 °C’de 2 dakika; 94 °C’de 30 sn, 54 °C’de 30 sn, 72 °C’de 30 sn 30 döngü; 72 °C’de 1 dakika ve 4 °C’de ∞ olarak uygulanmıştır.

PCR ürünleri ethidium bromide ilaveli %1’lik agaroz jelde 100 V’da 1 saat süreyle koşturularak, UV ışığı altında görüntülenmiştir.

3. Bulgular ve Tartışma

Antalya ilinin Finike, Serik, Aksu, Kaş ve Muratpaşa ilçeleri marul üretim alanlarında gerçekleştirilen arazi çalışmalarında kloroz, mozaik, deformasyon, nekroz ve baş büyüklüğünde azalma gibi genel olarak virüslerden kaynaklanan belirtiler gözlenmiştir (Şekil 1, 2). Benzer belirtiler farklı araştırmacılar tarafından da ifade edilmiştir (Yeşil ve Ağca, 2021; Akbaş ve ark., 2021).



Şekil 1. Yapraklarda nekroz ve deformasyon belirtisi



Şekil 2. Yapraklarda solgunluk, nekroz, bitkide baş büyüklüğünde azalma

Antalya ilinde sürvey alanlarından toplam 92 yaprak örneği alınarak DAS-ELISA testine tabi tutulmuştur. Toplanan örnek sayısı,

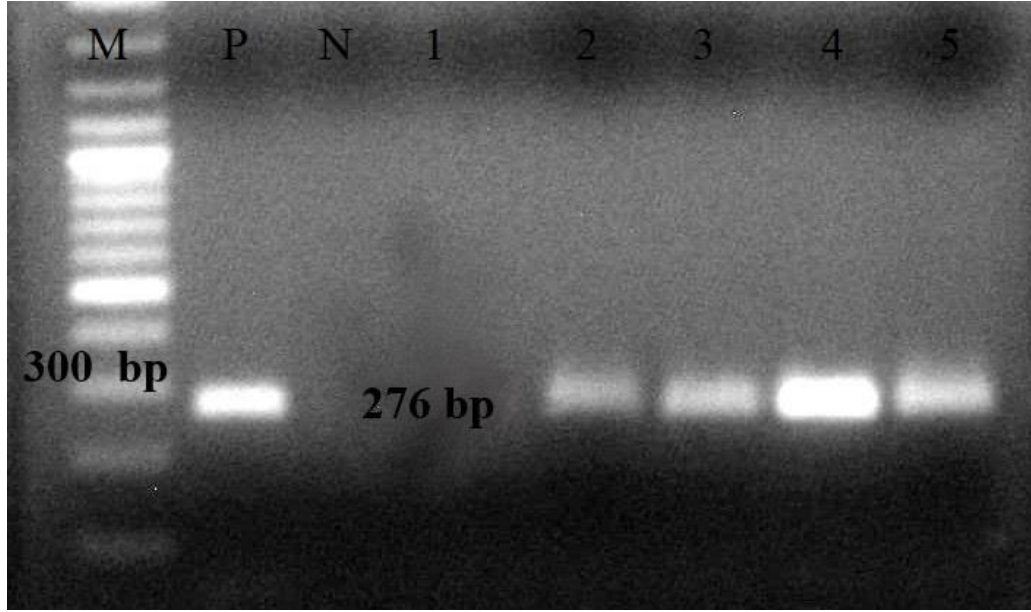
TSWV ile enfekteli örnek sayısı ve enfeksiyon oranları Tablo 1’de verilmiştir.

Tablo 1. DAS-ELISA testi sonucunda marul bitkilerinde belirlenen TSWV enfeksiyon oranı

Örnek Alınan Yer	Araziden alınan örnek sayısı	TSWV ile bulaşık örnek sayısı	Enfeksiyon oranı (%)
Finike	24	18	75
Serik	26	18	69.2
Muratpaşa	20	2	10
Aksu	11	0	0
Kaş	11	1	9.09
Toplam	92	39	42.39

Tablo 1’de DAS-ELISA test sonuçlarına göre Finike, Serik, Muratpaşa, Aksu ve Kaş üretim alanlarından toplanan 92 adet örneğin 24’ü Finike’den, 26’sı Serik’den, 20’si Muratpaşa’dan, 11 adeti Aksu ve yine 11 adet Kaş’tan toplanmıştır. Toplam 92 örnekten DAS-ELISA sonuçlarına göre 39 adet örnek TSWV ile enfekteli bulunmuştur. Enfeksiyon oranları, Finike’de % 75; Serikte % 69.2 Muratpaşa’da % 10 Kaş’ta ise %9.09 olarak tespit edilmiştir. Aksu’da ise TSWV enfeksiyonuna rastlanmamıştır.

Finike, Serik, Muratpaşa ve Kaş marul örneklerinden DAS-ELISA testi sonuçlarında pozitif çıkanlar ile Aksu’dan alınan bir örnek total RNA izole edilerek PCR çalışmalarında kullanılmıştır. RT-PCR çalışmalarına göre; DAS-ELISA testinde pozitif reaksiyon veren 4 izolat beklenen seviyede bant verirken; DAS-ELISA testi sonucunda negatif reaksiyon veren Aksu izolatında bant oluşumu gözlenmemiştir. Negatif kontrolde de herhangi bir bant oluşumu gerçekleşmemiştir.



Şekil 3. M: markır (100 bp DNA ladder; P: pozitif örnek; N: negatif örnek 1: Aksu örneği; 2: Finike örneği 3: Serik örneği 4: Muratpaşa örneği; 5: Kaş örneği

Kamberoğlu ve Alan (2011) Adana ve Mersin illeri marul üretim alanlarında yaptıkları çalışmada toplanan 336 örnekten 4'ünün TSWV ile enfekteli olduğunu bildirmiştir. Tanılama yöntemlerinden DAS-ELISA ve RT-PCR yöntemlerini kullanmışlar ve virüse özgü 811 bp büyüklüğünde bant elde etmişlerdir. Konya ili marul üretim alanlarında yapılan farklı bir çalışmada; 97 adet marul örneğinin testlenmesi sonucunda örneklerin %27.83'ünün TSWV ile enfekteli olduğu bulunmuştur (Yeşil ve Ağca; 2021). Ankara ilinde marullarda virüs hastalıklarının belirlenmesi ile ilgili çalışma da testlenen örneklerde TSWV'ne rastlanmamıştır (Ertunç ve Zelyüt, 2019). Akbaş ve ark., (2021) aynı bölgede yaptıkları çalışmada yine TSWV enfeksiyonuna rastlanmamışlardır.

Sertkaya (2012) Hatay ilinde marullarda yaptığı çalışmada TSWV'nün enfeksiyon oranını % 8.2 olarak belirlemiştir. Sertkaya (2015)'de aynı bölgede yaptığı farklı bir çalışmada da testlenen 53 adet marul örneğinde, TSWV enfeksiyon oranını % 5.6 olarak belirlemiştir.

Bozdoğan ve Kamberoğlu (2015), Antalya ili sebze üretim alanlarında TSWV tanılama çalışması ile ilgili yaptıkları çalışmada, TSWV'nün varlığını hem DAS-ELISA hem de

RT-PCR ile ortaya koymuşlardır. RT-PCR çalışmaları sonucunda 276 ve 514 bp büyüklüğünde bant elde etmişlerdir.

4. Sonuçlar

Virüs hastalıklarının mücadelesinde kullanılan herhangi bir pestisit bulunmamaktadır. Bu sebeple virüs hastalıklarının belirlenmesi, bitkilerin virüs enfeksiyonlarından korunmasında en önemli adımlardan birini oluşturmaktadır. Böcekler virüslerin taşınmasında vektör olarak çok etkilidirler. Bu nedenle virüs hastalıklarının yaygınlıklarının azaltılmasında; vektörünün biyolojisinin araştırılması, enfekteli bitkilerin ve hasat sonrası artıkların toplanması veya yakılarak imha edilmesi, virüsten arı üretim materyalinin kullanılması oldukça önemlidir.

Yapılan bu çalışma ile TSWV'nin maruldaki varlığı hem DAS-ELISA yöntemi hem de RT-PCR yöntemi ile ortaya konulmuştur. Bununla birlikte, virüs ve virüs benzeri patojenlerden kaynaklı marul bitkilerinin hepsinde TSWV etmeni tespit edilmemiştir. Böylece, bu belirtiler farklı virüs hastalıklarının olabileceğini, bazılarının TSWV etmeni ile birlikte şiddetli enfeksiyonlara neden olabileceğini işaret etmektedir. Dolayısıyla, gelecek çalışmalar

için bölgede farklı viral türlerin araştırılması önem arz etmektedir.

Yazarların Katkı Beyanı

Yazarlar makaleye eşit katkıda bulunduklarını, makalenin yayına hazır son halini gördüklerini/okuduklarını ve onayladıklarını beyan ederler.

Çıkar Çatışması Beyanı

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Determination of Antioxidant Activities of *Echinacea purpurea* (L.) Moench. Plant Extracts Harvested at Different Times

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Abstract

This research was carried out to determine the antioxidant activities of extracts obtained from plant parts of *Echinacea purpurea* L. (Moench) species harvested at different times. In the preparation of plantation, seeds for seedling production were planted in peat-containing pots on 24 April 2012. Rooted seedlings were planted in the experimental area in May. After planting, necessary cultural practices (irrigation, hoeing, etc.) of the plants were performed in 2013, 2014 and 2015. In this study, cone, leaf, flower and root parts of *Echinacea* plant harvested at different times between September 2014 and August 2015 were used. This study was conducted using the cone, leaf, flower, and root parts of the *echinacea* plant. While the cone, leaf and flower parts were harvested on 3 different dates, namely 24 July, 6 August and 19 August, the root part was harvested only once on 10 October. In this study, it was stated that the antioxidant activities of *Echinacea purpurea* species vary depending on the harvest period and the organs of the plant. The values of the highest total phenolic content and total flavonoid compound content were calculated. These values were determined as 59.107 mg GAE g⁻¹ extract and 1807.286 mg QE g⁻¹ extract, respectively. The lowest DPPH value was found to be 1.157 mg mL⁻¹. These datas were found for the flower parts of the plant from the first harvest time, the leaf parts from the second harvest period and the flower parts from the second harvest date, respectively.

Research Article

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1. Introduction

In today's societies, people work not only to eat, but also to eat healthy and improve their quality of life. In recent years, fields such as organic nutrition, herbal medicine, and alternative medicine are rapidly gaining ground and becoming widespread. Echinacea plant is also increasingly gaining ground as an important medicinal and aromatic plant. Studies have reported that approximately 25.000 plant species in the world are used for medicinal purposes (Kan, 2011). According to another study over than 50.000 species are utilized for medicinal purposes (Jamshidi-Kia et al., 2018). Asteraceae is one of the richest families of flowering plants and is represented by approximately 1000 genera and 20000 species on earth. In Türkiye, 134 genera and 1156 species belonging to this family have been identified (Davis et al., 1988). Echinacea (*Echinaceae purpurea* L.), a member of the Asteraceae family, is a medicinal plant. (Öner et al., 2023) One of the members of this family is the Echinacea species, which is very important economically.

Echinacea is a plant originating from North America. It is known by names such as "Cone Flower, Black Sampson, Red Sunflower" in English. In Türkiye, it is known by many different names such as "echinacea, purple hedgehog, hedgehog, thin-leaved lavender cocoon flower, samson root" (Mat, 2002). The word *Echinacea* is a Greek word derived from the word "echinos" meaning sea urchin or hedgehog. It is stated that the thorn-like flower structures on the flower base give the plant its name *Echinacea* (Mistrikova and Vaverkova, 2007).

Echinacea species are perennial herbaceous plants that reach 10-60 cm in height. The stem of the plant, which has taproot or fringe roots, is in a vertical position. The plant has a simple or branched stem structure. The plant is very resistant to drought and can regenerate itself (Mistrikova and Vaverkova, 2007). The leaves are oval-lance-shaped and have 3-5 veins. The center of the cone-headed flower is surrounded by radial flowers. The colors of radial flowers

vary from pink, white, purple, and red (Çalışkan and Odabaş, 2011).

Echinacea was the first introduced to the medical world by Meyer, a German doctor, in the 1870s (Hobbs, 1994). It is stated that the doctor, who uses the blood purifying medicine prepared from *E. agustifolia* roots in the treatment of many diseases such as rheumatism, migraine, pain, snakebite, wounds, indigestion, plant poisoning, poisonous snake bites, learned how to use the plant from the American Indians. This prepared medicine attracted the attention of Dr. John King and Pharmacist John Uri Lloyd, and subsequently the first scientific studies on echinacea were initiated by these two scientists. It is also noted that while initially only *E. angustifolia* roots were used, later *E. pallida* roots were also used (Mat, 2002). Extracts and preparations obtained from echinacea species have gained an important place in the herbal medicine market in many countries of the world, especially European countries and the United States (Upton and Graff, 2007). In 2019, three commonly used species, including *E. angustifolia*, *E. pallida* and *E. purpurea* had a market value of \$120 million in the US market, up 4.9 % compared to the previous year. In addition, it was stated that echinacea sales increased significantly by 90.9 % in the first half of 2020 in connection with the Covid-19 epidemic (Smith et al., 2020).

There are many studies have been indicated that the *Echinacea purpurea* plant has antioxidant properties. The antioxidant system is basically defined as a powerful mechanism that prevents the development of free radicals and peroxide reactions in the organism (Kobylynska and Tymochko, 2000). It has been reported that *Echinacea purpurea* can provide extra protection by maintaining normal redox status, especially when the body is exposed to infections (Chen et al., 2010; Merali et al., 2003). *Echinacea purpurea* contains important phenolic compounds. Kafaric and cichoric acids are two of these phenolic compounds. These phenolics are found in whole parts of the plant for instance,

flowers, leaves, roots, and stems. It is stated that the Asteraceae family in general and the echinacea plant in particular have antioxidant, anti-inflammatory, antiviral and immunostimulatory effects (Lee and Scagel, 2009). Studies have found that echinacea is an extremely powerful antioxidant in terms of rosmarinic acid, cichoric acid and caffeic acid derivatives, which are important antioxidants that suppress the negative effects of free radicals on metabolism (Jahanian et al., 2017). *Echinacea purpurea* powder was added to the diet of 320 (240 female, 80 male) Sudani ducks, 32 weeks old, under summer conditions and the results were investigated in terms of egg performance, serum lipid profile, antioxidant properties and semen quality. When the results were examined, egg number and mass, laying rate, feed consumption and feed conversion rate varied significantly according to the levels of *Echinacea purpurea* powder in the diet. With this study, it was determined that 2.5 g kg⁻¹ dietary *Echinacea purpurea* powder supplementation had beneficial effects on productivity and reproductive performance, as well as lipid profile and antioxidant status, and was economically valuable in breeding ducks in summer conditions (Awad et al., 2020). According to a study, it was determined that tinctures of the aerial parts of *Echinacea purpurea* based on 70 % ethanol exhibited antioxidant and antimicrobial activity against *Candida albicans* and *Saccharomyces cerevisiae* (Stanisavljevic et al., 2009). It has been stated that *Echinacea purpurea* tinctures can be used in the treatment of infectious diseases occurring in the oral cavity (Yezerska et al., 2022).

Studies on *Echinacea* species have shown that the roots of the plant are used in the treatment of many diseases such as rheumatism, migraine, plant poisoning, and snakebite (Mat, 2002). In a study conducted on the elemental components of *E. purpurea* grown in Serbia, it was stated that the flowers of the plant are rich in minerals such as Cu, Zn and Ni, and the leaves are rich in minerals such as Mg, Ca, Fe, Li and Sr (Razic, et al., 2003). In the light of all these findings, the importance

of examining different plant parts of echinacea is seen. In this study, extracts were prepared from the petals, leaves, central cone, and root parts of the *Echinaceae purpurea* L. plant harvested at different times, and the DPPH radical repellent effect of these extracts and the phenolic and flavonoid compounds were determined.

2. Materials and Methods

In the study, *E. purpurea* L. plants propagated from seeds were used as plant material.

2.1. General characteristics of the region where the trial was conducted

The plant samples used in the research were obtained from the echinacea plantation established in Gedikhasanlı Research and Application Center, which belongs to Yozgat Bozok University Faculty of Agriculture, in June 2012. In preparation for the plantation, seeds for seedling production were sown in viols containing peat on April 24, 2012. The seeds germinated in approximately 2 weeks and the seedlings were planted in the pre-planned trial area after they grew to an average height of 15 cm. Seedlings were planted in the trial area with a distance of 80 cm between rows and 50 cm on rows. The total trial area was prepared as 180 m².

The soil where the echinacea plantation is located is loamy in structure, slightly alkaline, salt-free, low in lime, organic matter, phosphorus and zinc, poor in nitrogen, deficient in iron content, sufficient in copper, manganese, and magnesium, and rich in potassium. Soil pH was measured as 7.6. Three different harvest times were determined for the petal, leaf, and central cone of the plant, and these were carried out on 24 July, 6 August, and 19 August 2015, respectively. The root part of the plant was harvested on a separate date, on October 10, 2015. The plant parts, collected separately according to harvest time and dried separately, were chopped into small pieces with the help of a grinder.

2.2. Preparation of extracts

Different parts of the *Echinacea purpurea* (L.) Moench plant such as leaves, flowers, cones, and roots were dried in the shade and ground with the help of a blender. 4 g of each sample obtained was weighed and 40 mL of methanol (1/10 w/v) was added. It was kept in the oven (Elektromag M 5040 P, Türkiye) at 40 °C for 24 hours. The resulting solutions were centrifuged (Universal320 R) at 9.000 rpm. With the help of solvent evaporator (Buchi, Germany) has been removed. After determining the amounts of the extracts obtained, methanolic extracts were obtained. The extracts were stored at +4 °C until analyzed.

2.3. Determination of DPPH radical scavenger activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, a known radical, was used to determine the free radical activities of the extracts. The amount of extract that neutralized a certain amount of DPPH radicals was determined and comparisons were made between samples. To determine the DPPH radical scavenging

activity, the concentration was prepared by dissolving 4 mg DPPH in 100 ml of methanol. This study was conducted using extracts dissolved in methanol. 8 mg ml⁻¹ extract solution was prepared as the main stock and then 8 different concentrations were obtained by dilution. 3.2 ml of DPPH radical and 200 µl of extract solutions of different concentrations were added to each sample and incubated for 30 minutes at room temperature in the dark. After incubation, absorbance values were measured at 517 nm for each sample. Spectrophotometric measurements to determine DPPH radical scavenging activity were carried out using the PerkinElmer Lambda 25 UV/VIS spectrophotometer device. Following this process, 50 % inhibition values were calculated for each sample. Ascorbic acid was used as the standard antioxidant. For control, methanol equal to the amount of extract solution was added to the test tube. Each experiment was carried out with 3 replications and each replication was carried out twice. The following formula was used to determine the % DPPH radical scavenger (Gezer et al., 2006).

$$\% \text{DPPH radical scavenger} = [(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}}] \times 100$$

Antioxidant changes causing 50 % inhibition were obtained by linear regression from the graph drawn with the calculated % inhibition value against different concentrations. The results were expressed as mg mL⁻¹.

2.4. Determination of total phenolic content (folin method)

During this study, the Folin-Ciocalteu Reagent (FCR) method was used to determine the total phenolic content of the extracts (Singleton et al., 1999). For the study, 200 µl of the samples prepared with methanol (2 mg ml⁻¹) was taken and 9 ml of distilled water was added. After adding 200 µl of Folin-Ciocalteu reagent, it was waited for 3 minutes and added to the test tube with the chemical containing 600 µl Na₂CO₃ (20 %), making a total of 10 ml. The samples were incubated at room

temperature and in the dark for 2 hours, then absorbance values were measured at 760 nm. Spectrophotometric measurements were made with the help of PerkinElmer Lambda 25 UV/VIS spectrophotometer device and total phenolic content was determined. To create the standard calibration curve, gallic acid dissolved in distilled water was used and 200 µl of methanol was added to each test tube. 0.1 mg ml⁻¹ gallic acid was prepared as the main stock and 9 different concentrations were obtained by dilution. Total phenolic substance of all plant extracts was calculated as mg gallic acid equivalent GAE g⁻¹ extract according to the gallic acid standard table. Each experiment was performed in 3 replicates.

2.5. Determination of total flavonoid content

Flavonoids protect cells from free radicals as potent antioxidants (Şenkal, 2020). Total

amounts of flavonoid compounds in the extracts were calculated by the method of Arvouet-Grand et al (Arvouet-Grand et al., 1994). 2 mg ml⁻¹ was prepared from the extract solution dissolved in methanol as the main stock. During the preparation of the experiment, 100 µl of 10 % aluminum nitrate and 100 µl of 1M potassium acetate were taken and the extract was added to make the final volume of the plant extract 100 µg ml⁻¹. The final concentration of the experiment was completed with 99 % ethanol to 5 ml. The absorbance values at 417 nm were measured for the samples kept in the dark and at room temperature for 40 minutes. For the quercetin standard, 0.5 mg ml⁻¹ was prepared as the head stock and 8 separate concentrations were acquired by dilution. Total flavonoid substance content is expressed as mg quercetin equivalent QE g⁻¹ extract. Spectrophotometric measurements to determine total flavonoid content were carried out using the PerkinElmer Lambda 25 UV/VIS spectrophotometer device. Each experiment was carried out in three replicates.

2.6. Statistical analyses

The data discussed in the study were evaluated in the TARIST program according to the Random Blocks Trial Design and variance

analysis was performed. Differences in variance analysis were checked with the Least Significant Difference (LSD) test (Açıkgöz and Gökçöl, 2004).

3. Results

3.1. Extraction efficiency of samples

The extraction efficiencies of the petal, leaf, central cone and root parts of the Echinacea plant at different harvest times were compared. The difference between the samples was found to be statistically insignificant. Extraction yields were calculated based on 4 mg dry matter amount. The amount and percentage of extract obtained from the petal parts of echinacea at the end of the first and second harvest periods were found to be the same, but a decrease was observed in the values obtained in the third harvest period. In the leaf, higher values were observed in the third harvest period compared to the first and second harvest periods. Although the highest value from the central cone was obtained in the third harvest period, the lowest value was observed in the second harvest period. The root part of the plant was harvested once at the end of the vegetation time, and 0.344 mg of extract was obtained from the root harvest.

Table 1. Extraction yields of leaves, petals, central cone and root parts of *Echinacea purpurea* L. (Moench) plant

Samples ¹	Amount of Extract Obtained (g)	Percentage of Extract Obtained (%)
F1	0.452	11.3
F2	0.452	11.3
F3	0.401	10.0
L1	0.290	7.3
L2	0.290	7.2
L3	0.332	8.3
C1	0.524	13.1
C2	0.507	12.7
C3	0.533	13.3
R	0.344	8.6

¹F: Flower, L: Leaf, C: Cone, R: Root 1,2,3: Harvest Time, Amount of Dry Matter: 4 g

3.2. Total flavonoid, total phenolic and dpph radical scavenging activity

Total flavonoid, total phenolic and DPPH values of plant parts of the Echinacea plant harvested at different times were calculated.

Values were found by taking the average of 3 repetitions. The differences between the mean values of DPPH radical scavenging activity and phenolic content of the samples were found to be statistically significant at the 1 % level, and the total phenolic content was found

to be significant at the 5 % level. The highest value in total phenolic substance content was reached with 59.10 mg GAE g⁻¹ extract from the flower parts of the first harvest period, while the lowest value was observed with 21.94 mg GAE g⁻¹ extract from the flower parts of the third harvest period. The highest value in total flavonoid content was obtained from the leaf parts with 1807.28 mg QE g⁻¹ extract in the second harvest period, while the lowest

value was recorded as 68.23 mg QE g⁻¹ extract in the root harvest period. The highest DPPH content was 8.09 mg ml⁻¹ in the root period, and the lowest was 1.15 mg ml⁻¹ in the flowers in the second harvest period. It was determined that antioxidant activity decreased as the DPPH value increased. The value of ascorbic acid was measured as 0.07 mg. All values of the findings obtained are presented in Table 2.

Table 2. Total phenolic, total flavonoid and DPPH values of extracts obtained from plant parts of *Echinacea purpurea* L. (Moench) species

Samples	Total phenolic (mg GAE g ⁻¹ extract)	Total flavonoids (mg QE g ⁻¹ extract)	DPPH (mg ml ⁻¹)
F1	59.107 ^{A1}	305.381 ^D	3.807 ^D
F2	38.358 ^B	168.873 ^E	1.157 ^G
F3	21.947 ^F	104.428 ^G	3.597 ^D
L1	41.250 ^B	1426.333 ^G	2.257 ^F
L2	31.470 ^{CD}	1807.286 ^F	2.487 ^{EF}
L3	23.222 ^F	1284.429 ^G	2.630 ^E
C1	26.028 ^{EF}	112.365 ^G	2.250 ^E
C2	30.875 ^{CD}	142.523 ^F	7.057 ^B
C3	27.559 ^{DE}	120.619 ^G	4.097 ^C
R	32.406 ^C	68.238 ^H	8.090 ^A
AA ²			0.070 ^H
LSD (0.05)	4.114	19.913	0.276

¹The difference between means marked with the same letter is statistically insignificant at the 5% level. ² Ascorbic Acid

According to the analysis of variance, the differences observed between the DPPH radical scavenging activities of leaves, petals, central cone, and root parts obtained from three different harvest times were found to be statistically significant at the 0.01 % level.

Flower parts were in the same group at the 5 % importance level in the first and third harvest periods. In addition, the difference between the cone parts in the first harvest period and the leaf parts in the third harvest period was found to be statistically insignificant (Table 2).

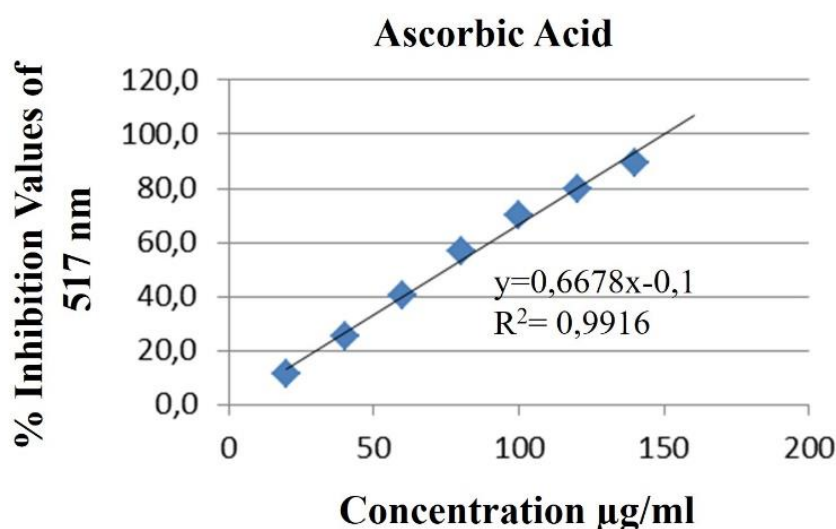


Figure 1. Percentage inhibition of dpph radical by standard ascorbic acid according to concentration

Total phenolic content of plant extracts of *Echinacea purpurea* L. (Moench) species harvested in different periods were calculated as mg GAE g⁻¹ extract. According to the analysis of variance, the differences recorded between the total phenolic content values of leaves, petals, cone, and root parts obtained from three different harvest times were found to be statistically significant at the 0.01 % level. The grouping values of total phenolic content values of plant parts obtained from three different harvest periods of the *Echinacea purpurea* L. (Moench) species are shown in Table 2. As a result of statistical analysis, cones, and leaves in the second harvest period were in the same group. In

addition, the flowers in the second harvest period and the leaves in the first harvest period were in the same group. As a result of the evaluations, the highest value in terms of total phenolic content was acquired from the flower parts in the first harvest period with 59.10 mg GAE g⁻¹, and the lowest value was obtained from the flower parts in the third harvest period with 21.94 mg GAE g⁻¹. As a result of the statistical analysis, no divergence was observed between the cone and leaf parts of the plant in the second harvest period. The absorbance value R² of the gallic acid standard curve of *Echinacea purpurea* L. (Moench) species at 760 nm was calculated as 0.9989. The specified values are shown in Figure 2.

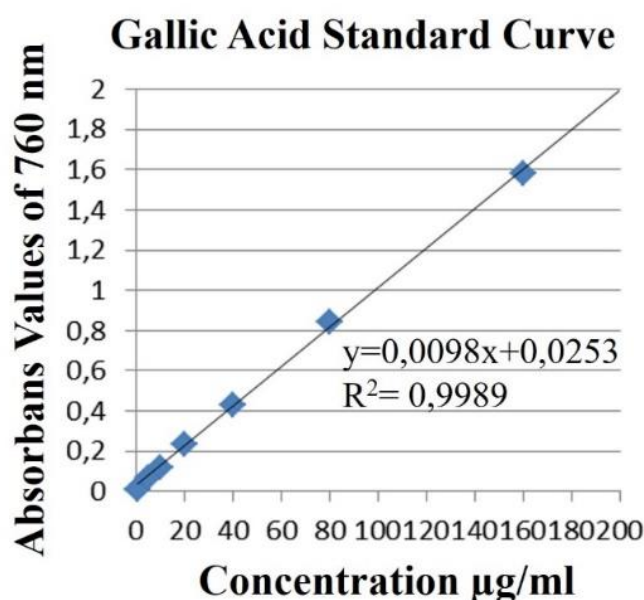


Figure 2. Standard curve for phenolic content determination in *Echinacea purpurea* L. (Moench)

Total flavonoid content values of plant extracts gained from different harvest periods of *Echinacea purpurea* L. (Moench) species were determined as mg QE g⁻¹ extract. According to the analysis of variance, the differences recorded between the total flavonoid content values of leaves, petals, cones, and root parts obtained from three different harvest times were found to be statistically significant at the 0.05 % level (Table 2). As a result of the evaluations, the highest value in terms of total flavonoid content was obtained from the second period

leaf harvest with 1807.28 mg QE g⁻¹, and the lowest data was obtained from the root harvest with 68.23 mg QE g⁻¹. As a result of statistical analysis, the cone and flower parts of the first harvest period and the cone and flower parts of the third harvest period were in the same group. The absorbance values of the Quercetin Standard Curve of *Echinacea purpurea* L. (Moench) species at 417 nm were found to be R² 0.9994 and it specified in Figure3. In Figure 4, data expressing the changes in total phenolic, total flavonoid and DPPH values are presented.

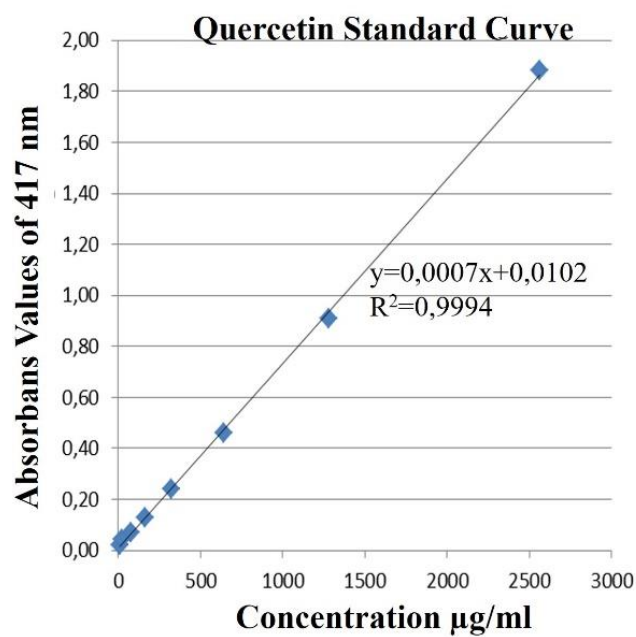


Figure 3. Flavonoid substance content quercetin standard curve graph

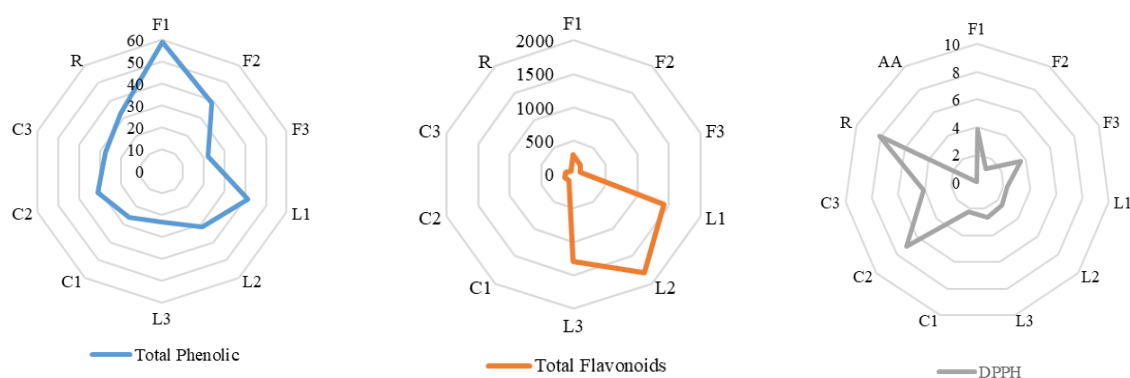


Figure 4. Changes in total phenolic, total flavonoid and DPPH values of the samples

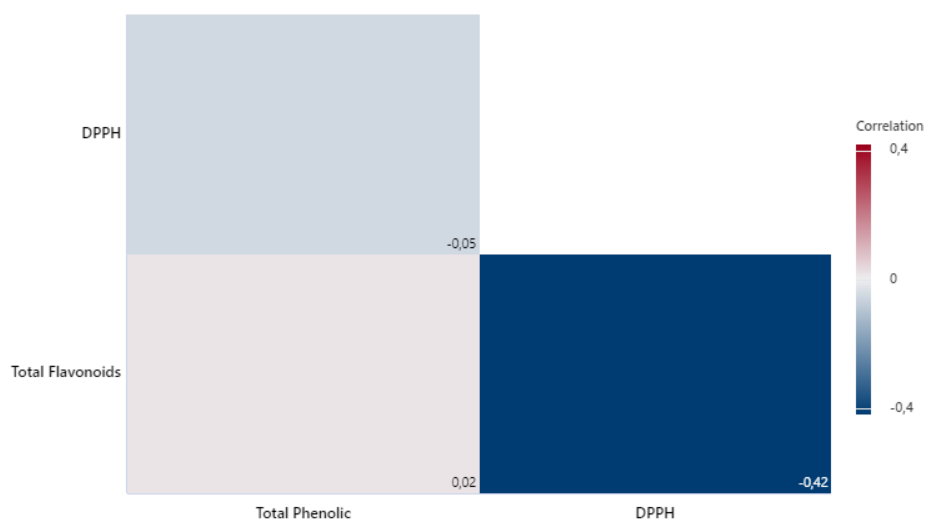


Figure 5. Correlation between total phenolics, total flavonoids and DPPH

Correlation Coefficient: 0.00-0.10 negligible correlation, 0.10-0.39 weak correlation, 0.40-0.69 moderate correlation, 0.70-0.89 strong correlation, 0.90-1.00 very strong correlation (Schober et al., 2018). Phenolic and flavonoid molecules are important antioxidant components responsible for neutralizing free radicals as they can donate hydrogen atoms to free radicals. In addition, it is stated that they have ideal structural properties for free radical scavenging (Amarowicz et al., 2004). Different studies indicate that total phenolic and flavonoid content has a linear correlation with antioxidant capacity (Shrestha and Dhillon, 2006). In our study, a moderate correlation was observed between DPPH and total flavonoids. No significant relationship was determined between total flavonoids and total phenolics and DPPH and it shown in Figure 5.

4. Discussion and Conclusion

Echinacea purpurea plant contains important phenolic compounds. Secondary metabolites such as phenolic compounds are of great importance for the continuity of the medicinal properties of the *Echinacea purpurea* plant (Cao and Kindscher, 2016). One of the important points regarding medicinal plants is to evaluate the effect of environmental conditions on the quality of the plant. In addition, studies on medicinal plants have focused on finding the conditions under which the plant reaches the highest point in terms of secondary metabolites (Daniel, 2016). Flower, leaf, root and stem parts may contain these phenolics (Lee and Scagel, 2009). According to studies conducted on the echinacea plant, it has been stated that the highest phenolic acid content is observed in the leaves and flowers and reaches the highest point (6.6 %) in the full flowering phase. In addition, the researcher found that the chemical compound contents of the echinacea flower may differ depending on the parts of the plant and that the highest caffeic acid concentration is in the flowers and roots (Foster, 1991). Caffeic acid derivatives such as echinocside, cichoric acid, caftaric acid, cynarin and chlorogenic acid are among the

important phenolic compounds for *Echinacea purpurea* (Cao and Kindscher, 2016). These compounds can have positive effects on the immune system thanks to their antioxidant and antimicrobial properties. Caffeic acid concentrations may vary depending on the organ in which it is found, environmental conditions and development period. In general, the concentration of caffeic acid derivatives is higher in roots than those in leaves and stems in *Echinacea purpurea* (Billah et al., 2019). Hu and Kitts (2000) reported in their study that methanol extracts of freeze-dried *E. purpurea* roots exhibited antioxidant activity. In this study, the highest phenolic content value of *E. purpurea* was obtained from the petals in the first harvest period. On the other hand, different organs of the plant exhibited different antioxidant capacities at different harvest times. Researchers stated that the total phenolic substance content in ethanol extracts obtained from *E. purpurea* was 11.0 ± 1.0 gallic acid equivalent on dry matter and that DPPH exhibited radical scavenging activity (Lee et al., 2009). In this study mentioned, the total phenolic substance of methanolic extracts obtained from *E. purpurea* was recorded as 21.9 ± 59.1 gallic acid equivalent on dry matter. This value is higher than reported by researchers. As a result of a study, it was specified that the DPPH radical scavenging activity determination was 85.1 % in 0.5 mg mL^{-1} extract and the IC₅₀ value was 0.23 mg mL^{-1} . As a result of a study published in 2015, the total phenolic compound content of *Echinacea purpurea* extracts was found to be 10.57 % GAE and the IC₅₀ value was 15.67 µg/mL . It has been stated that *Echinacea purpurea* extracts show strong antioxidant activity (Facino et al., 1995; Jukić et al., 2015). In this study, the IC₅₀ value of the samples was recorded between $1.157\text{--}8.090 \text{ mg mL}^{-1}$. In conclusion, *Echinacea purpurea* L. plant has strong antioxidant capacity. When compared in terms of DPPH properties, the flower parts of the plant showed the highest activity. Considering the DPPH analysis, it was stated that the first harvest period was suitable for the flower and leaf parts and the second harvest period was suitable for the cone. It was

observed that the first harvest period was suitable for flower and leaf parts and the second harvest period was suitable for cones in terms of phenolic compounds. In terms of flavonoid compounds, the highest value was obtained from the leaf parts in the second harvest period. In addition, the organ of the plant with the highest flavonoid content was found on the leaves. Factors such as plant organs and harvest time cause differences in the antioxidant activities of *Echinacea purpurea* plants. It has been stated by many researchers that the differences in antioxidant activities of *Echinacea purpurea* species vary depending on factors such as genotype, climate, and growing conditions.

Declarations of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicting Interests

The authors declare no conflict of interest.

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Investigation of Some Antibiotic Residue Levels in Honey Produced in Ardahan Province

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Abstract

Beekeeping has been a major agricultural activity since ancient times. The importance of beekeeping is still present in our country, just like in other parts of the world. Honey, beeswax, pollen, propolis, royal jelly, and bee venom are the products produced by beekeeping. Honey is particularly important for human nutrition among these. It is crucial that the honey is free of antibiotics and other residues for this reason. The purpose of this study is to determine the level of residues of certain antibiotics (tetracycline and sulfonamide group) in honey produced in Ardahan province. As part of the research, honey samples from beekeepers in Ardahan and its counties (Göle, Çıldır, Hanak, Damal, and Posof) were collected in August 2023. 90 honey samples were collected from 15 different apiaries operating in each settlement. The residue level of tetracycline and sulfonamide group antibiotics in the honey samples collected directly from the producers was determined using the ELISA method. In this study, tetracycline and sulfonamide group antibiotic residues were not found in any of the 6 centers sampled, namely Ardahan, Göle, Çıldır, Hanak, Damal, and Posof. The research concluded that the honey that is produced in Ardahan province is safe for human health. It is thought that it would be useful to provide training to beekeepers on the subject in order to maintain healthy honey (free of antibiotics and other residues) production in Ardahan province.

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1. Introduction

Beekeeping is a significant agricultural activity that has been practiced since ancient times. As in the rest of the world, beekeeping maintains its importance in our country. Our country's rich plant flora and diversity play a major role in the management of beekeeping (Ekici and Yazıcı, 2018; Öztürk, 2021). Beekeeping produces honey, beeswax, pollen, propolis, royal jelly, and bee venom. Bee creates honey from flower nectar, which is a natural product that is widely consumed throughout the world (Demirhan and Demirhan, 2022). This is why it has a special place and importance in human life (Kadirhan et al., 2019). Honey is a natural and nutritious food that is consumed by humans (Ağaoğlu et al., 2020). In addition, honey has been used in the treatment of many diseases, particularly those affecting the digestive and respiratory systems, since ancient times (Mahmud et al., 2015; Ekici and Yazıcı, 2018). The estimated annual production of honey worldwide is around 1.4 million tons (Aydemir Atasever and Yüksel, 2022). Asian countries contribute a significant amount of this production (about 40 %). Our country follows China in terms of world honey production and produces significant amounts of honey (Mahmoudi et al., 2014; Aydemir Atasever and Yüksel, 2022).

In the structure of honey, there are various vitamins, minerals, carbohydrates, amino acids, enzymes, and proteins (Kutlu et al., 2017). Because of the nutrients it contains, it is used in the production of creams used in the food and cosmetics industries (Ağaoğlu et al., 2020). Honey, which is obtained in a natural environment, can be contaminated with a range of harmful substances found in the environment (air, water, and soil) and plants (Mahmoudi et al., 2014). These harmful substances are transported to the hives by honeybees. Furthermore, the use of chemicals by beekeepers has a significant impact on the contamination of honey (Mahmoudi et al., 2014; Aydemir Atasever and Yüksel, 2022). It is reported that the most important environmental pollutants affecting bee

products are pesticides, aflatoxins, heavy metals, bacteria, and radioactivity (Mahmoudi et al., 2014; Özkan et al., 2015). Bee diseases are a major factor that affects beekeeping negatively. Serious economic losses can occur as a result of bee diseases. The most significant diseases that affect beekeeping are American foulbrood, European foulbrood, and varroa (Aydemir Atasever and Yüksel, 2022).

The residue in honey is caused by the use of unnecessary and insufficient doses of antibiotics in the fight against these diseases (Aydemir Atasever and Yüksel, 2022). Humans may experience health problems if they consume honey that contains antibiotic residue (Kutlu et al., 2017; Ağaoğlu et al., 2020). Some of the important health problems that can be seen include anaphylactic shock, allergic reactions, bacterial resistance, intestinal flora disruption, nervous and hematopoietic system diseases. It is reported that there is a possibility of carcinogenic and teratogenic effects (Apić et al., 2015; Kutlu et al., 2017; Ağaoğlu et al., 2020; Aydemir Atasever and Yüksel, 2022). The consumption of honey that contains antibiotic residues, particularly by children, the elderly, and sick individuals, can result in serious health problems (Kumar et al., 2020). As a result of these adverse effects, the use of antibiotics in apiculture is prohibited in our country as well as in European countries. The honey's maximum residue limits have not been determined due to this reason (Apić et al., 2015; Özkan et al., 2015; Ağaoğlu et al., 2020; Demirhan and Demirhan, 2022).

Beekeepers use antibiotics of the tetracycline group to treat and protect bee diseases. (Especially American foulbrood and European foulbrood) (Apić et al., 2015; Hosseinpour et al., 2021; Aydemir Atasever and Yüksel, 2022; Şahin et al., 2024). Oxytetracycline is commonly used for this purpose (Mahmoudi et al., 2014). Drugs in this group (such as tetracycline, chlortetracycline, oxytetracycline, and doxycycline) have a broad spectrum. They have bacteriostatic effects on gram-positive, gram-negative, aerobic, and anaerobic bacteria. They inhibit

protein synthesis in susceptible bacteria (Ağaoğlu et al., 2020). Other anti-bacterial medicines used in apiculture are sulfonamides. There are numerous drug derivatives in this group. Some of them include sulfadiazine, sulfamethazine, sulfadoxine, sulfabenzamide, sulfachlorophidazine, sulfathiazole, sulfamethoxazole, sulfadimethoxine, and sulfizoksazole. The drugs in this group have in bacteriostatic effect. They demonstrate their effects by replacing PABA (para amino benzoic acid), which is involved in the synthesis of folic acid, necessary for the growth and development of bacteria (Özkan et al., 2015; Kutlu et al., 2017).

According to Turkish Statistical Institute 2022 data, the production of honey in Turkey is 118,297.464 tons. In Ardahan province, there are 658 registered beekeeping enterprises. It is reported that the quantity of honey produced in the province of Ardahan is 244.963 tons (Anonymous, 2023). These data indicate that beekeeping in Ardahan is an important agricultural activity that contributes to the honey production of our country. Caucasian breed bee (*Apis mellifera caucasica* L.) breeding is carried out in Ardahan province (Cengiz and Yazıcı, 2018). It is also known as the genetic center of this breed. *Pseudomonas aeruginosa* bacterium was found in dead bees and honeycomb samples in a study conducted in Ardahan province (Kadirhan et al., 2019). In other research, it is stated that foulbrood, nosema, and lime disease are the most common diseases in Ardahan province (Cengiz and Yazıcı, 2018). The presence of bacterial diseases of bees in the province of Ardahan strengthens the possibility for beekeepers to use antibacterial drugs. The purpose of this research was to examine the residue levels of tetracycline and sulfonamide group antibiotics

in honey produced in Ardahan and its surrounding counties (Göle, Çıldır, Hanak, Damal, and Posof).

2. Materials and Methods

The research material consisted of honey samples obtained by beekeeping producers in Ardahan and its counties (Göle, Çıldır, Hanak, Damal, and Posof) in August 2023. The samples were taken from 15 different beekeepers operating in each settlement center. As a result, 90 honey samples were collected from 6 settlement centers. After harvesting, the samples that were collected directly from beekeepers (about 15 g) were placed in sterile disposable falcon tubes (50 ml) and the lid was tightly closed. The honey samples were taken from each hive to ensure homogeneity. The samples were then brought to the laboratory. Until they were tested in the laboratory, they were stored at room temperature to protect them from humidity and light. The level of antibiotic residue in the honey samples was determined using the ELISA method. Tetracycline (Cat. No: CSB-E12090f, CusaBio, U.S.A) and Sulfonamide (Cat. No: CSB-E12094f, CusaBio, U.S.A) analysis kits were utilized for this purpose. The samples were prepared and analyzed according to the instructions given by the manufacturer. At the end of the analysis, the standards and the optical density of the samples were read on the ELISA reader (BioTek ELx800, USA) at 450 nm. The optical densities obtained from samples were compared to those obtained from standard solutions, and the residues of tetracycline and sulfonamide were calculated.

3. Results and Discussion

In Table 1 below, tetracycline residue levels were presented in the honey samples obtained from Ardahan and its counties.

Table 1. Residues of tetracycline in the honey (ppb)

Region	The number of samples (n)	Tetracycline ^a Group
Ardahan	15	0 ± 0 #
Göle	15	0 ± 0 #
Çıldır	15	0 ± 0 #
Hanak	15	0 ± 0 #
Damal	15	0 ± 0 #
Posof	15	0 ± 0 #

a : Tetracycline, Minocycline, Rolitetracycline, Aureomycin, Demeclocycline, Terramycin, and Doxycycline

#: It was not detected within the test susceptibility limits (#: <0.05 ppb).

In Table 1 above, it is seen that the tetracycline group antibiotic residue could not be detected in any of the 90 honey samples taken from 6 regions (Ardahan, Göle, Çıldır,

Hanak, Damal, and Posof). In Table 2 below, the level of sulfonamide residue was presented in the honey samples obtained from Ardahan and its counties.

Table 2. Residues of sulfonamide in the honey (ppb)

Region	The number of samples (n)	Sulfonamide ^b Group
Ardahan	15	0 ± 0 *
Göle	15	0 ± 0 *
Çıldır	15	0 ± 0 *
Hanak	15	0 ± 0 *
Damal	15	0 ± 0 *
Posof	15	0 ± 0 *

^b : Sulfamerazine, Sulfadiazine, Sulfamonomethoxine, Sulfamethoxydiazine, Sulfamethazine, Sulfisomidine, Sulfadimethoxypyrimidine, Sulfadimoxine, Sulphamethoxazole, Sulfadoxine, Phthalylsulfathiazole, Sulfamethizole, Sulfaisoxazole, Sulfathiazole, Sulfaquinoxaline, Sulfamethoxypyridazine, Sulfaclozine, Sulfachloropyridazine, and Sulfabenzamide

: It was not detected within the test susceptibility limits (: <1 ppb).

Table 2 above shows that no sulfonamide group antibiotic residue was detected in any of the 90 honey samples collected from Ardahan and its counties (Göle, Çıldır, Hanak, Damal, and Posof). Beekeeping activity remains important in the agricultural sector in our country. Beekeeping's products (honey, propolis, royal jelly, beeswax, pollen, and bee venom) are a significant source of income. Pollination by bees has great benefits for agricultural products. As such, apiculture is of great importance to humans. Honey, which is a good source of nutrients for humans, is included in bee products. It has been used in the treatment of certain diseases since ancient times, in addition to its nutritional properties (Kadirhan et al., 2019). The digestive tract infections caused by *Shigella sonnei* in humans are among these diseases (Al-Masaudi et al., 2020). It is also reported for in vitro efficacy against dermatophytes such as *Trichophyton tonsurans*, *Microsporum canis*, *Aspergillus niger*, and *Candida albicans* (Mahmud et al., 2015).

Bee diseases adversely affect beekeeping and cause economic damage (Kadirhan et al., 2019). The diseases, particularly American foulbrood caused by *Streptococcus pluton* and European foulbrood caused by *Paenibacillus (Bacillus) larvae*, are both contagious and dangerous (Mahmoudi et al., 2014; Çakar and Gürel, 2019; Hosseinpour et al., 2021). The use of antibiotics for treating and preventing these

diseases can result in residue problems in honey. Such residues diminish the quality of honey and its market share (Mahmoudi et al., 2014). Additionally, when consumed as food, they can be harmful to human health. The risk increases due to the long half-life of antibiotic residues (Apić et al., 2015; Özkan et al., 2015; Kutlu et al., 2017).

Some of the antibiotics used in bees are tetracycline and sulfonamides (Mahmoudi et al., 2014; Özkan et al., 2015; Kutlu et al., 2017). Unconscious use of these drugs can lead to them being passed to humans through honey. Their presence can lead to bacterial resistance and other harmful effects in humans (Apić et al., 2015). *Crystalluria* and kidney dysfunction have been reported in humans due to sulfonamide derivatives. Besides adverse effects on the kidneys, allergic reactions, serum diseases, and mucosal lesions have been reported in sensitive individuals (Korkmaz et al., 2017). Pregnant women and children under 8 years of age should not take tetracycline group drugs as they can cause permanent color changes in their teeth (Ağaoğlu et al., 2020). The lack of antibiotic residues in honey is therefore needed to protect human health.

The fact that antibiotic residues have harmful effects on human health has prompted research on this subject worldwide and in our country. Antibiotic residues were found in 5 out of the 193 honey samples obtained in

Serbia (2.59 %) (Apić et al., 2015). An Indian study reported that oxytetracycline was detected in 15.3 % of 150 honey samples. In the same study, it is stated that 21.4 % of the samples contaminated with oxytetracycline are uncertified branded, 14.3 % are unbranded, and 10.2 % are certified branded honeys (Kumar et al., 2020). Tetracycline residues (mean $77.86 \mu\text{g kg}^{-1}$) were found in all 9 honey samples offered for sale in the United States, and it is stated that sulfonamide residue could not be detected in any of the samples (Sarkar et al., 2023). In Iran, 19 (14.07 %) honey samples were reported to contain tetracycline, while 20 (14.81 %) had sulfonamide residues (Mahmoudi et al., 2014).

The presence of antibiotics in honey has been investigated in many regions of our country. Of the 59 pine honey samples collected from the Aegean region (16 from Aydın, 36 from Muğla, and 7 from Balıkesir), it is reported that 35 contain tetracycline (6-42 ppb) residues and 31 contain sulfonamide (3-32 ppb) residues (Korkmaz et al., 2017). It is stated that 80 samples of honey sold in markets in Ankara province contain 7 different antibiotic residues. In the positive samples, there were residues of dihydrostreptomycin (58.75 %), streptomycin (22.5 %), erythromycin (13.75 %), sulfadimidine (10%), and enrofloxacin (2.5 %). In the same study, tetracycline and doxycycline were only found in one sample (Demirhan and Demirhan, 2022). Antibiotic residues were investigated in 60 samples, 30 of which were open and 30 of which were packaged, in Sivas region. It has been reported that 73.3 % of packaged honeys and 60 % of open honeys contain tetracycline residues (Ağaoğlu et al., 2020). In another study conducted on honey, it is stated that tetracycline residues at the level of 2.1-47.08 ppb (mean 9.33 ppb) were found in 37 (46.8 %) of 79 samples collected from Erzurum province (Aydemir Atasever and Yüksel, 2022). In another study, it was reported that 52 % of the 180 honey samples collected from Ardahan province contained sulfonamide residues (Özkan et al., 2015). Tetracycline and sulfonamide residues were not found in honey collected from 20 different regions in Bitlis

(Hizan) province (Kutlu et al., 2017) and 10 honey samples were collected from Muş province (Kutlu and Bengü, 2020). A study has demonstrated that tetracycline applied to hives can result in drug residues in honey in untreated hives (Martel et al., 2006).

In this study, tetracycline (Table 1) and sulfonamide (Table 2) group antibiotic residues could not be detected in a total of 90 honey samples, 15 from each of 6 different centers (Ardahan, Göle, Çıldır, Hanak, Damal, and Posof). The results obtained from this research are similar to the research results reported by Kutlu et al. (2017) and Kutlu and Bengü (2020). The fact that antibiotic residue cannot be detected in the honey can be explained by the fact that the producers in Ardahan province do not use antibiotics in bees. The results obtained from this study are not similar to the results of the research by Apic et al. (2015), Kumar et al. (2020), Sarkar et al. (2023), Mahmoudi et al. (2014), Korkmaz et al. (2017), Demirhan and Demirhan (2022), Ağaoğlu et al. (2020), Aydemir Atasever and Yüksel (2022), and Özkan et al. (2015) reporting antibiotic residues in honey. It is thought that the reasons for this may be the occurrence of different bee diseases in each region, the knowledge levels of beekeepers, and the habits of using antibiotics.

4. Conclusion

In conclusion, antibiotic residues of tetracycline and sulfonamide groups could not be detected in the honey produced in Ardahan area. This situation is crucial for the province of Ardahan, which is considered the genetic center of the Caucasian bee race. The honey that is produced in the Ardahan region is sold not only in the region but also in other regions. Therefore, the fact that it contains no antibiotic residues is beneficial to human health. It is believed that these findings will have a positive impact on the market share of Ardahan honey. It is very important to maintain healthy honey production (without antibiotics and other residues) in Ardahan area. The protection of honey against various pollutants is essential for human health. It is suggested to take some

measures for this. Regular checks on the sales of veterinary drugs and antibiotic residue levels in honey are necessary for this purpose. Furthermore, individuals who are engaged in beekeeping need to be educated on the subject. It is expected that these measures will contribute to national and international food security by promoting organic and healthy honey production.

Ethical Approval

The Ministry of Agriculture and Forestry (Ardahan Provincial Directorate of Agriculture and Forestry - dated 19.06.2023 and E-29486769-325.99-10277578) approved this research before it started.

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Identification of Plant Growth Promoting Rhizobacteria (PGPR) Isolated from Lavender (*Lavandula angustifolia* L.) and Determination of Their Antifungal Activity against *Fusarium oxysporum*

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Abstract

Plant growth promoting rhizobacteria (PGPR) have a variety of beneficial activities, including improved nutrient absorption, phytohormone regulation, and biological control of phytopathogens. Sixteen isolates from the *Lavandula angustifolia* L. rhizosphere were screened for their morphological, biochemical, and plant growth promoting properties in this study. Isolates were identified using the MALDI-TOF MS method. Additionally, its antagonistic properties against the phytopathogen *Fusarium oxysporum* were evaluated. Among all isolates, 10 isolates (QLN-1, QLN-2, QLN-5, QLN-6, QLN-7, QLN-8, QLN-11, QLN-13, QLN-14, QLN-15) fixed nitrogen, 11 isolates (QLN-2, QLN-4, QLN-5, QLN-6, QLN-7, QLN-8, QLN-12, QLN-13, QLN-14, QLN-15, QLN-16) dissolved inorganic phosphate, 8 isolates (QLN-1, QLN-2, QLN-6, QLN-7, QLN-9, QLN-12, QLN-14, QLN-16) produced siderophore, 9 isolates (QLN-2, QLN-3, QLN-4, QLN-6, QLN-7, QLN-8, QLN-12, QLN-15, QLN-16) produced IAA and 6 isolates (QLN-5, QLN-8, QLN-9, QLN-14, QLN-15, QLN-16) produced HCN. Isolate QLN-4 showed the highest antifungal activity against *Fusarium oxysporum* with an inhibition rate of 57.14 %. Isolate QLN-8 isolate (24.76 %) showed the weakest effect against *F. oxysporum*. Although research on PGPRs has increased recently, research on lavender is still limited. Our study reveals that local bacterial isolates obtained from lavender rhizosphere can be used as microbial fertilizers and biocontrol agents.

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1. Introduction

Lavender, meaning to wash in Latin, is a shrub belonging to the Lamiaceae family. Lavender oil, which has antibacterial and antioxidant properties, contains linalool, terpinen-4-ol, linalyl acetate, ocimene, acetate lavandulol, and cineole. The content of lavender essential oil varies depending on the type of lavender, the climate and soil structure of the region where it grows, and its productive and morphological characteristics (Denner, 2009). Lavender is grown all over the world, and the aromatic oils obtained from its flowers are frequently used in aromatherapy, baked goods, candles, cosmetics, perfumes, shampoos, and soaps (Çakar et al., 2021). It is thought that lavender extract prevents dementia, slows the growth of cancer cells, and is good for skin diseases. Additionally, various studies have revealed that lavender exhibits multiple pharmacological effects, such as sedative, anticonvulsant, analgesic, and local anesthetic roles (Rehman et al., 2020; Kozuharova et al., 2023). The most commonly used type of lavender is English lavender (*Lavandula angustifolia*). *L. angustifolia*, which has high commercial value and various applications, especially in the fields of medicine and pharmacy, needs to be grown naturally (Batiha et al., 2023).

Chemical fertilizers causing harm to the environment and human health, along with the excessive price increases, have necessitated the development of sustainable approaches in the cultivation of medicinal and aromatic plants like lavender. Thus, the significance of alternative environmentally friendly fertilizers is once again highlighted by this circumstance. PGPR are bacteria that colonize the plant's rhizosphere and support plant growth directly or indirectly. They promote plant growth through various mechanisms, such as nitrogen fixation, phosphate solubilization, ACC deaminase production, facilitating the uptake of N, P, K elements, and phytohormones like IAA. Indirectly, PGPRs induce systemic resistance (ISR), parasitism, antibiosis, and the production of metabolites (such as hydrogen cyanide) and antimicrobial metabolites

(Akçura and Çakmakçı, 2023). PGPRs utilize soil nutrients for plant growth, produce many regulators, protect plants from phytopathogens, improve soil structure, and reduce harmful compounds like pesticides. Therefore, rhizobacteria are crucial microorganisms for soil fertility and sustainable crop production (Riaz et al., 2021).

Although research on PGPRs has increased recently, it is still limited. Recent studies suggest that developing microbial formulations with local isolates showing activity in different ecosystems and plant species should be increased. Therefore, developing microbial fertilizers that protect the environment and comply with sustainable agriculture can significantly reduce chemical fertilizer use. This study aimed to identify bacteria in the *Lavandula angustifolia* rhizosphere using MALDI TOF MS and to determine their plant growth-promoting properties and antifungal activities against *Fusarium oxysporum*.

2. Materials and Methods

2.1. Sample collection

Rhizospheric soil samples were collected in June 2023 from *L. angustifolia* L. in the Medicinal Plants Garden of the Department of Field Crops, Faculty of Agriculture (39°57'44.2"N, 32°51'36.7"E) Ankara University. The sample was taken from the plant rhizosphere at 3 different locations at a depth of 10 cm. Each soil sample was labeled and brought to the bacteriological Laboratory and studied under aseptic conditions.

2.2. Isolation of rhizobacteria

Rhizospheric bacteria were isolated from 1 g of dried soil samples by serial dilution method. Soil samples were homogenized in 10 ml of sterile isotonic saline water. Each 1 g of soil sample was mixed in 9 ml of 0.85 % saline (NaCl) sterile water and then homogenized in a shaker for 1 hour. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6} . These dilutions were spread on nutrient agar (NA) solidified Petri dishes and incubated at 28 °C. After incubation, different colonies were

selected and planted in Petri dishes containing NA medium until a pure colony was obtained.

A total of 16 pure isolates were obtained after incubation in Luria-Bertani Broth (LB)

and stored in stock solution at -85 °C for use in research. Figure 1 shows the flowchart of the process from isolating bacteria in the rhizospheric soil sample to determine their plant growth-promoting properties.

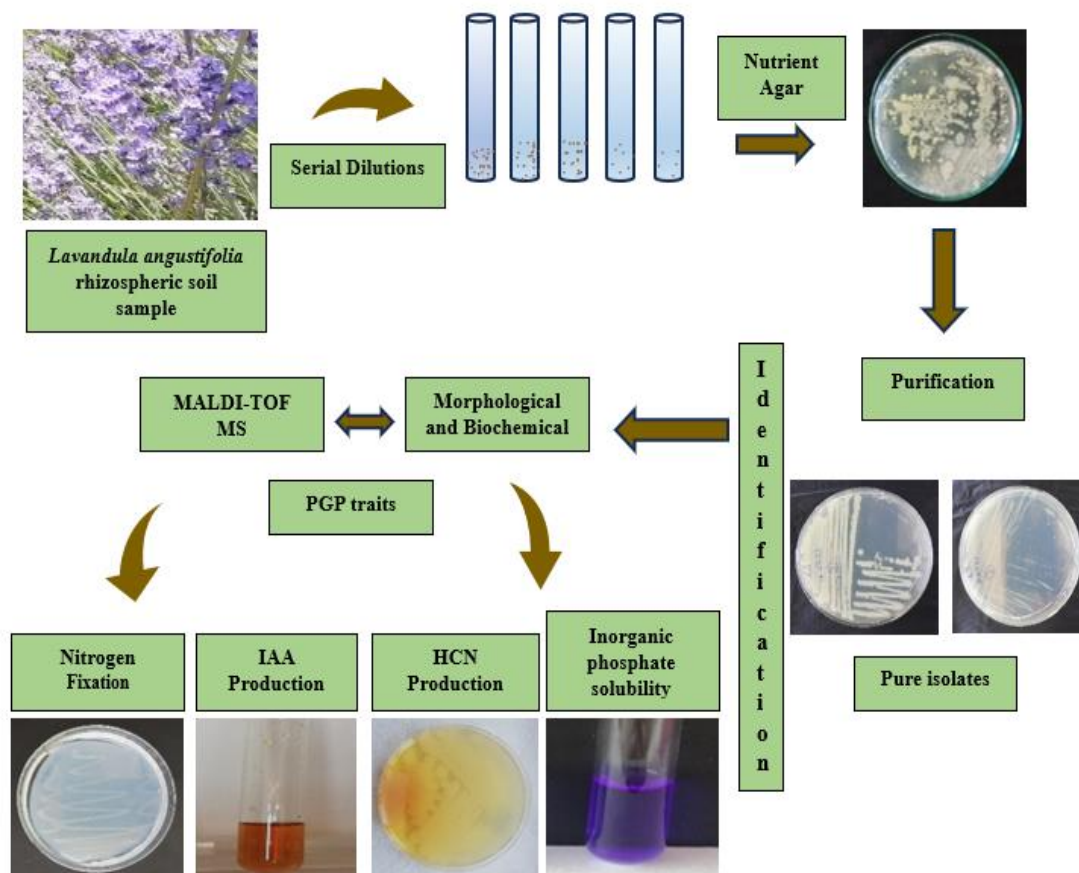


Figure 1. Flowchart representation of the process used to detect the characteristics of isolates from *L. angustifolia* rhizospheric soil

2.3. Identification of bacterial isolates

2.3.1. Biochemical and morphological characterization of isolates

Physiological, biochemical tests and Gram staining of the bacterial isolates were examined using methods described by Palleroni et al. (1984).

2.3.2. Identification of isolates with MALDI-TOF MS

MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) was used for bacterial identification. Microorganisms are identified by their unique molecular fingerprints by the MALDI Biotyper CA System. In this method,

protein profiles of microorganisms' biomolecules (such as protein, peptide, sugar, and polymer) are ionized and then passed through an electric and/or magnetic field. These profile spectra are compared graphically to reference microorganisms in the system's database to accurately identify them by genus and species (Sivri and Öksüz, 2019).

2.4. Screening of the isolates for plant growth promoting (PGP) properties

2.4.1. Determination of nitrogen fixing capacity

Determination of the nitrogen fixation abilities of the isolates was done according to the protocol specified by Wilson and Knight

(1952) and Park et al. (2005). The isolates were streaked on NA medium and incubated at 28 °C for 24 hours. Thereafter incubation, the fresh isolates were incubated for 4 days at 28±2 °C by inoculating the Petri dishes with solid Burk's N-free medium (0.05 g L⁻¹ Na₂SO₄, 10 g L⁻¹ glucose, 0.2 g L⁻¹ CaCl₂, 0.41 g L⁻¹ KH₂PO₄, 0.52 g L⁻¹ K₂HPO₄, 0.0025 g L⁻¹ Na₂MoO₄ · 2H₂O, 0.1 g L⁻¹ MgSO₄ · 7H₂O, 0.005 g L⁻¹ FeSO₄ · 7H₂O, and 1.8 g L⁻¹ agar 1 L dsH₂O) by line sowing method. After adjusting the pH of the medium to 7±0.1, it was autoclaved at 121 °C for 15 minutes. Three time intervals were determined for nitrogen fixation activity (+++: development after 6 h, ++: development after 12 h, +: development after 24 h).

2.4.2. Determination of siderophores-producing isolates

Chrome Azurol S (CAS) agar media was used to identify the production of siderophores (Schwyn and Neilands, 1987). The isolates grown in 24-hour fresh culture were inoculated on CAS agar plates and incubated for 24-48 hours at 30±2 °C. Following the incubation period, the production of siderophores was confirmed to be positive by the appearance of an orange halo surrounding the colony on CAS agar plates. For siderophore activity, Three time intervals were determined (+++: color change after 12h, ++: color change after 24h, +: color change after 36h).

2.4.3. Determination of HCN-producing isolates

The HCN production assay was carried out according to Bakker and Schippers (1987). Bacteria were inoculated on NA medium and filter papers impregnated with picric acid (0.5 % picric acid, 2 % sodium carbonate), which were placed on the edge of the Petri dishes. The Petri dishes were tightly closed with paraffin and incubated at 28±2 °C for 4 days. After incubation, yellow-colored picric acid-impregnated papers turned brown, which was considered a positive result. For HCN activity, three time intervals were determined (+++: color change after 6h, ++: color change after 12h, +: color change after 24h).

2.4.4. Determination of isolates producing indole-3-acetic Acid (IAA)

The Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce IAA. Bacterial cultures were grown for 48 hours at 36±2 °C. These fresh cultures were centrifuged for 30 min at 3000 rpm. The supernatant (2 ml) was combined with 4 ml of the Salkowski reagent (50 ml, 35 % perchloric acid, 1 ml 0.5 M FeCl₃ solution) and two drops of orthophosphoric acid. Pink appearances indicate that IAA is present. The presence of IAA in the culture supernatant was determined spectrophotometrically (Multiskan SkyHigh Microplate Spectrophotometer) at 530 nm. Three time intervals were determined for IAA activity: (+++: color change after 1 h, ++: color change after 6 h, and +: color change after 12 h).

2.4.5. Determination of isolates' inorganic phosphate dissolving capacity

The inorganic phosphate dissolving capacities of the isolates were determined qualitatively according to the protocol described by Mehta and Nautiyal (2001). 25 microliters of fresh pure bacterial culture were inoculated in tubes containing 5 ml of NBRIP-BPB medium (Brom phenol supplemented National Botanical Research Institutes's phosphate growth medium) (0.2 g L⁻¹ KCl, 10 g L⁻¹ glucose, 5 g L⁻¹ Ca₃(PO₄), 2.25 g L⁻¹ MgSO₄ · 7H₂O, 2.5 g L⁻¹ MgCl₂ · 6H₂O, 0.1 g L⁻¹ (NH₄)₂SO₄ and 0.025 g L⁻¹ BPB). It was incubated at 180 rpm at 30±2 °C for 3 days. The discoloration of the blue color of the NBRIP-BPB medium after incubation indicated that the isolates were phosphate solubilizers.

2.5. Assessment of extracellular enzyme production

Screening for amylase production was done as per the methodology of Smibert and Kreig (1994). Starch agar medium (yeast extract 5 g, NaCl 10 g, tryptone 10 g, starch 5 g, agar 15 g, pure water 1000 ml) was prepared to determine amylase activity. After planting on starch agar medium, it was placed in an oven set at 30 °C and incubated for 1 day. At the end of the

incubation, iodine solution (I₂ 1 g, KI 2 g, pure water 300 ml) was dropped onto the Petri dishes and waited for 5 minutes to examine the zone formation. Zone formation around the colony indicates the presence of amylase. The protease production of the isolates was determined according to the protocol described by Smibert and Kreig (1994). Agar (skim milk agar) medium containing milk was prepared for the protease production test, bacteria were cultivated using the streak seeding method and incubated at 37 °C for three days. Zones around the colony formed after incubation showed the presence of protease. The pectinase activity of the isolates was determined according to the protocol described by Geetha et al. (2014). Pectinase enzymes were identified in an M9 medium supplemented with 4 grams of pectin per liter. The plates were incubated for two days at 28±2 °C. The appearance of a clear halo surrounding colonies is indicative of pectinase production. Colloidal chitin was created using the Rodriguez-Kabana (1983) method. The isolates were cultured for four days on a chitin medium at 28±2 °C. There were clear zones around the bacterial colonies, indicating the utilization of colloidal chitin.

2.6. Assessment of antifungal activity

The fungal isolate (*Fusarium oxysporum*) used in the study was obtained from the culture collection unit of Ankara University, Faculty of Agriculture, Department of Plant Protection. Using a potato dextrose agar (PDA) medium, all the isolates were tested for antifungal activity against *F. oxysporum*. Bacterial isolates were grown on a nutrient agar medium at 25 °C for 24h to obtain fresh cultures. A 6 mm mycelial disc of fungi, *F. oxysporum* was placed in the center of the plates and incubated at 28°C for 7 days. Bacterial isolates were drawn on the edge of the Petri dish with a swab and incubated in the dark at 25 °C for one week. As a control, only the pathogenic fungus isolate was placed in the middle of the Petri dish containing PDA, and the evaluation was made when the pathogen fungus isolates covered the control Petri dish. The diameter of the fungus in the application Petri dishes was

measured in mm. The percent inhibition rate of bacteria and fungus colony development was determined by Mari et al. (1996), it was calculated using the percentage of inhibition of radial development formula. For each isolate, the experiments were conducted with three replicates.

$$\% \text{ Inhibition} = (A-B) / (A-M) \times 100$$

A: Colony diameter of the pathogen in the control application

M: Diameter of micellar disc (6 mm)

B: Colony diameter of the pathogen in bacterial application

2.7. Data analysis

Data for antifungal activity were analyzed in three replicates for using JMP Pro 17.0 statistical software. Dependant variables with normal distribution were presented as mean ± Standart Devision (SD). The antifungal activity was measured using analysis of variance (ANOVA). The differences in the mean amounts of variables were determined using the Tukey test (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Identification of isolates

In the present study, 16 isolates were obtained from the rhizospheric soil samples of *L. angustifolia* in Ankara, Turkey. MALDI-TOF MS analysis identified 16 isolates from soil samples in the rhizosphere, including 7 Bacillus (*B. endophyticus* QLN-1, *B. cereus* QLN-3, *B. subtilis* QLN-4, *B. mycoides* QLN-9, *B. pumilus* QLN-12, *B. megaterium* QLN-13, *B. mojavensis* QLN-16), 4 Pseudomonas (*P. koreensis* QLN-2, *P. putida* QLN-5, *P. fluorescens* QLN-8, *P. extremorientalis* QLN-15), 1 Stenotrophomonas (*S. rhizophila* QLN-7), 1 Pantoea (*P. agglomerans* QLN-6), 1 Enterococcus (*E. faecium* QLN-10) and 2 Lactobacillus (*L. plantarum* QLN-11, *L. paracasei* QLN-14). Among all isolates, 10 showed Gram (+) reaction. The catalase test was positive for all isolates except for *E. faecium* QLN-10 and *L. plantarum* QLN-11, whereas the oxidase test was positive for 12 isolates except for *B. subtilis* QLN-4, *P. agglomerans* QLN-6, *B. mycoides* QLN-9, and *E. faecium* QLN-10. Except *P. agglomerans*

QLN-9, *E. faecium* QLN-10 and *L. plantarum* QLN-11, motility tests all others samples were positive. MALDI-TOF MS is an extremely useful tool for identifying bacteria at the genus, species, and strain levels. In previous studies, many researchers used the MALDI-TOF MS method to identify bacteria (Pierce et al., 2007; Stıngu et al., 2008; Vega-Castano et al., 2012). Recently, this method has gained popularity due to its high accuracy and rapid results. Çelikten and Bozkurt (2018) used the MALDI-TOF method to identify 120 bacteria they isolated from the wheat rhizosphere to investigate plant growth-promoting bacteria. Ünlü et al. (2023) used MALDI-TOF MS to identify bacterial strains isolated from the

alfalfa rhizosphere as belonging to the genera *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Lysinibacillus*, *Acinetobacter*, and *Enterobacter*. Similarly, Öksel et al. (2022) used the MALDI-TOF MS method to identify bacteria in wheat rhizospheres. The findings of this study revealed that *Bacillus* (44 %) *Pseudomonas* (25 %) and were the most common bacterial genera in *L. angustifolia* rhizosphere. The percentage distributions of bacteria obtained from the rhizosphere of *L. angustifolia* are shown in Figure 2 and morphological, biochemical results and MALDI TOF MS score values of the isolates are presented in Table 1.

Table 1. Morphological and biochemical traits of isolates

Isolates	Gram Characteristic		Biochemical Characteristics			MALDI-TOF MS Score Value
	Gram reaction	KOH 3%	Catalase	Oxidase	Motility	
<i>Bacillus endophyticus</i> QLN-1	+	-	+	+	+	2.043
<i>Pseudomonas koreensis</i> QLN-2	-	+	+	+	+	1.915
<i>Bacillus cereus</i> QLN-3	+	-	+	+	+	2.148
<i>Bacillus subtilis</i> QLN-4	+	-	+	-	+	1.745
<i>Pseudomonas putida</i> QLN-5	-	+	+	+	+	1.894
<i>Pantoeae agglomerans</i> QLN-6	-	+	+	-	+	1.907
<i>Stenotrophomonas rhizophila</i> QLN-7	-	+	+	+	+	1.935
<i>Pseudomonas fluorescens</i> QLN-8	-	+	+	+	+	2.077
<i>Bacillus mycoides</i> QLN-9	+	+	+	-	-	1.874
<i>Enterococcus faecium</i> QLN-10	+	-	-	-	-	1.931
<i>Lactobacillus plantarum</i> QLN-11	+	-	-	+	-	1.924
<i>Bacillus pumilus</i> QLN-12	+	-	+	+	+	1.965
<i>Bacillus megaterium</i> QLN-13	+	-	+	+	+	2.103
<i>Lactobacillus paracasei</i> QLN-14	+	-	+	+	+	1.842
<i>Pseudomonas extremorientalis</i> QLN-15	-	+	+	+	+	1.904
<i>Bacillus mojavensis</i> QLN-16	+	-	+	+	+	2.105

Note: * +, positive; -, negative

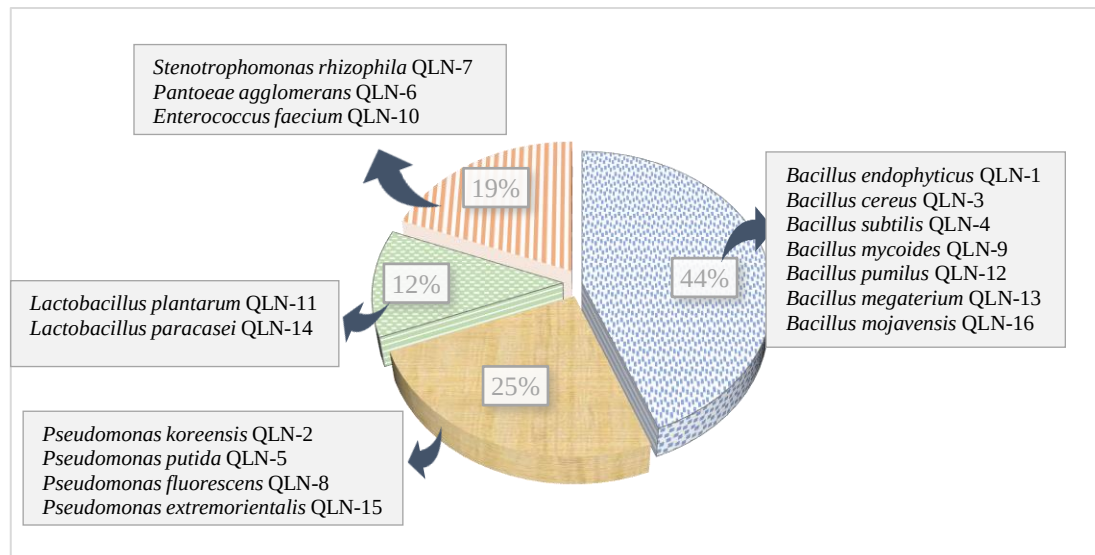


Figure 2. Percentages of bacteria isolated from *L. angustifolia* rhizosphere

3.2. Plant growth-promoting properties of isolates

PGPR supports plant growth directly or indirectly by colonizing plant roots and reducing the population of harmful microorganisms. The rhizosphere is the ecological niche with a rich nutrient presence between plant roots and soil microorganisms. These bacteria contribute to soil fertility and sustainability by producing IAA and siderophore for plants, solubilizing free phosphate, and fixing nitrogen. 16 isolates were tested for siderophores, phosphate solubility, nitrogen fixation, IAA, and HCN production abilities in vitro in the current study. Among all isolates, 62 % (QLN-1, QLN-2, QLN-5, QLN-6, QLN-7, QLN-8, QLN-11, QLN-13, QLN-14, QLN-15) fixed nitrogen, 37 % (QLN-5, QLN-8, QLN-9, QLN-14, QLN-15, QLN-16) produced HCN, 68 % (QLN-2, QLN-4, QLN-5, QLN-6, QLN-7, QLN-8, QLN-12, QLN-13, QLN-14, QLN-15, QLN-16) dissolved inorganic phosphate, 50 % (QLN-1, QLN-2, QLN-6, QLN-7, QLN-9, QLN-12, QLN-14, QLN-16) produced siderophores, and 56 % (QLN-2, QLN-3, QLN-4, QLN-6, QLN -7, QLN-8, QLN-12, QLN-15, QLN-16) produced IAA.

Nitrogen is one of the most important building blocks for plant growth and development. It is found in the structure of

nucleic acids, amino acids, vitamins, and many organic molecules of living beings. Plants cannot access N_2 , but N-fixing microorganisms can turn N_2 into ammonia, which plants can use right away, which reduces the use of N chemical fertilizer use and production costs (Khatoon et al., 2020). According to the available literature, *B. pumilus* (Cruz-Martín et al., 2015), *B. simplex* (Navid et al., 2023), and *B. subtilis* (Sharma et al., 2024) strains were identified to exhibit nitrogen fixation. In the present study, *Bacillus subtilis* QLN-4 fixed nitrogen. On the other hand, Zhao et al. (2011) reported that the *Bacillus cereus* MQ23 strain isolated from *Sophora alopecuroides* fixed nitrogen. In contrast, In the current study, *Bacillus cereus* QLN-3 did not fix nitrogen. Sezen et al. (2016) determined that 16 of the 180 bacteria isolated from the rhizosphere of wheat plants could fix nitrogen. Alnefai et al. (2020) determined that 8 isolates identified using MALDI-TOF MS showed strong nitrogen-fixing ability. Singh et al. (2023) determined that *P. koreensis* CY4 and *P. entomophila* CN11 isolated from sugarcane rhizosphere fixed nitrogen. Similarly, In the current study, *P. koreensis* QLN-2 fixed nitrogen (Table 2).

Many soil bacteria naturally synthesize the volatile secondary metabolite hydrogen cyanide (HCN). HCN disrupts cellular respiration in eukaryotic pathogens by

blocking the enzyme cytochrome c oxidase located in mitochondria. Additionally, it binds to and deactivates various other metalloenzymes, obstructing the mitochondrial electron transport chain and consequently stopping aerobic respiration. There are studies on the use of HCN-producing rhizospheric bacteria as biocontrol agents (Sehrawat et al., 2022; Thakur and Thakur, 2023). Bacteria produce hydrogen cyanide through bacterial cyanogenesis. The bacterial production of HCN has been linked to the suppression of growth and the killing of other organisms. Many bacterial species, especially *Pseudomonas* spp., and *Bacillus* spp., produce cyanide as a secondary metabolite. Ahmad et al. (2008) determined that among rhizospheric bacteria, 50 % of *Bacillus* isolates and 80 % of *Pseudomonas* isolates were positive for HCN production. Quessaoui et al. (2017) determined that *P. fluorescens* Q110B and *P. fluorescens* Q036B, obtained from tomato rhizosphere, produced HCN. Likewise, Halimursyadah et al. (2023) found that *P. fluorescens* produced HCN among the 37 isolates from the patchouli rhizosphere. In the present study, *P. fluorescens* QLN-8 also produced HCN. Singh et al. (2019) determined that *B. thuringiensis* SF 23, *P. aeruginosa* SF 44, *B. subtilis* SF 48, and *B. subtilis* SF 90 isolate produced HCN. Panchami et al. (2020) determined that *P. putida* Td4 obtained from cardamom rhizosphere produced high amounts ($82.5 \pm 2.38 \mu\text{g ml}^{-1}$) of HCN. Similarly, In the current study, *P. putida* QLN-5 also produced HCN (Figure 3).

Siderophores are low molecular weight organic substances produced by many bacteria. *Bacillus* species produce a wide array of siderophores such as bacillibactin, pyoverdine, pyochelin, and petrobactin, which are crucial for their survival. The production of siderophores by *Bacillus* species leads to competition with pathogens for the limited iron in the environment, thereby inhibiting the growth of phytopathogens. The majority of *Pseudomonas* and *Bacillus* strains in the rhizosphere can produce siderophores (Joseph et al., 2007). González-Sánchez et al. (2010) demonstrated that *B. cereus* and *B. subtilis*

obtained from avocado rhizosphere are capable of producing siderophores to promote the protection of avocado trees against *Rosellinia necatrix*. Pereira et al. (2016) reported that the endophytic *B. cereus* LR 1-11 strain isolated from *Lavandula dentata* produces siderophores. Guerrero-Barajas et al. (2020) determined that *B. mycoides* (A1, A2) isolated from avocado rhizospheric soil produced siderophore. In the present study, *B. endophyticus* QLN-1, *B. mycoides* QLN-9, *Bacillus pumilus* QLN-12, and *B. mojavensis* QLN-16 produced siderophore. Siderophores are iron chelating compounds synthesized by many *Pseudomonas* sp. Many studies are showing that *Pseudomonas* sp. produces siderophores (Saranraj et al., 2023; Qessaoui et al., 2024). Gu et al. (2020) determined that *P. koreensis* strain S150 isolated from tobacco rhizosphere produced siderophores. Interestingly, In the current study, *P. koreensis* QLN-2 produced siderophore. Subramaniam and Sundaram (2020) reported that *P. fluorescens* PSF02 isolated from agricultural soils also produced siderophores. Previous studies have shown that *Pantoea agglomerans* also produced siderophores (Hynes et al., 2008; Shariati et al., 2017). Similarly, In the present study, *P. agglomerans* QLN-6 produced siderophore.

IAA promotes the growth of longer roots with more root laterals, which are involved in nutrient uptake. Numerous reports have indicated that IAA produces PGPR species, which are present in the rhizosphere of numerous plants (Dashti et al., 2021; Arzoo et al., 2024). According to Sokolova et al. (2011), 80 % of bacteria isolated from the rhizosphere can produce IAA. IAA production has been determined in several PGPR genera, including *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pantoea*, and *Pseudomonas*. Devanathan et al. (2021) reported that *Bacillus* sp. in *Capsicum annum* L. rhizosphere synthesized high amounts of IAA. Khatami et al. (2023) determined that *Bacillus* sp., *Azotobacter* sp., and *Azospirillum* sp. produced IAA in lavender rhizospheres grown under salt stress. Similarly, In the current study, *B. cereus* QLN-3, *B. subtilis*

QLN-4, *B. pumilus* QLN-12, and *B. mojavensis* QLN-16 produced IAA. In recent study, Patel et al. (2024) reported that *P. fluorescens* isolated from the *Capsicum annuum* L. rhizosphere did not produce IAA. On the contrary, In the present study, *P. fluorescens* QLN-8 produced IAA (Figure 3).

Some bacteria known as phosphate solubilizing bacteria (PSB) can dissolve inorganic and organic phosphorus in the soil. These bacteria dissolve phosphate using various mechanisms, primarily producing acid. They release phosphorus into the soil through their organic and inorganic P solubility (Selçuk and Çakıcı, 2022). Some soil bacteria like *Pseudomonas* and *Bacillus* can dissolve insoluble phosphates, which has a significant impact on promoting plant growth. Solubilization of phosphate is one of *Bacillus*'s inherent characteristics (Li et al., 2023).

Mukhtar et al. (2017) determined that *B. safensis* PSB5 and *B. megaterium* PSB12 from wheat rhizosphere solubilized high phosphate levels (305.6, 217.2, and 148.1 $\mu\text{g ml}^{-1}$). According to Rawat et al. (2021), the most prevalent phosphate-solubilizing bacteria in the rhizosphere are *Bacillus*, *Enterobacter*, and *Pseudomonas*. Similarly, in the current study, *B. pumilus* QLN-12, *B. subtilis* QLN-4, *B. megaterium* QLN-13, *B. mojavensis* QLN-16, *P. koreensis* QLN-2, *P. putida* QLN-5, *P. fluorescens* QLN-8 and *P. extremorientalis* QLN-15 dissolved inorganic phosphate. Soares et al. (2023) demonstrated that *P. aeruginosa* UFT01 and *B. cereus* UFT42 dissolved inorganic phosphate but on the other hand, *B. cereus* QLN-3 did not dissolve inorganic phosphate in current study. Table 2 and Figure 3 presents the plant growth-promoting properties of the isolates.

Table 2. Plant growth promoting traits of the isolates

Isolates	Inorganic phosphate solubization	Nitrogen fixation	Siderophore production	HCN production	IAA production
<i>Bacillus endophyticus</i> QLN-1	-	+	+	-	-
<i>Pseudomonas koreensis</i> QLN-2	+	+++	+++	-	+++
<i>Bacillus cereus</i> QLN-3	-	-	-	-	+
<i>Bacillus subtilis</i> QLN-4	+	-	-	-	+
<i>Pseudomonas putida</i> QLN-5	+	++	-	++*	-
<i>Pantoea agglomerans</i> QLN-6	+	+++	+	-	+++
<i>Stenotrophomonas rhizophila</i> QLN-7	+	+	+	-	+
<i>Pseudomonas fluorescens</i> QLN-8	+	++	-	+	++
<i>Bacillus mycoides</i> QLN-9	-	-	+	+	-
<i>Enterococcus faecium</i> QLN-10	-	-	-	-	-
<i>Lactobacillus plantarum</i> QLN-11	-	+	-	-	-
<i>Bacillus pumilus</i> QLN-12	+	-	+	-	+
<i>Bacillus megaterium</i> QLN-13	+	+++	-	-	-
<i>Lactobacillus paracasei</i> QLN-14	+	+	+	+	-
<i>Pseudomonas extremorientalis</i> QLN-15	+	++	-	+	+
<i>Bacillus mojavensis</i> QLN-16	+	-	+	+	+

*For nitrogen fixation and HCN activity (+++: development after 6 hours, ++: development after 12 hours, +: development after 24 hours). **For siderophore activity: (+++: color change after 12h, ++: color change after 24h., +: color change after 36h). *** For IAA activity (+++: development after 1h, ++: development after 6 h, +: development after 12 h).

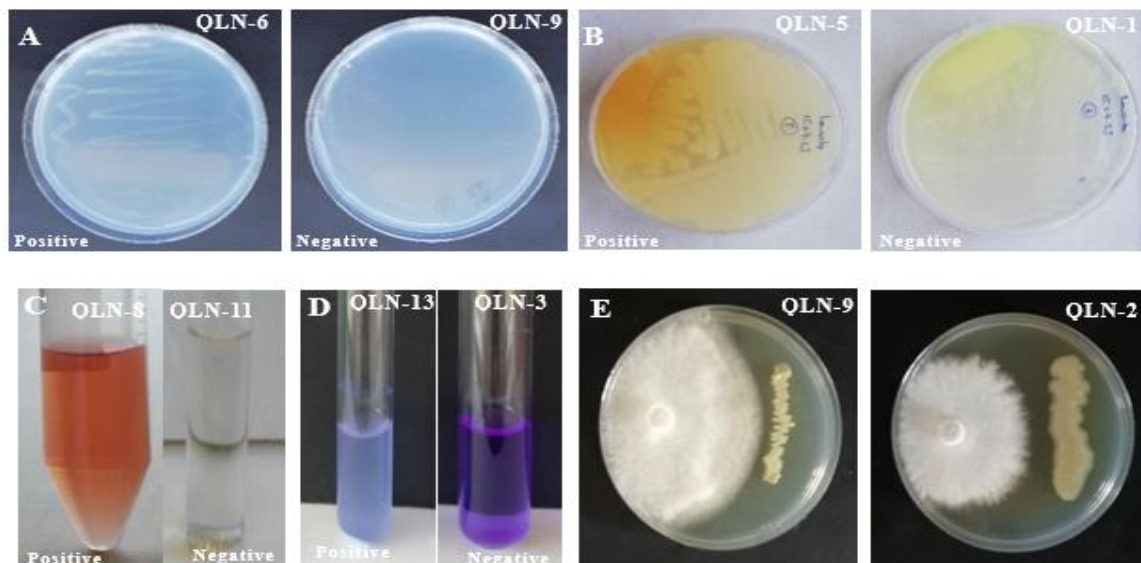


Figure 3. PGPR and antifungal activity test images of isolates (A: Nitrogen fixation B: HCN production C: IAA production D: Inorganic phosphate solubilization E: Antifungal test of isolates against *F. oxysporum*)

3.3. Antifungal activity

In the current study, it was determined that out of 16 isolates; 75 % have amylase; 56 % have protease; 62 % have pectinase; and 37 % have chitinase activity. *B. cereus* QLN-3, *B. subtilis* QLN-4, *P. fluorescens* QLN-8, and *B. pumilus* QLN-12 gave positive results for all extracellular enzyme tests. Plant growth-promoting rhizobacteria (PGPR) not only promote plant growth but also protect the plant against fungal pathogens by producing various extracellular enzymes such as chitinase, protease, and pectinase. Fungal cell walls contain chitin, glucan, cellulose, lipids, and proteins. Therefore, bacterial cellulases, lipases, and proteases play a crucial role in breaking down cell walls. These enzymes disrupt the structure of the fungal cell wall and eventually lead to the disintegration of the fungal cell. According to Dukare et al. (2020), extracellular enzymes stop the growth and development of the pathogen by preventing the

germination of fungal spores and germ tube extension. Many studies show that bacteria of the genus *Bacillus* and *Pseudomonas* produce these enzymes and kill different pathogens (Nagrle et al., 2023). Shin et al. (2023) reported that enzymes such as protease, pectinase, and xylanase help bacteria colonize plant tissues, establish symbiotic relationships with host plants, and thus show antifungal activity by inhibiting the growth of pathogenic fungi. Fathalla Mohammed (2020) reported that *B. subtilis* SBMP4, which has plant growth-promoting properties, produced high amounts of chitinase. In the current study, *B. subtilis* QLN-4 produced chitinase. According to Rathore et al. (2020), lytic enzymes such as β -1,3 glucanase and chitinase produced by *P. fluorescens* are an antagonistic feature of this strain against *Fusarium* sp. Our findings support this study. In the present study, *P. fluorescens* QLN-8 produced chitinase. Table 3 presents the extracellular enzyme production of isolates.

Table 3. Extracellular enzyme production of isolates

Isolates	Extracellular enzymes			
	Amylase test	Protease test	Pectinase test	Chitinase test
<i>Bacillus endophyticus</i> QLN-1	+	+	-	+
<i>Pseudomonas koreensis</i> QLN-2	-	-	+	-
<i>Bacillus cereus</i> QLN-3	+	+	+	+
<i>Bacillus subtilis</i> QLN-4	+	+	+	+
<i>Pseudomonas putida</i> QLN-5	-	+	+	+
<i>Pantoea agglomerans</i> QLN-6	+	-	+	-
<i>Stenotrophomonas rhizophila</i> QLN-7	-	+	-	-
<i>Pseudomonas fluorescens</i> QLN-8	+	+	+	+
<i>Bacillus mycoides</i> QLN-9	+	-	+	-
<i>Enterococcus faecium</i> QLN-10	-	+	-	-
<i>Lactobacillus plantarum</i> QLN-11	+	-	-	-
<i>Bacillus pumilus</i> QLN-12	+	+	+	+
<i>Bacillus megaterium</i> QLN-13	+	+	-	-
<i>Lactobacillus paracasei</i> QLN-14	+	-	-	-
<i>Pseudomonas extremorientalis</i> QLN-15	+	-	+	-
<i>Bacillus mojaviensis</i> QLN-16	+	-	+	-

Note: * +, positive; -, negative

The cultivation of medicinal and aromatic plants has significantly increased in recent years because of the high global demand for plant-based medicines and aromatic compounds. Medicinal and aromatic plants are significantly impacted by root rot, wilt, anthracnose, and dieback resulting from fungal and bacterial infections. These plants often have problems with various fungal diseases like leaf spots, blight, rust, powdery mildew, root rot, and dieback. Lavender wilt is caused by the fungus *F. oxysporum* (Özer et al., 2021). There have been many reports worldwide of *Fusarium* sp. infections in *Lavender* sp. (Vasileva, 2015; Soylu and Incekara, 2017; Li et al., 2024). In current study, Antifungal activity of isolates obtained from *L. angustifolia* rhizosphere was tested against *F. oxysporum* and the inhibition percentages varied between 24.76 % and 57.14 %. Among the isolates, *B. simplex* QLN-4 isolate showed the strongest antagonism against the pathogen with a high percentage inhibition value (57.14 %), followed by *Pseudomonas koreensis* QLN-2 isolate (52.38 %). The *P. fluorescens* QLN-8 isolate (24.76 %) showed the weakest effect against the pathogen (Table 4).

Bacillus spp. is regarded as a successful bacteria capable of synthesizing a diverse range of useful compounds. The production of antifungal metabolites by PGPRs such as *Bacillus* is a well-documented biocontrol agent

against phytopathogens (Majeed et al., 2018). Gajbhiye et al. (2010) reported that *B. subtilis* in the cotton rhizosphere inhibited mycelial growth by 50 % against *F. oxysporum*. Singh et al. (2017) determined that fifteen *B. subtilis* strains reduced *F. oxysporum* pathogen growth by varying rates ranging from 47 % to 85.5 %. In a recent study, Koumoutsis et al. (2004) reported that *B. amyloliquefaciens* FZB42 secretes fengycin and bacillomycin D, which have antagonist activity against *F. oxysporum*. Our findings are consistent with the other works. In the current study, *Bacillus subtilis* QLN-4 showed the maximum inhibition rate of 57.14 % (Table 4). According to Snook et al. (2009), *Bacillus mojaviensis* promotes plant growth by colonizing the rhizosphere and destroys plant pathogens by stimulating the immune response. Moreover, *B. mojaviensis* produces antifungal compounds such as fengycin and iturin. Diabankana et al. (2021) determined that *B. mojaviensis* PS17, which can produce chitinase, protease, cellulase, and β -glucanase, inhibited the development of *F. oxysporum*. Galitskaya et al. (2022) reported that lipopeptides produced by *B. mojaviensis* P1709 inhibited the growth of *F. oxysporum* by 93 %. In the current study, *Bacillus mojaviensis* QLN-16 showed an inhibition rate of 30.23 % against *F. oxysporum*.

Numerous studies have shown that *Pseudomonas*, which are commonly found in

soil and rhizosphere, prevent the growth of plant diseases by secreting various compounds (Koche et al., 2012; Wang et al., 2020; Yue et al., 2023). Rafikova et al. (2016) determined that *P. koreensis* IB-4 showed high antagonist activity against *F. oxysporum*. Similarly, in the current study, *P. koreensis* QLN-2 showed an high inhibition rate of 52.38 % against *F. oxysporum*. Priya et al. (2019) reported that *Pseudomonas* sp. inhibited *F. oxysporum* mycelial growth with an inhibition rate of 57.77 %. Moreover, Ali et al. (2020) determined that *P. putida* MS6, which has protease activity in the sunflower rhizosphere, showed a 79 % antagonist effect against *F. oxysporum*. Similarly, in the current study, *P. putida* QLN-5 showed an inhibition rate of 36.54 % against *F. oxysporum*. Rathore et al. (2020) determined that *P. fluorescens* Pf-5 showed 82.51 % growth inhibition against *Fusarium* sp. On the other hand, In the current study, *P. fluorescens* QLN-8 showed a low inhibition rate of 24.76 % against *F. oxysporum*. The plant growth-promoting properties of *Stenotrophomonas* spp. as well as their antagonistic behavior against soil-borne plant pathogens are well documented (Ryan et al. 2009). Schmidt et al. (2012) reported that *S.*

rhizophila DSM14405T significantly reduced disease severity against *Fusarium* spp. Aktas et al. (2023) determined that *S. maltophilia*, which can produce chitinase, inhibits mycelial development in *F. oxysporum*. In present study, *S. rhizophila* QLN-7 showed an high inhibition rate of 45.95 % against *F. oxysporum*.

Previous studies have shown that Enterococcus species not only have antifungal activities, but also increase shoot and root length, as well as fresh weight of plants like rice and melon by producing indole acetic acid and gibberellic acid (Lee et al., 2015). Mussa et al. (2018) determined that *E. casseliflavus* and *E. gallinarum* bacteria in rhizospheric soil samples inhibited *F. oxysporum* by 83 % and 78 %, respectively. Morales et al. (2020) reported that *E. mundtii* and *E. faecium* exhibited antifungal and antimicrobial activity by producing different compounds. Naik et al. (2023) reported that *E. faecium* MYSBC14 showed an inhibition percentage of 71.70 % against *Fusarium* sp. In present study, *E. faecium* QLN-10 showed a high inhibition rate of 45.23 % against *F. oxysporum*. Table 4 shows the percentage inhibition of the isolates against *F. oxysporum*.

Table 4. Antifungal activity test results of isolates against *F. oxysporum*

Isolates	Colony diameter of <i>F. oxysporum</i> (mm) Mean $\pm$ SD	Inhibition percentage (%) of <i>F. oxysporum</i>
<i>Bacillus endophyticus</i> QLN-1	58 $\pm$ 1.0 ^c	38.09
<i>Pseudomonas koreensis</i> QLN-2	46 $\pm$ 1.0 ^{de}	52.38
<i>Bacillus cereus</i> QLN-3	62.7 $\pm$ 2.33 ^{ab}	32.50
<i>Bacillus subtilis</i> QLN-4	42 $\pm$ 1.0 ^e	57.14
<i>Pseudomonas putida</i> QLN-5	59.3 $\pm$ 2.33 ^{bc}	36.54
<i>Pantoea agglomerans</i> QLN-6	65.6 $\pm$ 1.33 ^a	29.04
<i>Stenotrophomonas rhizophila</i> QLN-7	51.4 $\pm$ 4.33 ^d	45.95
<i>Pseudomonas fluorescens</i> QLN-8	69.2 $\pm$ 1.0 ^a	24.76
<i>Bacillus mycoides</i> QLN-9	49.5 $\pm$ 1.0 ^{de}	48.21
<i>Enterococcus faecium</i> QLN-10	52.0 $\pm$ 2.3 ^d	45.23
<i>Lactobacillus plantarum</i> QLN-11	67.2 $\pm$ 1.33 ^a	27.14
<i>Bacillus pumilus</i> QLN-12	52.8 $\pm$ 1.33 ^{dc}	44.28
<i>Bacillus megaterium</i> QLN-13	66.7 $\pm$ 2.33 ^a	27.73
<i>Lactobacillus paracasei</i> QLN-14	65.3 $\pm$ 1.33 ^a	29.40
<i>Pseudomonas extremorientalis</i> QLN-15	58.7 $\pm$ 2.33 ^{bc}	37.26
<i>Bacillus mojavensis</i> QLN-16	64.6 $\pm$ 1.33 ^a	30.23

*For Antifungal activity: $p < 0.01$; statistically significant level. a-e: The difference between the means shown by different letters in the same column is statistically significant (Mean $\pm$ SD: Mean $\pm$ Standard Deviation).

4. Conclusion

To our knowledge, the current study is the first study in Turkey to isolate PGPR from *L. angustifolia* rhizosphere. Over the last two decades, multiple studies have indicated PGPR strains in many plant rhizospheres, aid plant growth and development. PGPR plays roles in producing phytohormones, increasing nutrient availability, and protecting the plant against many pathogens. Research is scarce on determining the ecology of PGPR. There is a need to screen strategies for selecting the best local rhizobacterial strains for use as environmentally friendly biofertilizers to prevent long-term use of fungicides that cause environmental and ecological problems. Rhizobacteria isolated from *L. angustifolia* exhibit significant plant growth-promoting properties and antifungal activities. These isolates can serve as effective microbial fertilizers, offering an environmentally friendly alternative to chemical fertilizers and contributing to sustainable agriculture. Therefore, further research on PGPR is necessary to help create more effective local rhizobacterial strains that can function in several agroecological environments.

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Achene and Flower Characteristics of Some *Centaurea* Species Collected from Diyarbakır (Türkiye)

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Abstract

In this study, belonging to the genus *Centaurea* L. collected from Diyarbakır and its surroundings were investigated flower structures and the achene micro and macro morphology of 10 species [*C. solstitialis* L., *C. iberica* Trev. Ex Sprengel, *C. urvillei* ssp *nimrodus* (Boiss. & Hausskn.) Wagenitz, *C. consanguinea* DC. (E), *C. behen* L, *C. rigida* Banks & Sol., *C. balsamita* Lam., *C. kurdica* Reichardt (E), *C. stapfiana* (Hand.-Mazz.) Wagenitz, *C. virgata* Lam.]. Among the ten species studied, five have yellow flowers *C. solstitialis*, *C. behen*, *C. rigida*, *C. balsamita*, *C. stapfiana* and the other five *C. iberica*, *C. urvillei* ssp *nimrodus*, *C. consanguinea*, *C. kurdica*, *C. stapfiana*, *C. virgata* have pink-purple flowers. It has been found that phyllaries on the involucre bearing the flowers are typically characterized by horn-like spines or protrusions. As it is well-established that seed and fruit structures are key features used in taxonomy, achene and pappus structures were examined in this study, and measurements were taken with a digital caliper. The results of measurement and SEM imaging reveal that the macro structure of achene differs significantly from its micro structure. This suggests that traits are of great importance and can be used in taxonomic diagnosis.

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1. Introduction

Centaurea L. is the third most diverse genus of the Asteraceae family in terms of species diversity (Davis, 1975). According to the List of Plants of Türkiye, the genus *Centaurea* is represented by 194 taxa in Türkiye, while this number has reached 221 with the recently added new species and records (Uysal, 2012; Yıldırım, 2018; Negaresh and Rahiminejad, 2018; Duman et al., 2021, Özbek, 2021; Şirin et al., 2022; Uysal, 2022). Of these taxa, 133 are endemic and the endemism rate of the genus for Türkiye is 60 %. The size of this species creates significant confusion in taxonomy, and therefore serious problems are encountered in determining species (Susanna and Garcia-Jacas, 2007). Its natural vegetation cover is steppe and 33 % of its territory is covered with forest and heathland, 40 % with cultivated land, and 22 % with meadows and pastures. A protection decision was taken for *Ajuga xylorrhiza* in Çermik (Ertekin, 2013) and *Rosularia blepharophylla* (Hoşgören, 2018) endemic plants growing in Ergani. *Hesperis hedgei*, *Lathyrus trachycarpus*, *Paracaryum kurdistanicum*, and *Onobrychis silvanensis* are among other endemic plants identified in Diyarbakır (Ertekin, 2002). *C. stapfiana*, *C. urvillei* ssp *nimrodus*, *C. kurdica* and *C. consanguinea* species, which are the main materials of this study, are also endemic to the region (Güner et al., 2012).

Due to its rich species diversity, it presents major challenges when it comes to species identification (Ranjbar et al., 2013). Therefore, numerous studies on morphological, anatomical (Bona and Aras, 2008; Köse et al., 2010; Çelik et al., 2008), palynological (Erkara et al., 2012; Pehlivan, 1995), cytological (Ghaffari, 1999; Gömürge and Adıgüzel, 2001), and ecological characteristics (Türkoğlu et al., 2009; Çelik et al., 2005) of the genus *Centaurea* L. have been conducted by many researchers for many years. However, it should not be forgotten that fruit and seed characteristics are important not only in the genus *Centaurea* but also in all plant groups (Öztürk, 2020; Dumanoğlu, 2021).

The genus *Centaurea* L. is characterized by a capitulum-shaped flower. It has flowers in white, yellow, pink or purple. The hairy or spiny phyllaries they carry are usually arranged in several rows, usually with spiny or mucronate tips and pinnate margins. Achene-type fruits possess types of hairy pappus that facilitate the transportation of seeds by wind (Chuang and Ornduff, 1992). Achenes have a variety of shapes, sizes, patterns, and surface differentiation that are used to classify taxa (Roque and Funk, 2013; Özcan and Akıncı, 2019). These microstructural details of achenes enable taxa to be differentiated and their level of phylogenetic to be revealed. This practice is particularly suitable for families whose taxa are complex to identify (Bona, 2014). Hence, evaluation of fruit and seed traits has been deemed necessary by many researchers in recent years (Shabestari, 2013). It is particularly in recent years that studies of this type have taken on greater importance and have reached micromorphological depths. This necessity prompted us to undertake such a study (Köstekçi and Arabacı, 2015).

Despite the stereomicroscope alone being used for the traditional identification of plant species, some researchers disagree and rely on micro morphological characteristics to confirm the identification. This is because parameters related to fruit and seed micro-structure are of great importance in the differentiation and evolutionary identification of taxa (Candan et al., 2016). Therefore, in this study, both stereomicroscopy and scanning electron microscopy were used for imaging. Because, while only morphological distinctions are observed in stereomicroscopic examinations of seeds, precise determination of species is made in electron microscopy examinations. Thus, erroneous or suspicious determinations are avoided.

2. Materials and Methods

Plant samples were collected from their natural habitats in Diyarbakır and surrounding roads during the flowering and ripe fruit periods. The collected samples were meticulously dried using herbarium techniques and preserved in the Botanical Herbarium

(DUF) of the Department of Biology, Dicle University (Table 1). The flowers were photographed using a Nikon d7100 body and 18x140 lens. Meanwhile, Achen samples were imaged with a Leica MC179 HD camera and an FEI QUANTA 250 electron microscope connected to a Leica Z16 APO stereomicroscope.

Diyarbakır, the study area, is a city situated in the Southeastern Anatolia Region of Turkey, around a high plateau along the banks of the Tigris River. In the city, where the continental climate prevails, summers are extremely hot and dry, while winters are cold, harsh, and rainy. Steppe, the natural vegetation of the Southeastern Anatolia Region, is also dominant in Diyarbakır (Figure 1).

Table 1. Localities where the species were collected

Plant name	Herbarium number	Locality
<i>C. balsamita</i>	6999	Dicle University-Campus
<i>C. behen</i>	6700	Diyarbakır-Silvan 15. km
<i>C. consanguinea</i>	6998	Diyarbakır-Eğil
<i>C. iberica</i>	6701	Diyarbakır-Bismil 32. km
<i>C. kurdica</i>	6996	Diyarbakır-Çınar 20. km
<i>C. rigida</i>	6702	Silvan-Diyarbakır 41. km
<i>C. solstitialis</i>	6703	Ergani-Çermik 25. km.
<i>C. stapfiana</i>	6997	Ergani
<i>C. urvillei</i> ssp. <i>nimrodus</i>	6704	Ergani-Mountain Ziyaret
<i>C. virgata</i>	6705	Çermik-Sinek stream

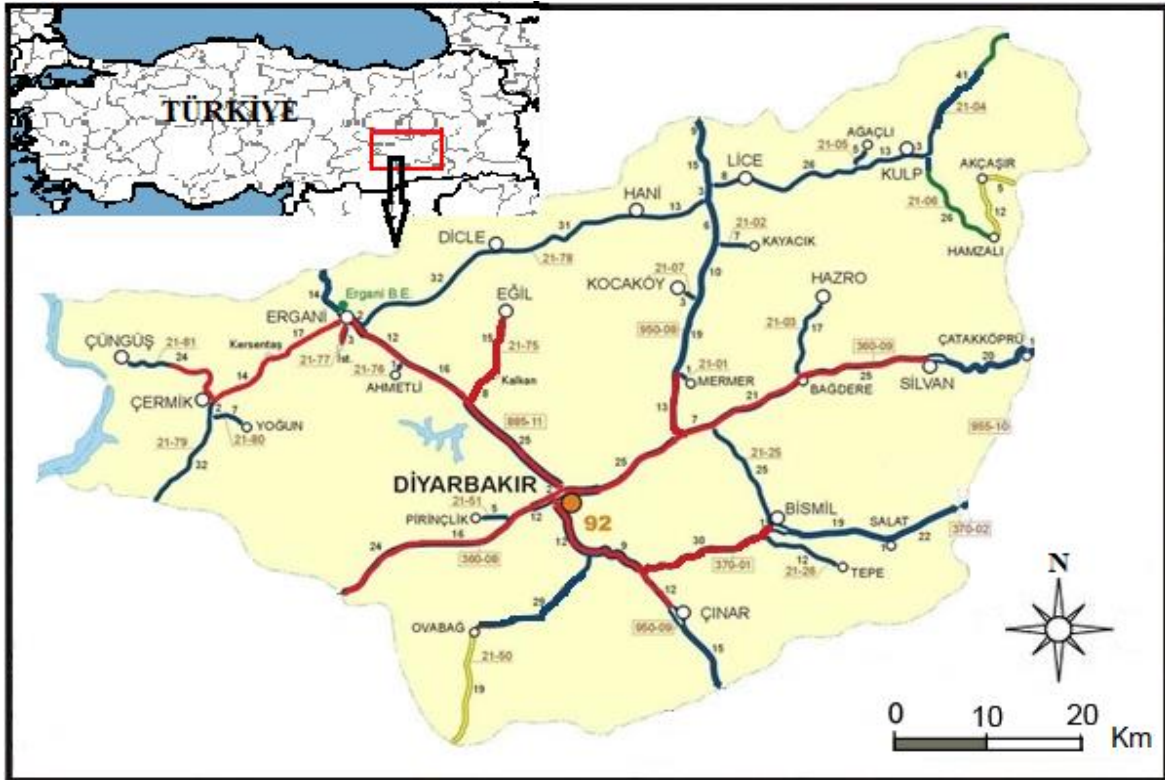


Figure 1. Diyarbakır working area map

The province covers an area of 15.168 km² and is 660 meters above sea level. In the city where the continental climate prevails, summers are extremely hot and dry, while winters are cold, harsh, and rainy (www.diyarbakir.bel.tr.).

3. Result and Discussion

3.1. Flowers

The inflorescence is capitulum-shaped, characteristic of the family. Capitulum is the inflorescence bearing numerous sessile flowers on a swollen main axis (Figure 2). It has been observed that the phyllaries of *Centaurea* species end with horn-like protrusions in the form of spines.

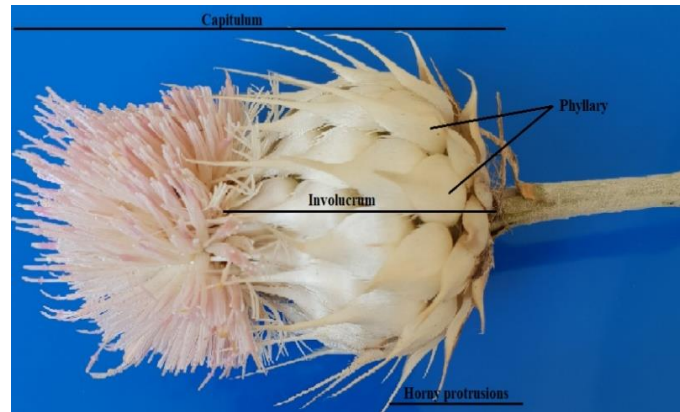


Figure 2. Flower structure of the genus

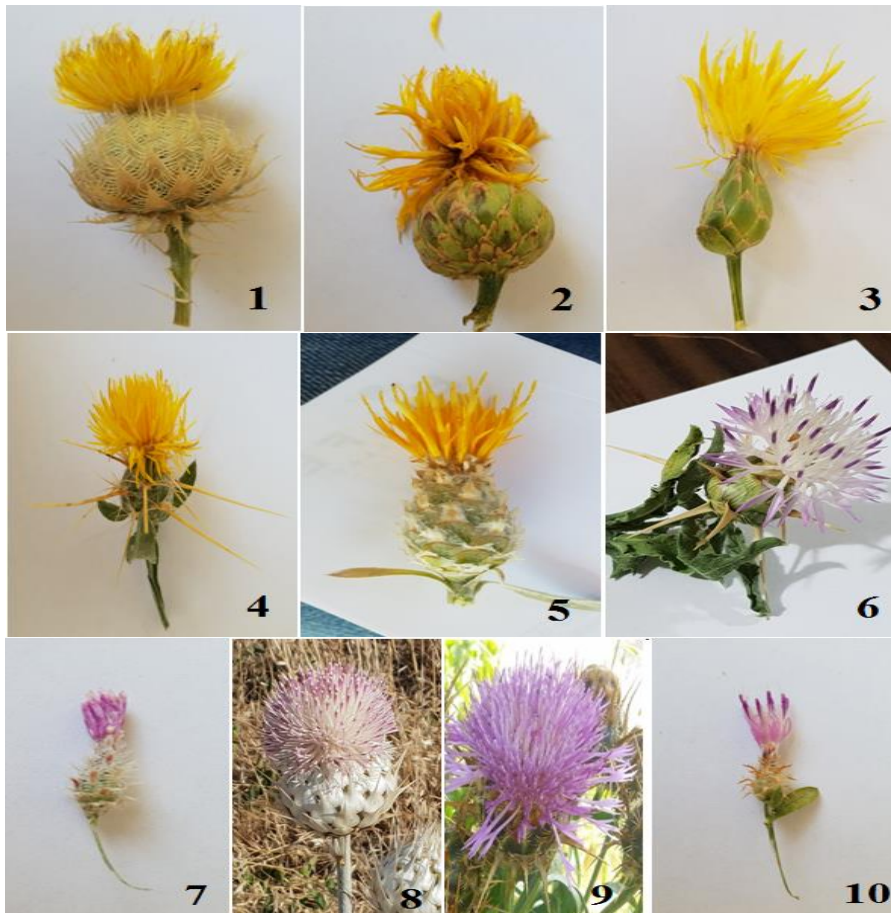


Figure 3. Flowers of 1-*C. balsamita*, 2-*C. behen*, 3-*C. rigida*, 4-*C. solstitialis*, 5-*C. stapfiana* yellow, 6-*C. iberica*, 7-*C. consanguinea*, 8-*C. kurdica*, 9-*C. urvillei* ssp. *nimrod*, 10-*C. virgata* species have been shown to be pink-purple in color

3.2. Stereomicroscope images of achenes

C. balsamita Lam: The phyllaries on the bulging involucre bearing yellow flowers are surrounded by stiff, beard-like stinging hairs. The brown-colored achene is decorated with black lines. The pappus is composed of sparse, shiny, and thin to long hairs. The hilum bears a small red additional structure belonging to the calyx called the appendage. This appendage is significant for species identification (Figure 4.1).

C. behen L.: The phyllaries on the yellow-flowered bulging involucre are pyramids without spines and green in color. Achene is yellowish-white in color and hairless. The pappus bears numerous, long, and soft broom-like bristles. The hilum is in the center and at the base (Figure 4.2).

C. consanguinea DC.: It is endemic. It has a long thin involucre and pink-purple flowers. The thornless phyllaries are surrounded by brown and soft-textured hairs. The achene is swollen, brown with yellow stripes, and obtuse. The outer papillae are denser and thinner than the inner ones. The hilum is on one side and is small (Figure 4.3).

C. iberica Trev. Ex Sprengel: It bears white-purple flowers. The phyllaries end in thick and long spines that hang outwards. Achene is creamy-yellow with brown spots, obtuse. The pappus is sparse and consists of linear hairs. The hilum is curved inwards (Figure 4.4).

C. kurdica Reichardt: It is an endemic species. The flowers are pink-purple, sometimes white. The bright white involucre is very large and bears hard, white spines. Achene and pappus, brown-yellow, obtuse. The hilum is close to one side (4.5).

C. rigida Banks & Sol.: Its general appearance is very similar to the *C. behen* species. *C. rigida* differs in that the flower-bearing involucre is smaller and cylindrical. Fillaries, as in *C. behen*, also do not bear thorns or hairs. Achene is very small, brown-red, and densely hairy. Pappus shows a very sparse structure (Figure 4.6).

C. solstitialis L.: Flowers are yellow. Phyllaries bear long, stiff spines. The base of the spines is surrounded by stiff hairs. The achene is brown-yellow in color and hairless. The outer pappus hairs are yellow compared to the inner ones. The hilum is located on one side (Figure 4.7).



Figure 4. Stereomicroscope microphotographs of achenes: 1-*C. balsamita*, 2-*C. behen*, 3-*C. rigida*, 4-*C. solstitialis*, 5-*C. stapfiana*, 6-*C. iberica*, 7-*C. consanguinea*, 8-*C. kurdica*, 9-*C. urvillei* ssp. *nimrodii*, 10-*C. virgate*

C. stapfiana (Hand.-Mazz.) Wagenitz: It is an endemic species. The flowers are yellow and large. The phyllaries are hard but not spiny. Achenes are yellowish-white, glabrous, and rugose. Pappus are very long. The hilum is located on one edge of the protruding base. The pappus need to be shortened to obtain images when they are long enough to cover the achenes (Figure 4.8).

C. urvillei ssp. *nimrodii* (Boiss. & Hausskn.) Wagenitz: It is endemic. The flowers are pink, purplish red, or whitish. The phyllaries have large hard spines at the ends and hard stinging hairs at the base of these

spines. Achenes are yellowish-white, densely hairy, obtuse. The pappus is white and elongated. The hilum is on one side and surrounded by hairs (Figure 4.9).

C. virgate Lam.: It is very similar to the *C. consanguinea* species with its pinkish-purple flowers and involucre structure. However, the species is distinguished by a more rigid and outwardly curved phyllaries. The achene is yellowish-white with indistinct stripes, obtuse. Pappus hairs are sparse and very short. The hilum is on the side (Figure 4.10).

The achene characteristics of the species were examined and measurements were taken using a digital caliper with 5 samples each. The

arithmetic averages of the measurements were calculated and the results were shown in the table (Table 2).

Table 2. Fruit measurements of species

Species	Achene				Pappus	Appendage
	Length	Width	Colour	Shape		
<i>C. balsamita</i>	4.38	1.03	Brown-black	Oblong	4.39	+
<i>C. behen</i>	4.60	2.06	Yellowish white	Ovoid-Oblong	6.69	-
<i>C. consanguinea</i>	1.45	0.7	Yellow stripes on brown	Obovoid	3.47	-
<i>C. iberica</i>	3.42	1.80	Mottled brown on creamy yellow	Ovoid-Oblong	1.42	-
<i>C. kurdica</i>	6.88	3.07	Brown-yellow	Oblong	7.25	-
<i>C. rigida</i>	3.62	1.38	Brown	Ovoid	5.58	-
<i>C. solstitialis</i>	2.28	1.09	Brown-yellow	Lanceolate	2.40	-
<i>C. stapfiana</i>	8.12	1.88	White	Oblong	17.13	-
<i>C. urvillei</i> ssp. <i>nimrod</i> <i>dis</i>	5.06	2.80	Yellowish white	Ovoid-Oblong	13.87	-
<i>C. virgata</i>	3.24	1.25	Yellow	Obovat-Oblong	1.74	-

3.3. Scanning electron microscopy (SEM) images

C. balsamita Lam: The surface of the achene is composed of small, almost cylindrical, short cells interlocking with each other, with the appearance of fish scales. Cells are brightly colored, distinctly circumscribed, and rounded at the tip (Figure 5.1).

C. behen L: The cells covering the surface of the achene are thin, rectangular, elongated, and have wide margins. Because of the width of these margins, the cells appear hollow (Figure 5.2).

C. consanguinea DC.: The cells forming the surface of the achene are elongated oblong and covered with fine hairs. These cells with indistinct margins appear to be intertwined (Figure 5.3).

C. iberica Trev. ex Sprengel: The surface of the achene is covered with sparse hairs, and the cells on it are thin and elongated with indistinct walls (Figure 5.4).

C. kurdica Reichardt: The Achene surface is sparsely hairy. The cells covering the surface

are shiny, elongated, and clearly circumscribed (Figure 5.5).

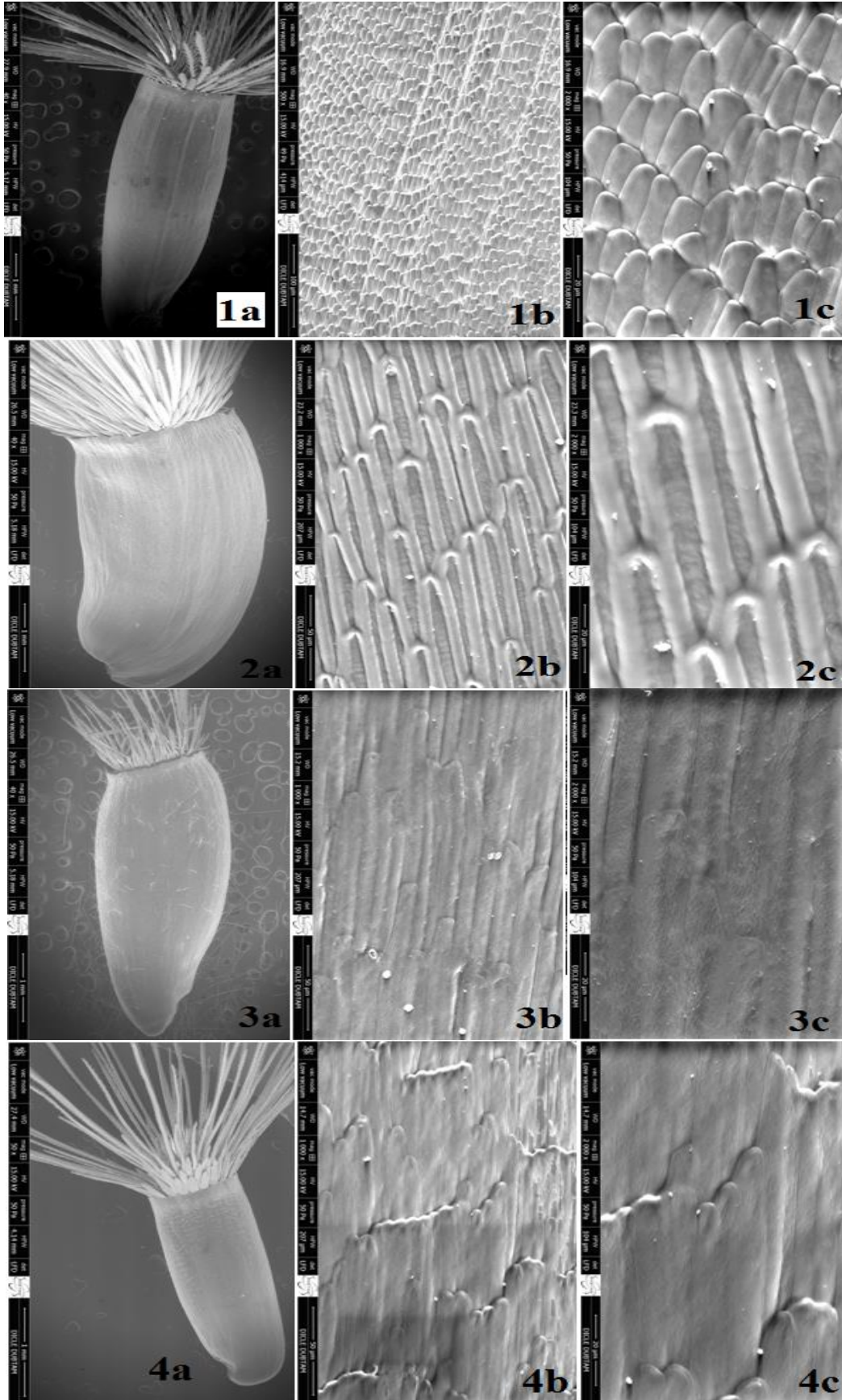
C. rigida Banks & Sol.: The condition of the hairs on the surface varies periodically. The edges of the cells on the surface are quite indistinct. Sometimes it appears as a single layer (Figure 5.6).

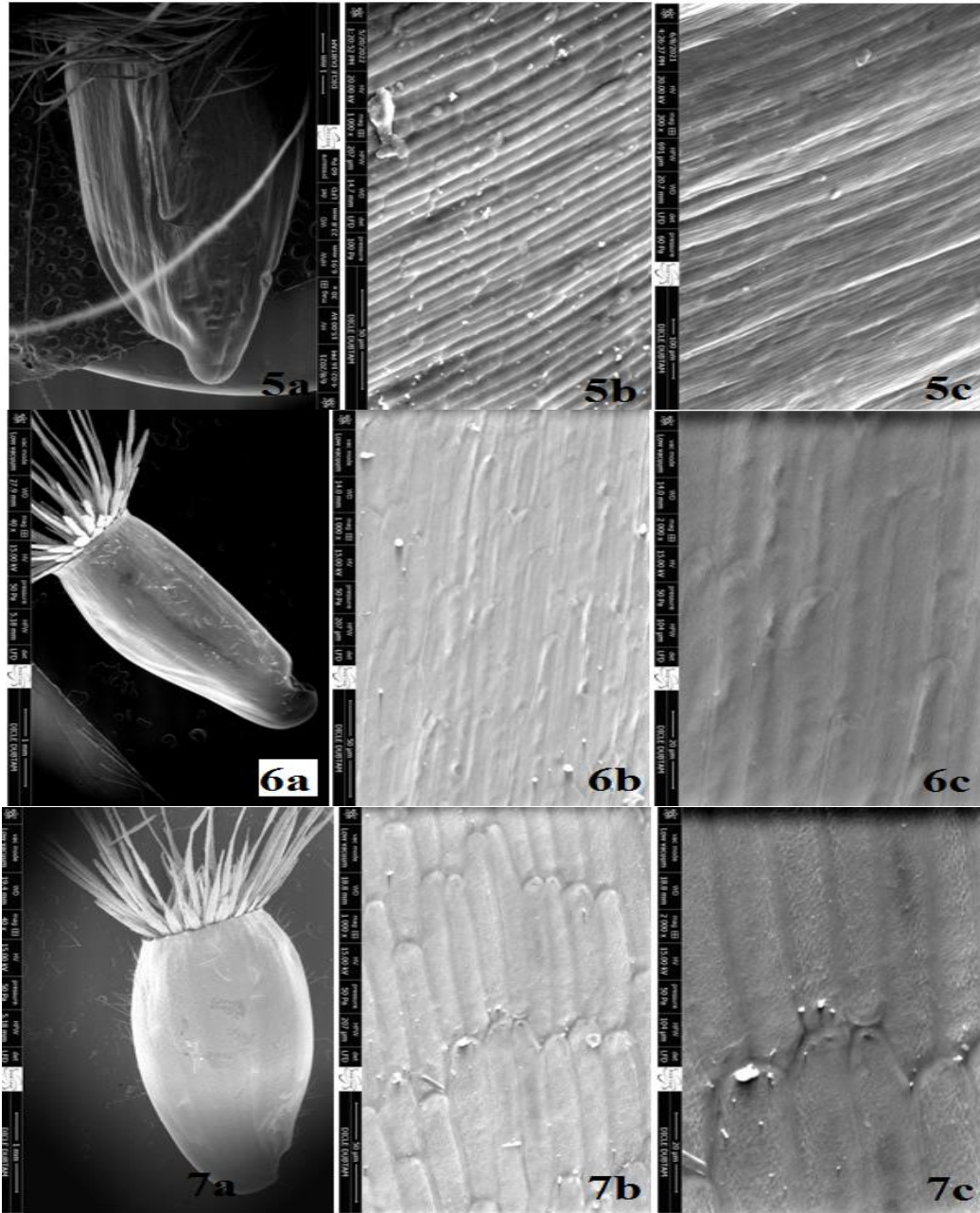
C. solstitialis L.: Achene surface cells are covered with cylindrical cells. The arrangement of these cells resembles the waves of the sea. These fluctuations produce irregular stratifications (Figure 5.7).

C. stapfiana (Hand.-Mazz.) Wagenitz: The cells on the achene are shiny and, as in *C. behen*, the cell margins are wide and appear to be higher than the cell center (Figure 5.8).

C. urvillei ssp. *nimrod*
dis (Boiss. & Hausskn.) Wagenitz: The seed surface is covered with hairs dense enough to cover the cells in certain areas. Compared to others, the cells are longer and the cell margins are distinct (Figure 5.9).

C. virgata Lam: The surface is short and sparsely hairy. Cell margins are distinct (Figure 5.10).





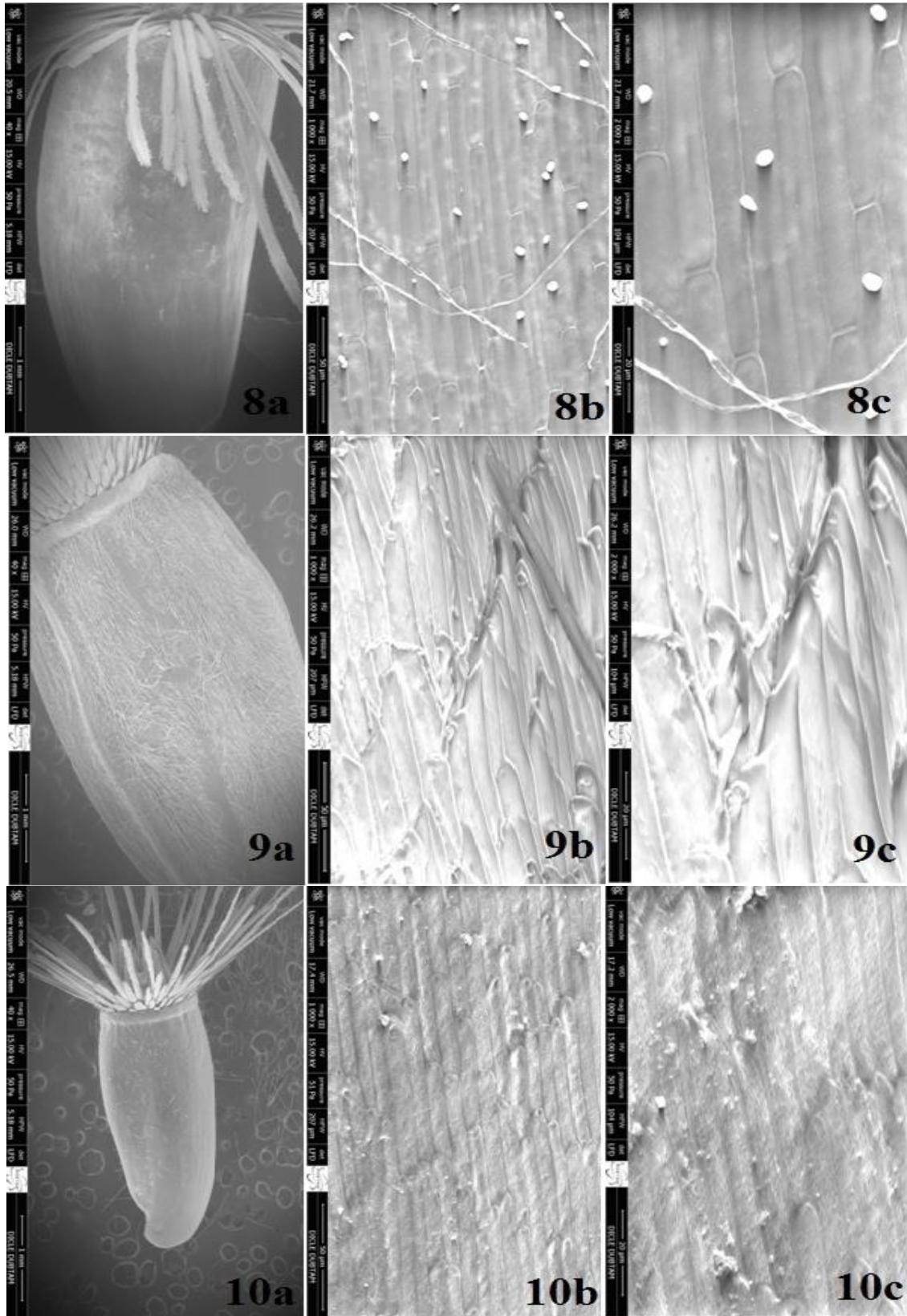


Figure 5. Scanning Electron Microscopy (SEM) microphotos of achenes; 1-*C. balsamita*, 2-*C. behen*, 3-*C. rigida*, 4-*C. solstitialis*, 5-*C. stapfiana*, 6-*C. iberica*, 7-*C. consanguinea*, 8-*C. kurdica*, 9-*C. urvillei* ssp. *nimrod*, 10-*C. virgata*

4. Discussion

Numerous studies have been conducted on this genus due to its difficulty in identification. In particular, as more studies have been conducted on achene morphology, it has been observed that there are significant differences between taxa and that these differences can be utilized for taxonomy. The achene morphology of 23 species of the genus *Centaurea* L. was studied. Many qualitative and quantitative differences were observed in the achenes of the species they studied (Aksoy et al., 2010; Bancheva and Gorgorov, 2010; Bona, 2015).

One of the characters used for identification is the presence of pappuses. The presence of a pappus is considered a structural adaptation for wind transport and is of great importance in identification (Kulkarni, 2013). Pappus also can change its morphology in the presence of moisture in various ways that aid germination. The shape change can adjust the rate of abscission, allowing germination to increase or decrease depending on the favorable conditions Greene and David (2005). Loss or significant reduction of the pappus is not only important for identification but also for adaptation to changing environmental factors and habitats. Moreover, the fact that the length of the pappus is less than the length of the achene is considered a result of adaptation to arid environments (Paria ve China, 1998; Abid and Kayzer, 2009).

In this study, pappus was found in all samples. Pappuses were sparse and shorter than achene length in *C. virgata*, *C. iberica*, and *C. consanguinea*, close to each other in *C. balsamita* and *C. solstitialis* and longer in the other 4 species. Especially in *C. stapfiana* and *C. kurdica* species, pappus lengths were very long, whereas in *C. behen* and *C. urvillei* ssp *nimrodus* they were very dense.

Another taxonomically important character is hairiness (Uysal et al., 2005). In *C. rigida* and *C. urvillei* ssp *nimrodus*, the surface of the achene is covered with dense hairs. Likewise, it has been reported that the hairiness of the species also indicates drought. In *C. iberica*, *C. urvillei* ssp *nimrodus*, *C. kurdica* and *C. solstitialis* the phyllaries end in horn-shaped

elongated spines. Sparse trichomes were observed in the *C. iberica* achene (Bıyıklıoğlu et al., 2018).

Furthermore, another important feature in the taxonomy of the genus is the presence of appendages. The appendage is present in the *C. balsamita* species, though the remaining 9 lack appendages. The data obtained in this study prove the significance of achene and pappus size, color, and shape differences in systematics. *C. urvillei* ssp. *nimrodus* and *C. stapfiana* are endemic species and their achene characteristics were reported for the first time in this study.

5. Conclusion

The aim of the study was to demonstrate the significance of the micro and macro morphological characteristics of achenes for systematic differentiation. Similarly, in this study, some of the characteristics used to differentiate taxa, such as achene size, color and shape, pappus length and color, and the presence of appendages or hairs, were used. Based on the results of the investigation, oval or oblong achene shapes were found to be common among the taxa studied. Analysis of the SEM micrographs revealed ornamentation on some achene surfaces and different states of hairiness, and both sides of the achenes were laterally flattened. In conclusion, this study supports the need for the diagnostic use of achene surface patterns.

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Isolation and Identification of Phosphate Solubilizing Bacteria (PSB) from the Rhizosphere of *Thymus vulgaris* L.

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Abstract

Phosphate-solubilizing bacteria dissolve insoluble form of phosphate in the soil with different mechanisms, converting them into a form that can be used, and replaced by chemical fertilizers providing beneficial use of a natural reserve. Although research on phosphate solubilizing bacteria has increased recently, research on the thyme rhizosphere is still limited. It is necessary to investigate different rhizospheric local bacteria that can solubilize phosphate and replace chemical fertilizers. 42 bacterial isolates were obtained from the rhizosphere of Thyme (*Thymus vulgaris* L.) in this study. Among these isolates, 13 phosphate-solubilizing bacterial isolates were selected which formed a transparent (halo) region around the colonies on Pikovskaya's Agar (PKA) plates. Isolates were identified using the MALDI-TOF MS method. The morphological, biochemical and IAA production of these isolates as well as quantitative measurements of phosphate solubilization of the isolates in NBRIP broth medium was evaluated. The highest efficiency was noted from *Bacillus pumilus* PCB-6 with solubilization value of 326.8 ppm. This was followed by *Acinetobacter calcoaceticus* PCB-3 with solubilization value of 313.8 ppm and *Pantoea agglomerans* PCB-4 with solubilization value of 307.4 ppm, respectively. Among the Phosphate solubilizing bacterial isolates, P solubilization index was defined as between 1.6 and 4.2 on PKA agar medium. Additionally, the highest IAA production, at 18.43 µg ml⁻¹, was obtained from *Pantoea agglomerans* PCB-4. This was followed by *Bacillus pumilus* PCB-6 with 17.40 µg ml⁻¹ and *Acinetobacter calcoaceticus* PCB-3 with 16.71 µg ml⁻¹. This study clearly shows that selected local isolates can be used as effective phosphate-based microbial fertilizers.

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microbial fertilizer

1. Introduction

Phosphorus, is a crucial element for plant growth, development, and seed formation, and is a key component of many biological molecules, including nucleic acids and enzymes. Biological oxidation plays a key role in metabolic reactions such as photosynthesis and respiration. Phosphorus is stored in plant seeds and pollen as phytate. It is the primary source of inositol in plants. Soil phosphorus deficiency causes plants to slow down root formation, limit photosynthetic activity, and decrease tolerance to phytopathogens (Aktas and Toğay, 2022). Although phosphorus is abundant in the soil in both organic (such as phytate and nucleotides) and inorganic forms (such as $\text{Ca}_3(\text{PO}_4)_2$), plants cannot use it directly because they do not dissolve. Plants can only take up phosphorus from the soil in the form of phosphate anions. Orthophosphorus (H_2PO^- and HPO_4^{-2}) is the primary inorganic form of phosphorus that plants can absorb and utilize. Inorganic phosphate fertilizers are commonly added to agricultural soils to increase available phosphorus and plant yield. The applied phosphorus is a highly reactive molecule, it forms complexes with cations like Fe^{+3} , Ca^{+2} , Al^{+3} , and Mg^{+2} in the soil. Therefore, in general only less than 30 % of the applied P fertilizers is utilized by the plants and rest of this phosphorus converts into a form that can not be used by plants and contributes to environmental pollution. To enhance P uptake efficiency, innovative and sustainable eco-friendly alternatives, such as microbial fertilizer applications, are necessary (Selçuk and Çakıcı, 2022).

Many microorganisms (like bacteria, fungi, and algae) are well-known for their ability to efficiently solubilize phosphorus and maintain soil health. The most well-known efficient phosphate-solubilizing groups are *Bacillus*, *Pseudomonas*, *Rhizobium*, *Penicillium*, *Aspergillus*, *Actinomycetes*, and Arbuscular Mycorrhizal (Haile et al., 2022). Bacteria are key microorganisms that regulate soil phosphate levels. Despite the extremely limited capacity of plants to absorb

phosphorus, phosphate-solubilizing bacteria in their immediate surroundings and a small part of the rhizosphere offer plants a chance to take up phosphorus directly. Some Plant Growth-Promoting Rhizobacteria (PGPR) are particularly effective in solubilizing highly insoluble tricalcium phosphate, hydroxyapatite, and rock phosphate (Akçura and Çakmakçı, 2023; Cheng et al., 2023).

Phosphate-solubilizing bacteria play a vital role in agriculture due to their ability to enhance plant growth and improve nutrient uptake. By solubilizing phosphorus in the soil, these bacteria make this essential nutrient more readily available to plants, leading to increased growth and development. Additionally, the improved availability of phosphorus can enhance the uptake of other nutrients by plants, further supporting their nutritional requirements. Moreover, plants treated with phosphate-solubilizing bacteria Show to exhibit increased resistance to various environmental stresses, such as drought and disease. This highlights the significant impact that these bacteria can have utility in improving agricultural productivity and sustainability, and enhance play a vital role soil fertility, promoting plant growth, and improve nutrient uptake (Billah et al., 2019). The environmental and health hazards posed by chemical fertilizers, coupled with their soaring costs, have underscored the need for sustainable methods in cultivating medicinal and aromatic plants such as Thyme. Consequently, the importance of alternative, eco-friendly fertilizers has been brought to the forefront by these concerns. This study aimed to identify the local bacterial community in the thyme rhizosphere using MALDI TOF MS and evaluate its potential as a phosphate solubilising microbial fertilizer.

2. Materials and Methods

2.1. Collection of soil samples

Rhizospheric soil samples were collected on May 2023 from *T. vulgaris* in the Medicinal Plants Garden of the Department of Field Crops, Faculty of Agriculture (39°57'44.2"N, 32°51'36.7"E) Ankara University. Following aseptic procedures, the soil samples were

promptly collected from the rhizosphere soil of *Thymus vulgaris* L. at a depth of 10 cm, by collecting them in sterile plastic bags and transferred to the laboratory for further

analysis. Figure 1 shows the flowchart of the process from isolating bacteria in the Thyme rhizospheric soil sample to determine their phosphate solubilizing abilities.

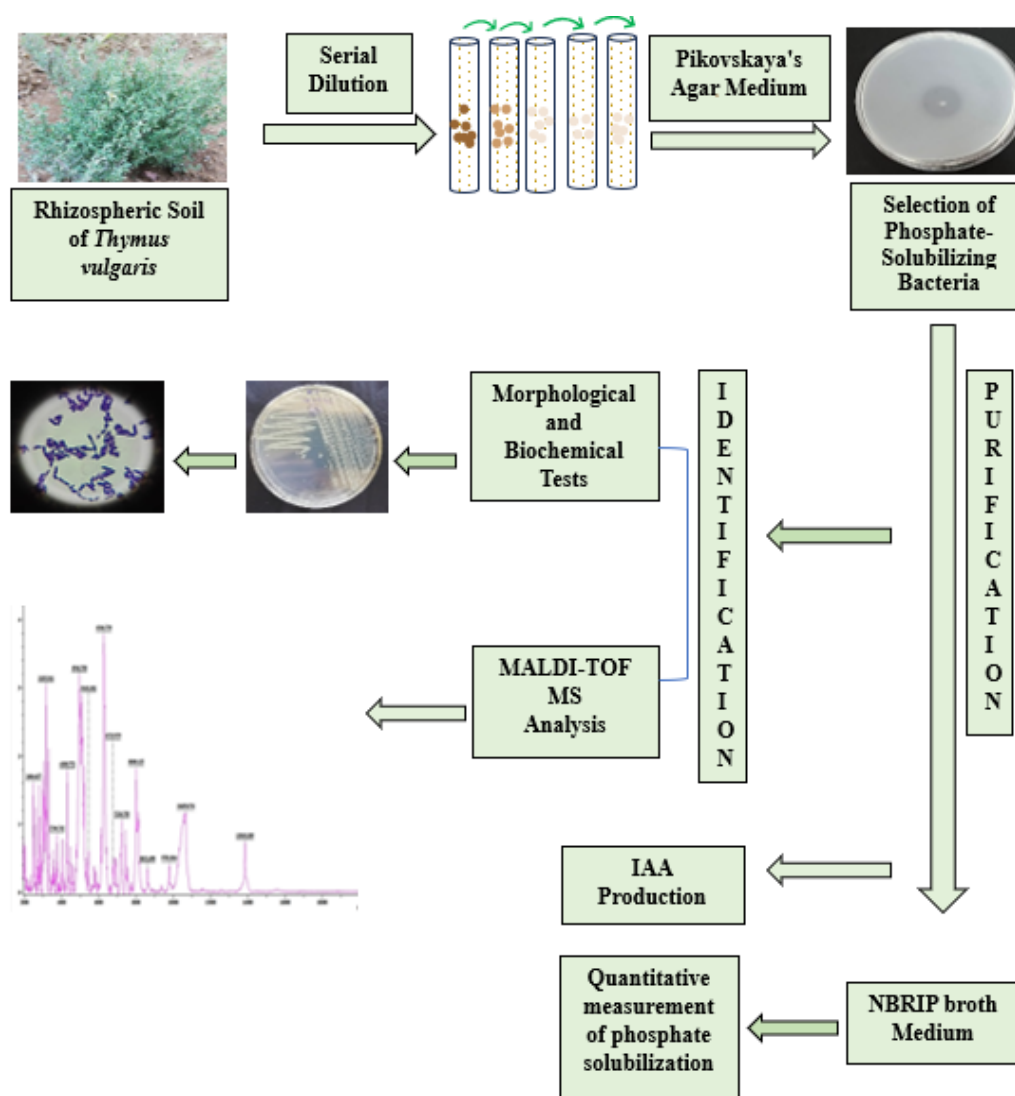


Figure 1. Flow chart for identification of isolates in Thyme rhizospheric soil samples

2.2. Isolation of phosphate solubilizing bacteria

Phosphate solubilizing bacteria were isolated from 1 g of rhizospheric soil samples using the serial dilution method. Soil samples were homogenized in 10 ml of sterile isotonic saline water. The soil samples (1 g) were mixed with 9 ml of 0.85 % saline (NaCl) sterile water and then homogenized in a shaker for 10 min. Each rhizospheric soil sample was diluted from 10^{-1} to 10^{-6} . These dilutions were spread on Pikovskaya's Agar (PKA) (0.2 g L^{-1} NaCl,

10 g L^{-1} glucose, 0.2 g L^{-1} KCl, 5 g L^{-1} $\text{Ca}_3(\text{PO}_4)_2$, 0.5 g L^{-1} $(\text{NH}_4)_2\text{SO}_4$, 0.1 g L^{-1} $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g L^{-1} $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g L^{-1} yeast extract, 0.002 g L^{-1} $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 1000 ml distilled water) and incubated for 5 days at 30°C . The formation of a clear halo zone around the colonies on PKA agar plates indicated the presence of phosphate solubilizing bacteria. To obtain pure cultures, single colonies with clear zones were transferred to plates containing the same medium. Pure phosphate solubilizing bacteria

colonies were spot inoculated at the center of the Pikovskaya agar medium. After 10 days of incubation at 30 °C, the zones of phosphate solubilization around the colonies were measured. The experiments were performed in triplicate. The purified isolates were maintained on nutrient agar plates at 4 °C, and

duplicates of each isolate were preserved in 40% glycerol stocks at -30 °C. The solubilization index (SI) was determined using measurements taken after seven days of growth from a point inoculation on PKA medium at 28 °C (Meena et al. 2015).

$$SI = \frac{(\text{Colony diameter} + \text{Halo zone diameter})}{\text{Colony diameter}}$$

2.3. Quantitative measurement of phosphate solubilization

The phosphate solubilizing efficiency of 13 isolates that previously created a transparent (halo) zones on Pikovskaya Agar was assessed using the methodology developed by Barton (1948). NBRIP broth (5 g L⁻¹ Ca₃(PO₄), 2.5 g L⁻¹ MgCl₂.6H₂O, 10 g L⁻¹ glikoz, 2.25 g L⁻¹ MgSO₄.7H₂O, 0,1 g L⁻¹ (NH₄)₂SO₄, and 0.2 g L⁻¹ KCl) was used to quantitatively determine the phosphate solubilizing abilities of phosphate-solubilizing isolates. For this purpose, 0.1 ml of fresh isolate (10⁸ CFU ml⁻¹) was inoculated in triplicate into test tubes containing 10 ml of NBRIP growth medium and incubated at 30 °C at 180 rpm for 7 days. After incubation, the tubes were centrifuged at 5000 rpm for 10 min, and then the supernatant of each culture was analyzed for phosphate concentration in ppm. The experiments were performed in triplicate. Non inoculated medium was used as control.

2.4. Determining IAA production by phosphate-solubilizing bacteria

The Sarwar and Kremer (1995) protocol was used to assess the isolates' capacity to produce IAA. Bacterial cultures were cultivated for 48 hours at 30±2 °C. These freshly prepared cultures were centrifuged for 30 minutes at 3000 rpm. The supernatant (2 ml) formed after centrifugation was mixed with 4 ml of the Salkowski reagent (reagent (50 ml, 1 ml 0.5 M FeCl₃ solution, 35 % perchloric acid) and two drops of orthophosphoric acid. Pink appearances indicated presence of IAA. The presence of IAA in the culture supernatant was determined spectrophotometrically

(SHIMADZU UVmini-1240 Spectrophotometer) at 530 nm.

2.5. Morphological and biochemical characterization of phosphate-solubilizing bacteria

The morphological characterization of 13 phosphate-solubilizing bacteria (PCB) was carried out using color, motility, and Gram staining assays, while the biochemical characterization was performed using catalase and oxidase tests. The catalase and oxidase test of the isolates was determined according to the protocol described by Clarke and Cowan (1952). For the catalase test, 2 drops of 30% hydrogen peroxide were dropped on the colonies taken with a sterile loop and the emergence of gas bubbles was observed. The observation of gas bubbles indicated a positive result. For the oxidase test, 1 % tetramethyl-p-phenylenediamine was dropped on the colonies using a sterile loop, and a change in color to blue indicated a positive result.

2.6. Identification of phosphate-solubilizing bacteria

The MALDI TOF MS method was used to identify 13 isolates with phosphate solubilizing ability. Microorganisms were identified by their unique molecular fingerprints using the MALDI Biotyper CA System. In this method, the protein profiles of microorganism biomolecules (such as proteins, peptides, sugars, and polymers) were ionized and passed through an electric and/or magnetic field. These profile spectra were compared graphically to reference microorganisms in the database of the system to accurately identify

them by genus and species (Sivri and Öksüz, 2019).

2.7. Data analysis

Data on phosphate solubilization efficiency and solubilization index (SI) were analyzed in triplicate with JMP Pro 17.0 statistical software. Dependant variables with normal distribution were presented as mean $\pm$ Standart Devision (SD) (Genç and Soysal, 2018).

3. Results and Discussion

3.1. Morphological and biochemical characterization

13 of the 42 isolates isolated from the thyme rhizosphere were determined to form a

transparent (halo) region around the colonies on Pikovskaya's Agar (PKA) medium in this study. These isolates were selected for morphological, biochemical and phosphate quantification. Among the 13 isolates, 6 were Gram positive; and 7 showed Gram-negative reactions. The catalase test was positive for all isolates except for PCB-5, whereas the oxidase test was positive for 8 isolates except for PCB-2, PCB-3, PCB-4, and PCB-10. All isolates were positive in motility test and the colony color was mostly white. Morphological and biochemical characteristics of rhizobacterial isolates are given in Table 1.

Table 1. Morphological and biochemical traits of isolates

Isolates No	MALDI-TOF MS results	Gram Stain Test	Motility	Colony color	Biochemical		Characteristics
					Catalase	Oxidase	
PCB-1	<i>Pseudomonas koreensis</i>	-	+	white	+	+	
PCB-2	<i>Bacillus subtilis</i>	+	+	cream	+	-	
PCB-3	<i>Acinetobacter calcoaceticus</i>	-	+	cream	+	-	
PCB-4	<i>Pantoeae agglomerans</i>	-	+	light yellow	+	-	
PCB-5	<i>Stenotrophomonas rhizophila</i>	-	+	white	-	+	
PCB-6	<i>Bacillus pumilus</i>	+	+	cream	+	+	
PCB-7	<i>Lactobacillus paracasei</i>	+	+	white	+	+	
PCB-8	<i>Bacillus mojavensis</i>	+	+	white	+	+	
PCB-9	<i>Bacillus pumilus</i>	+	+	cream	+	**	
PCB-10	<i>Pantoea agglomerans</i>	-	+	light yellow	+	-	
PCB-11	<i>Bacillus mojavensis</i>	+	+	white	+	+	
PCB-12	<i>Pseudomonas libanensis</i>	-	+	light yellow	+	+	
PCB-13	<i>Pseudomonas chlororaphis</i>	-	+	white	+	+	

Note: * +, positive; -, negative ** Not detected

3.2. Identification of isolates by MALDI-TOF-MS method

MALDI-TOF MS analysis identified 13 isolates from soil samples in the rhizosphere, including 5 *Bacillus* (PCB-2, PCB-6, PCB-8,

PCB-9, and PCB-11), 3 *Pseudomonas* (PCB-1, PCB-12, and PCB-13), 2 *Pantoeae* (PCB-4, and PCB-10), 1 *Stenotrophomonas* (PCB-5), 1 *Lactobacillus* (PCB-7) and 1 *Acinetobacter* (PCB-3) (Figure 1).

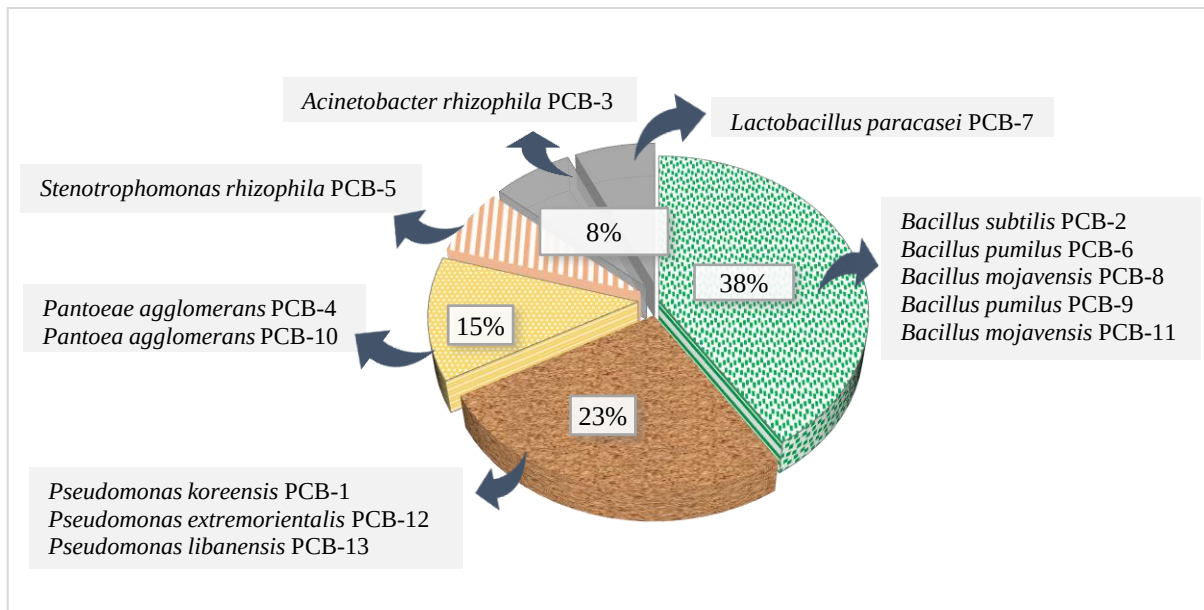


Figure 1. Percentage distribution of phosphate-solubilizing bacteria

MALDI-TOF MS is an extremely useful tool for identifying bacteria at the genus, species, and strain levels. Recently, this method has gained popularity due to its high accuracy and rapid results. In previous studies, many researchers used the MALDI-TOF MS method to identify phosphate-solubilizing bacteria (Lacava et al., 2021; Omar, 2022). Muthuri et al. (2012) identified forty-three phosphate-solubilizing endophytic bacteria strains, including *Pseudomonas*, *Bacillus*, and *Klebsiella*, in bananas using the MALDI TOF method. Çelikten and Bozkurt (2018) used the MALDI-TOF method to identify 120 bacteria they isolated from the wheat rhizosphere to investigate plant growth-promoting bacteria. Martínez-Hidalgo et al. (2021) determined that the phosphate-solubilizing bacteria in canola rhizospheres, which they identified with the MALDI TOF method, mostly belonged to the *Paenibacillus* and *Pseudomonas* genera. Similarly, Öksel et al. (2022) used the MALDI-TOF MS method to identify bacteria in wheat rhizospheres. The findings of this study revealed that *Bacillus* (38 %) *Pseudomonas* (23 %) were the most common bacterial genera

in *T. vulgaris* rhizosphere. Dip et al. (2024) determined that the phosphate-solubilizing bacteria in the root zones of *Sporobolus indicus* and *Panicum coloratum*, identified using the MALDI TOF method, mainly belonged to the *Enterobacter* and *Pseudomonas* genera.

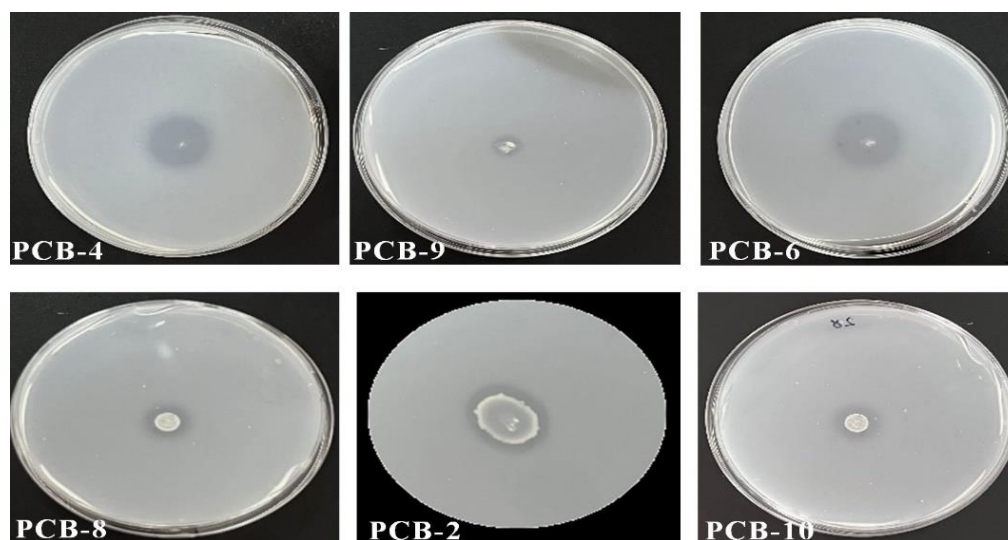
3.3. Phosphate solubilization and IAA production properties of isolates

In current study, P solubilization index of PSB strains are defined to be between 1.6 to 4.2 on PKA agar medium (Figure 2). On the other hand, in the NBRIP broth medium a rate of 84.3 to 326.8 ppm was detected. According to the phosphate solubilizing abilities of phosphate solubilizing bacteria in NBRIP broth medium, the highest value was obtained from *Bacillus pumilus* PCB-6 with 326.8 ppm. This was followed by *Acinetobacter calcoaceticus* with 313.8 ppm and *Pantoea agglomerans* PCB-4 with 307.4 ppm, respectively. The phosphate solubilizing activities and solubilization index (SI) of the isolates were shown in Table 2.

Table 2. Phosphate solubilization activities and solubilization index (SI) of the isolates

Isolates No	Phosphate solubilizing activity (ppm)	Solubilization index (SI)
<i>Pseudomonas koreensis</i> PCB-1	287.6±5.92 ^c	2.4±0.26 ^{cd}
<i>Bacillus subtilis</i> PCB-2	233.5±1.93 ^e	2.8±0.26 ^{bc}
<i>Acinetobacter calcoaceticus</i> PCB-3	313.8±7.45 ^b	3.5±0.36 ^{ab}
<i>Pantoea agglomerans</i> PCB-4	307.4±3.68 ^b	3.8±0.40 ^a
<i>Stenotrophomonas rhizophila</i> PCB-5	84.3±2.10 ^h	1.8±0.36 ^{de}
<i>Bacillus pumilus</i> PCB-6	326.8±5.10 ^a	4.2±0.26 ^a
<i>Lactobacillus paracasei</i> PCB-7	233.1±1.45 ^c	1.4±0.36 ^c
<i>Bacillus mojavensis</i> PCB-8	221.3±1.90 ^f	1.7±0.17 ^{de}
<i>Bacillus pumilus</i> PCB-9	197.4±1.68 ^g	1.6±0.5 ^{de}
<i>Pantoea agglomerans</i> PCB-10	242.7±2.10 ^e	1.8±0.1 ^{de}
<i>Bacillus mojavensis</i> PCB-11	206.6±1.60 ^g	1.7±0.1 ^{de}
<i>Pseudomonas libanensis</i> PCB-12	262.1±4.16 ^d	2.3±0.26 ^{cd}
<i>Pseudomonas chlororaphis</i> PCB-13	281.5±2.47 ^c	2.1±0.17 ^{cde}

*For Data Analysis: $p < 0.001$; statistically significant level. a-i: The difference between the means shown by different letters in the same column is statistically significant. (Mean ± SD: Mean±Standard Deviation)

**Figure 2.** The transparent zones formed by phosphate solubilizing bacteria on PKA agar medium

IAA is a hormone that promotes cell elongation, division, and differentiation in plants, as well as root system development, nutrient uptake, and overall plant growth. Plant hormone producing bacteria increase root surface area, leading to greater nutrient uptake, as reported by Bai et al. (2003). Since PGPRs in the rhizosphere produce IAAs, plant roots are more influenced by these IAAs from PGPRs, which boosts root system growth and increases the root surface area in contact with the soil (Randive et al., 2024). IAA synthesis is common among soil and plant-associated bacteria. Asra et al. (2024) reported that

PGPRs in the rhizosphere can support plant growth by producing IAA. In this study, it was determined that indigenous PSB strains isolated from thyme rhizome can produce IAA at different rates except *Stenotrophomonas rhizophila* PCB-5. According to the findings of the current study, the highest IAA production was obtained in *Pantoea agglomerans* PCB-4 with 18.43 $\mu\text{g ml}^{-1}$. This was followed by *Bacillus pumilus* PCB-6 with 17.40 $\mu\text{g ml}^{-1}$ and *Acinetobacter calcoaceticus* PCB-3 with 16.71 $\mu\text{g ml}^{-1}$, respectively (Figure 3A and 3B).

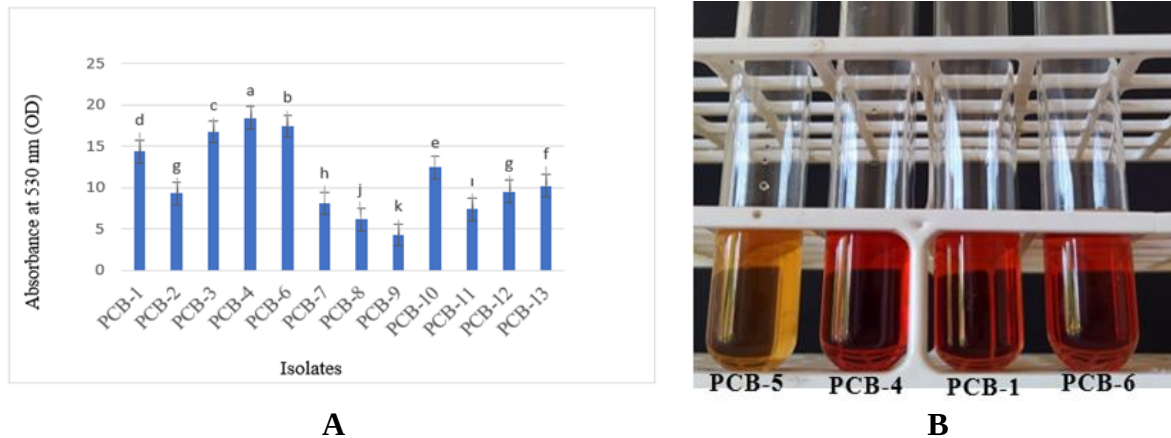


Figure 3. A. IAA production by isolates on NBRIP medium B. Pink appearances formed by some IAA producing isolates

In previous studies, researchers found similar results regarding phosphate solubilization and IAA production of strains isolated from different plant rhizospheres. Panhwar et al. (2012) determined that *Bacillus* sp. PSB9 isolated from aerobic rice in Malaysian rice fields was an effective phosphate solubilizer and IAA producer. Rfaki et al. (2020) reported that among the phosphate-solubilizing bacteria isolated from the wheat rhizosphere, the strain producing the highest amount of IAA was *Pseudomonas* sp. B26 with 21.2 mg ml^{-1} , while the strain producing the least amount of IAA was *Serratia* sp. B107 with 2.9 mg ml^{-1} . According to Sharma et al. (2021), 80 % of bacteria isolated from the rhizosphere can produce IAA. Khatami et al. (2023) reported that rhizospheric *Bacillus* sp. synthesized high amounts of IAA. On the other hand, In the current study, *Bacillus subtilis* PCB-2 ($9.29 \text{ } \mu\text{g ml}^{-1}$), *Bacillus mojavensis* PCB-8 ($6.16 \text{ } \mu\text{g ml}^{-1}$) and *Bacillus pumilus* PCB-9 ($4.27 \text{ } \mu\text{g ml}^{-1}$) produced IAA with low potential.

Audipudi et al. (2012) tested the phosphate solubilization abilities of bacteria using solid and liquid medium and determined that their phosphate solubilization abilities ranged 80 to 100 ppm. Pramanik et al. (2017) utilized MALDI-TOF MS for the identification of the K5 strain as *Klebsiella pneumoniae*, which demonstrated phosphate solubilization at 80.25 ppm. Mei et al. (2021) documented that the strain *Pantoea agglomerans* IALR1325, isolated from flora in the Appalachian

Mountains of Central Virginia, USA, exhibited a high phosphate dissolution rate of $372.8 \text{ } \mu\text{g ml}^{-1}$. Kirui et al. (2022) determined that among 71 phosphate-solubilizing bacteria from different agricultural regions in Kenya, the bacteria with the highest phosphate solubilization index of 5.883 was *Burkholderia cepacia*.

Aliyat et al. (2022) reported that bacteria taken from the phosphate mining area had the ability to dissolve different phosphate forms (tricalcium phosphate, aluminum phosphate and iron phosphate), and the highest phosphate solubilization index (SI) belonged to *Pantoea agglomerans* CB19 with 4.79. In another study, Ma et al. (2023) documented that the solubilization index (SI) of *Pantoea rhizosphaerae* MQR6T, a phosphate-solubilizing bacterium isolated from the rhizosphere of the *A. truncatum* in China, ranged from 3.20 to 3.98. It was determined that *Pantoeae agglomerans* PCB-4 had a high (307.4 ppm) phosphate solubilization ability in the current study.

The presence of a significantly elevated population of phosphate solubilizing bacteria in the rhizosphere, as opposed to non-rhizospheric soil, has been well-established in previous studies (Linu et al., 2019; Isiya, 2024). These bacteria, primarily pseudomonas bacteria, solubilize phosphate through various mechanisms, especially acid production. According to Billah et al. (2019), phosphate-solubilizing bacteria solubilize phosphate in

the soil through the release of several organic acids and enzymes and convert it into a form that is available to the plant. Amri et al. (2023) determined 28 phosphate-solubilizing bacteria from soil samples collected from different regions of Tunisia. They found that the solubilization index ratios of these bacteria ranged 2.14 to 3.51, with the highest SI ratio belonging to *Pseudomonas fluorescens*. It was determined that the phosphate solubilization index in the *Pseudomonas* genus was 2.1-2.4 in the present study. These results align with the findings of Roychowdhury et al. (2019), and Blanco-Vargas et al. (2020), which demonstrated that the solubilization index among various bacterial isolates, including *Pseudomonas* spp., ranged 2.56-4.50. They also showed that the formation of halo zones by these bacteria on growth plates is due to the production of organic acids, thus identifying them as effective phosphate solubilizers. Kaur et al. (2022) documented that 19 phosphate-solubilizing bacteria, isolated from the potato rhizosphere and identified using the MALDI-TOF-MS method, exhibited phosphate solubility ranging between 115 -747 $\mu\text{g ml}^{-1}$. Ben Zineb et al. (2020) reported that *Pseudomonas koreensis* LT62, which they isolated from Tunisian agricultural soil, had a phosphate solubilization ability of 325.21 $\mu\text{g ml}^{-1}$ in NBRIP broth medium. Murgese et al. (2020) determined that *Pseudomonas koreensis* TFD26 isolated from the rhizosphere of *Cucumis melo* solubilized phosphate. Valli et al. (2023) reported that among 200 bacterial strains isolated from rice rhizosphere, *Pseudomonas koreensis* 69RS strain solubilized three forms of inorganic phosphate (AlPO_4 , $\text{Ca}_3(\text{PO}_4)_2$ and $\text{Fe}(\text{PO})_4$) in vitro. Similarly, In the present study, it was determined that *Pseudomonas koreensis* PCB-1 had a phosphate solubilization ability of 287.6 ppm in NBRIP broth medium. Aula et al. (2023) determined that 12 out of 17 isolates from Indonesian mangrove soil could solubilize phosphate, with *Pseudomonas libanensis* TL-8 emerging as a promising phosphate solubilizer with a PSI value of 2.82. Similarly, the phosphate solubilization index of *Pseudomonas libanensis* PCB-12 was

determined as 3.2 in the present study. Yu et al. (2011) determined that *Pseudomonas chlororaphis* (W24) strain, among the 34 isolates they isolated from the walnut rhizosphere, dissolved tricalcium phosphate (TCP) in solid and liquid medium. Similarly, *Pseudomonas chlororaphis* PCB-13 solubilized phosphate in both liquid (281.5 ppm) and solid medium (SI: 2.1) in the current study.

Rawat et al. (2021), has mentioned that the most prevalent inorganic phosphate-solubilizing bacteria in the rhizosphere are *Bacillus*, *Enterobacter*, and *Pseudomonas*. Previous studies reported that among phosphate-solubilizing bacteria, *Bacillus* was an effective phosphate solubilizer (Swain et al., 2012; Abdelmoteleb and Gonzalez-Mendoza, 2020). Solubilization of phosphate is one of *Bacillus*'s inherent characteristics (Li et al. 2023). Mukhtar et al. (2017) determined that *B. safensis* PSB5 and *B. megaterium* PSB12 from wheat rhizosphere solubilized high phosphate levels (305.6, 217.2, and 148.1 $\mu\text{g ml}^{-1}$). Prajakta et al. (2019) reported that *Bacillus mojavensis* PB-35 had a phosphate solubilization ability of 86.88 among 95 rhizobacterial strains isolated from soybean rhizosphere. In the current study, it was determined that *Bacillus mojavensis* PCB-8 with 221.3 ppm and *Bacillus mojavensis* PCB-11 with 206.6 ppm had a higher phosphate solubilization ability. Jiang et al. (2020) isolated 23 phosphate solubilizing bacteria, most of which belong to *Bacillus* sp., from the peanut rhizosphere and determined that it has a high potential to dissolve calcium phosphate (65- 496 mg L^{-1}).

Wang et al. (2020) reported that the *Bacillus subtilis* BPM12 strain in the corn rhizosphere dissolved phosphate with a ratio of 189.1 $\mu\text{g ml}^{-1}$. Gupta et al. (2022) determined that *B. subtilis* PS4, isolated from 3 different rice fields, was the strain with the highest phosphate solubilization efficiency with a ratio of 50.9. It was determined that *Bacillus subtilis* PCB-2 had a phosphate solubilization ability of 233.5 ppm in NBRIP broth medium in the present study. Dipta et al. (2017) found that

Bacillus pumilus MK5 in the cauliflower rhizosphere has phosphate solubilizing abilities at different tri-calcium phosphate concentrations, with the highest phosphate solubilization being at 500 ppm. Abdel-Hamid et al. (2021) measured the diameters of the clear zones formed by the endophytic isolates *Bacillus velezensis* T13 and *Bacillus licheniformis* T11 on Pikovskaya agar medium, obtained from the roots of *Thymus vulgaris*. They found that the phosphate solubilizing activity of these diameters was 7.5 ± 0.3 mm and 8.9 ± 0.2 mm, respectively. Sanchez-Gonzalez et al. (2022) reported that *Bacillus pumilus* A3, among 5 strains in the potato rhizosphere, had a phosphate solubilizing potential of 246 mg L⁻¹. These findings are consistent with the results of the current study. In the present study, *Bacillus pumilus* PCB-6 was determined to have a high (326.8 ppm) phosphate solubilizing potential.

4. Conclusion

Low phosphorus in agricultural soils is an urgent problem affecting agricultural systems worldwide, significantly limiting crop productivity and leading to a significant decrease in crop yields. The supplementation of chemical P fertilizers to low phosphorus deficiency in soils causes serious damage to the environment by disrupting the ecosystem. In addition, plants can absorb less than 30 % of chemical phosphorus fertilizers. Therefore, it is an inevitable fact that alternative natural fertilizers are needed to eliminate phosphorus deficiency in soils. Phosphate-solubilizing bacteria contribute to the high availability of soluble phosphates that can be assimilated by plants by dissolving insoluble phosphate in the soil and alleviate the problem of immobilization of a large part of inorganic phosphates applied to the soil by chemical fertilizers. Several studies have shown that many rhizosphere PGPR strains act as phosphate solubilizers. Screening strategies are needed to select the best local rhizobacteria strains that are environmentally friendly to prevent the long-term use of chemical phosphorus fertilizers that cause environmental and ecological problems. The

use of phosphate-solubilizing local bacteria in different rhizospheres is crucial for sustainable agriculture as it reduces the need for chemical fertilizers, lowers fertilizer costs, and minimizes environmental harm. Although the isolation of bacteria from different thyme rhizospheres (*Thymus danenensis*) and their plant growth promoting properties have been investigated, there is no study on phosphate-solubilizing bacteria in the *Thymus vulgaris* rhizosphere. This is the first study on the isolation of phosphate solubilizing bacteria from the *Thymus vulgaris* rhizosphere. In this study, 13 bacterial isolates from the rhizosphere of Thymus (*Thymus vulgaris* L.) had good phosphate solubilization ability. When the phosphate solubilization abilities of these phosphate solubilizing bacteria were compared in NBRIP medium, the most effective one was *Bacillus pumilus* PCB-6 with 326.8 µg ml⁻¹. This was followed by *Acinetobacter calcoaceticus* PCB-3 with 313.8 µg ml⁻¹ and *Pantoea agglomerans* PCB-4 with 307.4 µg ml⁻¹, respectively. These selected local isolates should be evaluated in terms of competence, plant growth performance, and antifungal activity against different pathogens at greenhouse and field levels.

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İkinci Ürün Koşullarında Mısır-Maş Fasulye Üretiminde Farklı Ekim Sistemlerinin Silaj Kalite ve Fermentasyonuna Etkisi

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Özet

Birlikte üretim, bir veya daha fazla benzer olmayan bitkinin aynı sıralarda veya farklı sıralarda birlikte yetiştirilmesi olarak tanımlanmaktadır. Bu çalışma, Kahramanmaraş ekolojik koşullarda mısır+maş fasulyesi bitkilerinin aynı sıra üzerinde farklı ekim desenlerinin (saf mısır (% 100 mısır), saf maş fasulyesi (% 100 maş fasulyesi), 1M + 1MFS (% 50 mısır + % 50 maş fasulyesi), 1M + 1MFA (% 100 mısır + % 100 maş fasulyesi), 2M + 1MFA (% 100 mısır + % 50 maş fasulyesi), 3M + 1MFA (% 100 mısır + % 33 maş fasulyesi) ve 3M + 1MFS (% 75 mısır + % 25 maş fasulyesi) verim ve silaj kalitesine etkisini incelemek üzere gerçekleştirilmiştir. Deneme tesadüf bloklar deneme desenine göre üç tekrarlamalı olarak yürütülmüştür. Çalışma sonucunda toplam yeşil ot verimi 316-7430 kg da⁻¹, silaj kuru madde (T₆₀) oranı % 24.27-31.45, silaj pH'sı 3.81-4.86, laktik asit bakteri sayısı 4.54-5.74 (log₁₀ cfu g silaj⁻¹), maya sayısı 3.80-6.31 (log₁₀ cfu g silaj⁻¹) olarak belirlenmiştir. Silajların ham protein içeriğinin % 6.09-14.55, NDF içeriğinin % 43.62-50.44, ADF içeriğinin % 26.06-30.91 arasında değiştiği tespit edilmiştir. Araştırma sonucunda, birlikte üretim sistemi ile kaliteli kaba yem üretiminde artış sağlanmıştır. Elde edilen bulgular sonucunda ikinci ürün koşullarında yüksek ot verimi ve kaliteli bir silaj için 3M+1MFA ekim sistemi önerilmektedir.

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Yem verimi
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silaj

The Impact of Different Planting Systems in Maize-Mung Bean Production on the Silage Quality and Fermentation in Second Product Conditions

Abstract

Cultivation of one or more non-similar plants together in the same order or in different orders is called "intercropping". The study was conducted to study the impact of different cultivation patterns (sole maize (%100 mazie), sole mung bean (100 % mung bean), 1M + 1MFS (50 % maize + 50 % mung bean), 1M + 1MFA (100 % maize + 100 % mung bean), 2M + 1MFA (100 % maize + 50 % mung bean), 3M + 1MFA (100 % maize + 33 % mung bean) and 3M + 1MFS (75 % maize + 25 % mung bean) on the yield and silage quality of maize+mung beans on the same row in Kahramanmaraş ecological conditions. The trial randomized blocks were carried out in three repetitions according to the trial pattern. Total forage yield 315.48-7429.46 kg da⁻¹, silage dry matter (T₆₀) ratio 24.27-31.45 %, silage pH 3.81-4.86, lactic acid bacterial count 4.54-5.74 (log₁₀ cfu g silage⁻¹), yeast count 3.80-6.31 (log₁₀ cfu g silage⁻¹). Protein content of varied from 6.09 to 14.55 %, NDF content from 43.62 to 50.44 %, and ADF contents from 26.06 to 30.91 %. The research resulted in an increase in quality raw forage production, combined with the production system. As a result of the findings obtained, 3M+1MFA planting system is recommended for high forage yield and a quality silage in the second product conditions.

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1.Giriş

Hayvancılık işletmelerinin temel hedefi, sığır-dana karkas ağırlığını ve süt üretimini destekleyecek silaj verimini ve kalitesini korumaktır. Silajlık mısır (*Zea mays* L.), gelişmiş veya gelişmekte olan ülkelerde süt işletmelerinin taleplerini karşılayabilecek arzu edilen bir yem olarak önemli bir rol oynamıştır. Fakat buğdaygil bitkilerinin ham protein konsantrasyonunun baklagil bitkilerine kıyasla düşük olması nedeniyle süt veya et üretimi için ilave protein takviyesine ihtiyaç duyulmaktadır. Seydoşoğlu ve Gelir (2019) ve Arıkan ve ark. (2023), baklagiller ile tahıl bitkileri birlikte yetiştirilip silaj yapıldığında yüksek kalitede kaba yem üretimi elde edildiğini bildirmişlerdir. Birlikte ekim “intercropping” iki veya daha fazla farklı bitkilerin, aynı alanda aynı anda yetiştirilme sistemidir. Birlikte ekim sistemi ile tarımsal ürün çeşitliliği artar, toprak verimliliği korunur, herbisit, pestisit ve gübre kullanımı azalır, verimlilik artar ve yem kalitesi iyileşir. Diğer bir ifadeyle birlikte üretim, sürdürülebilir tarım hedeflerini izleyen bir sistemdir (Mousavi ve Eskandari, 2011).

Birlikte ekimin başlıca türleri arasında karışık ekim, sıraya ekim ve şeritvari ekim yer almaktadır (Ofori ve Stern, 1987). Sıraya ekim sisteminde bitkiler farklı sıraya ekilebileceği gibi aynı sıraya farklı oranlarda ekimi yapılabilmektedir. Birlikte üretim sisteminin temel hedefleri birim alandaki verim ve kaliteyi arttırmaktır. Maş fasulyesi (*Vigna radiata* (L.) Wilczek) Fabaceae familyasına ait olup, insan beslenmesi amacı ile yetiştiriciliği yapılmasına rağmen hayvan beslenmesinde kullanımı oldukça yaygındır (Mohan Naik ve ark., 2020). Maş fasulyesi, börülce ve guar gibi yıllık yazlık baklagiller, yem üretimi açısından iyi bir potansiyele sahiptir. Maş fasulyesi yüksek kuru madde verimi, yüksek ham protein içeriği, yüksek nitrojen sabitleme yeteneği, hızlı büyüme, kuraklığa toleransın yüksek olması nedeniyle gerekli kaba yemin bir kısmının sağlanmasında önemli bir rol oynayabilmektedir (Ghotbi ve ark., 2022). Ertekin ve Yılmaz (2022b), tarafından tatlı sorgum ve maş fasulyesinin birlikte üretim

sistemi ile silaj kalitesinin arttığı ve R1:2 (1 sıra maş fasulyesi + 2 sıra tatlı sorgum), MB14+SS14 (maş fasulyesi 14 bitki m², tatlı sorgum 14 bitki m²) birlikte üretim sisteminin ise en uygun karışım olduğu bildirilmiştir. Shaker-Koohi ve ark. (2014) tarafından yapılan bir çalışmada ise monokültür ekime kıyasla birlikte üretim sisteminde protein oranının arttığı bildirilmiştir. Gürel ve Okant (2020), mısır + börülce birlikte üretim sistemlerinin mısır koçan oranı hariç tüm özellikleri önemli derecede etkilediğini, birlikte üretim sistemlerinin toprak kaynakları sıkıntılı ülkelerde risk faktörünü azalttığını ve toprak verimliliğini koruduğunu bildirmişlerdir.

Bu çalışmanın temel amacı, Kahramanmaraş koşullarında silaj yapımı için mısır ve maş fasulyesinin yüksek verim ve kalitesine yönelik en iyi ekim desenini belirlemektir.

2.Materyal ve Yöntem

Bu çalışma, Kahramanmaraş koşullarında Doğu Akdeniz Geçit Kuşağı Tarımsal Araştırma Enstitüsüne (DAGTEM) 2023 yılı yaz sezonunda (Temmuz-Eylül) yürütülmüştür. Denemede Kahramanmaraş bölgesinde ikinci ürün olarak yetiştiriciliği de yapılabilen “Colonia” mısır çeşidi materyal olarak kullanılmıştır.

Deneme alanının 30 cm derinliğinden alınan toprak örneğine göre siltli-tınlı toprak sınıfında yer almaktadır. Toprağın yarayışlı fosfor (P₂O₅) miktarı 3.66 kg da⁻¹, yarayışlı potasyum miktarı (K₂O) 62.00 kg da⁻¹, organik madde miktarı % 1.03, elektriksel iletkenlik değeri 0.023 dS m⁻¹, kireç miktarı % 19.85 ve toprak pH'ı 7.80 olarak tespit edilmiştir.

Çalışmanın yürütüldüğü bölge, Doğu Akdeniz bölgesinde yer almaktadır. Kışları ılık ve yağışlı, yazları ise sıcak ve kurak bir Akdeniz iklim özelliği görülmektedir. Denemenin yürütüldüğü yıl ortalama sıcaklık miktarı uzun yıllara (1930-2023) kıyasla daha sıcak, toplam yağış miktarı ise daha düşük olarak gerçekleşmiştir. Ortalama sıcaklık değerleri Temmuz, Ağustos ve Eylül ayında sırasıyla 25.2 °C, 30.9 °C ve 26.8 °C, toplam

yağış miktarı sırasıyla 3.2 mm, 0.7 mm, 0.7 mm olarak gerçekleşmiştir (Anonim, 2024).

Saf ekim ve karışık ekim desenleri için deneysel uygulamalar saf mısır, saf maş fasulyesi, 1M + 1MFS, 1M + 1MFA, 2M + 1MFA, 3M + 1MFA, 3M + 1MFS aynı sıra üzerine 3 adet mısır ve 1 adet maş fasulyesi ekimi yapılmıştır. S kodlu olan ekim sistemlerinde mısır yerine maş fasulyesi ekimi yapılmıştır. Tablo 1'de birlikte ekim sistemlerine ait detaylı açıklama sunulmuştur. Ekim sıra arası 70 cm, sıra üzeri 15 cm olarak

planlanmıştır. Her parsel 4m uzunluğunda ve (0.70 cm x 4 sıra) 2.8 m genişliğinde tasarlanmıştır. Deneme tesadüf bloklar deneme desenine göre üç tekrarlamalı olarak yürütülmüştür. Deneme 9 kez damla sulama sistemi ile sulanmış, yabancı ot kontrolü için iki defa çapalama işlemi, kök boğaz doldurma işlemi yapılmıştır. Denemede 8 kg da⁻¹ P₂O₅ ve 18 kg N da⁻¹ olacak şekilde gübreleme yapılmıştır. Fosforlu gübrelerin tamamı, azotlu gübrelerin yarısı ekimle birlikte taban gübresi olarak, geri kalan kısmı ise bitkiler yaklaşık 40-50 cm boylandığında verilmiştir.

Tablo 1. Birlikte ekim sistemleri ile açıklamalar

Mısır ve fasulye ekim sistemleri	Açıklama	Ekim normları (bitki da ⁻¹)		Mısır ve fasulye ekim oranları
		Mısır	Maş fasulye	
Saf mısır	Mısır 70x15 cm sıklıkta ekilmiştir.	9.524	-	% 100 Mısır
Saf maş fasulye	Maş fasulyesi 70x15 cm sıklıkta ekilmiştir.	-	9.524	% 100 Maş fasulyesi
2M + 1MFA	Aynı sraya ekim yapılmıştır. Mısır bitki sıklığı hiç azaltılmadan, sıra üzerinde 2 mısırdan sonra 1 maş fasulye araya ekilmiştir. Mısırların sıra üzerindeki mesafesi 15 cm, maş fasulyelerin arasındaki mesafe ise 30 cm olacak şekilde ayarlanmıştır.	9.524	4.762	% 100 Mısır + % 50 Maş fasulyesi
3M + 1MFA	Aynı sraya ekim yapılmıştır. Mısır bitki sıklığı hiç azaltılmadan, sıra üzerinde 3 mısırdan sonra 1 maş fasulye araya ekilmiştir. Mısırların sıra üzerindeki mesafesi 15 cm, maş fasulyelerin arasındaki mesafe ise 45 cm olacak şekilde ayarlanmıştır.	9.524	3.175	% 100M + % 33 Maş fasulyesi
3M + 1MFS	Aynı sraya ekim yapılmıştır. Sıra üzerinde 3 mısırdan sonra, mısır yerine maş fasulye ekilmiştir. Yan yana iki mısır arasındaki sıra üzeri mesafesi 15 cm, arada maş fasulye olduğunda ise iki mısır arası mesafe 30 cm olmuştur. Maş fasulyelerin arasındaki mesafe ise 60 cm olacak şekilde ayarlanmıştır.	7.142	2.382	% 75 Mısır +% 25 Maş fasulyesi
1M + 1MFS	Aynı sraya ekim yapılmıştır. Sıra üzerinde 1 mısırdan sonra, mısır yerine maş fasulye ekilmiştir. İki mısır arası mesafe 30 cm, iki maş fasulye arası mesafe de 30 cm olacak şekilde ayarlanmıştır.	4.762	4.762	% 50 Mısır +% 50 Maş fasulyesi
1M + 1MFA	Aynı sraya ekim yapılmıştır. Mısır bitki sıklığı hiç azaltılmadan, sıra üzerinde 1 mısırdan sonra 1 fasulye araya ekilmiştir. İki mısır arası mesafe 15 cm, iki maş fasulye arası 15 cm olacak şekilde ayarlanmıştır.	9.524	9.524	% 100 Mısır +% 100 Maş fasulyesi

Hasat mısır bitkisinin gelişme dönemine göre belirlenmiş ve mısır danesindeki süt çizgisinin danenin üçte iki seviyesine indiği dönemde (hamur olum) hasat edilmiştir. Yeşil ot verimi için orta 2 sıra hasat edilmiş ve dekara verimi hesaplanmıştır. Hasat edilen tüm parseller içerisinde yabancı otlar ayrılmış ve parseli temsil edilecek örnekler bitki parçalayıcı-doğrayıcı makinada 2-3 cm uzunluğunda parçalanmıştır. Özel plastik vakumlanabilen paketlere yaklaşık 500 gram örnek katılarak, içerisindeki hava % 99.9

oranında alınıp, ağzı otomatik olarak yapıştırılmıştır. Silaj örnekleri güneş almayacak şekilde serin bir yerde bekletilmiştir. Ayrıca, kuru madde oranının belirlenebilmesi için 2-3 cm uzunluğunda parçalanmış örneklerden homojen bir şekilde yaklaşık olarak 100 g örnek tartılmış ve 70 °C'de etüvde sabit ağırlığa gelinceye kadar kurutulmuştur. Kimyasal analizler için kuru madde oranları belirlenen örnekler 1 mm elek çapındaki öğütme makinasında öğütülmüştür. Hücre duvarı bileşenlerinden; NDF ve ADF

içerikleri Ankom Fiber Analiz cihazında yapılarak hesaplanmıştır (Van Soest ve ark., 1991). Örneklerin, azot içeriği Kjeldahl metodu kullanılarak hesaplanmıştır (AOAC, 1990).

Fermantasyon kalitesinin belirlenmesi amacı ile taze materyal ($T_{0\text{gün}}$) ve olgunlaşmış silajların ($T_{60\text{gün}}$) pH ölçümü ve mikro organizma sayımları (laktik asit bakterileri, küfler, mayalar ve enterobakteriler) yapılmıştır. Her muamele grubundan 25 g örnek 225 ml Ringer solüsyonunda mikser ile yüksek devirde 1 dakika karıştırılmış ve Whatman 54 filtre kağıdından süzölmüştür. Elde edilen süzükten 1×10^{-1} düzeninde seyreltme serileri hazırlanmış, uygun besi yerlerine ekimleri yapılmıştır. Laktik asit bakterileri için MRS besi yeri, küfler ve mayalar için MEA besi yeri ve enterobakteriler için VRBD besi yeri kullanılmıştır. MRS ve MEA 37°C 'de 48 saat, VRBD besi yeri ise 33°C 'de 18 saat inkübe edildikten sonra mikro organizma sayımları yapılmıştır (Seale ve ark., 1990).

Veriler JMP istatistiki paket programı kullanılarak % 5 olasılık düzeyinde varyans analizine tabi tutulmuştur ve LSD testi kullanılarak anlamlı farklılıklar belirlenmiştir (Kalaycı, 2006).

3.Bulgular ve Tartışma

Silajlık mısır yeşil ot veriminin 4860-6941 kg da^{-1} arasında değiştiği, en yüksek verimin 1M + 1MFA ekim deseninden, en düşük verimin ise maş fasulyesi bitkisinin sıraya ekildiği 1M + 1MFS ve 3M + 1MFS ekim

sistemlerinden elde edilmiştir ($P<0.01$). Sıra üzerinde farklı yoğunluklarda ekimi yapılan mısır+maş fasulyesi birlikte ekim desenleri ile bitki verimlerinin arttığı belirlenmiştir. Benzer şekilde Soe ve ark. (2021), saf mısıra kıyasla birlikte üretim sistemi ile mısır yeşil ot veriminin arttığını bildirmişlerdir. Maş fasulyesi yeşil ot veriminin 316 kg da^{-1} ile 488 kg da^{-1} arasında değiştiği, en düşük maş fasulyesi yeşil ot veriminin saf ekimlerden elde edildiği belirlenmiştir ($P<0.01$). Mısır bitkisinin maş fasulyesini gölgelemesi ve maş fasulyesinin sarılcı yapısı nedeniyle bitki boylarının uzadığı, bu da ot verimine katkı sağladığı ifade edilebilir. Nitekim, Sencar ve ark. (1997), bitki sıklığı azaldıkça bitkilere ulaşan ışık yoğunluğunun arttığını, yoğun ışığın bitkilerin alt boğumlardaki odunlaşmayı teşvik ederek bitki boyunun kısalmasına neden olduğunu bildirmişlerdir. Bununla birlikte, birçok çalışmada baklagil ve buğdaygil bitkilerinin birlikte üretilmesi ile yeşil ot veriminin arttığı rapor edilmiştir (Khan ve ark., 2012; Aizaz ve ark., 2023).

Aynı sıraya farklı ekim desenlerinin toplam yeşil ot verimine etkisi istatistiki olarak önemli bulunmuştur ($P<0.01$). En yüksek toplam yeşil ot verimi 7430 kg da^{-1} ile 1M + 1MFA ekim deseninden elde edildiği, bunu 7085 kg da^{-1} ile 2M+1MFA ekim deseninin izlediği en düşük toplam yeşil ot veriminin ise 316 kg da^{-1} ile saf maş fasulyesi ekim deseninden elde edildiği belirlenmiştir. Benzer şekilde, Erdoğan ve ark., (2013) ve Zheng ve ark. (2020), birlikte üretim sistemi ile kaba yem veriminin artırılabilceğini bildirmişlerdir.

Tablo 2. Birlikte üretim ekim desenlerinin yeşil ot verimleri, kuru madde oranları ve pH değerine etkisi

Ekim Deseni	Yeşil ot verimi (kg da^{-1})		Toplam yeşil ot verimi (kg da^{-1})	KM(T_0)		
	Mısır	Fasulye		KM(T_0)	KM(T_{60})	pH(T_{60})
Saf Mısır	5960 d	-	5960 d	33.76 a	31.45 a	3.81 d
Saf Maş fasulyesi	-	316 b	316 f	27.57 d	24.27 e	4.86 a
1M + 1MFS	4860e	555 a	5415 e	31.95 bc	27.57 d	3.97 b
1M + 1MFA	6941 a	488 a	7430 a	32.81 ab	29.80 b	3.90 bc
2M + 1MFA	6624 b	460 a	7085 b	31.19 c	28.33 cd	3.97 b
3M + 1MFA	6265 c	452 a	6717 c	32.82 ab	30.48 ab	3.86 cd
3M + 1MFS	5096 e	477 a	5573 e	32.43 abc	28.64 c	3.89 c
Ortalama	5958	458	5499	31.79	28.65	4.06
F değeri	<0.0001**	0.0256*	<0.0001**	<0.0001**	<0.0001**	<0.0001**
LSD	253.66	120.88	248.96	1.54	0.98	0.95

*:p<0.05, **:p<0.01 istatistiki düzeyde önemli, KM: kuru madde oranı

Farklı ekim desenlerinin taze materyal ve olgunlaşmış silajların KM içeriğini istatistiki olarak etkilediği Tablo 2’de görülmektedir. Taze materyalin KM oranının % 27.57-33.76 arasında değiştiği, en yüksek KM içeriği saf mısır ekimlerinden elde edildiği, bu değeri 3M + 1MFA, 1M + MFA ekim desenlerinin izlediği belirlenmiştir. Çalışmada en düşük KM değeri saf maş fasulyesi ekimlerinden elde edilmiştir. Birlikte üretim KM içeriği saf mısır ekimine kıyasla daha düşük çıkmıştır. Benzer şekilde Azim ve ark. (2000) ve Kızılsimşek ve ark. (2020) tarafından gerçekleştirilen çalışmalarda saf mısır silajının karışım silajlarına göre KM içeriğinin daha yüksek olduğu bildirilmiştir. KM (T_{60}) değerleri incelendiğinde en yüksek değer saf mısır, en düşük değer ise saf maş fasulyesi ekimlerinden elde edilmiştir. Mısır maş fasulyesi birlikte ekim desenleri incelendiğinde ise en düşük KM (T_{60}) içeriği % 27.57 ile 1M + 1MFS ekim deseninden elde edilmiştir. En yüksek KM (T_{60}) içeriği 3M + 1MFA ekim deseninde belirlenmiştir. Baklagil bitkilerinin KM ve suda çözünen karbohidrat (SÇK) içeriklerinin düşük olması ve tamponlanma kapasitesinin yüksek olması nedeniyle silaj fermentasyon sonucunda KM kaybı görülmektedir. Dolayısı

ile taze materyal KM (T_0) içeriği 1M + 1MFA ile 3M + 1MFA aynı gruplarda yer almasına rağmen silaj fermentasyon sonucunda 1M + 1MFA grubunda daha fazla kuru madde kaybı gözlenmiştir. Bu durum maş fasulyesinin 1M + 1MFA silajında daha fazla oranda bulunması ile ilişkilendirilebilir. Elde edilen bulgular Kızılsimşek ve ark., (2020)’nın sonuçları ile uyumludur.

pH değerinin 3.81-4.86 arasında değiştiği en yüksek pH değerinin saf maş fasulyesinden elde edildiği, en düşük pH’ın ise saf mısır silajından elde edildiği belirlenmiştir. Çalışmada birlikte üretim sistemi ile maş fasulyesinin silaj pH değerinin iyileştiği belirlenmiştir. Birlikte üretim sisteminde en düşük pH değeri, mısırın ekim sisteminin azaltılmadığı, fasulyenin araya ekildiği 3M + 1MFA ekim sisteminden elde edilmiştir. Birlikte üretim sisteminde en yüksek pH değeri ise 1M + 1MFS ve 2M + 1MFA ekim sistemlerinden elde edilmiştir. Bu durum maş fasulyesinin bu ekim sistemlerinde fazla oranda olması ile ilişkilendirilebilir. Elde edilen bulgular Kızılsimşek ve ark. (2020)’nın sonuçları ile uyumludur.

Tablo 3. Birlikte üretim ekim desenlerinin mikroorganizma sayımlarına etkisi

Ekim Deseni	LAB sayısı (T_0)	LAB sayısı (T_{60})	Maya sayısı (T_0)	Maya sayısı (T_{60})	Küf sayısı (T_0)	Enterobakteri sayısı (T_0)
Saf Mısır	7.12 a	5.23 bc	5.43 c	4.00 bc	4.74 c	7.32 c
Saf Maş fasulyesi	5.99 d	5.74 a	6.80 a	4.90 bc	5.96 a	9.06 a
1M + 1MFS	6.21 cd	4.82 cd	6.36 ab	5.18 ab	5.85 a	7.86 bc
1M + 1MFA	6.50 bc	4.64 d	6.58 ab	4.57 bc	5.65 ab	7.88 bc
2M + 1MFA	6.53 bc	4.54 d	6.50 ab	6.31 a	4.65 c	8.21 b
3M + 1MFA	6.86 ab	4.65 d	6.34 ab	3.80 c	5.62 ab	8.17 b
3M + 1MFS	6.21 cd	5.40 ab	6.12 b	4.36 bc	5.28 b	8.18 b
Ortalama	6.49	5.00	6.30	4.73	5.39	8.10
F değeri	0.0005**	0.0003**	0.0017**	0.0143**	<0.0001**	0.0017**
LSD	0.39	0.42	0.50	1.24	0.44	0.59

**: $p < 0.01$ istatistiki düzeyde önemli

Mikroorganizma sayımlarına ait sonuçlar Tablo 3’de verilmiştir. Farklı ekim desenlerinin LAB sayısı, maya sayısı, küf sayısı ve enterobakteri sayısı üzerine etkisinin istatistiki olarak önemli olduğu belirlenmiştir ($P < 0.01$). Çalışmada taze materyale ait laktik asit bakteri sayısı incelendiğinde en yüksek LAB sayısını 7.12 ($\log_{10}$ cfu g^{-1} taze materyal)

ile saf mısırdaki ve en düşük LAB sayısı ise 5.99 ($\log_{10}$ cfu g^{-1} taze materyal) ile saf maş fasulyesinde belirlenmiştir. Bu nedenle birlikte üretim sistemi ile silajların LAB sayısında azalma görülmüştür. Fermentasyon sonucunda silajların LAB sayısı ise 4.54-5.74 ($\log_{10}$ cfu g^{-1} silaj) arasında değiştiği, en yüksek değerin saf maş fasulyesi silajlarında tespit edilmiştir.

En düşük LAB sayısı ise istatistiki olarak aynı grupta yer alan 1M + 1MFA, 2M + 1MFA ve 3M + 1MFA ekim desenlerinden elde edildiği belirlenmiştir.

Taze materyalin maya içeriği 5.43-6.80 ($\log_{10}$ cfu g⁻¹ taze materyal) arasında değiştiği, en yüksek maya sayısının saf maş fasulyesi ekiminden elde edildiği, en düşük maya sayısına ise saf mısırın sahip olduğu belirlenmiştir. Çalışmada maya sayısı bakımından 1M + 1MFS, 1M + 1MFA, 2M + 1MFA ve 3M + 1MFA ekim desenlerinin aynı gruplarda yer aldığı belirlenmiştir. Olgunlaşmış silajların maya sayısının 3.80-6.31 ($\log_{10}$ cfu g⁻¹ silaj) arasında değiştiği, en yüksek maya sayısının 2M + 1MFA silajından elde edildiği, bu değeri 5.18 ($\log_{10}$ cfu g⁻¹ silaj) ile 1M + 1MFS silajının izlediği, en düşük maya sayısının ise 3M + 1MFA silajından elde edildiği belirlenmiştir. Taze materyalin küf sayısı 4.65 ile 5.96 ($\log_{10}$ cfu g⁻¹ taze materyal) arasında değiştiği, en yüksek küf sayısının 5.96 ($\log_{10}$ cfu g⁻¹ taze materyal) ile saf maş fasulyesi ve 5.85 ($\log_{10}$ cfu g⁻¹ taze materyal) ile 1M + 1MFS ekimlerinden elde edildiği, bu değeri 5.65 ($\log_{10}$ cfu g⁻¹ taze materyal) ile 1M + 1MFA ve 5.62 ($\log_{10}$ cfu g⁻¹ taze materyal) 3M + 1MFA ile ekim deseninin izlediği belirlenmiştir. En düşük küf sayısı 4.74 ($\log_{10}$ cfu g⁻¹ taze materyal) ile saf mısır ve 4.65 ($\log_{10}$ cfu g⁻¹ taze materyal) ile 2M + 1MFA ekim deseninden elde edilmiştir. Ortalama enterobakteri sayısı 8.10 ($\log_{10}$ cfu

g⁻¹ taze materyal) olarak belirlenmiş ve en yüksek enterobakteri sayısı 9.06 ($\log_{10}$ cfu g⁻¹ taze materyal) ile saf maş fasulyesinde belirlenmiştir. Olgunlaşmış silajlarda küf ve enterobakteri görüntüsü kaydedilememiştir.

Başarılı bir silaj fermentasyonu, bitkinin kuru madde içeriğine, besin içeriğine ve mikroflorasına bağlı olarak değişmektedir (Moon, 1981). LAB sayısının fazla olması siloda laktik asit üretimini hızla artırarak ortam asitliğini sağlar, böylece pH hızla düşer. Genellikle baklagil bitkilerinin laktik asit bakteri sayısı kaliteli bir fermentasyon için yeterli değildir (Akday ve ark., 2023). Öte yandan, LAB sayısı yüksek olsa dahi baklagil bitkilerinin bünyelerinde yeteri kadar fermente edecek ürünün (SÇK) bulunmaması, laktik asit üretimini kısıtlamaktadır (Kızılsimşek ve ark., 2016). Ayrıca maş fasulyesinin maya, küf ve enterobakteri sayısının da oldukça yüksek olması silaj kalitesini olumsuz etkilemektedir. Çalışmada maş fasulyesinin mısır ile birlikte üretilmesi ile silajlarda istenmeyen maya, küf ve enterobakteri sayılarında bir azalma, silajlarda istenen LAB sayılarında ise artış gözlemlenmiştir. Özellikle 3M + 1MFA ekim sisteminde en düşük maya sayısı ve enterobakteri sayısı, yüksek LAB sayısı belirlenmiştir. Ertekin ve Yılmaz (2022b) tarafından yapılan bir çalışmada, maş fasulyesi ile sorgumun birlikte ekilmesiyle LAB sayılarının iyileştiği bildirilmiştir.

Tablo 4. Birlikte üretim ekim desenlerinin silajın ham protein, NDF ve ADF içeriğine etkisi

Ekim Deseni	HP	NDF	ADF
Saf Mısır	6.09 e	50.44 a	30.91 a
Saf Maş fasulyesi	14.55 a	43.62 b	26.06 b
1M + 1MFS	9.39 b	49.38 a	29.11 a
1M + 1MFA	8.23 cd	49.09 a	29.87 a
2M + 1MFA	8.65 bc	49.54 a	30.47 a
3M + 1MFA	7.55 d	48.99 a	30.42 a
3M + 1MFS	7.64 cd	48.80 a	30.49 a
Ortalama	8.87	48.55	29.62 a
F değeri	<0.0001**	0.0068**	0.0251*
LSD	1.05	2.99	2.67

*:p<0.05, **:p<0.01 istatistiki düzeyde önemli, HP: ham protein oranı, NDF: nötr deterjan lif, ADF: asit deterjan lif

Tablo 4’de silajların ham protein, NDF ve ADF içeriklerine yer verilmiştir. Farklı ekim desenlerinin, silajların potansiyel beslenme değerini istatistiki olarak önemli derecede

etkilediği belirlenmiştir. Silajların HP oranlarının % 6.09-14.55 arasında değiştiği, en yüksek HP içeriğinin saf maş fasulyesi silajından tespit edildiği belirlenmiştir. Bu

değeri % 9.39 ile 1M + 1MFS ekim deseni izlemiştir. En düşük HP içeriği ise saf mısırdaki tespit edilmiştir. NDF oranlarının % 43.62-50.44, ADF oranlarının % 26.01-30.91 arasında değiştiği, en düşük NDF ve ADF değerlerinin saf maş fasulyesi silajında elde edildiği belirlenmiştir. Çalışmada NDF ve ADF içerikleri bakımından saf mısır silajı ve diğer ekim desenlerinden elde edilen silajlar istatistiki olarak aynı gruplarda yer almıştır.

Baklagil bitkilerinin ham protein içeriği yüksek, NDF ve ADF içerikleri ise düşüktür. Bu nedenle, mısır + maş fasulyesi birlikte üretim silajlarının saf mısır silajlarına kıyasla daha yüksek HP içeriğine sahip olduğu belirlenmiştir. Benzer sonuçlar Öten ve ark. (2016), Kızılsimşek ve ark. (2017), Kızılsimşek ve ark. (2020), Ertekin ve Yılmaz (2022a) ve Kurt ve Aydemir (2023) tarafından bildirilmiştir.

4.Sonuç

Saf ekimlere kıyasla mısır+maş fasulyesinin birlikte yetiştirilmesi ile yüksek ot verimi elde edilmiştir. Özellikle, mısır bitkisinin ekim sıklığının hiç azaltılmadığı, maş fasulyesinin de mısır aralarına ekildiği ekim sistemlerinde daha yüksek ot verimleri elde edilmiştir. Bununla birlikte, birlikte üretim sistemi ile maş fasulyesinin KM içerikleri ve pH değerlerinde iyileşme görülmüştür. Çalışmada maş fasulyesinin mısır ile birlikte üretilmesi ile silajlarda istenmeyen maya, küf ve enterobakteri sayılarında bir azalma, silajlarda istenen LAB sayılarında ise artış gözlemlenmiştir. En yüksek yeşil ot verimi 1M + 1MFA, fermentasyon sonrası en yüksek KM içeriği, en düşük pH değeri, en düşük maya sayısı 3M + 1MFA ekim sisteminden elde edilmiştir. En yüksek protein içeriği 1M + 1MFS ekim sisteminde elde edilmiş, NDF ve ADF değerleri arasında bir farklılık oluşmamıştır. Araştırma sonucunda yüksek ot üretimi için 1M + 1MFA ekim sistemi, hem yüksek ot verimi hem de kaliteli bir silaj üretimi için 3M + 1MFA ekim sistemi önerilmektedir. Ot verimi ve silaj için 1M + 1MFA ekim sistemi kullanılacaksa katkı maddeleri (LAB

inokulasyonu, melas, vb.) eklenmesi önerilmektedir.

Yazarların Katkı Beyanı

Yazarlar makaleye eşit katkıda bulunduklarını, makalenin yayına hazır son halini gördüklerini/okuduklarını ve onayladıklarını beyan ederler.

Çıkar Çatışması Beyanı

Tüm yazarlar, bu çalışma için herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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The Biological Activities of *Hypericum perforatum* L.

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Abstract

Representing the Hypericaceae family, *Hypericum perforatum* L. offers antidepressant, wound-healing, antioxidant, antiviral, and analgesic properties for burns, bruising, and edema. With the demise of many current treatment choices and the worrying levels of antibiotic resistance produced by dangerous bacteria, new therapy approaches are required. This study focused on *H. perforatum* plant's total phenolic content, antioxidant capability, and antibacterial activity. The fatty acid composition and phytochemical constituents in *H. perforatum* were also analyzed by GC-MS. The antibacterial activity of the plant's methanol extract was evaluated using disc diffusion method. Microorganisms utilized in the antibacterial activity assay included the fungus *Candida albicans*, bacteria strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. It was determined that *H. perforatum*'s methanol extract exhibited remarkable antagonistic activity against the examined pathogenic microorganisms with the lowest and strongest antibacterial effects against *S. typhimurium* and *P. aeruginosa*, respectively. The Folin-Ciocalteu phenol reagent method was used to quantify the phenolic compounds, and *H. perforatum* methanol extract was shown to have a total phenolic compound content of $1538.98 \pm 11.88 \mu\text{g GAE mL}^{-1}$. The antioxidant capability was evaluated with three different methods. The methanol extract of the plant exhibited a $1552.74 \pm 3.85 \mu\text{mol TE mL}^{-1}$ DPPH radical scavenging activity while it showed $2876.92 \pm 15.46 \mu\text{mol AAE mL}^{-1}$ and $3307.84 \pm 45.87 \mu\text{mol TE mL}^{-1}$ PFRAP and CUPRAC values, respectively. The GC-MS analysis revealed that palmitic acid and linolenic acid were the predominant fatty acid species, along with the major phytochemical constituents were found as linalyl acetate and alpha-pinene.

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1. Introduction

Hypericum perforatum L. (St. John's Wort) is the most broadly grown plant among the *Hypericum* species, with yellow flowers, stemless, oval and linear, hairless and generally woody structure at the base (Burunkaya et al., 2021). Perennial *H. perforatum* is extensively found in temperate and tropical regions of America, Europe, Asia, Africa and Australia and grows naturally in areas up to 2500 m above sea level (Silva et al., 2021).

Hypericum is one of the oldest plant groups belonging to the Hypericaceae family, containing approximately 500 species that have been widely used in folk medicine for centuries (Crockett et al., 2011, Grafakou et al., 2022). This genus includes more than 100 taxa in Türkiye. They are classified into 19 sections, including 45 species endemic to Türkiye (Boga et al., 2016). Due to its many uses, such as treating skin injuries and burns, as well as stomach ulcers, bile disorders, bronchial and genitourinary tract inflammation, colds, migraines, headaches, obesity, and diabetes mellitus, it is a highly attended herbal folk medicine (Božin et al., 2013; Tokgöz et al., 2020).

The presence of bioactive substances, such as xanthones, phloroglucinols, flavonoids, biflavones, and naphthodianthrones is thought to be responsible for *H. perforatum*'s therapeutic effects. Additional biological activity of *H. perforatum* extracts include wound healing, antidiabetic, antiviral, antibacterial, antitumoral, and antioxidant properties (Kapoor et al., 2023). Pharmacological research has shown that several extracts made from the stem and flowers of *Hypericum* species have antibacterial, antidepressant, antioxidant, and antinociceptive properties (Eruygur et al., 2019). Plants, which are used medicinally, have recently attracted great attention due to the increasing need for raw materials as sources of organic bioactive compounds. These plants have been studied for antimicrobial activity against various microorganisms (Tala et al., 2015).

Phloroglucinol derivatives have demonstrated antifungal and antibacterial effects against some bacterial strains, such as *Bacillus cereus*, *Staphylococcus aureus*, *Nocardia garden*, and *Bacillus subtilis*. They are commonly found in the lipophilic fractions of several *Hypericum* species. The use of some *Hypericum* species in the treatment of microbiological diseases is becoming increasingly widespread. Recent research has shown that medicinal plants may have antibacterial properties, which makes it crucial to employ them in the treatment of both infectious and some chronic illnesses (Sherif et al., 2023; Tusevski et al., 2024).

Scientific research is required to determine the pharmacological and chemical characteristics of the bioactive chemicals found in *Hypericum* species, as well as to cultivate the plant in order to preserve and produce large quantities of these compounds (Çırak and Kurt, 2014). St. John's wort is consumed in different forms (tea, oil, etc.), but studies on the phenolic compound contents, antioxidant capacity and antimicrobial effects of *H. perforatum* grown in Türkiye remain limited.

The purpose of this study was to determine the biological activity of *H. perforatum*. For this purpose, methanol extract of *H. perforatum* was investigated in terms of total phenolic compounds, antioxidant, and antimicrobial activity. In addition, some phytochemical constituents and fatty acid composition of the hexane extract of the plant were determined by GC-MS.

2. Materials and Methods

H. perforatum plants used in this study were obtained commercially from Niğde Province and dried at 40 °C. The dried plant samples with a constant weight were then ground and stored under laboratory conditions before extraction.

2.1. Extract preparation

One gram of dried plant sample was mixed with 30 mL methanol and homogenized at 9000 rpm for 10 min by a Daihan HG-15D homogenizer. The mixture was then incubated

in an ultrasonic bath (Sonorex, Bandelin) at 40 °C for 30 min. All the plant debris was precipitated by centrifugation at 4000 rpm for 20 min. Next, the supernatant was passed through Whatman No. 1 filter paper. A rotary evaporator (HeiVap Value, Heidolph) was used to extract the solvent until it was completely dry. The residue was re-suspended in enough methanol to obtain an extract with a concentration of 100 mg mL⁻¹. The total phenolic compounds (TPC), antioxidant and antimicrobial effect assays were performed using this extract and its dilutions.

2.2. Content of total phenolics

Total phenolic compounds of *H. perforatum* methanol extract were determined by Folin Cocalteu's phenol reagent method (Singleton et al., 1999; Torunoğlu et al., 2024). Briefly; 100 µL extract was added to Folin Cocalteu's phenol reagent, shaken and incubated at ambient temperature for 10 min. Following the addition of 0.71 M Na₂CO₃ solution, the mixture has remained at ambient temperature for an additional 90 minutes in the dark. The absorbances of the blue color formed in the mixture and the blank solution without the extract were measured by a spectrophotometer (MultiSkan GO, Thermo) at 765 nm. All measurements were performed as triplicates. A gallic acid standard curve was established for the assessment of the results in µg GAE mL⁻¹ extract.

2.3. Antioxidant activity

2.3.1. DPPH radical scavenging assay

DPPH (2,2 diphenyl-1-picrylhydrazyl) radical scavenging activity assay was performed to evaluate the antioxidant capability of the methanol extract of *H. perforatum*. DPPH has a maximum light absorption at 517 nm (Blois, 1958; Shimada et al., 1992). DPPH solution (0.1 mM) and a 5-step serial dilution of *H. perforatum* methanol extract (1, 2, 3, 4, and 5 mg mL⁻¹) were prepared with methanol. After adding 100 µL of extract from each dilution to 2.9 mL of DPPH solution, the mixture was agitated rapidly and remained at ambient temperature for 15 min in the dark. After the samples were

incubated, the absorbances were measured at 517 nm using a spectrophotometer (MultiSkan GO, Thermo). The DPPH solution was used as a blank. A standard curve was established using the scavenging activity of various concentrations of Trolox. All the measurements were performed as triplicates and the results were presented as means and standard deviations in µmol TE mL⁻¹ extract.

2.3.2. Potassium ferricyanide reducing antioxidant power (PFRAP) assay

The antioxidative activity of the sample, which reacts to generate potassium ferrocyanide (K₄[Fe(CN)₆]) by combining with potassium ferricyanide (K₃[Fe(CN)₆]), can be linked to a rise in the absorbance of ferric ferrocyanide, a complex with a maximum absorbance at 700 nm (Xiao et al., 2020). The PFRAP test was carried out in accordance with Mokrani et al. (2016) report. The ascorbic acid standard curve was established for data evaluation and the results were represented as µmol AAE mL⁻¹ extract.

2.3.3. Cupric ion reducing antioxidant capacity (CUPRAC) assay

When substances that function as electron donors are present, the color of the neocuproine and Cu(ii)Cl₂ mixture changes from bright yellow to orange, signifying that the sample has the ability to reduce cupric (Cu⁺²) ions to cuprous (Cu⁺) ions. With minor adjustments, the CUPRAC assay was performed in accordance with the original technique of Apak et al. (2004). At 450 nm, each sample's absorbance was measured in relation to a blank solution. To assess the antioxidant capacity of *H. perforatum* extract at varying doses, the Trolox standard curve was developed, and the results were represented as µmol TE mL⁻¹ extract.

2.4. Antimicrobial activity

The antimicrobial activity of *H. perforatum* methanol extract was evaluated by disc diffusion method against test microorganisms. *Pseudomonas aeruginosa* DSMZ 50071, *Salmonella typhimurium* SL1344, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus*

subtilis DSMZ 1971 and *Candida albicans* DSMZ 1386 were used as test microorganisms. The microbial strains were cultured for 24 hours at 36 °C in Luria-Bertani (LB) broth medium. The turbidity of the microorganisms was regulated to 0.5 McFarland standard before the assay (Taşkaya et al., 2023).

LB agar medium was used to plate 100 µL of microbial cultures. After that, 20 µL aliquots of the *H. perforatum* methanol extract with 25, 50, and 100 mg mL⁻¹ concentrations were transferred to sterile paper discs that had a 6 mm diameter and were placed on the agar medium. The extraction solvent (methanol) was used as negative control and the discs containing vancomycin (30 µg/disc) and gentamycin (10µg/disc) were used as positive control. After a 24-hour incubation period at 36°C, the diameters of the inhibition zones that developed around the discs were measured on the plates. Each experiment was carried out in triplicate, and the measurements were given in millimeters.

2.5. GC-MS analysis

Although the main bioactive constituents such as hypericin, hyperforin and their derivatives of *Hypericum* species are generally determined and characterized by liquid chromatography (Nait-Si and Fourneron, 2004; Akdeniz et al., 2020; Zhang et al., 2022), GC-MS is preferred to investigate some aromatic compounds and fatty acids (FA). To determine aromatic compounds and FA composition of *H. perforatum* one g powdered plant sample was homogenized with 25 mL of hexane and incubated at room temperature in the dark overnight. Additional incubation in an ultrasonic bath for 30 min was also performed. The homogenate was then filtered through a filter paper and concentrated using a rotary evaporator. Methyl esterification was maintained by 2 M KOH in methanol and 1 N HCl. The upper layer of the extract was analyzed in GC-MS after being dried by anhydrous Na₂SO₄ and passed through a syringe filter.

GC-MS analysis was performed with a QP2010 Ultra GC-MS (Shimadzu) containing a Restek Rxi 5MS column (30 m x 0.25 mm x

0.25 µm). One µL extract was injected into the GC with a split ratio of 1:40 using AOC 2.0 autosampler. The temperature profile of GC was set according to Canpolat and Canpolat (2023). All compounds extracted from *H. perforatum* were identified by comparing their mass spectra with those from the Wiley mass spectra library (W9N11) and the Flavor and Fragrance Natural and Synthetic Compounds Library (FFNSC 1.2).

3. Results and Discussion

3.1. Total phenolic compounds and antioxidant activity

The biological activities of *H. perforatum* are well documented in terms of antidepressant (Bukhari and Dar, 2013), neuroprotective (Özdemir et al., 2016), antiepileptic (Ivetic et al., 2011), antimicrobial and antiviral (Chen et al., 2019), antitumor (Yi et al., 2015), and antioxidant (Radulović et al., 2007; Sagratini et al., 2008; Öztürk et al., 2009) activities which are frequently related with the content of secondary metabolites such as hypericin, hyperforin, and pseudohypericin along with the phenolic compounds (Zdunić et al., 2011; Budantsev et al., 2021). The total phenolic compound content of *H. perforatum* methanol extract at a concentration of 5 mg mL⁻¹ was ascertained by Folin-Ciocalteu's phenol reagent method and found as 1538.98±11.88 µg GAE mL⁻¹.

Many scientific studies have shown that antioxidants reduce cellular damage, support defense and help prevent oxidative damage to cellular components (Wong et al., 2006; Çilesiz et al., 2023). The antioxidant activity of *H. perforatum* methanol extract was also investigated using three different techniques. In DPPH radical scavenging assay, DPPH can be transformed into its reduced form as DPPH-H by antioxidants acting as hydrogen donors which is determined by colorimetry with a decreased absorbance value at 517 nm. The highest DPPH scavenging activity in this study was determined as 1552.74±3.85 µmol TE mL⁻¹ at the extract concentration of 5 mg mL⁻¹. The high antioxidant activity of *H. perforatum* is generally associated with the content of

phenolic compounds that donate H^+ to DPPH (Orčić et al., 2011).

Three *Hypericum* species, namely *H. perforatum*, *H. origanifolium*, and *H. scabrum*, were investigated in terms of total phenolic contents and antioxidant activities by Seyrekoğlu et al. (2022) indicating that the total phenolic contents of three species were found as 128.82 ± 6.31 ; 137.03 ± 9.67 and 148.31 ± 4.57 mg GAE g^{-1} DW, respectively, while the IC_{50} values of ethanol extracts against DPPH were determined as 3.17 ± 0.23 ; 3.79 ± 0.27 and 3.65 ± 0.4 μg mL^{-1} , respectively. They stated that the IC_{50} values of the ethanol extracts in the DPPH assay were higher than those of BHT and ascorbic acid, which were used as standard compounds, indicating the high antioxidant capability of the *Hypericum* species.

The DPPH radical scavenging activity of *H. perforatum* collected from İzmir, Türkiye was investigated along with some other biological

activities. It was reported that the methanol extract at a concentration of 100 mg mL^{-1} exhibited a DPPH radical scavenging activity of 32 % (Okmen et al., 2017). However, the study conducted by Zheleva-Dimitrova et al. (2010) indicated that the DPPH radical scavenging activity of *H. perforatum* collected from Bulgaria was 77.6 %.

In another study investigating the effect of solvent concentration, temperature, and extraction time on extract yield, total phenolic content, and antioxidant activity of *H. perforatum*, it was indicated that the most effective DPPH radical scavenging activity was determined by the extract obtained using 100 % ethanol (EtOH) while it was followed by the extract obtained using 30 % EtOH (Seyrekoğlu and Temiz, 2020). The study also stated that 30 °C and 40 min were found as optimal temperature and extraction time values for the highest yield extracts with the highest DPPH radical scavenging activity.

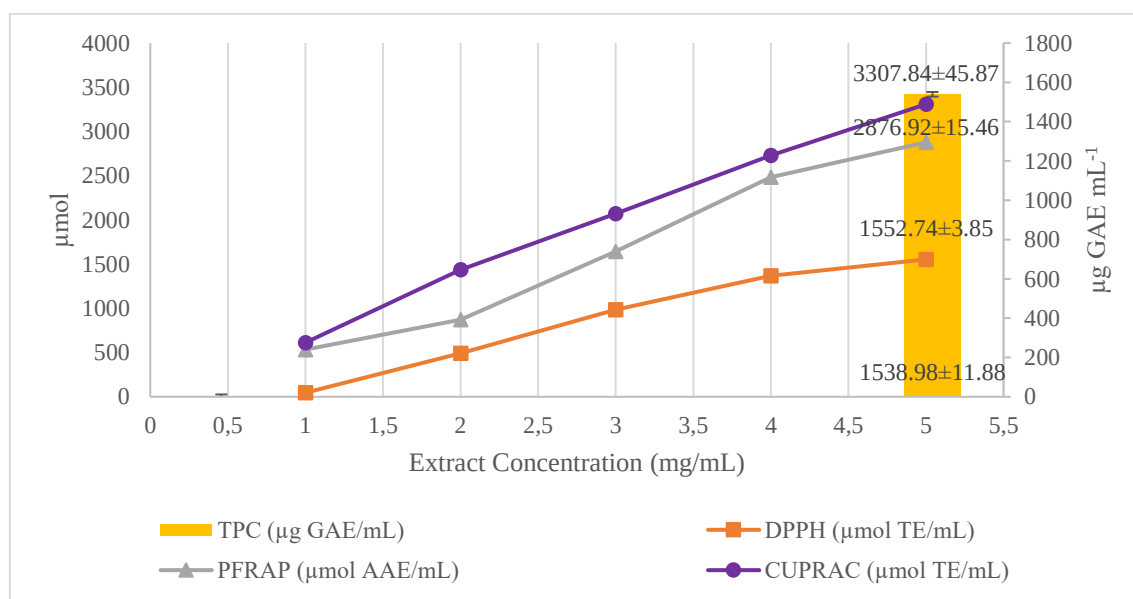


Fig 1. The total phenolic compounds and antioxidant activity (DPPH, PFRAP, and CUPRAC) of *H. perforatum* methanol extract with various concentrations

The antioxidant activity of *H. perforatum* was also evaluated in terms of metal ion reducing capability via potassium ferricyanide reducing power (PFRAP) assay and cupric ion reducing antioxidant capacity (CUPRAC) assay. PFRAP assay depends on the reducing power of the substances present in the extract which act as electron donors to reduce ferric

(iii) ions to ferrous (ii) ions. Similarly in the CUPRAC assay cupric (ii) ions are reduced to cuprous (i) ions hydrogen donation by antioxidant substances that are present in the extract (Kırmızı Sönmez et al., 2022). Fig 1 indicated that methanol extract of *H. perforatum* at 5 mg mL^{-1} concentration exhibited a PFRAP value of 2876.92 ± 15.46

$\mu\text{mol AAE mL}^{-1}$ and a CUPRAC value of $3307.84 \pm 45.87 \mu\text{mol TE mL}^{-1}$ in this study. Maltaş et al. (2013) investigated the antioxidant and antimicrobial activities of the methanol extracts of three *Hypericum* species and reported that *H. perforatum* exhibited CUPRAC and FRAP values of 19.67 ± 0.71 and $59.73 \pm 0.78 \text{ mg TEAC g}^{-1}$ extract, respectively. It was also stated that antioxidant activity of *H. perforatum* was well correlated with the phenolic content of the sample which was determined as $10.01 \pm 0.89 \text{ mg GAE g}^{-1}$ extract. In another study that investigated biological activities of the water extracts from some plants used as folk remedies in Türkiye, Sarıkürkçü et al. (2020) reported that *H. perforatum* water extract with a $181.02 \pm 1.47 \text{ mg GAE g}^{-1}$ total phenolic content performed CUPRAC and FRAP values of 268.81 ± 5.32 and $92.52 \pm 0.11 \text{ mg BHA g}^{-1}$ extract, respectively.

It was considered that the difference in the antioxidant activities of *H. perforatum* between the literature, and this study; might be related to the geographical, meteorological, and environmental conditions of the sampling area along with the investigated plant part, the growth phase of the plant, solvent, extraction conditions (time, temperature, technique, etc.) and the modifications in the experimental methods.

3.2. Antimicrobial activity

The antimicrobial activity of methanol extract of *H. perforatum* with different concentrations was investigated by disc diffusion method against six strains including one fungal strain, Gram-negative and Gram-positive bacteria.



Figure 2. Antimicrobial activity of *H. perforatum* methanol extract against *P. aeruginosa* DSMZ 50071 (left) and *S. aureus* ATCC 25923 (right).

According to Table 1, the results revealed that the highest antimicrobial activity was exhibited by 100 mg mL^{-1} methanol extract against *P. aeruginosa*, a Gram-negative bacterium, followed by *S. aureus*, a Gram-positive bacterium. The findings are in agreement with studies reporting antimicrobial activity of all *Hypericum* extracts against *P. aeruginosa* (Bariş et al., 2011; Gül et al.,

2021). According to the studies conducted with pathogen bacteria including Gram negative (*P. aeruginosa*, *E. coli*) and Gram positive (*S. aureus*, *B. subtilis*) the extracts obtained with various solvents from *Hypericum* species exhibited remarkable antibacterial activity against these strains (Türker et al., 2018; Özkan et al., 2018).

Table 1. Inhibition zone diameters of *H. perforatum* methanol extract measured against test microorganisms

Test Microorganisms	Inhibition Zone Diameters (mm)				
	<i>H. perforatum</i> (mg mL ⁻¹)			VA	CN
	25	50	100		
<i>E. coli</i> ATCC 25922	8.67±0.47	11.33±0.47	13.67±0.47	12	20
<i>S. typhimurium</i> SL1344	7.33±0.47	8.33±0.94	12.00±0.82	8	21
<i>P. aeruginosa</i> DSMZ 50071	13.33±1.25	16.67±1.25	18.67±0.94	nd	20
<i>S. aureus</i> ATCC 25923	10.33±0.47	13.67±0.47	17.33±0.47	17	22
<i>B. subtilis</i> DSMZ 1971	9.67±0.94	11.67±0.48	14.33±0.94	9	20
<i>C. albicans</i> DSMZ 1386	10.67±0.47	12.33±0.47	14.67±0.47	nd	nd

*Results indicate means ± standard deviations of three independent experiments.

*VA: Vancomycin (30µg/disc), CN: Gentamycin (10µg/disc)

Table 1 also indicated that all concentrations of *H. perforatum* extract showed antimicrobial activity against all test organisms at varying levels. The antibacterial effects of *Hypericum* extracts have been the subject of numerous investigations showing different levels of antibacterial activity (Del Monte et al., 2015; Tusevski et al., 2016; Sherif et al., 2023). Saddiqe et al. (2020) reported that extracts of several *Hypericum* species obtained by various solvents, including methanol, have demonstrated similar antibacterial effects against some pathogen bacterial strains including *Enterobacter aerogenes*. The study also revealed that the antibacterial activity varied significantly depending on the plant species and solvent type.

According to a study examining antibacterial activity using antibiotic-resistant clinical isolates, *H. perforatum* extracts demonstrated superior antibacterial activity against a considerable fraction of Gram-positive bacteria than the susceptibility limits of broad-spectrum antibiotics (Kısa et al., 2023). In the study that investigated the antibacterial activity via disc diffusion method of *H. capitatum* extracted with different solvents, it was reported that all extracts showed antibacterial effect against test microorganisms at various levels (Boga et al., 2016). It was also stated that methanol extract had higher activity against test microorganisms (Boga et al., 2016). Hoş and Tunç (2016) also

investigated the antibacterial activity of *H. calycinum* using the extracts obtained from different plant parts (root, leaves and flowers) and reported that the highest inhibitory effect was observed against *Streptococcus mutans*. They also stated that the flower extracts showed better antibacterial activity against test microorganisms than the root and leaf extracts. Additionally, the chemical components, extract yield, and antimicrobial activity might all be impacted by solvents with varying polarities.

3.3. GC-MS results

The GC-MS analyses of *H. perforatum* hexane extract resulted in the determination of 15 compounds including 6 fatty acid methyl esters and 9 phytochemical compounds (Table 2). Palmitic acid (13.92±0.81 %), linolenic acid (11.915±0.125 %), stearic acid (8.465±0.445 %) and linoleic acid (2.44±0.21 %) were found as predominant fatty acids. 1,2-Benzenedicarboxylic acid (1.2±0.1 %) and 4-Pentenoic acid, 4-methyl- (0.685±0.015 %) were also determined. The fatty acid profile of *H. perforatum* consisted of saturated fatty acids compared to unsaturated ones (Table 2). The fatty acid profile was compatible with the literature as it was reported that the extracts obtained from different parts (roots, flowers and aerial parts) of various *Hypericum* species could show saturated or unsaturated fatty acid constituents as predominants (Türkoğlu et al., 2015; Boga et al., 2016; Akdeniz et al., 2020).

Table 2. Compounds obtained from *H. perforatum* hexane extract by GC-MS analysis

No	Compound	RT	MW	CF	RC
1	alpha-Pinene	7.72	136	C10H16	8.145±0.165
2	4-Pentenoic acid, 4-methyl-, methyl ester	7.944	128	C7H12O2	0.685±0.015
3	Cymene	10.463	134	C10H14	1.02±0.08
4	dl-Limonene	10.596	136	C10H16	1.32±0.03
5	Eucalyptol	10.685	154	C10H18O	1.635±0.055
6	Linalool	12.816	154	C10H18O	6.42±0.27
7	Camphor	14.216	152	C10H16O	1.305±0.025
8	endo-Borneol	14.883	154	C10H18O	0.62±0.04
9	Verbenone	16.195	150	C10H14O	1.135±0.125
10	Linalyl acetate	17.523	196	C12H20O2	10.55±0.48
11	1,2-Benzenedicarboxylic acid, diethyl ester	26.795	222	C12H14O4	1.2±0.1
12	Palmitic acid, methyl ester	36.231	270	C17H34O2	13.92±0.81
13	Linoleic acid, methyl ester	39.949	294	C19H34O2	2.44±0.21
14	Linolenic acid, methyl ester	40.084	292	C19H32O2	11.915±0.125
15	Stearic acid, methyl ester	40.603	298	C19H38O2	8.465±0.445

*RT: Retention time (min); MW: Molecular weight; CF: Chemical formula; RC: Relative concentration (mean ± standard deviation %).

Linalyl acetate (10.55±0.48 %), alpha-pinene (8.145±0.165 %), and linalool (6.42±0.27 %) were found as the major phytochemical compounds along with cymene, limonene, eucalyptol, camphor and verbenone in the hexane extract of *H. perforatum*. Previous studies on essential oil profiles and phytochemical constituents on various *Hypericum* species showed similarities with our findings (Bağcı and Yüce, 2011; Çırak and Bertoli, 2013).

4. Conclusions

The analytical methods used to determine antioxidant and antimicrobial activity may provide specific but limited information about these features. In this study, the antioxidant and the antimicrobial activity of the methanol extract obtained from *H. perforatum* were ascertained by three different methods (DPPH radical scavenging activity, PFRAP and CUPRAC) and disc diffusion method, respectively. GC-MS analysis was also conducted to determine the phytochemical composition of the *H. perforatum* hexane extract. It can be said that this study on this plant has contributed to the use of natural sources as antioxidant and antimicrobial agents with high efficiency and fewer side effects. Since, Türkiye is one of the richest sources in terms of plant diversity due to its geographical advantages, research on finding, extracting and identifying novel antioxidant and

antimicrobial agents should be promoted, particularly for *Hypericum* genus with many endemic species.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article and that they have read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that they have no conflict of interest for this study.

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Effect of Adding Different Rates of Bee Pollen to Quail Rations on Performance and Carcass Parameters

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Abstract

This study was conducted to determine the effects of different amounts of pollen added to quail (*Coturnix coturnix japonica*) diets on fattening performance, some carcass, and internal organ parameters. In this study, 625 one-day-old mixed-sex Japanese quail (*Coturnix coturnix japonica*) chicks were used as animal material. The quail chicks were randomly divided into 5 main groups of 125 chicks each and each main group was divided into 5 subgroups of 25 chicks. According to the feed groups; the control group (C) was fed with the basic ration, while the trial (Trial I, II, III, and IV) groups were formed by adding pollen to the basic ration at different rates (0.25, 0.50, 0.75 and 1.00 %). In the 42-day study, quails were reared in cages with dimensions of 96x46x25 cm. At the end of the research, the PI group was found to be significantly higher than the C group in terms of live weight and live weight gain ($P<0.001$), while no statistical difference was detected between the groups in terms of feed consumption. Among the carcass parameters, slaughter weight ($P<0.001$), hot and cold carcass weight ($P<0.01$), and breast weight ($P<0.05$) were found to be statistically higher in the PI group than in the C group. Among the visceral organ weights, the PI group was determined to be higher than the C group in terms of intestine weight. As a result, considering the overall experiment, it is recommended to use 0.25 % pollen in quail rations due to its positive effect on fattening performance, some carcass parameters, and intestinal weight.

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1. Introduction

In order to meet the protein needs of the increasing world population, there has been a significant increase in the production of farm animal products, especially poultry and pigs (OECD-FAO, 2023). In particular, poultry meat and meat products are preferred one step ahead because they are more economical than other farm animals, easier to raise and obtain, have lower cholesterol and fat content than red meat, and consumer habits are shifting towards white meat (Grom, 1990; Yücesoy and Kaya, 2022). One of the most preferred reasons for commercial quail farming is that it reaches slaughter weight in a short time of 42 days and is rich in nutritional value and essential fatty acids (Griggs and Jacob, 2005; Szczesna, 2006; Ergün et al., 2014).

Antibiotics have been used for many years to control all kinds of external factors that may cause suppression of the immune system and to increase fattening performance, especially in the process of reaching slaughter weight in a short period of 42 days in commercial poultry (chicken and quail) fed with high nutritional composition (Leeson and Summers, 2008). With the banning of antibiotics in the European Union and Turkey in 2006, researchers have intensified their research on natural alternative feed additives that can show the effect of antibiotics (Tuncer, 2007; Bolacali and Irak, 2017). For this purpose, research is being conducted on phytobiotics, probiotics, prebiotics, and bee products such as pollen and propolis, which are natural products that can be an alternative to antibiotics (Griggs and Jacob, 2005; Ergün et al., 2014).

Bee pollen is rich in active ingredients such as proteins, amino acids, carbohydrates, lipids and fatty acids, phenolic compounds, enzymes and coenzymes, vitamins, and bio-elements (Campos et al., 2008; Komosinska-Vassev et al., 2015). Although the nutritional content of bee pollen varies depending on the flora around the hive, it has a rich nutritional composition (Silva et al., 2006; Campos et al., 2008). The average protein level of bee pollen is 20-30 %, and approximately 10 % of this protein consists of essential amino acids such

as lysine, methionine, histidine, threonine, tryptophan, leucine, isoleucine and phenylalanine. The total carbohydrate level in bee pollen is on average 15-50 %, and especially fructose and glucose constitute approximately 25 % of the total carbohydrate (Roulston, 2000; Villanueva et al., 2002; Almeida-Muradian et al., 2005; İlçeli and Yıldız, 2021). Pollen, which is rich in essential fatty acids (EFA) such as linoleic, γ -linoleic, and archaic acids, has an average crude oil content of 5-10 % and contains significant amounts of phospholipids and phytosterols. Bee pollen also contains approximately 0.04-3 % of active compounds such as flavonoids, leukotrienes, catechins, and phenolic acids (Asafova et al., 2001; Villanueva et al., 2002; Campos et al., 2008; İlçeli and Yıldız, 2021).

The use of bee products as apitherapeutic products dates back to ancient times; it has been reported to have anti-oxidant, antimicrobial, anti-fungal, anti-viral, anti-inflammatory, immune stimulation, and fattening performance-enhancing effects (Kroyer and Hegedus, 2001; Almaraz-Abarca et al., 2004; Sarıkaya et al., 2008; Eraslan et al., 2009; Liu et al., 2010; Pascoal et al., 2014; Dimitriou et al., 2023). In previous studies, pollen addition to poultry diets improved fattening performance and carcass yield (Babaei et al. 2016; Sarıkaya et al., 2017; İlçeli and Yıldız, 2021; Sevim, 2021), increases immunoglobulin levels and has an immunostimulating effect (De Oliveira et al., 2013; Hosseini et al., 2016), protects against heat and stress (Haščík et al., 2015) and improves meat quality (Sevim, 2021).

Although it has been determined that there have been studies on the use of pollen as a feed additive in poultry in previous years, there is limited information on the extent to which pollen can be added to the ration as a performance-enhancing feed additive alternative to antibiotics. In this study, the effect of pollen addition to quail rations at different rates (0.25, 0.50, 0.75, and 1 %) on fattening performance and carcass parameters was examined.

2 Materiel and Methods

2.1 Experimental chickens, diets, and design

In this study, 625 one-day-old different-sex Japanese quail (*Coturnix coturnix japonica*) chicks were used as animal material. The quail chicks were randomly divided into 5 main groups of 125 chicks each and each main group was divided into 5 subgroups of 25 chicks. Quails were fed with growth feed (day 1-42) whose nutrient content was prepared according to NRC (1994). Table 1. According to the feed groups; the control group (C) was fed with the basic ration, while the trial (PI, PII, PIII, and PIV) groups were formed by adding pollen to the basic ration at different rates (0.25, 0.50, 0.75 and 1.00 %). In the 42-day study, quails were reared in cages with dimensions of 96x46x25 cm. During the experiment, necessary heating and 24-hour lighting were provided and feed and water were provided ad libitum.

After weighing the hatching weight of the quails, their live weights were determined by weekly weighings, and live weight gain (LWG) was calculated. Feed was given to the quails by weighing and feed consumption (FC) of the quails was determined by deducting excess feed from the total feed given as a result of weekly weighings. Feed consumption was divided by live weight gain (FCR: FC/LWG) and feed conversion ratio (FCR) was calculated. On the 42nd day of the study, in order to determine the slaughter and carcass parameters, a total of 120 quails were slaughtered separately from the experimental groups, 4 quails from each subgroup and 20 quails from each main group, with live weights close to the group averages. In terms of slaughter and carcass parameters, hot and cold carcass weight, foot, thigh, breast, wing, liver, heart, intestine, neck, back, and other weights were determined.

Table 1. Composition and nutritional content of quail feed used in the research.

Ingredient	Initial-Growing Period (days 1-42), %	Nutrient Content on a Dry Matter Basis, %	Values
Maize	45.30	Dry matter	89.85
Wheat	8.91	Metabolic energy kcal kg ⁻¹ **	2903
Vegetable oil	1.60	Crude protein	24.08
Soybean Meal (48 %, HP)	30.00	Crude fat	3.41
Fish Meal (64 %, HP)	3.00	Crude fiber	4.65
Sunflower Meal (32 %, HP)	9.00	Crude ash	5.64
Limestone	1.16	Calcium	0.81
Vit. & Min. premix*	0.25	P	0.38
Salt	0.35	Na	0.20
DCP	0.20	Cl	0.28
Antioxidant	0.08	Met+Sis	0.85
L-Threonine	0.15	Lysine	1.30
		Threonine	1.02
		Tryptophan	0.31

*: Per diet: 13,000 IU vitamin A, 3,500 IU vitamin D3, 100 mg vitamin E, 3 mg vitamin K3, 3 mg vitamin B1, 8 mg vitamin B2, 6 mg vitamin B6, 30 mg vitamin B12, 30 mg Niacin, 8 mg calcium-D-panthothenate, 2 mg folic acid, 70 mg vitamin C, 70 mg D-biotin, 200 mg choline chloride, 2 mg canthaxanthin, 0.75 mg apocarotenoid acid ester, 120 mg Mn, 100 mg Zn, 90 mg Fe, 16 mg Cu, 1.5 mg I, 0.75 mg Co, 0.30 mg Se

**: Found by calculation (Jurgens, 1996).

2.2. Statistical Method

Determination of the difference between trial groups was determined according to the General Linear Model procedure (One-Way

Anova) in the SPSS package program. Duncan's test was used for multiple comparisons of groups. The statistical significance level was accepted as $P < 0.05$.

3. Results

In this study, live weight values of quails to which pollen was added to the ration at various periods are presented in Table 2. It was

determined that adding pollen to quail rations as a feed additive significantly increased live weights in PI and PII groups compared to group C at the 5th ($P<0.05$) and 6th weeks ($P<0.001$).

Table 2. Effect of pollen addition to quail rations on weekly (wk) live weights of quails, (g).

Items	Control (M±SE)	PI (M±SE)	PII (M±SE)	PIII (M±SE)	PIV (M±SE)	P
Hatching	7.19±0.01	7.18±0.01	7.19±0.01	7.18±0.01	7.19±0.01	-
1st wk	34.46±0.25	35.19±0.37	34.63±0.40	34.66±0.20	33.88±0.10	-
2nd wk	68.93±0.53	70.15±0.35	69.92±0.27	68.46±0.64	68.34±0.64	-
3rd wk	102.09±1.44	106.82±1.72	104.34±1.35	102.41±1.65	100.89±1.82	-
4th wk	132.33±2.07	137.92±2.41	134.85±2.47	134.48±3.28	130.57±2.65	-
5th wk	165.07±3.31 ^{ab}	170.59±2.67 ^a	168.38±2.12 ^a	162.94±2.63 ^{ab}	159.64±2.40 ^b	*
6th wk	196.66±1.49 ^b	202.95±1.92 ^a	198.49±1.97 ^{ab}	196.34±2.15 ^b	190.16±0.51 ^c	***

C: Control; P I: 0.25% Pollen; P II: 0.50% Pollen; PIII: 0.75% Pollen; PIV: 1 % Pollen added.
-: Non significant ($P>0.05$); *: $P<0.05$; *** $P<0.001$. M: Mean; SE: Standard Error

The findings of the study on live weight gain (LWG), daily feed consumption (FC), and feed conversion ratio (FCR) for the initial (day 1-21), growth (day 21-42), and general experiment (day 1-42) periods are presented in Table 3. It was determined that the addition of pollen to the ration as a feed additive did not affect terms of LWG, FC, and FCR in the initial and growth periods.

In the overall study, it was determined that the addition of pollen as a feed additive improved the daily LWG ($P<0.001$) values in quails. Throughout the study, the highest LWG value and the best FCR performance were determined in the PI groups. There was no statistical difference between the groups in terms of the FC ($P>0.05$).

Table 3. Effect of pollen supplementation to quail diets on daily live weight gain (g), daily feed intake (g/day/head), feed conversion ratio (g).

Items	Control (M±SE)	PI (M±SE)	PII (M±SE)	PIII (M±SE)	PIV (M±SE)	P
Initial Period (Day 1-21)						
LWG	4.52±0.07	4.74±0.08	4.62±0.06	4.54±0.08	4.46±0.09	-
FC	11.37±0.35	11.56±0.19	11.48±0.31	11.37±0.39	11.61±0.43	-
FCR	2.52±0.04	2.43±0.04	2.48±0.06	2.51±0.09	2.60±0.06	-
Growth Period (Day 22-42)						
LWG	4.50±0.08	4.58±0.11	4.48±0.76	4.47±0.09	4.25±0.08	-
FC	23.24±0.56	22.77±0.56	23.26±0.52	23.21±0.37	22.36±0.26	-
FCR	5.16±0.08	4.98±0.05	5.18±0.09	5.19±0.11	5.26±0.06	-
Overall Experiment (Day 1-42)						
LWG	4.51±0.03 ^b	4.66±0.05 ^a	4.55±0.05 ^{ab}	4.50±0.05 ^b	4.35±0.01 ^c	***
FC	17.31±0.20	17.17±0.33	17.37±0.38	17.30±0.33	16.99±0.20	-
FCR	3.84±0.03	3.68±0.04	3.81±0.06	3.84±0.08	3.90±0.04	-

C: Control; P I: 0.25% Pollen; P II: 0.50% Pollen; PIII: 0.75% Pollen; PIV: 1 % Pollen added. M: Mean; SE: Standard Error
-: Non significant ($P>0.05$); *: $P<0.05$; *** $P<0.001$.

In the study, a statistical difference was found between the groups in terms of slaughter

($P<0.001$), hot carcass ($P<0.01$), cold carcass ($P<0.01$) and breast ($P<0.05$) weight. The

highest value in these parameters was found in the PI group. In terms of visceral organ weights, it was determined that there was no statistical difference between the groups in parameters other than intestine weight

($P>0.05$). Unlike these, a statistical difference was found in intestine weight ($P<0.05$), while the highest intestine weight was observed in the PI group ($P<0.05$).

Table 4. Effect of pollen addition to the diet on slaughter and carcass weights of quails (g).

Parameter	Control (M±SE)	PI (M±SE)	PII (M±SE)	PIII (M±SE)	PIV (M±SE)	P
Slaughter	189.16±0.50 ^b	196.72±0.38 ^a	193.06±0.76 ^{ab}	190.17±0.88 ^b	185.33±0.59 ^c	***
Hot carcass	142.56±1.00 ^b	152.24±1.32 ^a	149.85±1.59 ^{ab}	144.52±1.71 ^b	140.85±2.66 ^c	**
Cold carcass	141.03±1.52 ^b	150.85±1.90 ^a	147.85±1.62 ^{ab}	143.01±1.98 ^b	138.87±3.07 ^c	**
Leg	32.71±0.81 ^b	37.56±1.45 ^a	33.64±1.18 ^b	31.25±2.80 ^b	31.74±1.22 ^b	-
Breast	50.38±1.34 ^b	53.88±1.58 ^a	52.81±1.85 ^{ab}	51.08±3.26 ^b	45.60±2.13 ^c	*
Wing	12.10±0.36	12.98±0.27	12.68±0.42	12.27±1.34	11.91±0.52	-
Back	17.02±0.91	18.20±1.03	17.84±0.77	17.26±1.85	16.76±1.40	-
Neck	10.42±0.36	11.14±1.22	10.92±1.32	10.56±1.28	10.26±1.45	-
Other	18.59±1.08	19.86±1.32	19.47±1.08	18.83±1.68	18.29±1.07	-
Heart	1.83±0.36	1.95±0.27	1.91±0.40	1.85±0.20	1.80±0.31	-
Liver	3.07±0.40	3.29±0.36	3.22±0.26	3.11±0.19	3.02±0.23	-
Intestinal	4.76±2.03 ^{ab}	5.02±1.45 ^a	4.84±0.63 ^{ab}	4.62±1.69 ^{bc}	4.42±1.23 ^c	*
Gizzard	3.84±0.21	4.10±0.26	4.02±0.31	3.89±0.39	3.78±0.23	-
Abdominal fat	2.36±0.64	2.53±0.35	2.44±0.10	2.39±0.18	2.32±0.35	-

C: Control; P I: 0.25% Pollen; P II: 0.50% Pollen; PIII: 0.75% Pollen; PIV: 1 % Pollen added. -: Non significant ($P>0.05$), *: $P<0.05$; ** $P<0.01$; *** $P<0.001$. M: Mean; SE: Standard Error

4. Discussion

Bee pollen, which is rich in nutrients, contains most of the essential nutrients required for growth and development (Villanueva et al., 2002; Capcarova et al., 2012; Petruska et al., 2020), not only promotes growth, but also improves the immune system of poultry, protects the health of the intestinal system and improves the quality and safety of animal products (Liu et al., 2010; Hosseini et al., 2016; Sevim, 2021; İlçeli and Yıldız, 2021; Al-Kahtani et al., 2022). There are also studies in the literature reporting that bee pollen has positive effects on performance (Frag Soha and Rayes, 2016; El-Medany et al., 2017; İlçeli and Yıldız, 2021).

In this study, it was determined that the addition of bee pollen to quail rations did not affect LW in the first 4 weeks of the study, which is compatible with the findings of Sevim (2021). In this study, the LW findings of PI and PII groups at 6 weeks were significantly higher than group C, which was consistent with the

results of Attia et al. (2014) and Farag Soha and Rayes (2016).

Table 3 shows data on growth performance. Considering the starting and growth period in this study, it was determined that there was no statistical significance between the groups in terms of LWG, FC, and FCR values, which was compatible with research on quail and broiler chickens (Haščík et al., 2016; Sevim, 2021). Considering the overall experiment, the PI group was found to be significantly higher in LWG than the C group, which was similar to the findings of other studies on poultry (Attia et al., 2014; Hosseini et al., 2016). Considering the overall experiment, the PI group was found to be significantly higher in LWG than the C group, which was similar to the findings of other studies on poultry (Attia et al., 2014; Hosseini et al., 2016). In this study, it was determined that the lowest LWG and FCR values were in the PIV group, to which 1 % pollen was added to the rations, and the feed consumption of the same group

decreased numerically. Although the reason why the addition of 1 % pollen to the ration negatively affects fattening performance is not fully explained, it can be thought that it negatively affects fattening performance due to some components it contains. It was determined that the result that there was no statistical difference between the groups in terms of FC at any period of the study was compatible with the reports of İlçeli and Yıldız (2021).

While no difference was detected between groups in terms of feed consumption throughout the study, the best FCR value was detected in the PI group ($P < 0.05$). In the study conducted by İlçeli and Yıldız (2021) on quails, they reported that the addition of 3, 6, 9, 12, and 15 g of pollen to the ration had no effect on LW, LWG, and FC, but the addition of 6 g of pollen significantly increased the FCR value compared to the C group. Unlike this result, Sarıkaya et al. (2018) reported that the addition of 0.25 and 0.50 % pollen to quail diets did not affect the overall LWG, FC, and FCR values. It is thought that one of the reasons for the inconsistency between studies is due to the difference in pollen used. In addition, animal material (such as using quails of different genders), trial design, and environmental differences can also be considered among the factors affecting the research.

As seen in Table 4, the slaughter, hot and cold carcass, breast, and intestinal weights of the PI group, to which 0.25 % pollen was added to their diet, were determined to be significantly higher than the C group, while the lowest value was found to be in the PIV group. Since breast meat is a commercially important product in poultry meat, the increase in breast meat in the PI and PII groups to which pollen was added is gaining importance. The finding that no statistical significance was detected in terms of other carcass and internal organ parameters was confirmed by Sarıkaya et al. (2018) (except for abdominal fat weight in Sarıkaya et al. 2018) and Canogullari et al. (2009) was found to be compatible with the research findings.

Farag Soha and Rayes (2016) report that adding different amounts of pollen to broiler and quail diets has positive effects on some carcass parameters and visceral organs. Unlike these reports, Canogullari et al. (2009) and Haščik et al. (2015) report that adding pollen to the diet has no effect on carcass parameters. Haščik et al. (2015) found that the addition of propolis to the broiler diet did not affect the carcass ratio, breast ratio, and liver ratios, but increased the leg ratio. Haščik et al. (2016) found that adding propolis to the broiler diet did not affect slaughter weight, carcass weight, carcass ratio, heart ratio, liver ratio, and gizzard ratio, but reduced abdominal fat weight. In this study, it was determined that there was an increase in intestinal weight in the PI group compared to the control. The increase in intestinal weight can be explained by studies reporting that supplementing bee pollen into the diets of birds can improve the initial development of the gastrointestinal tract and the digestive process (Toman et al., 2015; Haščik et al., 2017; Nemauluma et al., 2022). Fazayeli-Rad et al. (2015) reported that adding 20 g/kg pollen to broiler diets increased intestinal weight, and also increased the villus length and crypt depths of groups to which pollen was added at different rates to the diet. According to this result, it is thought that the high fattening performance of the PI group, whose diet was supplemented with pollen throughout the experiment, may be related to the positive effect of pollen on the digestive system.

5. Conclusions

As a result, it is recommended to use 0.25 % pollen in quail diets in poultry rations due to its positive effect on overall fattening and performance parameters and some carcass parameters and intestinal weight. However, when the findings of this and other studies on the use of pollen as a feed additive are evaluated; More detailed studies are needed on its effects on metabolism, considering the bioactive substances it contains.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Ethical Committee Approval

This research was conducted based on the permission numbered 2017-04 of Siirt University Animals Research Local Ethic Committee (SİU-DEHAM) (Ethics Committee decision dated 15.12.2017 and numbered 07.07.2017).

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Anfis Based Reference Evapotranspiration (ET₀) Estimation Using Limited and Different Climate Parameters

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Abstract

This study aims to estimate the reference evapotranspiration (ET₀) with adaptive neuro-fuzzy inference system (ANFIS). The calculation of ET₀ requires climate data such as maximum and minimum temperature, wind speed, sunshine duration and humidity. Sometimes it may not be possible to access the climate data. In the study, ET₀ is estimated with ANFIS by using fewer input parameters. Moreover, it is proved that it is possible to calculate ET₀ via ANFIS by using some other climate parameters such as temperature-humidity-wind index (THW), air pressure, wind chill, which have no direct effect on the calculation of ET₀. In the study, five scenarios were created and evaluated with a statistical performance indicator. In Scenario 1, ET₀ was estimated using THW, air pressure and air density. The relationship between the ET₀ estimated by ANFIS and the calculated ET₀ was found to be 0.76 (R²). In Scenario 2, THW, solar energy and solar radiation were used and R² was found to be 0.66. Scenario 3 used THW and wind chill, resulting in an R² of 0.43. In Scenario 4, THW and maximum temperature were used and R² was 0.87. In Scenario 5, THW and humidity were used and the R² with ET₀ was 0.84.

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1. Introduction

In the effective use of irrigation water in plant cultivation, climate, soil, plant and water factors are evaluated together in the production process (Seydoşoğlu, 2018; Keten and Değirmenci, 2020; Özdoğan Çavdar et al., 2021). These factors can also be used to calculate reference evapotranspiration and crop evapotranspiration, which are very important for the irrigation schedule. Evapotranspiration is described as the loss of water to atmosphere through evaporation from the plant and soil surface. Estimating water potential plays an important role in water management and has a significant impact on plant production (Rajagopal et al., 2022). Precise estimation of reference evapotranspiration (ET_0) enables reliable management of scarce water resources. It is also one of the important stages in identify crop water consumption (Roy et al., 2022; Roy et al., 2021; Roy, 2021; Wu et al., 2021; Roy et al., 2020; Zeinolabedini Rezaabad et al., 2020). ET_0 is a dynamic, complex, unstable and non-linear hydrological process with important effect on efficient water resources planning and long-term management (Alam et al., 2024; Goyal et al., 2023; Lu et al., 2023). In addition, evapotranspiration is an important parameter used in hydrology and irrigation schedules. Reference evapotranspiration is particularly important. Estimating ET_0 changes is useful for analyzing the management of water resources (El-Kenawy et al., 2022).

Gupta et al. (2024) different soft computing models for ET_0 prediction were compared. Artificial neural network (ANN), wavelet-associated ANN (WANN), ANFIS and multiple nonlinear regression (MNLR) models were investigated. The Gamma test technique was used for the selection of appropriate climatic parameter. The results showed that the ANN-10 model was more successful in predicting ET_0 compared to the other models. Solar radiation was identified as the most sensitive input parameter, while actual vapor pressure was the least sensitive variable.

Kartal (2024) used an ANN model developed by the Levenberg-Marquardt method to predict monthly ET_0 values in Elazığ province. Data such as precipitation, relative humidity, wind speed, temperature and sunshine duration were used to predict ET. The results showed that the proposed model provided high accuracy (R values between 0.9898 and 0.9995) in Elazığ, Keban, Baskil and Ağın regions. Moreover, the MAPE values of the model were quite low and consistent with Hargreaves' method ($R^2 = 0.996$). These findings provide important information for water resources and agricultural irrigation management.

Qin et al. (2024) emphasizes the importance of ET_0 estimation for sustainable water resources management. Support vector regression, Bayesian linear regression, Ridge regression and Lasso regression models were used for ET_0 estimation. However, prediction errors occurred in these models due to data noise or overlearning. Hybrid models have been developed to reduce these errors and improve prediction accuracy. Before constructing the hybrid models, each model was weighted according to variance reciprocal and information entropy weighting methods. As a result, the information entropy-based hybrid model showed the best performance in the medium-term (1-30 days) ET_0 forecast in the Northern Plains of China.

Bidabadi et al. (2024) aims to predict ET_0 with incomplete meteorological data at Sirjan and Kerman synoptic stations in the arid regions of Iran. ANN, ANFIS and ANN with gray wolf optimization (ANN-GWO) models are tested with Penman-Monteith FAO-56 data. The ANFIS, ANN and ANN-GWO methods outperformed the Hargreaves method with more readily available data such as maximum and minimum temperature. ANFIS outperformed the other methods for all data combinations. The best results were obtained at Kerman and Sirjan using maximum and minimum temperature and ET_0 data of the neighboring station. As a result of this evaluation, the root mean square error (RMSE) was found to be 0.33 and 0.36.

Duhan et al. (2023) was conducted to develop and evaluate the performance of distinct machine learning (ML) models for ET_0 prediction. The models included Multiple linear regression (MLR), Least square-support vector machine (LS-SVM), ANNs and ANFIS. Using 50 years of meteorological data, ET_0 was predicted and the LS-SVM model showed the best performance ($R^2 = 0.998$). The ET estimated by LS-SVM was compared with MODIS satellite data and MODIS was found to overestimate ET . After applying the correction factor, the R^2 value of the satellite data was 0.74 and the RMSE value was 1.19 mm. It was stated that ML and satellite-based ET estimates would be useful for water budgeting at local and regional level to manage water scarcity.

Yan et al. (2023) proposed an innovative deep learning (DL) approach, HS-LSTM, for ET_0 prediction with limited daily climate data. The method was based on the Hargreaves-Samani (HS) model and Long short-term memory (LSTM) neural network, and ET_0 was predicted using only daily maximum and minimum air temperatures. The model outperformed traditional ML algorithms and other HS-based models in terms of accuracy. Tested in the Songliao Basin (Northeast China), the HS-LSTM method significantly reduced errors compared to other techniques. It was indicated that it can provide effective results for future ET_0 forecasts based only on air temperature data. This study presents a new strategy that provided more accurate ET_0 forecasts with limited meteorological data.

Zereg and Belouz (2024), investigated daily ET_0 prediction with limited measurement data using the Support vector regression (SVR) machine learning algorithm for ET_0 prediction. Ten years of data collected at the Dar-El-Beidha meteorological station in Algeria was used, and the Julian Day (J) is included as an input for the first time. Various SVR models were developed using data such as maximum, minimum and mean temperature, mean relative humidity, wind speed, sunshine duration. The results shown that SVR models were more successful in predicting ET_0 than

traditional empirical equations, radial basis function neural networks (RBFNN) and ANFIS. SVR models performed in the range of RMSE 0.28-0.72 mm day⁻¹, R^2 0.86-0.98 and MAPE 7-19 %. These findings provided useful solutions for ET_0 estimation in data deficient regions.

Küçüktopcu et al. (2023) addressed the problem that machine learning (ML) models such as ANN, generalised neural regression networks (GRNN) and ANFIS may give inconsistent results in solving linear problems and proposed more accurate evapotranspiration (ET_0) prediction models by hybridising these models with ARIMA. The results of the models developed using daily ET_0 data collected between 2010-2020 in Samsun province reduced the root mean square error (RMSE) of the ARIMA-GRNN model by 48.38 %. It also showed that it reduced the ARIMA-ANFIS model by 8.56 % and the ARIMA-ANN model by 6.74 %. These hybrid models could provide more accurate forecasts for areas that need reliable ET_0 forecasts, such as agriculture and water management.

Adnan et al. (2021) proposed a hybrid heuristic algorithm (HA), an innovative technique in the field of machine learning, that improved the accuracy of ET_0 estimation, which was of great importance for regional water management, agricultural planning, and irrigation design. However, the new hybrid HA techniques, the Moth-Flame Optimisation Algorithm (MFO) and the Water Cycle Optimisation Algorithm (WCA), had rarely been applied for ET_0 estimation in previous literature.

Bakhtiari et al. (2022) utilized four data-aided methods (ANFIS, ANN, the M5 tree model), and support vector machine (SVM) for ET_0 prediction in the South Caspian region. The success of the models is check against the FAO-56 Penman-Monteith method using climate data such as solar radiation, average air temperature, average relative humidity and wind speed for the period 1991-2020. The results shown that the ANFIS model provides the highest accuracy, especially in the validation phase, and performs best when all

climate variables were used. It was suggested that the ANFIS model was also effective with fewer climate variables when T-mean and R-s are used.

Hadadi et al. (2022) evaluated the performance of models hybridised with ANFIS and two biomimetic optimisation algorithms (shuffled frog leaping algorithm-SFLA and grey wolf optimisation-GWO) for the prediction of monthly actual evapotranspiration (AET) in the Neishaboor basin (Iran) from 2001 to 2010. The models were tested using eight scenarios involving three groups of inputs: meteorological, remote sensing and hybrid-based predictors. The results showed that remotely sensed predictors, such as the Soil moisture deficit index (SWDI), significantly improved the accuracy and achieved the highest error reduction. The ANFIS models hybridised with SFLA and GWO algorithms achieved a 12 % and 14 % reduction in error rate, respectively. The performance of the models showed that the models indicated the highest accuracy in the first third.

Quej et al. (2022) evaluated the accuracy of three artificial intelligence (AI) models such as support vector machines (SVM), adaptive neuro-fuzzy inference system (ANFIS) and categorical boosting (CatBoost) for ET_0 prediction at five different locations in Mexico.

The Penman-Monteith FAO-56 equation was used as a reference. Three different input combinations (temperature, precipitation, relative humidity) were tested. SVM models showed the best performance, outperforming ANFIS and CatBoost models. Relative humidity was found to improve model accuracy and temperature-based AI models performed better than the Hargreaves-Samani method. It is concluded that AI models can be an effective alternative to traditional methods.

Literature studies have also shown that accurate estimation of daily ET_0 is essential to improve real-time irrigation scheduling and decision-making for water resource allocation (Valipour et al., 2023; Sharafi et al., 2023). The aim of this study is to accurately predict ET_0 with a few input parameters using a machine learning based approach such as ANFIS. In order to achieve this objective, five scenarios with infrequently used meteorological data were developed and ANFIS was trained and then tested on these scenarios. In the proposed study, inputs such as THW, air pressure, air humidity, wind chill, which are not included in the reference evapotranspiration calculation formula and therefore do not directly affect the reference evapotranspiration, are used and the models are evaluated with five scenarios. A block diagram showing a summary of the proposed study is given in Figure 1.

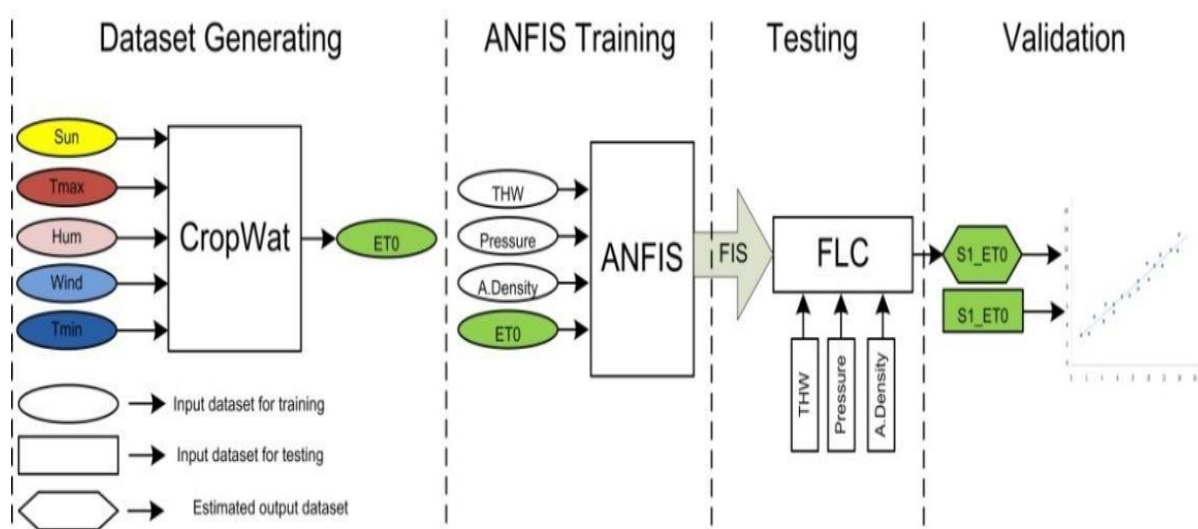


Figure 1. Summary block diagram describing the structure of the proposed work

2. Materials and Methods

2.1. Meteorological data of the studied area

This study was carried out in the fields of Eastern Mediterranean Gateway Agricultural Research Institute in Kahramanmaraş. Although Kahramanmaraş province is located in the Eastern Mediterranean Region, it has different climate characteristics due to its location at the transition point of many regions. Typical Mediterranean climate characteristics (cold and rainy winters, hot and dry summers) are observed in the region where the study was carried out. The altitude of the study area is 467 m above sea level. Meteorological data were obtained from the Davis Brand Vantage Pro-2 model climate station built in the field. The data were taken hourly from the climate station. The data were obtained from the climate station during July-August-September. In total, a data set was used consisting of 1912 rows. Out of this data set, 1612 rows were used for training and 300 rows were used for testing.

2.2. Evapotranspiration and traditional calculation methods

ET_0 is an important parameter used to

characterise the water cycle of ecosystems (Zhu et al., 2024; Araghi et al., 2020). In other words, ET_0 is the amount of water lost per unit area under certain weather conditions in a given region. Since it has an important role in determining the water requirement of plants, it has a critical role in agriculture. Accurate estimation of reference evapotranspiration is important in scheduling agricultural irrigation, crop consumption and hydrological modelling (Katipoğlu, 2023). In addition, reference evapotranspiration is one of the main constituents of the hydrological cycle. Its estimation is of great importance in agricultural transactions of all crops, especially in irrigation (Aghelpour and Norooz-Valashedi, 2022). Suitable irrigation scheduling and agricultural water management essential a exact estimation of the crop water demand. In practice, ET_0 is first predicted and then used to calculate the evapotranspiration of each crop (Mehdizadeh et al., 2021). The evapotranspiration cycle can be explained by the block diagram was given in Figure 2.

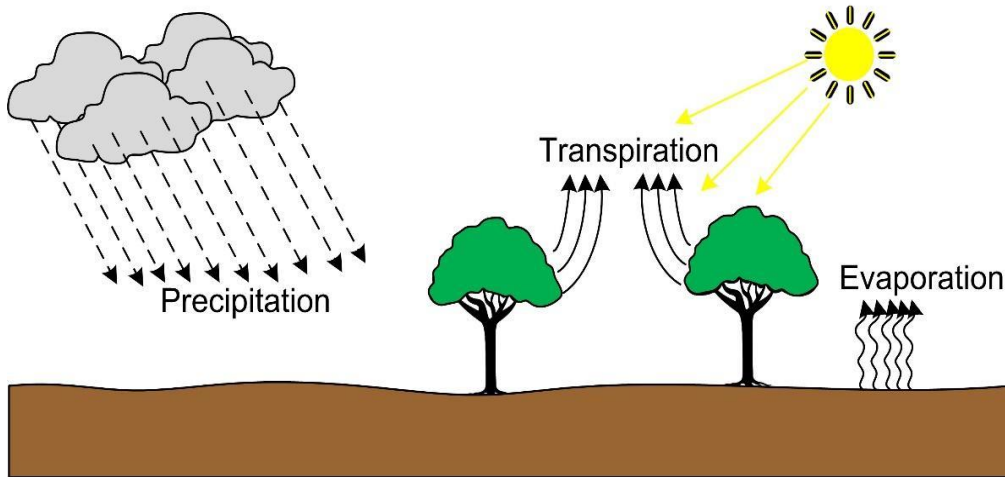


Figure 2. Block diagram explaining the occurrence of evapotranspiration

Many empirical methods have been developed to determine the reference evapotranspiration and many meteorological data are used in these methods. The most

widely used of these is the Penman Monteith method (Allen et al., 1998). Using the Penman Monteith method, the reference evapotranspiration is calculated by Equation 1.

$$ET_0 = \frac{0.408\Delta (R_n - G) + \frac{900}{T} \gamma u_2 \delta e}{\Delta + \gamma(1 + 0.34u_2)} \quad (1)$$

Where:

- ET_0 : Reference evapotranspiration (mm day^{-1})
 Δ : Rate of change of saturation specific humidity with air temperature (Pa K^{-1})
 R_n : Net irradiance ($\text{MJ m}^{-2} \text{day}^{-1}$)
 G : Ground heat flux ($\text{MJ m}^{-2} \text{day}^{-1}$)
 T : Air temperature (2m) (K)
 u_2 : Wind speed (2m) (m s^{-1})
 δe : Vapor pressure deficit (kPa)
 γ : Psychrometric constant ($\gamma \approx 66 \text{ Pa K}^{-1}$)

Based on the Penman Monteith method, ET_0 can also be determined by software such as Cropwat. In this study, the actual ET_0 value was calculated with Cropwat. In order to determine ET_0 with Cropwat, climate parameters such as maximum temperature and minimum temperature, wind speed, humidity and sunshine duration should be known. Nowadays, researches are being carried out to accurately estimate ET_0 with climate parameters other than the parameters required for the use of software such as Cropwat.

2.3. Design of ANFIS model

In recent years, a lot of machine learning tools have been enhanced with the expect to replace traditional empirical models for ET_0 prediction that require difficult data collection and calibration (Chia et al., 2021). One of them is ANFIS, a hybrid machine learning technique

involving artificial neural networks and fuzzy logic controller (Üneş et al., 2020). ANN is a powerful tool for modelling various complex and nonlinear systems. Moreover, their combination with fuzzy logic solves problems at a higher speed (Del Cerro et al., 2021). ANFIS represents a multilayer model that trains input parameters and output variables and provides the most efficient prediction matching between input and output (Alizamir et al., 2020). In other words, ANFIS learns all the relationships between the input parameters (P_1 and P_2) and the output variable and generates the function (f) of the desired output value. In addition, ANFIS has the ability to realise and detect the phenomenon without the need for mathematical management equations (Seifi ve Riahi, 2020). This can be expressed by Equation 2.

$$\begin{aligned}
 & \text{IF } P_1 \text{ is } A_1 \text{ and } P_2 \text{ is } B_1 \text{ then } f \\
 & \quad = \rho_1 P_1 + q_1 P_2 + r_1 \\
 & \text{IF } P_1 \text{ is } A_2 \text{ and } P_2 \text{ is } B_2 \text{ then } f \\
 & \quad = \rho_2 P_1 + q_1 P_2 + r_2 \\
 & \quad \vdots \\
 & \quad \vdots \\
 & \quad \vdots \\
 & \text{IF } P_1 \text{ is } A_n \text{ and } P_2 \text{ is } B_n \text{ then } f \\
 & \quad = \rho_n P_1 + q_n P_1 + r_n
 \end{aligned} \tag{2}$$

In all these aspects, ANFIS is defined as an approach that includes machine learning and fuzzy logic methods used in solving non-linear

and complex engineering problems. The block diagram of the ANFIS model to be used in the proposed study is given in Figure 3.

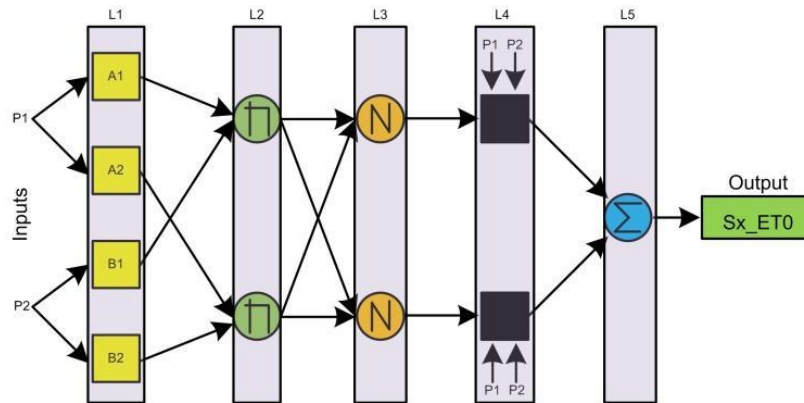


Figure 3. Block diagram of the ANFIS model to be used in the proposed study

The actual ET_0 value was calculated with Cropwat software. For this purpose, maximum temperature, minimum temperature, humidity, sunshine duration and wind speed dataset were used as input. The ANFIS model was trained with the actual ET_0 data obtained. The ANFIS model was run with the untrained test dataset and estimated ET_0 values were obtained. Finally, the actual ET_0 and estimated ET_0 were compared on scatter plots.

3. Results and Discussion

In this section, the results of the proposed

study are discussed. Accordingly, within the scope of the study, 5 different scenarios were created by combining climate parameters that are not directly effective in ET_0 calculation. For each scenario, a data set consisting of 1612 rows was created to be used in training. The training of ANFIS was carried out over these datasets. The test data set consists of 300 rows for each scenario. The scenarios, the input parameters used in these scenarios and the RMSE values in ANFIS training are given in Table 1.

Table 1. Input parameters and RMSE values of the scenarios

Scenario	Input parameters for ET_0 estimation	ANFIS minimal training RMSE
S1	THW, Air Pressure, Air Density	2.954612
S2	THW, Solar Energy, Solar Radiation	2.379339
S3	THW, Wind Chill	2.638500
S4	THW, Max. Temperature	1.844835
S5	THW, Output Humidity	2.067438

According to Table 1, in Scenario 1, ET_0 was estimated using THW, air pressure and air density as input parameters. The relationship between the ET_0 estimated by ANFIS and the actually calculated ET_0 was found as 0.76 (R^2). In Scenario 2, THW, solar energy and solar radiation were used as input parameters. The R^2 between predicted and actual ET_0 was found to be 0.66. In Scenario 3, THW and wind chill parameters were used. As a result,

R^2 was 0.43. In Scenario 4, THW and maximum temperature were used as input and R^2 was 0.87. Finally, in Scenario 5, the input parameters are THW and humidity. The R^2 of the prediction made with these two inputs with the actual ET_0 was found to be 0.84. The scatter plots of the estimated ET_0 value obtained as a result of ANFIS against the actual ET_0 value of the scenarios are given in Figure 4.

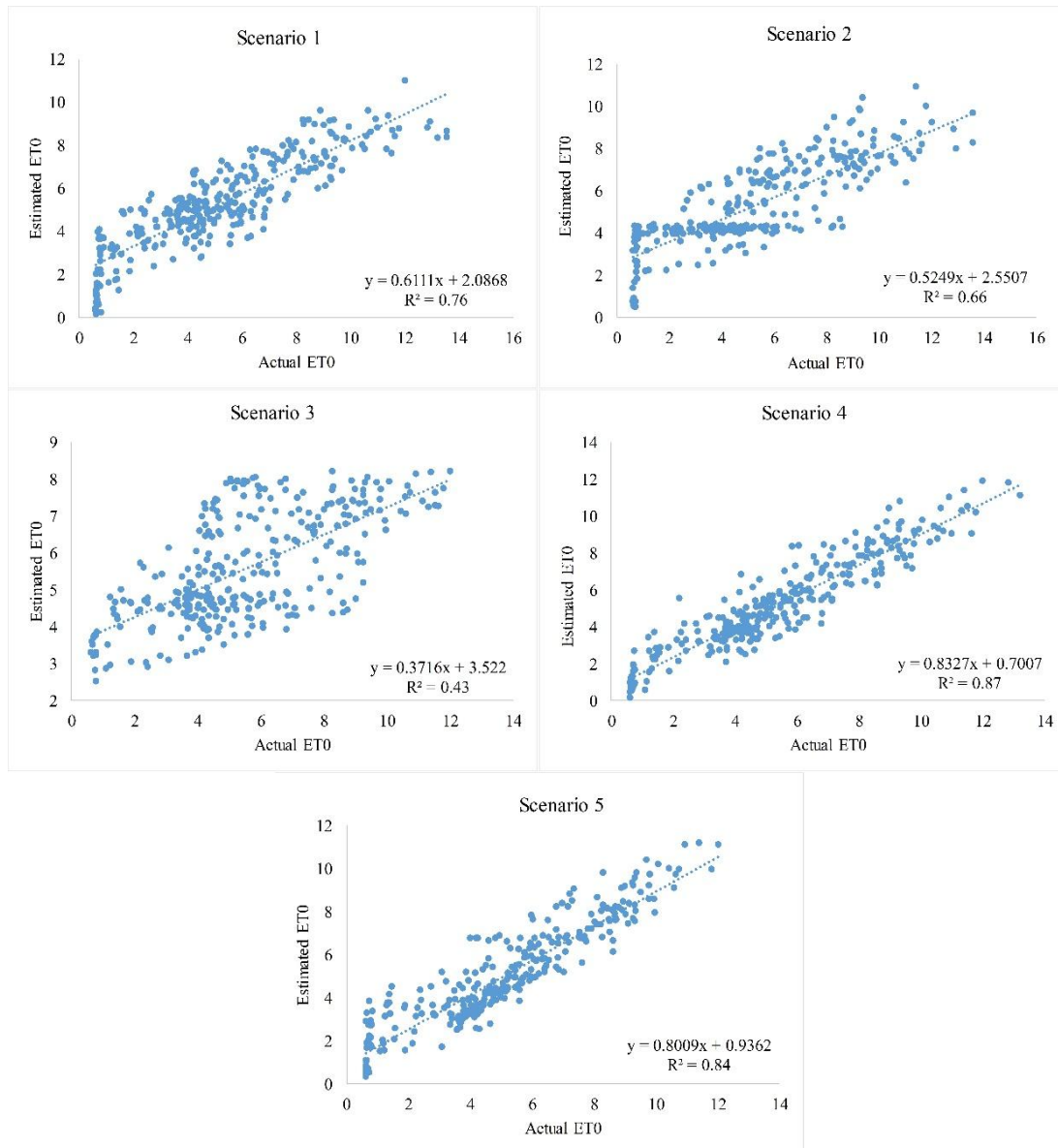


Figure 4. Scatter plots of actual and predicted ET_0 values for five scenarios

When the graphs given in Figure 4 are analysed, it is understood that Scenario 4 has the highest R^2 of actual ET_0 and ET_0 estimated by ANFIS among all scenarios. In addition, the scenario with the lowest ANFIS minimal training RMSE value is the scenario 4. Three input parameters were used in Scenarios 1 and 2. In Scenarios 3, 4 and 5, two input parameters were used. Özel and Büyükyıldız (2019) used average temperature, maximum temperature, minimum temperature, wind speed, relative humidity, monthly total precipitation, vapour pressure and atmospheric pressure as input to ANFIS to predict monthly evaporation in Karaman. When they used eight sets of climate data as input in their study, they found R^2 to be 0.82. Among

these data, when they used only average and maximum temperature as input, they obtained R^2 as 0.72. Ladlani et al. (2014) used six different climate data such as average temperature, maximum temperature, minimum temperature, wind speed, relative humidity, sunshine duration as input to ANFIS by classifying them with various scenarios to predict daily ET_0 in Algeria. They found the highest R^2 as 0.83 in the scenario where all six inputs were used. Terzi et al. (2006), they used climate parameters such as air temperature, water temperature, relative humidity, solar radiation, wind speed and air pressure as inputs to predict the evaporation of Lake Eğirdir (Isparta, Turkey) using ANFIS. They obtained

coefficient of determination of 0.98, RMSE of 0.24, and average performance error of 4.6 %, which are consistent with the evaporation values calculated by Penman method. The results of our proposed study and the results obtained by other researchers in the literature were generally consistent with each other. Cobaner (2011) compared three empirical models (CIMIS Penman, Hargraves and Ritchie) with an artificial neural network based approach using the ANFIS model. As a result, they concluded that the ANFIS model provides high accuracy with less computation. (Kisi et al., 2014) compared ANFIS models with empirical models (Valiantzas equations, Turc, Hargreaves and Ritchie) and their calibrated versions. The comparison results show that the three- and four-input ANFIS models outperform the respective empirical equations in modeling ET₀, while the calibrated two-parameter Ritchie and Valiantzas equations outperform the two-input ANFIS models. (Seifi et al., 2020) gamma test was used to select the best input vectors and the ET₀ predictions of the LSSVM-GT model with different kernels (RBF, linear, polynomial) were compared with ANN-GT, ANFIS-GT and empirical equations. Gamma test showed that the most important climate variables are minimum and maximum air temperature and wind speed. The LSSVM model outperformed ANFIS and ANN models when the same meteorological inputs were used. Moreover, LSSVM, ANFIS and ANN models outperformed empirical equations such as Blaney-Criddle and Hargreaves-Samani.

4. Conclusions

The results obtained from the study and the results of the researchers show that even with a limited number of input parameters, it is possible to estimate ET₀ with a high accuracy with ANFIS. In addition, it is possible to obtain a high accuracy ET₀ value with ANFIS even with parameters that are calculated empirically (equation-based) and not included in the ET₀ calculation. The study and the ANFIS-based ET₀ estimation studies in the literature have shown that average, maximum and minimum temperature have an important role in ET₀

estimation. It has been observed that the number of parameters and data of the dataset provided as input parameters to ANFIS significantly increases the accuracy of the prediction value obtained as a result of ANFIS.

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Şeftali Posasının Yonca Silajlarında Karbonhidrat Kaynağı Olarak Kullanılma Olanakları

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Özet

Bu çalışmada, farklı dozlarda şeftali posası ilavesinin yonca silajlarının kimyasal kompozisyon ve fermentasyon özellikleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Araştırma kapsamında, katkı maddesi olarak şeftali posası, % 0 (kontrol), % 5 ve % 10 düzeylerinde yonca silajlarına eklenmiştir. Araştırmadan elde edilen verilerin değerlendirildiğinde, şeftali posası katkısı silajların kuru madde ve ham protein içeriğini düşürmüştür ($P<0.001$). Ayrıca % 5 şeftali posası ilavesi, kontrol grubuyla karşılaştırıldığında NDF ($P<0.05$) ve ADF ($P<0.01$) içeriklerinde düşüşe neden olmuştur. Laktik asit ($P<0.05$), asetik asit ($P<0.001$), propiyonik asit ($P<0.001$), bütirik asit ($P<0.01$) içerikleri kontrol grubunda en düşük değerde iken, % 5 şeftali posası katkılı grupta pH içeriği en düşük düzeyde olduğu tespit edilmiştir ($P<0.05$). Araştırma sonunda, yonca silajları üzerindeki etkilerinin farklı dozlar ve/veya ekstraksiyon yöntemleri kullanılarak değerlendirilmesi gerektiği sonucuna varılmıştır.

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Anahtar Kelimeler

Şeftali posası
yonca
silaj
fermentasyon
kalite

Possibilities of Using Peach Pulp as a Carbohydrate Source in Alfalfa Silages

Abstract

In this study, it was aimed to determine the effects of peach pulp addition at different doses on the chemical composition and fermentation characteristics of alfalfa silages. In this study, peach pulp was added to alfalfa silages at 0 % (control), 5 % and 10 % levels. When the data obtained from the study were evaluated, peach pulp addition decreased the dry matter and crude protein content of the silages ($P<0.001$). In addition, 5% peach pulp addition caused a decrease in NDF ($P<0.05$) and ADF ($P<0.01$) contents compared to the control group. Lactic acid ($P<0.05$), acetic acid ($P<0.001$), propionic acid ($P<0.001$), butyric acid ($P<0.01$) contents were the lowest in the control group, while the pH content was the lowest in the group with 5 % peach pulp ($P<0.05$). At the end of the study, it was concluded that the effects on alfalfa silages should be evaluated using different doses and/or extraction methods.

Research Article

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quality

1. Giriş

Şeftali, Türkiye'de meyve suyu endüstrisinde yüksek miktarda kullanılan meyvelerden birisidir. Türkiye'de 2021 yılında 916.300 ton şeftali üretimi yapılmıştır (TÜİK, 2022). Türkiye şeftali üretimi bakımından dünyada 5. sırada yer almaktadır. Şeftali, çeşitli fenolik, karetoneoid, vitamin A, potasyum açısından zengin bir meyvedir (Manzoor ve ark., 2012). Şeftali, dünyada en çok tüketilen ve yüksek ekonomik değere sahip çekirdekli meyvelerden birisidir (Zhao ve ark., 2015; Vulic ve ark., 2022). Meyve suyu endüstrisinde işlenen meyvelerden geri kalan atıklara posa denir (Gowman ve ark., 2019). Meyve posası, yüksek biyoaktif bileşikler ve lif içerdiğinden dolayı iyi bir fonksiyonel gıda kaynağı olarak kabul edilmektedir (O'shea ve ark., 2012). Gıda sanayinde işlenen meyvelerden yaklaşık olarak % 25 oranında posa ortaya çıkmaktadır (Nawirska ve Kwaśniewska, 2005). Hasat döneminin kısa olması ve yüksek miktarda üretilmesi nedeniyle ortaya çıkan meyve posası miktarı fazladır (Beres ve ark., 2017). Meyve posalarının üretime dahil edilmesi çevresel ve ekonomik kaynakların korunması için önemlidir (Vorobyova ve Skiba, 2021). Meyve posalarının silaj katkı maddesi olarak kullanımı son yıllarda ilgi çeken bir konu olmuştur (Ülger ve Özdemir, 2023). Meyve posaların silaj olarak kullanılması çevre kirliliğinin azaltılmasına yardımcı olmasının yanı sıra yeni alternatif yem kaynakları ortaya çıkmış olacak ve ayrıca yem olarak tahıl üretiminin üzerindeki baskı azalmış olacaktır (Abarghuei ve ark., 2010; Hu ve ark., 2015). Ayrıca silaj fermentasyonu sırasında yoncanın proteolizi ve son yıllarda yaşanan iklimsel değişikliklerden dolayı kurutma sorunu yaygın bir problemlerdir. Bu nedenle melas ve meyve posaları gibi kolay çözünebilir karbonhidrat kaynakları katılarak bu sorun aşılabilmektedir (Vulic ve ark., 2022). Ancak şeftali posasının yem katkı maddesi olarak kullanımı çok fazla yaygın değildir. Yalçınkaya ve ark. (2012), şeftali posasının kuru madde, ham kül, organik

madde, ham protein, ham yağ ve ham selüloz düzeylerini sırasıyla % 13.96; % 0.60; % 13.36; % 1.62; % 3.11 ve % 33.74 olarak tespit etmişlerdir.

Çalışmada, ruminantların beslenmesinde önemli bir yere sahip olan yonca bitkisinden, silaj yapımı aşamasında yaşanan sorunların giderilmesi için şeftali posasının farklı düzeylerde ilave edilerek silajların besin madde içerikleri ve bazı fermentasyon özelliklerine olan etkisi araştırılmıştır.

2. Materyal ve Yöntem

2.1. Silajlık materyallerin hazırlanması ve silolama dönemi

Araştırma silajlarının ana materyalini oluşturan yonca bitkisi (*Medicago sativa* L.) çiçeklenme döneminde hasadı yapılarak laboratuvara getirilmiştir. Taze yoncaya bir gün soldurulma işlemi uygulanmış ve silaj yapma makinesi ile 1-3 cm uzunluğunda parçalama gerçekleştirilmiştir. Araştırma grupları: (i) Kontrol (Şeftali posası katkısız); (ii) : %5 şeftali posası; (iii) %10 şeftali posası olacak şekilde düzenlenmiştir. Şeftali posası, parçalanmış yonca bitkisine belirtilen düzeylerde homojen olarak ilave edilmiştir. Silajlar, laboratuvar ortamında oda sıcaklığında 60 gün boyunca 3 tekerrür olacak şekilde ağzı sıkıca kapatılmış anaerob kavanozlarda fermentasyona bırakılmış ve 60 günlük silolama dönemi sonunda numuneler açılarak analizleri yapılmıştır.

2.2. Kimyasal analizler

Fermentasyon sonrası açılan numuneler kimyasal analizler için hazırlanmıştır. Kuru madde analizi etüvde 60 °C sıcaklıkta numune ağırlığı sabit tartıma gelinceye kadar kurutulmuştur (AOAC, 1989). Kuru madde tespiti için kurutulan bu numuneler kimyasal analizler için 1 mm elekten geçecek şekilde laboratuvar değirmeninde (IKA MF.10) öğütülmüştür. Öğütülen bu numuneler, analizler için hava almayacak şekilde kilitli poşetlerde saklanmıştır. Analiz sonuçları kuru madde bazında değerlendirilmiştir.

Silajların Dumas yöntemiyle ham protein; 550 °C'de 4-6 saat kül fırınında yakılarak ham kül ve eter ekstraksiyon yöntemi (ANKOM XT15) ile ham yağ AOAC (1989)'da belirtilen yöntemlere göre yapılmıştır. Nişasta analizi polarimetrik yöntemle ve enerjiyle ilgili değerler hesaplama yolu yapılmıştır. Nötr deterjanda çözünmeyen lif (NDF) ve asit deterjanda çözünmeyen lif (ADF) analizleri ise Van Soest ve ark. (1991) bildirdiği metoda göre ANKOM 2000 tam otomatik fiber analiz cihazı kullanılarak gerçekleştirilmiştir.

2.3. Fermentasyon özellikleri

Altmış günlük inkübasyon sonucu açılan silajlarda pH, asetik, propiyonik, bütirik ve laktik asit düzeyleri belirlenmiştir. Bunun için 40 g silaj örneği 360 ml saf su ilave edilerek blenderde karıştırılmıştır. Elde edilen sıvı süzülerek 40 ml örnek ve 400 ml'ye tamamlanmıştır. Bu sıvı tekrar Whatman 54 filtre kâğıdından süzülerek santrifüj edilmiştir. Örnekler analiz edilene kadar -20 °C'de saklanmıştır. Laktik asit analizi için Lepper'in kısaltılmış metodu kullanılmıştır (Akyıldız,

1984). Asetik, propiyonik ve bütirik yağ asitleri (UYA) analizleri gaz kromatografisinde (Model, Agilent, 7890, ABD) analiz edilmiştir. pH ölçümleri için silaj numunesi %20 olacak şekilde saf su ile karıştırılarak, karışımdan elde edilen süzütünün pH seviyesi, pH metre ile ölçülmüştür.

2.4. İstatistiksel analizler

Çalışmaya ilişkin istatistikler, tek yönlü varyans analizi (One-way ANOVA) prosedürü kullanılarak Minitab 16.1 yazılımı ile gerçekleştirilmiştir. Homojen alt kümelerdeki gruplar için farklılık tespiti %95 güven aralığında Tukey çoklu karşılaştırma testi kullanılarak gerçekleştirilmiştir.

3. Bulgular ve Tartışma

3.1. Taze yoncanın ham besin madde kompozisyonu

Silolama öncesi 4. biçimde hasat edilen yonca bitkisinin besin madde analizleri yapılarak Tablo 1'de verilmiştir.

Tablo 1. Yoncanın silolama öncesi ham besin madde kompozisyonu

Parametreler	Taze Yonca
Kuru Madde, %	20.05
Ham Protein, % KM	21.13
Ham Yağ, % KM	2.40
NDF, % KM	35.62
ADF, % KM	27.13
HK, % KM	15.88
Metabolik Enerji Mcal kg ⁻¹	2.40
Nişasta	2.38

% KM: % Kuru madde; NDF: Nötral deterjanda çözünmeyen lif, ADF: Asit deterjanda çözünmeyen lif.

Dördüncü biçimdeki taze yoncanın kuru maddesi % 20.05, ham protein oranı % 21.13, ham yağ içeriği % 2.40, ham kül oranı % 15.88 olarak belirlenmiştir. Çalışmada yoncanın silolama öncesi NDF ve ADF değerinin % 35.62 ve % 27.13 olduğu görülmüş, ME değerinin 21.40 Mcal kg⁻¹, nişasta değerinin de % 2.38 olduğu tespit edilmiştir.

Yonca bitkisinin besin maddesi içeriği ülkemizde ve dünyada yapılan çalışmalarla benzerlik göstermektedir. Nitekim Canbolat ve ark. (2010), taze yoncanın kuru madde (KM), ham kül (HK), nötr deterjanda çözünmeyen lif

(NDF), asit deterjanda çözünmeyen lif (ADF), ham protein (HP) ve ham yağ (HY) değerlerinin sırasıyla % 25.10; 7.13; 53.70; 37.81; 19.35 ve 6.34 olduğunu ifade ederken; Agarussi ve ark. (2019), soldurulmamış yoncanın KM'sini % 13.04; soldurulmuş yoncanın ise % 23.54 olarak tespit etmiştir. Ertekin ve Kızılsimşek (2020), silaj yapılmadan önceki taze yoncanın HP, HK, NDF, ADF ve pH'ını sırasıyla %18.33; % 8.24; % 49.86; % 31.17 ve 6.06 olarak tespit ederken; Huo ve ark. (2021), taze yoncanın % 32.5 KM, % 31.6 organik madde, % 17.3 HP,

% 42.3 NDF, % 23.7 ADF, % 5.97 ADL, % 0.98 HY içerdiğini belirlemiştir. Kang ve ark. (2021), silolama öncesi yoncanın % 17 HP, % 26.3 ham selüloz, % 46.0 NDF, % 39.9 ADF ve 16.92 MJ kg⁻¹ toplam enerji içerdiğini ifade

ederken; Lazarević ve ark. (2023), % 33.90 KM, % 20.46 HP içerdiğini belirtmiştir.

Farklı oranlarda şeftali posası ilavesinin yonca silajlarının kalitesine etkisine ait analiz sonuçları Tablo 2’de sunulmuştur.

Tablo 2. Şeftali posası katkılı yonca silajlarının kimyasal kompozisyonu

Parametreler	Şeftali posası düzeyleri, %			
	Kontrol	5	10	P Değeri
Kuru Madde %	20.61 ^a	19.49 ^b	19.32 ^b	0,000
Ham Protein, % KM	22.70 ^a	20.46 ^b	20.04 ^b	0.000
Ham Yağ, % KM	2.60	3.51	3.14	0.073
Ham Kül, % KM	16.43	15.25	16.04	0.379
Nişasta, % KM	2.50	2.76	2.42	0.243
ADF, % KM	27.82 ^a	18.82 ^b	23.50 ^{ab}	0.003
NDF, % KM	35.84 ^a	26.92 ^b	31.45 ^{ab}	0.040
ME (Mcal kg ⁻¹)	2.19	2.47	1.96	0.184
NE _L	1.17	1.31	1.19	0.151

^{a-b}: Aynı satırdaki farklı harfler istatistiksel olarak farkı göstermektedir (P<0.01).

^{a-b}: Aynı satırdaki farklı harfler istatistiksel olarak farkı göstermektedir (P<0.001).

ADF: Asit deterjanda çözünmeyen lif; NDF: Nötr deterjanda çözünmeyen lif; ME: Metabolik enerji; NE_L: Net enerji laktasyon; % KM: % Kuru madde.

Araştırma silajlarının kuru madde içerikleri önemli farklılıklar (Tablo 2), kontrol grubunda % 20.61 tespit edilen kuru madde içeriği, % 5 ve % 10 şeftali posası katılan gruplarda % 19.49 ve 19.32 olarak bulunmuş ve şeftali posası ilavesiyle silajların kuru madde içeriği düşmüştür (P<0.001).

Yonca gibi baklagil yemlerinden, yüksek tampon kapasiteleri ve düşük suda çözünabilir karbonhidrat konsantrasyonları nedeniyle genellikle iyi kalitede silaj yapımının kolay olmadığı bilinmektedir (Owens ve ark., 1999). Yüksek kaliteli silaj için gerekli laktik asit fermentasyonu sağlanamadığı durumda, istenmeyen mikroorganizmalar çoğalmakta ve silajlarda bozulmalar meydana gelmektedir (Bolsen ve ark., 1996). Araştırma sonunda, düşük kuru madde içeriğine sahip şeftali posası katkısının silajlardaki düzeyleri arttıkça kuru madde içeriklerinin azaldığı belirlenmiştir. Sonuç olarak, şeftali posasının düşük kuru madde içeriğinden kaynaklanabileceği silajların kuru madde içeriklerine herhangi bir olumsuz etkisinin bulunmadığı, yeterli laktik asit bakterilerinin gelişmesini teşvik ettiği ve ortamda istenmeyen unsurların gelişimini durdurarak kuru madde kayıplarını azalttığı söylenebilir. Araştırma silajlarının ham yağ, ham kül, nişasta, ME ve NE_L içerikleri

bakımından görülen farklılıklar önemli bulunmamıştır (P>0.05). Besharati ve ark. (2017), yonca silajlarına elma posası katkısıyla ME ve NE_L içeriklerinin arttığını bildirmişlerdir. Nişasta, yapısal olmayan karbonhidratlardan olup; depo polisakkaritlerden birisidir. Nişasta, laktasyondaki süt inekleri için başlıca enerji kaynağı olarak kullanılmaktadır. Araştırmamızda yonca silajının sahip olduğu nişasta düzeyi % 2.50 bulunmuş, şeftali posası katılan gruplarda nişasta düzeyleri % 2.76 ve % 2.42 olarak bulunmuş ve gruplar arasındaki farklılık önemsiz çıkmıştır (P>0.05). Yonca silajının ham kül değeri, % 16.43 olarak bulunurken, % 5 şeftali posası katılan grupta % 15.25; % 10 şeftali posası katılan grupta da % 16.04 olarak tespit edilmiştir. Yonca silajına şeftali posası katılması, silajın ham kül değerini azaltsa da gruplar arasında bir farklılığa yol açmamıştır (P>0.05). Özdemir ve Ülger (2022), şeftali posası silajının ham kül düzeyini % 5.21 olarak tespit ederken; Yalçınkaya ve ark. (2012), % 0.61 olarak saptamışlardır. Mevcut araştırmada yonca silajlarının ham yağ düzeyleri, gruplar arasında istatistiki bir farklılaşmaya yol açmamış (P>0.05), kontrol grubunun ham yağ düzeyi % 2.60; şeftali posası katılan silajların ham yağ düzeyleri de % 3.14-3.51 olarak tespit

edilmiştir. Araştırmada kontrol grubundaki yonca silajının ham protein değeri % 22.70 iken, şeftali posası katkılı gruplarda sırasıyla ham protein değeri, % 20.46 ve % 20.04 olarak tespit edilmiş, gruplar arasında farklılıkların istatistiksel olarak önemli olduğu belirlenmiştir ($P<0.001$). Şeftali posası ilavesinin yonca silajının ham protein içeriğini azalttığı söylenebilir. Bununla birlikte, şeftali posası katkısının düzeyleri arttıkça silajlarda bozulma belirtisi olan bütirik asit varlığını artırması araştırma silajlarında yüksek düzeyde olmasa da proteolizis gerçekleştiğine işaret etmektedir. Arslan Duru (2019) yonca silajına lavanta posası eklenmesiyle ham protein içeriğinin düştüğünü ifade etmiştir.

Yonca silajlarına % 5 şeftali posası katkısıyla NDF ($P<0.05$) ve ADF ($P<0.01$) içerikleri düşmüştür. ADF içeriği, kaba yemlerin sindirilme derecesi hakkında bilgi veren önemli bir kriterdir. Ayrıca, NDF içeriği de hayvanın tüketebileceği yem miktarını yansıtmaktadır. % 5 şeftali posası ilavesiyle düşen NDF ve ADF içerikleri, laktik asit bakteri faaliyetlerinin silajlardaki hücre duvarı bileşenlerinin parçalanabilirliklerini artıracak kadar hızlandırdığına işaret etmektedir. Başka

bir deyişle silaj fermentasyonu sırasında mikroorganizmaların asidik hidrolizi ve/veya fibrinolitik enzim üretiminin bir sonucu olduğu düşünülmektedir. Tablo 3’de belirtildiği üzere % 5 şeftali posası içeren silajların pH içeriklerinin düşük olması ve laktik asit içeriklerinin yüksek olması bu görüşü destekler niteliktedir.

Tablo 3 incelendiğinde, % 10 şeftali posası katkısının kontrol grubuyla karşılaştırıldığında, yonca silajlarının pH’sını düşürdüğü belirlenmiştir ($P<0.05$). Fermentasyonun dönemlerinde silo ortamındaki pH değerinin düşüş hızı, kaliteli silaj eldesi için oldukça önemlidir. Silolama döneminde katkı maddesi ilave edilmesinin başlıca nedenleri, özellikle zor silolanabilen taze materyallerle gerçekleştirilen silajlarda istenmeyen ve patojen mikroorganizmaların gelişimini engellemek/baskılamak ve dolayısıyla daha önce belirtildiği gibi silajların pH değerlerini en kısa zamanda düşürerek fermentasyon son ürünlerinin yoğunluğu azaltmaktır. Ke ve ark. (2015), yonca silajlarına elma posası ilavesiyle pH’nın düştüğünü ancak üzüm posası ilavesiyle değişmediğini bildirmişlerdir.

Tablo 3. Şeftali posası katkılı yonca silajının fermentasyon özellikleri

Parametreler	Şeftali posası düzeyleri, %			
	Kontrol	5	10	P Değeri
Laktik asit (g kg ⁻¹)	1.55 ^b	2.13 ^a	1.84 ^{ab}	0,032
Asetik asit (g kg ⁻¹)	4.54 ^b	11.68 ^a	11.18 ^a	0,000
Propiyonik asit (g kg ⁻¹)	0.22 ^c	0.45 ^b	0.84 ^a	0,000
Bütirik asit (g kg ⁻¹)	0.10 ^b	0.28 ^a	0.33 ^a	0,005
pH	6.67 ^a	6.42 ^b	6.57 ^{ab}	0.021

^{a-b}: Aynı satırdaki farklı harfler istatistiksel olarak farkı göstermektedir ($p<0,05$).

^{a-b}: Aynı satırdaki farklı harfler istatistiksel olarak farkı göstermektedir ($p<0,01$).

^{a-c}: Aynı satırdaki farklı harfler istatistiksel olarak farkı göstermektedir ($p<0,001$).

Şeftali posası ilavesi, araştırma silajlarının laktik asit ($P<0.05$), asetik asit ($P<0.001$), propiyonik asit ($P<0.001$) ve bütirik asit ($P<0.01$) içeriklerini artırmıştır. İyi silajlarda laktik asit birincil asit olmalıdır. Bu asit, silajda bulunan diğer asitlerden (asetik, propiyonik ve bütirik asitler) daha güçlüdür ve bilindiği gibi silo ortamında genellikle pH değerinin düşmesinden sorumludur. İyi bir silajda toplam silaj asitlerinin en az % 65 -70’i laktik asitten

oluşması istenmektedir (Kung, 2010; Cvetnic ve ark., 2023). Yonca silajındaki laktik asit düzeyi mevcut çalışmada 1.55 g kg⁻¹ olarak bulunurken; şeftali posası silajı katkılı gruplarda bu değer 1.84 ve 2.13 g kg⁻¹ olarak tespit edilmiştir. Yonca silajına şeftali posası katılması, silajın laktik asit düzeyinde artışa yol açmıştır. Silaj kalitesi için önemli bir kriter olan laktik asit düzeyi katkısız silajlarda genel olarak düşük, katkılı silajlarda ise yüksek

olarak bulunmaktadır. İyi kalitede silaj eldesindeki en önemli kriterlerden biri olan laktik asitin silajları koruyacak minimum varlığının sağlanması gerekmektedir. Araştırma silajlarında % 5 şeftali posası ilave edilen grupta laktik asit üretiminin gerçekleştiği gözlemlense de bütün gruplar dikkate alındığında yavaş gelişen fermentasyon koşulları nedeniyle yeterli koruyucu etkiyi sağlama da sınırlı kalacağı anlaşılmaktadır. Guler ve ark. (2006), elma posası ilavesinin yonca silajlarının laktik asit konsantrasyonlarını artırdığını ifade etmişlerdir.

Asetik asit, silajın hava aldığı ve bozulduğunun bir göstergesidir (Mut ve ark., 2020). Asetik asit, silajda ikinci en yüksek konsantrasyona sahip olan asittir ve genellikle % 1-3 arasında değişmektedir (Cvetnic ve ark., 2023). Silajdaki asetik asit içeriği aynı zamanda kuru madde içeriğinden de etkilenmektedir. Kuru madde arttıkça asetik asit içeriği azalmaktadır. Mevcut çalışmada, organik asitlerden asetik asit söz konusu olduğunda, yonca silajında asetik asit düzeyi (4.54 g kg^{-1}), şeftali posası katılan gruplardaki asetik asit düzeylerinden (11.18 ve 11.68 g kg^{-1}) düşük bulunmuştur. Şeftali posası silajı ile ilgili yapılan bir çalışmada şeftali posası silajındaki asetik asit düzeyi 11.90 g kg^{-1} KM olarak tespit edilmiştir (Yalçınkaya ve ark., 2012).

Silajların fermentasyon aşamasında laktik asit bakterilerinin en önemli rakibi, bütirik asit bakterileri olduğu bilinmektedir. Silo yemlerinde, Clostridial fermentasyon gerçekleştiğinde, yoğun bütirik asit üretimi nedeniyle önemli düzeyde besin madde kaybı oluşmaktadır. Bu bakteri grubu, laktik asit bakterilerinin tükettiği ortamdaki karbonhidratları kullanarak gereksinim duydukları besin maddelerini azaltırlar ve/veya tamamen tüketirler (Ni ve ark., 2018). Bütirik asit, laktik asit bakterileri ve maya büyümesi üzerindeki engelleyici etkisinden dolayı silajda istenmemektedir (Cvetnic ve ark., 2023). Ayrıca silajlarda amonyak azot yoğunluğu, ortamda var olan proteaz aktivitesi ve mikrobiyal yoğunluğun özellikle bütirik asit

bakterilerinin etkilerinden kaynaklanmaktadır. Yüksek pH seviyelerinde hem bütirik asit hem de proteolitik enzimleri aktiftir. Araştırma silajlarında artan bütirik asit içeriği, mevcut silajlarda proteolizisin silajların bozulmasını teşvik etmeyecek ve pH değerini yükseltmeyecek düzeyde başa bir deyişle sınırlı da olsa şekillendiğine işaret etmektedir. Araştırma silajları organik asit içerikleri bakımından genel olarak değerlendirildiğinde, silajlardaki organik asit kompozisyonu, yonca gibi zor silolanabilen yeşil yemler için beklenen düzeyde olduğu söylenebilir. Şeftali posasının karbonhidrat desteği sağlayarak silajlardaki organik asit profilini pozitif yönde etkilediği görülmektedir. Canbolat ve ark. (2010), üzüm posası katkısının yonca silajlarında laktik asit ve propiyonik asit içeriğini artırdığını, asetik asit ve bütirik asit içeriğini düşürdüğünü belirtmişlerdir.

4. Sonuç

Silolama, gıda sanayii atıklarından hayvan beslemede yan ürün olarak kullanımı yonca gibi zor silolanan değerli yem bitkilerinin kalitesini artırmada uygun bir yol olduğu düşünülmektedir. Özellikle son yıllarda kurutmada yaşanan problemler dikkate alındığında silolamanın önemi daha da artmıştır. Şeftali posasının işlenmesinin yonca silajlarının kimyasal bileşimi ve bazı fermentasyon özellikleri üzerinde etkili olduğu, ancak kaliteli silajlar elde etmek için silajlar üzerindeki etkilerinin farklı dozlar ve/veya ekstraksiyon yöntemleri kullanılarak değerlendirilmesi gerektiği sonucuna varılmıştır.

Yazarların Katkı Beyanı

Yazarlar, makaleye eşit katkıda bulunduklarını, makalenin yayına hazır son halini gördüklerini/okuduklarını ve onayladıklarını beyan ederler.

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Türkiye’de Ayran Üretim Modelinin Mevsimsel Zaman Serileri Analizi ile Oluşturulması

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Özet

Bu çalışma, Türkiye’de ayran üretim miktarının aylık serisinin modellenmesi amacıyla yapılmıştır. 2010 Ocak 2024 Nisan dönemi aylık üretim serisinin modellenmesinde ARIMA (p, d, q) (P, D, Q)_s modeli kullanılmıştır. Uygun modelin seçiminde, ARIMA (p, d, q) (P, D, Q)_s modellerinden hesaplanan kalıntıların bağımsız olup olmadıkları göz önüne alınmıştır. Bu nedenle modelin uygunluğu için Ljung-Box Q(r) istatistikleri ve p (olasılık) değerleri saptanmıştır. Aylık üretim serisinin modeli oluşturulmuş ve p değeri % 5’ten daha büyük olduğundan (p=0.145) model uygun kabul edilmiştir. Serinin durağanlığı incelendiğinde, serinin düzeyde durağan olmadığı ve birinci farkı ile birinci mevsimsel farkı alınarak durağan hale geldiği görülmüştür. Yapılan analizler neticesinde, ARIMA (0, 1, 2) (0, 1, 0)₁₂ modelinin tahmin için uygun olduğu ortaya konmuştur. Yani ikinci dereceden bütünleşik mevsimsel hareketli ortalama modeli elde edilmiştir. Modelin katsayıları 0.277 ve 0.255 olarak hesaplanmıştır. Modele ait denklem ise, $Z_t = Z_{t-1} + Z_{t-12} - Z_{t-13} - 0.277e_{t-1} - 0.255e_{t-2} + e_t$ şeklindedir. Önerilen model kullanılarak 2024 Mayıs-2025 Aralık dönemi aylık ayran üretimi öngörüsü hesaplanmıştır. Öngörü sonucunda, 2024 Mayıs-2025 Aralık dönemi üretim miktarının 77529-99349 ton arasında olacağı beklenmektedir. Sonuç olarak, incelenen aylık ayran üretimi değerlendirildiğinde genel olarak Mart-Ağustos ayları arasında üretimde artış, Eylül-Şubat ayları arasında ise üretimde azalma eğilimi devam etmiştir. 2025 yılı öngörü sonuçları da bu durumu desteklemektedir.

Araştırma Makalesi

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Anahtar Kelimeler

Zaman serileri
mevsimsellik
durağanlık
ayran
üretim

The Forming with Seasonal Time Series Analysis of the Ayran Production Model in Türkiye

Abstract

This study was conducted to model the monthly series of ayran production amount in Türkiye. ARIMA (p, d, q) (P, D, Q)_s model was used in modeling the monthly production series for the period January 2010 April 2024. In selecting the appropriate model, whether the residuals calculated from ARIMA (p, d, q) (P, D, Q)_s models were independent or not was taken into consideration. For this reason, Ljung-Box Q(r) statistics and p (probability) values were determined for the suitability of the model. The model of the monthly production series was created and the model was considered appropriate since the p value was greater than 5 % (p= 0.145). When the stationarity of the series was examined, it was seen that the series was not stationary at level and became stationary by taking its first difference and first seasonal difference. As a result of the analysis, it was revealed that the ARIMA (0, 1, 2) (0, 1, 0)₁₂ model is suitable for prediction. In other words, a second-order integrated seasonal moving average model was obtained. The coefficients of the model were calculated as 0.277 and 0.255. The equation of the model is $Z_t = Z_{t-1} + Z_{t-12} - Z_{t-13} - 0.277e_{t-1} - 0.255e_{t-2} + e_t$. Using the proposed model, monthly ayran production forecast for the period May 2024-December 2025 was calculated. As a result of the forecast, the production amount for the 2024 May-2025 December period is expected to be between 77529-99349 tons. As a result, when the monthly ayran production examined was evaluated, there was generally an increase in production between March and August, and a decreasing trend in production continued between September and February. The forecast results for 2025 also support this situation.

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1. Giriş

Süt bileşimindeki; laktoz, süt yağı, esansiyel yağ asitleri, biyolojik değeri yüksek süt proteinleri (yüksek kaliteli protein), esansiyel amino asitler, fosfolipitler, özellikle kalsiyum ve fosfor başta olmak üzere bazı önemli mineraller (magnezyum, potasyum, çinko), başta B₂ vitamini (riboflavin), B₁₂ vitamini (kobalamin), A vitamini ve/veya β karoten olmak üzere B₁ vitamini (tiyamin), B₃ vitamini (niyasin) ve B₆ vitamini (piridoksin) gibi vitaminler ve daha birçok bileşeni yeterli ve dengeli bir şekilde içermesinden dolayı hayati öneme sahip bir gıdadır (Çakmakçı, 2020). Ayrıca içeriğinde; immünoglobulin proteinleri, hormonlar, enzimler, büyüme faktörleri, sitokinler, nükleotidler, peptitler ve diğer biyoaktif peptitler de bulunmaktadır (Metin, 2017; Serdar Eymirli ve ark., 2019). Bu nedenle de süt ve/veya süttten üretilen fermente süt ürünleri (ayran, yoğurt, kefir, kıymız vb.) sağlıklı ve dengeli beslenmenin her zaman çok önemli bir parçası olmaya devam etmiş/etmektedir (Çakmakçı, 2020). Süt ve süttten üretilen fermente süt ürünleri sadece insanların beslenme ihtiyaçlarını karşılamak için değil, aynı zamanda osteoporoz (kemik erimesi), kemik yumuşaması, diş çürüğü, obezite, kardiyovasküler hastalıklar, zayıf gastrointestinal sağlık, hipertansiyon ve kolorektal kanser gibi çeşitli rahatsızlıkların önlenmesi için de tüketilebilmektedir (Demirgül ve Sağdıç, 2018). Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliğine (Tebliğ No: 2022/44) göre Fermente süt ürünü, “Sütün uygun mikroorganizmalar tarafından fermentasyonu ile pH değerinin koagülasyona yol açacak veya açmayacak şekilde düşürülmesi sonucu oluşan ve içermesi gereken mikroorganizmaları yeterli sayıda canlı ve aktif olarak raf ömrü sonuna kadar bulunduran süt ürünü” ifade etmektedir. Starter kültür ilave edilerek üretilen ayran, yoğurt, kefir, kıymız vb. fermente süt ürünleri genel olarak *Streptococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* ve *Acetobacter* vb. bakteri cinslerine ait türler ile *Saccharomyces*, *Kluyveromyces* vb. maya cinslerine ait türlerin fermentasyonu sonucu elde edilmiş ürünlerdir. Fermentasyon, süttün tadını değiştirirken

ürünün raf ömrünü arttırmaktadır. Laktik asit bakterileri ve mayalar, fermente süt ürünlerinin lezzet, tekstür ve besin değerlerine katkıda bulunarak üretiminde çok önemli bir rol oynamaktadırlar.

İlk defa Orta Asya'da Türkler tarafından bulunan ve sonra da bütün dünyaya yayılmış olan yoğurt ve bir yoğurt içeceği olan ayran (Chandan ve ark., 2017), Türk kültüründe çok önemli bir yere sahip geleneksel fermente süt ürünleridir. Yoğurt ve ayran, günümüzde süte (inek sütü başta olmak üzere koyun, keçi ve manda sütü) spesifik starter kültür olarak *Streptococcus thermophilus* ve *Lactobacillus delbrueckii* subsp. *bulgaricus*'un birlikte ilave edilmesiyle hazırlanan ve Türkiye'de çok değerli olan fermente süt ürünleridir (Fazilah ve ark., 2018; Salık ve Çakmakçı, 2022). Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliğine (Tebliğ No: 2022/44) göre Ayran, “Yoğurda su ilave edilerek veya bileşimi ayarlanan süte spesifik starter kültür olarak *Streptococcus thermophilus* ve *Lactobacillus delbrueckii* subsp. *bulgaricus*'un birlikte ilave edilmesiyle hazırlanan fermente süt ürünü” ifade etmektedir. Türkiye'de geleneksel olarak üretimi yöreden yöreye farklılık gösteren ayran, büyük işletmelerde endüstriyel olarak tam yağlı, yarım yağlı ve yağsız olarak üretilmektedir. Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliğine (Tebliğ No: 2022/44) göre Ayran, “tam yağlı ayran: süt yağı $\geq$ %1.8; yarım yağlı ayran: % 1.2 > süt yağı $\geq$ % 0.8; yağsız ayran: süt yağı $\leq$ % 0.5” olarak sınıflandırılmıştır. Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliğine (Tebliğ No: 2022/44) göre Ayranın kimyasal bileşimi “(Kütlece %), süt proteini: en az % 2.0; tuz: en fazla % 0.8; titrasyon asitliği (laktik asit olarak): en az % 0.5 en fazla % 1.0” olarak belirtilmiştir. Önemli bir fermente süt ürünü olan ve uzun yıllar boyunca Orta Asya ve Anadolu'da üretilen ayran, özellikle Türk toplumunun beslenmesi açısından çok önemlidir. Antimikrobiyal ve terapötik özelliklere sahip olması, sindiriminin kolaylığı, ferahlatıcı etkisi, basit yöntemlerle yoğurttan üretilmesi ve üstün besleyici değeriyle, ayran Türk toplumunun zevkle tükettiği milli içeceklerinden biridir (Patır ve

ark., 2006; Çetin ve ark., 2014). Ayrıca ayran; kalsiyum, fosfor, magnezyum ve potasyum mineralleri ile B₂, B₁₂, A, D ve K vitaminlerini içermesinden dolayı beslenme açısından çok önemli bir kaynaktır (Ötleş ve Nakilcioğlu Taş, 2019; Salık ve Çakmakçı, 2022).

Dünya genelinde çok yaygın olarak tüketilen ayran geleneksel ve endüstriyel olarak üretilmektedir. Türkiye’de daha çok geleneksel olarak yoğurdun belirli oranda su ile seyreltilerek tuz ilave edilmesi metodu ile yapılan ayran üretimi tercih edilmektedir. Ayrıca, bazı büyük kapasiteli süt ürünleri (yoğurt) işletmeleri su ilavesi ile seyreltilen sütün (bileşimi ayarlanmış sütün) fermente edilmesi metodu ile endüstriyel olarak ayran üretimi yapmaktadırlar (Şanlı ve ark., 2011). Türkiye’de ilkel teknoloji ile geleneksel olarak üretilen ayranlarda standart bir üretim yöntemi yoktur. Çünkü, geleneksel olarak üretilen ayranların ham maddeleri ve üretim yöntemleri farklı olabilmektedir. Ayrıca, geleneksel olarak üretilen ayranlar daha çok küçük kapasiteli işletmelerde modern teknolojiden ve hijyen koşullarından yoksun olarak üretilmektedir. Bu nedenle piyasadaki geleneksel olarak üretilen ayranların kimyasal bileşimi, mikrobiyolojik özellikleri ve kalitelerinin birbirinden çok farklı olması kaçınılmazdır (Patır ve ark., 2006). Bunun sonucu olarak da Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliğine uygun standartlarda ayran üretimi mümkün olmamaktadır. Ancak, günümüzde gelişen teknoloji ile beraber Ayran Türk Gıda Kodeksi Fermente Süt Ürünleri Tebliğine (Tebliğ No: 2022/44) göre endüstriyel olarak iki metotla üretilmektedir. İlk üretim yoğurda belirli oranlarda su ve tuz ilave edilerek, ikinci üretimde ise bileşimi (kurumadde miktarı) ayarlanan süte spesifik starter kültür olarak *Streptococcus thermophilus* ve *Lactobacillus delbrueckii* subsp. *bulgaricus*’un birlikte ilave edilerek sütün fermente edilmesiyle üretilmektedir. Bu nedenle, Türkiye’de gelişen modern teknoloji ile beraber endüstriyel olarak çok büyük kapasiteli işletmelerde hijyen koşullarına da dikkat edilerek kimyasal bileşim, mikrobiyolojik özellik ve kalite bakımından Türk Gıda Kodeksi Fermente Süt Ürünleri

Tebliğine uygun standartlarda ayran üretimi mümkün olacaktır. Ayrıca, Türkiye’de endüstriyel olarak ayran üretimi artmaya devam edecektir.

Türkiye’de ayran, içme sütü (pastörize, sterilize veya UHT içme sütleri) ve yoğurttan sonra en çok üretilen 3. süt ürünüdür. Türkiye’de toplam ayran üretim miktarı her yıl düzenli bir artış göstermiştir. Ayrıca, ferahlatıcı bir özelliği olan ayranın toplam üretim miktarı özellikle ilkbahar ve yaz aylarında (Mart-Ağustos ayları arasında) artmış, sonbahar ve kış aylarında (Eylül-Şubat ayları arasında) ise azalmıştır. Türkiye’de 2020 yılında toplam ayran üretim miktarı 587 bin 831 ton (588 bin ton), 2021 yılındaki toplam ayran üretim miktarı 716 bin 882 ton (717 bin ton) (Tüik, 2022), 2022 yılındaki toplam ayran üretim miktarı 766 bin 278 ton (766 bin ton) (Tüik, 2023), 2023 yılındaki toplam ayran üretim miktarı ise 829 bin 548 ton (830 bin ton) olarak tespit edilmiştir (Tüik, 2024). Bu tespit edilen sonuçlara göre; Türkiye’de 2021 yılında toplam ayran üretim miktarı 2020 yılına göre %22 artmış (Tüik, 2022), 2022 yılında toplam ayran üretim miktarı ise 2021 yılına göre % 6.9 artmış (Tüik, 2023), 2023 yılında toplam ayran üretim miktarı ise 2022 yılına göre % 8.3 artmıştır (Tüik, 2024). Türkiye’de yıllar itibarıyla toplam ayran üretim miktarındaki artışa paralel olarak toplam ayran tüketim miktarı da her yıl düzenli bir artış göstermiştir. Ayrıca, ayranın toplam üretim miktarı gibi toplam tüketim miktarı da özellikle ilkbahar ve yaz aylarında (Mart-Ağustos ayları arasında) artmış, sonbahar ve kış aylarında (Eylül-Şubat ayları arasında) ise azalmıştır. Türkiye’de 2021 yılında kişi başına düşen yıllık ayran tüketim miktarı 10 kg olarak hesaplanmıştır (Anonim, 2021). Türkiye süt arz ve talep dengesi dikkate alındığında toplam süt arzı 2002-2006 döneminde 2004 yılındaki azalma haricinde sürekli artış göstermiştir. Toplam süt arzı aynı dönemde % 6.4 oranında artarak, 10.9 milyon tondan 11.6 milyon tona yükselmiştir. Üretim ise aynı dönemde % 8.1 oranında artarak 10.6 milyon ton olmuştur. Süt arzında kendine yeterlilik oranı % 90.8 olup, incelenen dönem içinde % 90’ın üzerinde olmuştur. Süt ve süt

ürünleri pazarı tamamen yurtiçi piyasa koşullarına bağımlı olarak gelişim göstermiştir (Anonim, 2007).

Bu çalışmanın amacı Türkiye’de süt ürünleri içinde önemli yeri olan ayran üretiminin zaman serileri analizi ile modellenmesi ve ileriye yönelik öngörüsünün yapılmasıdır.

2. Materyal ve Yöntem

Araştırmanın materyali 2010 Ocak-2024 Nisan dönemine ait ayran üretim miktarına

ilişkin verilerden oluşmuştur. Veriler Türkiye İstatistik Kurumu (Tüik, 2024)’nın “Süt ve Süt Ürünleri Üretim Miktarı (Ton)” başlığı altında “Ayran” üretim miktarıdır. Mevsimsel zaman serileri; mevsimsel otoregresif (SAR), mevsimsel hareketli ortalama (SMA) ve mevsimsel otoregresif hareketli ortalama (SARMA) modelleri olarak sınıflandırılır. Derecesi P, periyodu s olan $SAR(P)_s$, mevsimsel otoregresif modeli,

$$(1 - \Phi_1 B^s - \Phi_2 B^{2s} - \dots - \Phi_P B^{Ps})Z_t = \varepsilon_t \quad (1)$$

şeklinde olmaktadır. Burada $i=1, 2, \dots, P$ olmak üzere Φ_i mevsimsel otoregresif modelin katsayısıdır (Yaffee ve McGee, 2000). Burada, Φ : Mevsimsel otoregresif modelin parametre katsayısı, B: Gerileme operatörü (Backward

shift operatör), B^s : Birinci periyot, B^{2s} : İkinci periyot ve B^{Ps} : P’ninci periyot. Derecesi Q ve periyodu s olan mevsimsel hareketli ortalama modeli $SMA(Q)_s$,

$$\Theta_Q(B^s) = 1 + \Theta_1 B^s + \Theta_2 B^{2s} - \dots + \Theta_Q B^{Qs} \quad (2)$$

şeklinde (Milenkoviç ve ark., 2018). Burada, s: Periyot, Θ : Mevsimsel hareketli ortalama model parametre katsayısı, Q: Mevsimsel hareketli ortalama modelin

derecesi, B: Gerileme operatörü, B^s : Birinci dereceden mevsimsel hareketli ortalama modeli, B^{2s} : İkinci dereceden mevsimsel hareketli ortalama modeli olarak ifade edilir.

Mevsimsel otoregresif hareketli ortalama yani $ARIMA(p, d, q)(P, D, Q)_s$ modeli

$$(1 - \phi_1 B - \phi_2 B^2 - \dots - \phi_p B^p)(1 - \Phi_1 B^s - \Phi_2 B^{2s} - \dots - \Phi_P B^{Ps})(1 - B)^d(1 - B^s)^D X_t = (1 - \theta_1 B - \theta_2 B^2 - \dots - \theta_q B^q)(1 - \Theta_1 B^s - \Theta_2 B^{2s} - \dots - \Theta_P B^{Qs})e_t \quad (3)$$

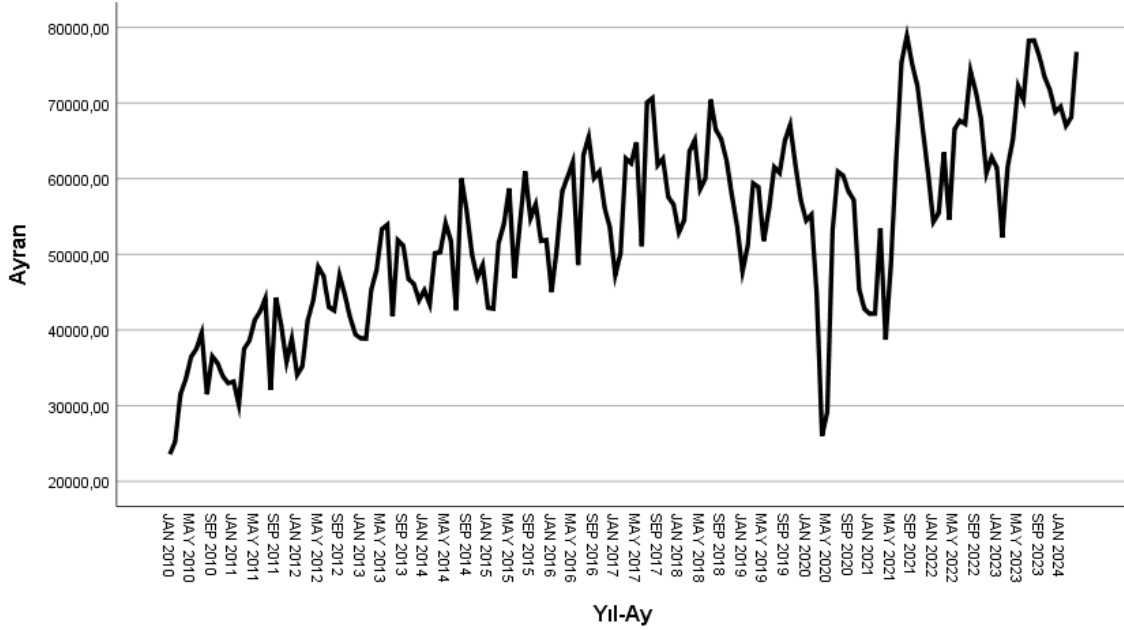
şeklinde yazılabilir (Kadılar, 2009). Burada, p: Otoregresif model derecesi, d: Fark, q: Hareketli ortalama model derecesi, P: Mevsimsel otoregresif model derecesi, D: Mevsimsel fark, Q: Mevsimsel hareketli ortalama model derecesi, s: Periyot, $\phi_1 B$: Birinci dereceden otoregresif model katsayısı, $\phi_2 B^2$: İkinci dereceden otoregresif model katsayısı, $\phi_p B^p$: p’ninci dereceden otoregresif model katsayısı, $\Phi_1 B^s$: Birinci dereceden

mevsimsel otoregresif model katsayısı, $\Phi_2 B^{2s}$: İkinci dereceden mevsimsel otoregresif model katsayısı, $\Phi_P B^{Ps}$: P’ninci dereceden mevsimsel otoregresif model katsayısı, $\theta_1 B$: Birinci dereceden hareketli ortalama model katsayısı, $\theta_2 B^2$: İkinci dereceden hareketli ortalama model katsayısı, $\theta_p B^q$: q’nüncü dereceden hareketli ortalama model katsayısı, $\Theta_1 B^s$: Birinci dereceden mevsimsel hareketli ortalama model katsayısı,

$\Theta_2 B^{2s}$: İkinci dereceden mevsimsel hareketli ortalama model katsayısı, $\Theta_p B^{Qs}$: Q'nüncü dereceden mevsimsel hareketli ortalama model katsayısıdır. İstatistik analizler SPSS 26 paket programı ile yapılmıştır.

3. Bulgular ve Tartışma

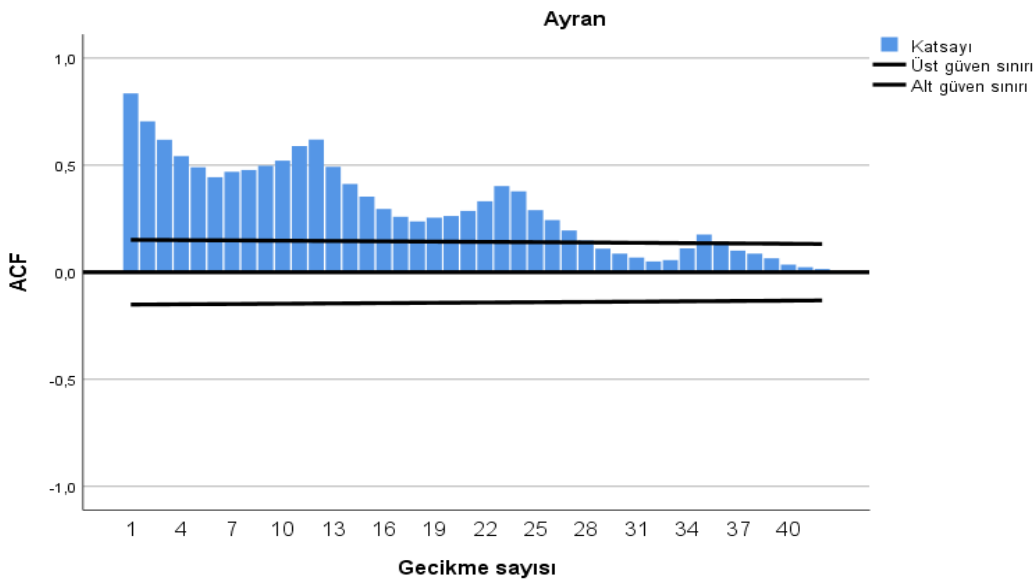
Ayran serisine zaman serileri analizi uygulandığında, 2010 Ocak-2024 Nisan dönemi mevsimsel zaman serisi grafiği Şekil 1'de oluşturulmuştur.



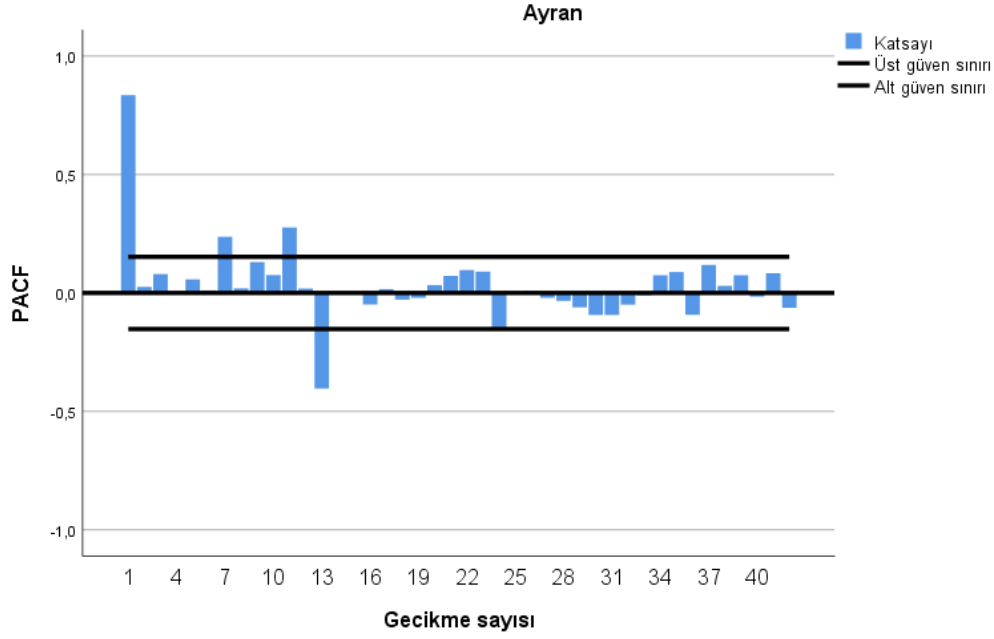
Şekil 1. Türkiye'de ayran üretim miktarı 2010 Ocak-2024 Nisan zaman serileri grafiği

Şekil 1'de görüldüğü gibi ayran üretim miktarı serisinde trend vardır. Bunu daha net ortaya koymak için serinin otokorelasyon fonksiyonu

(ACF) ve kısmi otokorelasyon fonksiyonu (PACF) grafikleri sırasıyla Şekil 2'de ve Şekil 3'te verilmiştir.



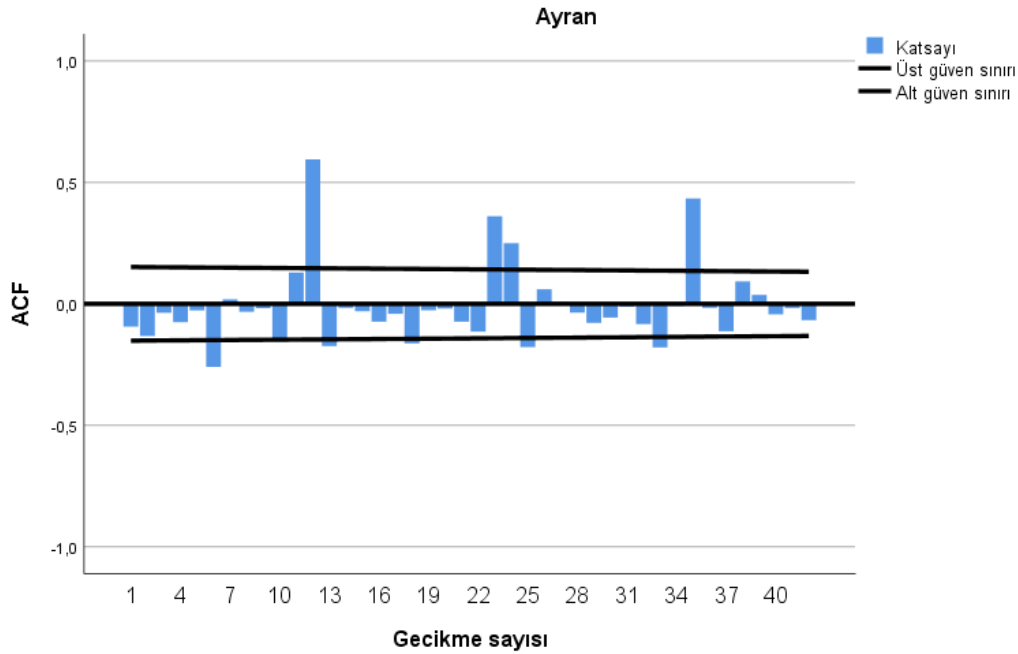
Şekil 2. Serinin ACF fonksiyonu



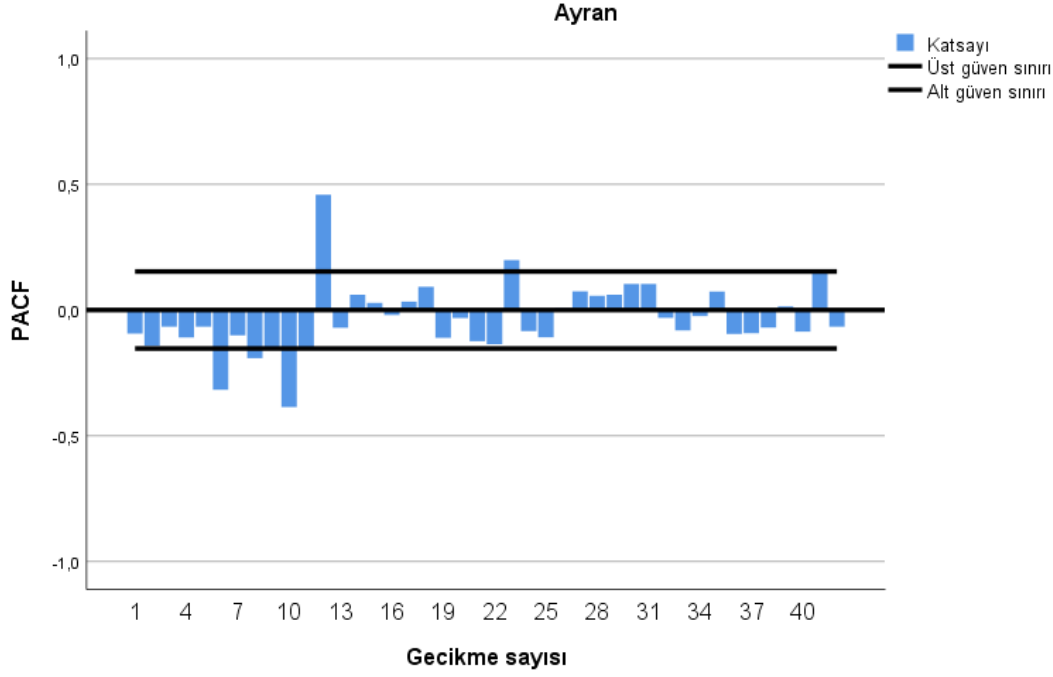
Şekil 3. Serinin PACF fonksiyonu

Şekil 2’de ACF grafiğine göre seride trend görülmektedir. Serinin trendden arındırılması için birinci farkı alınmıştır. Birinci fark

serisinin ACF ve PACF grafikleri sırasıyla Şekil 4’te ve Şekil 5’te verilmiştir.



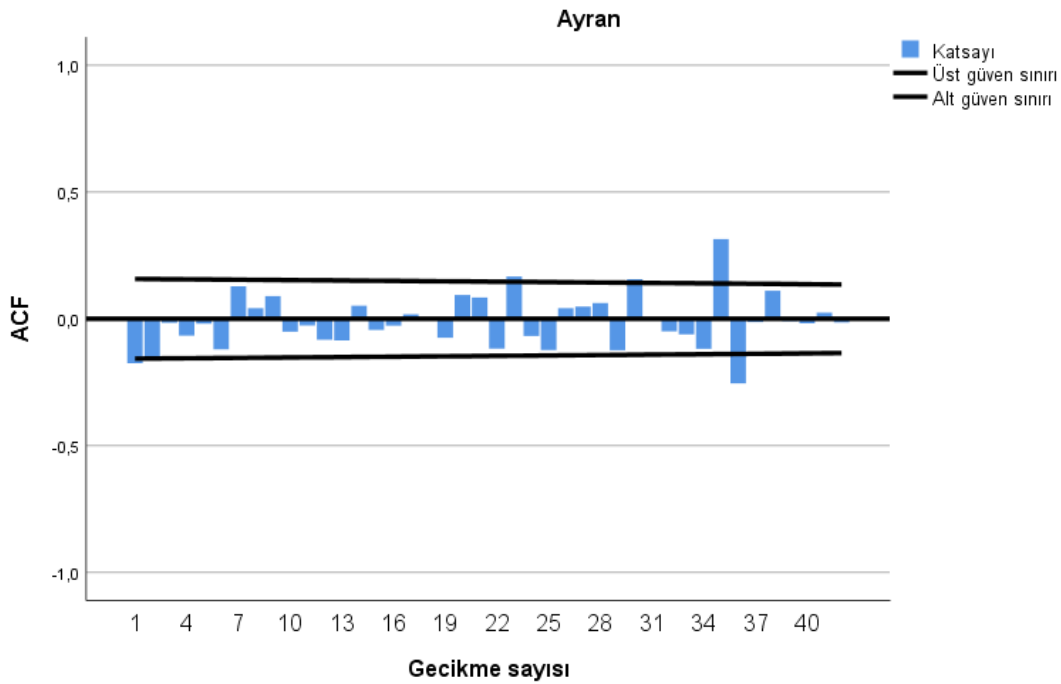
Şekil 4. Serinin birinci farkının ACF fonksiyonu



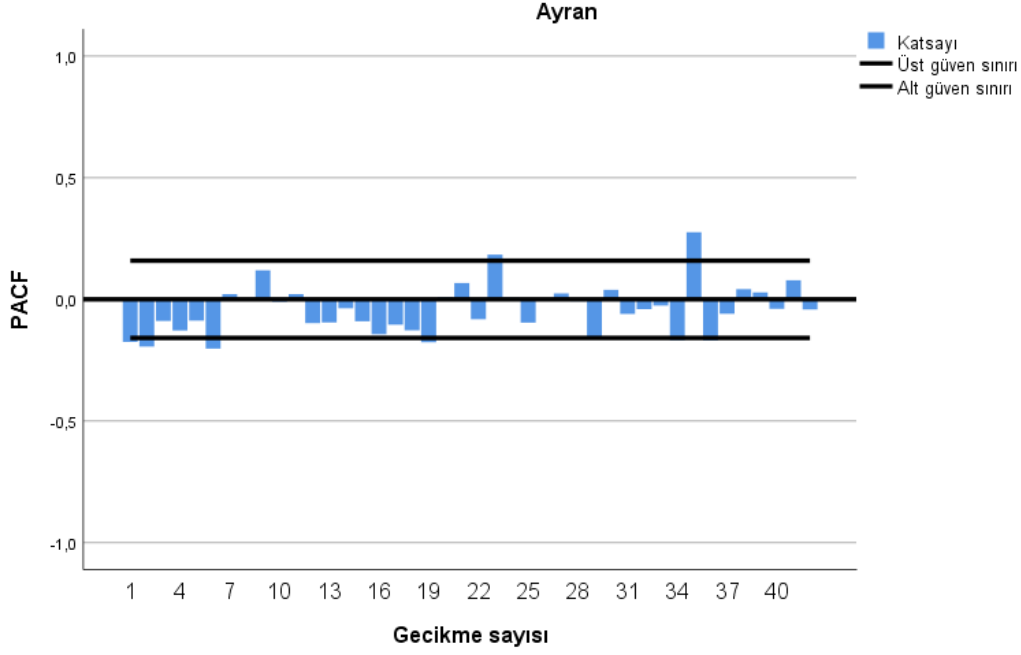
Şekil 5. Serinin birinci farkının PACF fonksiyonu

Şekil 4'te gösterilen ACF grafiğinde 12. ve 24. gecikme değerleri, Şekil 5'e ait olan PACF grafiğinde 12. gecikme değeri güven sınırını aşmıştır. Burada periyot olan 12, 24, ... gibi gecikme uzunlukları dikkate alınmıştır. Dolayısıyla seride mevsimsel trend vardır.

Seriye mevsimsel trendden arındırmak ve durağan hale getirmek için serinin mevsimsel farkı alınmalıdır. Birinci mevsimsel farkı alınan serinin ACF ve PACF grafikleri sırasıyla Şekil 6'da ve Şekil 7'de sunulmuştur.



Şekil 6. Serinin birinci mevsimsel farkının ACF fonksiyonu



Şekil 7. Serinin birinci mevsimsel farkının PACF fonksiyonu

Birinci farktan sonraki birinci mevsimsel farkı alınmış serinin ACF ve PACF grafiklerinde 2. gecikmeden sonra gecikme değerleri sıfıra yaklaşmıştır (Şekil 6 ve Şekil 7). ACF grafiğindeki 2. gecikme sıfıra daha hızlı yaklaşmıştır (Şekil 6). ACF grafiğinde ilk 2 gecikme güven sınırını aştığından ve 2. gecikmeden sonra daha hızlı sıfıra yaklaştığından dolayı “birinci dereceden

bütünleşik mevsimsel hareketli ortalama modeli” olarak adlandırılan bir model ortaya çıkmıştır. Aylık seri olduğundan bu serinin periyodu 12’dir. Dolayısıyla $p=0$, $d=1$, $q=2$, $P=0$, $D=1$, $Q=0$ ve $s=12$ ’dir. Elde edilen model tipi ARIMA (0, 1, 2) (0, 1, 0)₁₂ şeklindedir. ARIMA (0, 1, 2) (0, 1, 0)₁₂ modeli aşağıdaki gibi ifade edilir. ARIMA (0, 1, 2) (0, 1, 0)₁₂ modeli

$$\begin{aligned} (1 - B)(1 - B^{12})Z_t &= (1 - \theta_1 B - \theta_2 B^2)e_t \\ (1 - B - B^{12} + B^{13})Z_t &= (1 - \theta_1 B - \theta_2 B^2)e_t \\ Z_t - Z_{t-1} - Z_{t-12} + Z_{t-13} &= e_t - \theta_1 e_{t-1} - \theta_2 e_{t-2} \\ Z_t &= Z_{t-1} + Z_{t-12} - Z_{t-13} - \theta_1 e_{t-1} - \theta_2 e_{t-2} + e_t \end{aligned} \quad (4)$$

şeklinde açık olarak yazılabilir. Modele ait özet istatistikleri bilgileri Tablo 1’de sunulmuştur.

Tablo 1. Model istatistikleri

Model Fit statistics								Ljung-Box Q (18)		
Stationary R-squared	R-squared	RMSE	MAPE	MAE	MaxAPE	MaxAE	Normalized BIC	Statistics	DF	Sig.
0.100	0.757	5586.535	7.439	3853.087	90.161	23448.271	17.352	21.943	16	0.145

Tablo 1’de görüldüğü gibi, modele ait Bayesci Bilgi Kriteri (BIC)=17.352 bulunmuştur. Modelin uygunluğu Ljung-Box Q testi ile incelendi ve 21.943 olarak bulunmuştur,

$p=0.145>0.05$ olduğundan model uygun görülmüştür. Modelin parametre tahmini katsayıları Tablo 2’de sunulmuştur.

Tablo 2. ARIMA (0, 1, 2) (0, 1, 0)₁₂ modeli parametreleri

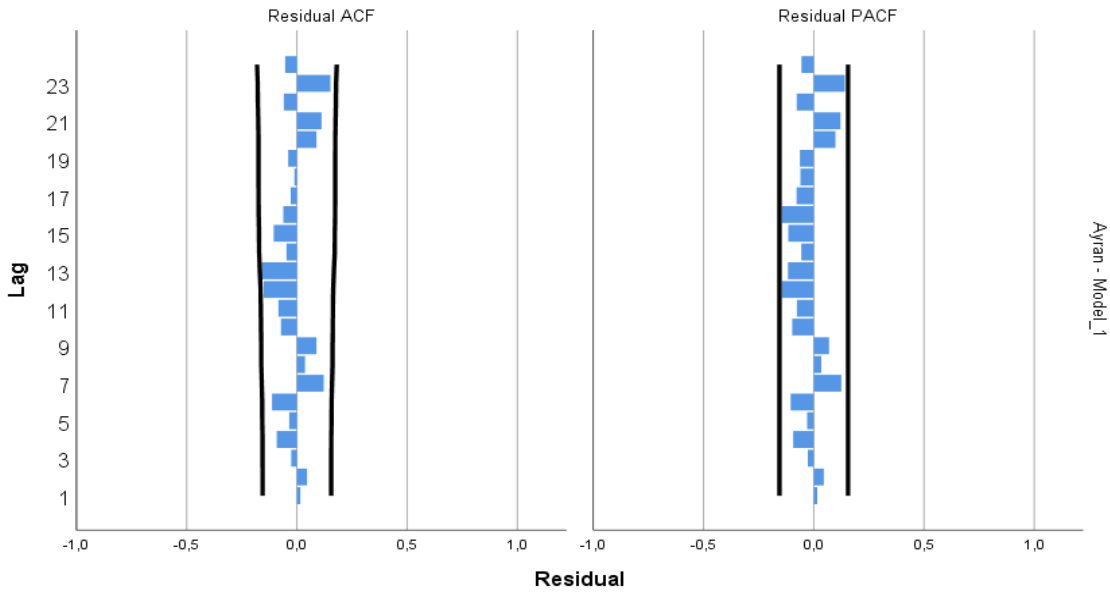
Parametre	Katsayı	SE	t	p
Sabit	19.788	209.674	0.094	0.925
Fark	1			
MA (1)	0.277	0.078	3.548	0.001
MA (2)	0.255	0.078	3.269	0.001
Mevsimsel fark	1			

Tablo 2’de gösterilen modelin parametre katsayılarına göre ARIMA (0, 1, 2) (0, 1, 0)₁₂ modeli aşağıdaki gibi ifade edilmiştir.

$$(1 - B)(1 - B^{12})Z_t = (1 - 0.277B - 0.255B^2)e_t \quad (5)$$

$$Z_t = Z_{t-1} + Z_{t-12} - Z_{t-13} - 0.277e_{t-1} - 0.255e_{t-2} + e_t \quad (6)$$

Modele ait hata terimlerinin ACF ve PACF grafikleri Şekil 8’de sunulmuştur.



Şekil 8. Hata terimlerinin ACF ve PACF grafikleri

Şekil 8’deki ACF ve PACF grafiklerinde gecikmelere ait ilişki miktarları güven sınırları içinde yer aldığından Akgürültülü bir seridir. Bir başka deyişle Beyaz Gürültü (White Noise) serisidir. Bu durumda

Türkiye’de ayran üretim miktarı için ileriye yönelik ayran üretim miktarının öngörüsü yapılmıştır ve Tablo 3’te sunulmuştur. Öngörü hesaplamasında elde edilen

$$Z_t = Z_{t-1} + Z_{t-12} - Z_{t-13} - 0.277e_{t-1} - 0.255e_{t-2} + e_t \text{ zaman serisi modelinde}$$

Z_{t-1} : Ayran üretim değerinin bir gecikmeli değeri yani son veri 2024 Nisan olduğundan bunun bir önceki değeri 2024 Mart ayı ayran üretimi,

Z_{t-12} : Ayran üretim değerinin son veriden 12 ay önceki değeri,

Z_{t-13} : Ayran üretim değerinin son veriden 13 ay önceki değeri,

e_{t-1} : Modele ait hata teriminin bir gecikmeli değeri,

e_{t-2} : Modele ait hata teriminin iki gecikmeli değeri yerine konarak hesaplanır ve öngörü değeri elde edilir.

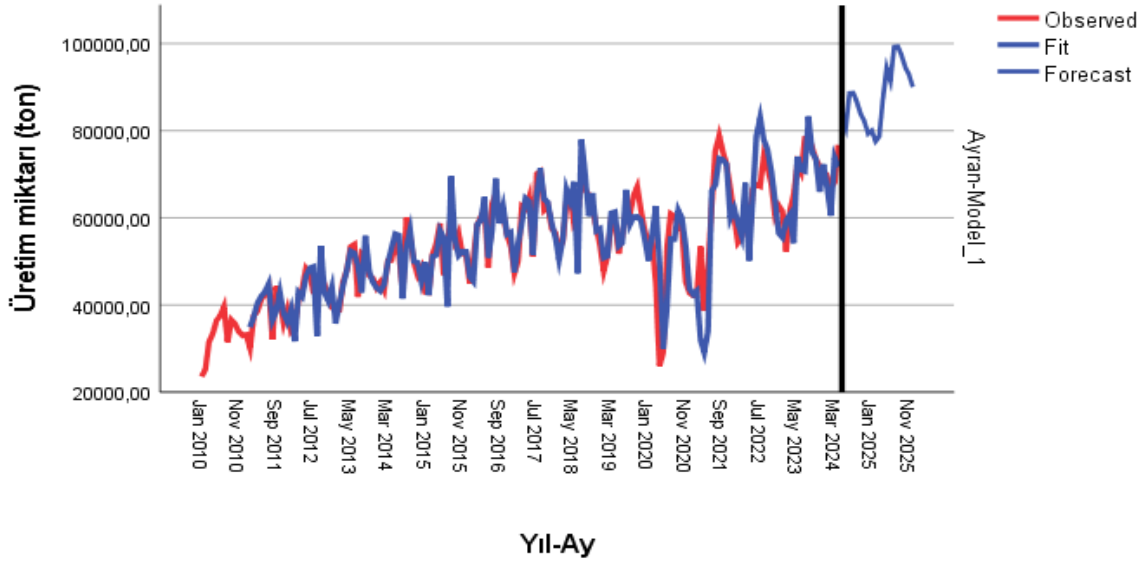
Orijinal serinin, tahmini ve öngörüsü ile birlikte grafiği Şekil 9’da gösterilmiştir.

Tablo 3. İleriye yönelik ayran üretimi (ton) öngörüsü

Zaman	Öngörü
Mayıs 2024	83809
Haziran 2024	80824
Temmuz 2024	88627
Ağustos 2024	88694
Eylül 2024	86576
Ekim 2024	83830
Kasım 2024	82156
Aralık 2024	79305
Ocak 2025	80027
Şubat 2025	77529
Mart 2025	78653
Nisan 2025	87337
Mayıs 2025	94405
Haziran 2025	91439
Temmuz 2025	99262
Ağustos 2025	99349
Eylül 2025	97250
Ekim 2025	94524
Kasım 2025	92870
Aralık 2025	90039

Tablo 3 incelendiğinde 2024 Mayıs-2025 Aralık dönemi öngörüsü sonucunda en yüksek üretim miktarı 2025 yılı Ağustos ayında 99349

ton olarak beklenmektedir. Bunu 99262 ton üretimle 2025 yılı Temmuz ayı ve 97250 ton üretimle 2025 yılı Eylül ayı izlemektedir.



Şekil 9. Ayran üretimi orijinal, tahmini ve öngörü değerleri

Şekil 9’da orijinal gözlem değerleri ile tahmini değerler uyum içindedir ve birbirine çok yakındır. Süt ve süt ürünleri üzerine yapılmış çalışmaların birinde, Türkiye’de Ocak 2010 ile Aralık 2023 dönemine ait 156 aylık süt ve süt ürünlerinin mevsimsel zaman serileri analizi yapılmıştır. Analiz sonucunda, toplanan inek sütü üretimi için SARIMA (0, 1, 0) x (0, 1, 1)₁₂, içme sütü üretimi için SARIMA (0, 1, 0) x (0, 1, 1)₁₂, ayran üretimi için SARIMA (1, 1, 1) x (1, 1, 0)₁₂, yoğurt üretimi için SARIMA (0, 1, 1) x (0, 1, 1)₁₂, tereyağı üretimi için SARIMA (1, 1, 1) x (0, 1, 1)₁₂ ve kaymak üretimi için SARIMA (0, 1, 1) x (1, 1, 0)₁₂ modelleri uygun görülmüştür ve değerlendirilmiştir (Doğar ve ark., 2024). Bahsedilen bu çalışma ile bizim çalışmamızdan elde edilen sonuçlar farklılık göstermiştir.

Diğer bir çalışmada, Bingöl kent merkezindeki tüketicilere 2018 yılında süt ve bazı süt ürünleri tüketim miktarı hakkında anket uygulanmıştır. Yapılan anket sonuçlarına göre, tüketicilerin aylık olarak 8.80 kg açık süt, 5.70 kg pastörize süt, 4.70 kg paket süt, 4.50 kg peynir, 1.50 kg tereyağı, 9.80 kg yoğurt ve 6.50 kg ayran tükettikleri saptanmıştır (Karakaya ve Kızıloğlu, 2018). Bu sonuçlara göre, ayranın tüketiciler tarafından tercih edilen bir ürün olduğu ortaya çıkmıştır.

4. Sonuçlar

Türkiye’de 2010 Ocak-2024 Nisan dönemini içeren 14 yıl 4 aylık (toplam 172 ay) ayran üretimi üzerinde geleceğe yönelik tahminler mevsimsel zaman serileri yöntemi ile analiz edilmiştir. Seriler durağan olmayıp, birinci farkı ve mevsimsel birinci farkı alındıktan sonra durağan hale gelmiştir. Yapılan analizle ayran üretiminin ARIMA (0, 1, 2) (0, 1, 0)₁₂ modeline uyduğu görülmüştür. Modelin uygunluğu Ljung-Box Q testi ile belirlenmiştir. Serilerin hatalarının Akgürültülü olduğu görülmüştür. ARIMA (0, 1, 2) (0, 1, 0)₁₂ modeline göre yapılan öngörü ile ayran üretimi 2024 Mayıs-2025 Aralık döneminde 77529-99349 ton arasında seyredecektir. Bu ayran üretim miktarları sürekli artan olmayıp inişli çıkışlı bir seyir içinde olacaktır. Ayran üretiminin genel olarak ilkbahar ve yaz aylarında (Mart-Ağustos ayları arasında) artan, sonbahar ve kış aylarında (Eylül-Şubat ayları arasında) ise azalan bir seyir içinde olacağı beklenmektedir.

Yazarların Katkı Beyanı

Tüm yazarlar çalışmaya eşit derecede katkıda bulunmuş olup; makalenin son halini okuyup onayladıklarını beyan ederler.

Çıkar Çatışması Beyanı

Tüm yazarlar bu çalışma için herhangi bir çıkar çatışması olmadığını beyan ederler.

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Stimulatory Effects of Different Seed Priming Treatments on Germination and Early Seedling Growth of Sugar Beet

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Abstract

As known, seed germination constitutes the first stage in plant production, and the rapidness of this trait is important for the agriculture of the sugar beet. Particularly, it is crucial to realize after the winter and cool weather. The main aim of the study was to accelerate seedling growth by germinating them observe their effects on plant emergence, and improve seed viability using Serenada, Aranka, and Lider sugar beet varieties treated with hydropriming, hormonal priming, and organic priming for 6, 12, and 18 h under room temperature and humidity conditions. The study showed that the behavior of treated seeds was significantly different compared to the control treatment. The germination percentage, mean germination time, shoot length and root length ranged between 85.50-95.00 (%), 2.80-4.49 (days), 6.34-9.82 (cm), and 3.73-9.12 (cm) respectively. Seedling fresh and dry weight changed between 57.88-89.66 (mg plant⁻¹), and 5.02-12.31 (mg plant⁻¹) in the same order. The best results in germination percentage, mean germination time, root length, and maximum seedling dry weight were obtained after seaweed treatments for 18 h, for seedling length seedling fresh weight. It is concluded that growth parameters are genotype-dependent. Used genotypes in this experiment showed different responses for the investigated agronomic characters (with the priming treatments), and they can be used in any local sugar beet programs for the investigated traits with a carefully selected depending on the objectives of the study.

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1. Introduction

Sugar beet (*Beta vulgaris* L.) crop plants have always been of strategic importance to Türkiye (Topak et al., 2010) since this plant is used in wide sectors (candy industries, biscuits, waffle, etc.) in agriculture as sugar powder, hybrid, and monogerm seeds. On the other hand, germination ability and emergence percentage/homogeneity of the seeds are very important factors in its production. Similarly, cultivation is rather important since the germination of sugar beet grown in drought and semi-drought-hit areas tends to be irregular and can span over a long period.

Generally speaking, the germination period of a seed is the most important stage for the cultivation of that plant (Turan and Samur, 2024), which constitutes the first stage in crop production and prevents optimum germination and emergence depending on factors such as environmental factors (soil moisture, temperature, soil salinity, etc.) and seed structure (seed maturity, hard-shelled species, genetic structure, etc.) (Bareke, 2018). Germination is the most important stage of seedling formation plays and an important role in plant production. As known, priming is beginning to play an important role among new technologies in seed development (Chomontowski et al., 2020). The main agronomic purpose of this application is to accelerate germination, and plant emergence to improve seed viability and accelerate seedling growth. Under appropriate temperature and humidity conditions, many methods, such as hydropriming, halopriming, osmopriming, hormopriming, soil matrix priming, and biopriming, are mainly used to examine the effects of seed treatments on germination (Rhaman et al., 2020). Primed seed gives earlier, more uniform, and sometimes greater seedling, establishment, and growth (Mondal and Bose, 2021).

Liquid seaweed extracts improve nutrient and water uptake by favorably affecting the root development of plants (Dutta et al., 2019; Ali et al., 2021). They also increase the plant canopy by accelerating the formation of chlorophyll; therefore, liquid seaweed extracts

provide more protein, carbohydrates, and other substances, making plants resistant to diseases and pests, and environmental stresses such as extreme heat, frost, insufficient sunlight, drought, and excessive cold (Chaturvedi et al., 2022). They are a source of macro and micronutrients for plants and can increase yield by up to >30.0 %. Liquid seaweed extracts enhance resistance against viruses and reduce nematode damage. They increase the effectiveness of pesticides by about 25.0 %, improve quality, and increase the market and export value of products (Blunden et al., 1992). With seaweed extract, it has been determined that the plant shows resistance against stress factors; with robust root development seedling growth and development (Matsiyak et al., 2011). There is increasing interest in the use of biostimulants such as seaweed extracts as a crop cultivation technique to influence vegetative growth and increase fruit yield without adversely affecting plant quality (Hernández-Herrera et al., 2023). The beneficial effects of seaweed extracts can be attributed to chemical compounds such as minerals, amino acids, vitamins, and phytohormones that enrich soils and are therefore widely used in agriculture to achieve their desired potential (Yakhin et al., 2017). Seaweed (*Ascophyllum nodosum*) extract, is one of the organic preparations, known to increase plant growth by promoting germination in seeds (Güneri, 2023). In many previous studies, it has been determined that seaweeds have positive effects on early seedling stage (Ağrağaç and Zorer Çelebi, 2022).

Another priming agent is kinetin, and it is one of the leading plant growth regulators (Othman et al., 2021), and affects cell differentiation by initiating the activation of enzymes necessary for vital reactions, leading to increased cell division, cell elongation, and growth, and increasing RNA activation (Wu et al., 2021; Sharma et al., 2022). Additionally, the effectiveness of this technology in increasing and harmonizing the percentage of germination and field emergence, improving seedling strength and growth characteristics, and thus increasing crop yield (Bindraban et

al., 2015; Jaiswal et al., 2022; Sundareswaran et al., 2023). Kinetin hormone plays an important role in plant growth and development (Hamayun et al., 2015). Research on the effects of this hormone on seed germination reveals different results in various plant species (Mensah et al., 2020). Generally, it has been observed that kinetin promotes seed germination and accelerates the germination process. The effects of kinetin on seed germination are also related to its regulation of the division and growth processes of plant cells (Soliman, 2019). Seeds are stimulated by soaking with natural or synthetic compounds to increase their ability to withstand various stress factors, to promote germination strength with uniformity. Therefore, the current study aimed to compare the germination behaviors of sugar beet seeds after hydropriming, hormonal priming (kinetin), and organic priming (seaweed) for different durations of time.

2. Materials and Methods

2.1. Materials

Pure monogerm seeds of three sugar beet varieties Serenada, Aranka, and Lider were used in the experiment, and they were obtained from Ankara Sugar Mills (Private) Ltd. Etimesgut, Türkiye

2.2. Methods

The seeds were treated for 6, 12, and 18 h before starting the experiment, the seeds of all varieties were soaked in 5 % sodium hypochlorite solution for sterilization with distilled water (3 × 5 minutes) each and dried to their initial weight.

- Control treatment (The seeds of each variety were used without any treatment)
- Hydropriming (distilled water),
- Seaweed (5 g l⁻¹) was preferred for organic application.

The commercial name **L OCEAN** solid seaweed was used as a seaweed liquid source.

- Kinetin (0.25 mg l⁻¹) was preferred as a hormonal treatment.

Seeds primed with kinetin and seaweed were rinsed three times with distilled water for 5 min to remove traces of the respective chemicals from the surface of the seeds and then dried to

their initial weight. This research was replicated four times with 50 seeds in each replicate to assess the viability level of a total number of 200 seeds for each parameter. The counted number of seeds in each treatment was placed on a three-layered filter paper moistened with 20 mL of pure water. The rolled papers were packed in sealed plastic bags and incubated at 24 ± 1 °C in an incubator in the dark to prevent moisture loss and promote seed germination. The seeds with a radicle length of 2 mm were counted as germinated (Day and Kocak-Sahin, 2023).

The mean germination percentage is calculated by formula (Formula 1). Mean germination time was noted every 24 h following (Anonymous, 2017) with the help of formula (Formula 2). Where (n) is the number of total seeds germinated in 24 h and (D) is the number of days counting from the start of the germination experiment.

$$GP = \frac{\text{Germinated seeds at final day}}{\text{Total seeds}} \times 100 \quad (1)$$

$$MGT = \sum(Dn) / \sum n \quad (2)$$

At the end of 14 days, shoot length, root length, and seedling fresh weight were measured taking twenty seedlings randomly taken from each replicate (Day et al., 2024; Kulan et al., 2021). The dry weight was recorded after the samples were obtained in an oven at 72 °C for a period of 48 hours (Day et al., 2024).

2.3. Statistical analysis of data

The experimental results have been tested by analysis of variance, using a Completely Randomized Design one factorial experiment with four replications using the JMP-12 statistical program (Anonymous, 2015) to compare the differences among treatment means using Duncan's multiple range test (DMRT).

3. Results and Discussion

Seed germination is the most significant and vital stage in crop production. Losses in crop production are experienced due to low seed-quality germination and stress. Primed seeds of

each variety showed variable effects on germination percentage, mean germination time, shoot length, root length, seedling fresh weight, and seedling dry weight compared to the control treatment.

3.1. Germination percentage

Germination percentage showed a statistically significantly different germination percentage of cultivars after different priming treatments ($p \leq 0.01$). Priming treatments with each variety were divided into three groups. The performance of each variety in every group was compared with the control treatment. Minimum seed germination was noted in control treatments, where varieties Serenada, Aranka, and Lider indicated germination percentages of 85.50 %, 87.00 %, and 86.00 %.

The highest germination percentage was obtained from seaweed (18 h) treatment in cv. Serenada (95.00 %) and cv. Lider (93.50 %). A minimum germination percentage was noted after hydropriming (18 h) treatment in cv. Aranka (92.50 %). They had a positive effect on GP in all treatments with an increase in the time of priming. Variety Serenada, Aranka, and Lider had germination percentages of 90.00-92.00 %, 91.50-92.50 %, and 90.50-92.00 % after hydropriming. The Germination percentage of these three varieties improved from 91.50-95.00 %, 91.00-92.00 %, and 92.00-93.50 % after treatment with seaweed extract. Cv. Serenada, Aranka, and Lider had Germination percentage of 89.00-91.00 %, 88.00-92.00 %, and 91.50-93.00 % after Kinetin treatment (Table 1).

Table 1. Effects of differential priming on germination percentage (%) in sugar beet varieties

Priming Treatment	Duration (h)	Varieties		
		Serenada	Aranka	Lider
Control	0	85.50 ^{c**}	87.00 ^{b**}	86.00 ^{b**}
	6	90.00 ^b	91.50 ^{ab}	90.50 ^a
Hydropriming	12	91.00 ^{ab}	92.00 ^{ab}	91.50 ^a
	18	92.00 ^{ab}	92.50 ^a	92.00 ^a
Priming with Seaweed extract	6	91.50 ^{ab}	91.00 ^{ab}	92.00 ^a
	12	92.50 ^{ab}	91.50 ^{ab}	93.00 ^a
	18	95.00 ^a	92.00 ^{ab}	93.50 ^a
Priming with Kinetin	6	89.00 ^{bc}	88.00 ^{ab}	91.50 ^a
	12	89.00 ^{bc}	90.00 ^{ab}	92.00 ^a
	18	91.00 ^{ab}	92.00 ^{ab}	93.00 ^a

**All values shown by small letters in a column are significantly different using Duncan's multiple range test at $p < 0.01$ level of significance

3.2. Mean germination time

A significant interaction was noted among the mean germination time (day) of different cultivars after different priming treatments ($p \leq 0.01$). Regardless of the three cultivars and the duration of their treatments, each variety took the longest time to germinate the seeds under control treatment. The maximum mean germination time was found for cv. Serenada (4.34 days), cv. Aranka (4.49 days) and cv. Lider (4.36 days). The minimum mean germination time was obtained from kinetin (18 h) treatment in cv. Serenada (3.35 d) while it was obtained from seaweed (18 h) treatment

in cv. Aranka (2.80 days) followed by cv. Lider (2.85 days).

Mean germination time reduced priming at different times when compared with the control treatment. Hydropriming treatments reduced seed germination duration from 3.76-3.80 days, 3.27-3.49 days, 3.47- 3.79 days in cv. Serenada, Aranka, and Lider respectively (Table 2).

Priming for different durations with seaweed extracts also reduced the germination time. It ranged from 3.84-3.56 days, 3.35-2.80 days, 3.82- 2.85 days in cv. Serenada, Aranka, and Lider respectively. A partial reduction in germination duration was noted after kinetin

treatment, it varied between 3.93-3.35 days, 4.00-3.22 days, and 3.90- 3.48 days in cv. Serenada, Aranka, and Lider respectively. The

fastest germination time was noted on all three varieties after 18 h treatment (Table 2).

Table 2. Effects of differential priming on mean germination time (day) in sugar beet varieties

Priming Treatment	Duration (h)	Varieties		
		Serenada	Aranka	Lider
Control	0	4.34 ^{a**}	4.49 ^{a**}	4.36 ^{a**}
Hydropriming	6	3.80 ^{bc}	3.49 ^{bc}	3.79 ^{ab}
	12	3.78 ^{bc}	3.36 ^{cd}	3.60 ^{a-c}
	18	3.76 ^{bc}	3.27 ^{cd}	3.47 ^{bc}
Priming with Seaweed extract	6	3.84 ^{a-c}	3.35 ^{cd}	3.82 ^{ab}
	12	3.60 ^{bc}	3.36 ^{cd}	3.28 ^{bc}
	18	3.56 ^{bc}	2.80 ^d	2.85 ^c
Priming with Kinetin	6	3.93 ^{ab}	4.00 ^{ab}	3.90 ^{ab}
	12	3.47 ^{bc}	3.49 ^{bc}	3.68 ^{a-c}
	18	3.35 ^c	3.22 ^{cd}	3.48 ^{a-c}

**All values shown by small letters in a column are significantly different using Duncan's multiple range test at $p < 0.01$ level of significance

3.3. Shoot length

A statistically significant interaction was noted after each priming treatment when compared with the control treatment ($p \leq 0.01$) for each variety. The maximum shoot length was obtained from the seaweed treatment (18 h) with 9.56 cm in cv. Serenada, while the lowest shoot length was obtained from the control treatment with 6.34 cm. In the Aranka

variety, the maximum shoot length was obtained from hydropriming (18 h) treatment at 9.82 cm and the lowest shoot length was obtained from control treatment at 7.30 cm. In the Lider variety, the highest shoot length was obtained from seaweed extract (18 h) and hydropriming treatment (18 h) at 9.23 cm, and the minimum shoot length was obtained from the control treatment with a length of 6.53 cm. (Table 3).

Table 3. Effects of differential priming on shoot length (cm) in sugar beet varieties

Priming Treatment	Duration (h)	Cultivars		
		Serenada	Aranka	Lider
Control	0	6.34 ^{d**}	7.37 ^{bc**}	6.53 ^d
Hydropriming	6	7.98 ^{bc}	7.69 ^{bc}	7.18 ^{cd**}
	12	9.05 ^{a-c}	9.61 ^a	8.85 ^{ab}
	18	9.56 ^a	9.82 ^a	9.23 ^a
Priming with Seaweed extract	6	8.03 ^{bc}	7.76 ^{cb}	6.73 ^d
	12	9.30 ^{ab}	8.80 ^{a-c}	8.60 ^{a-c}
	18	9.40 ^{ab}	9.06 ^{ab}	9.23 ^a
Priming with Kinetin	6	7.76 ^{cd}	7.30 ^c	7.55 ^{b-d}
	12	8.19 ^{a-c}	8.51 ^{abc}	9.11 ^{ab}
	18	8.42 ^{a-c}	8.57 ^{a-c}	9.21 ^a

**All values shown by small letters in a column are significantly different using Duncan's multiple range test at $p < 0.01$ level of significance

3.4. Root length

A statistically significant interaction was noted for root length ($p \leq 0.01$). Minimum root length was noted using seeds without any pretreatment (control treatment), regardless of the sugarbeet varieties. The maximum root length was obtained from the seaweed

treatment at 8.26 cm in the Serenada, while the lowest root length was obtained from the control treatment at 3.72 cm. In the Aranka, the maximum root length was obtained from hydro treatment with a root length of 8.70 cm, and the minimum root length of 5.09 cm was obtained from the control treatment. The Lider showed a maximum root length of 9.12 cm.

Table 4. Effects of differential priming on root length (cm) in different sugar beet varieties

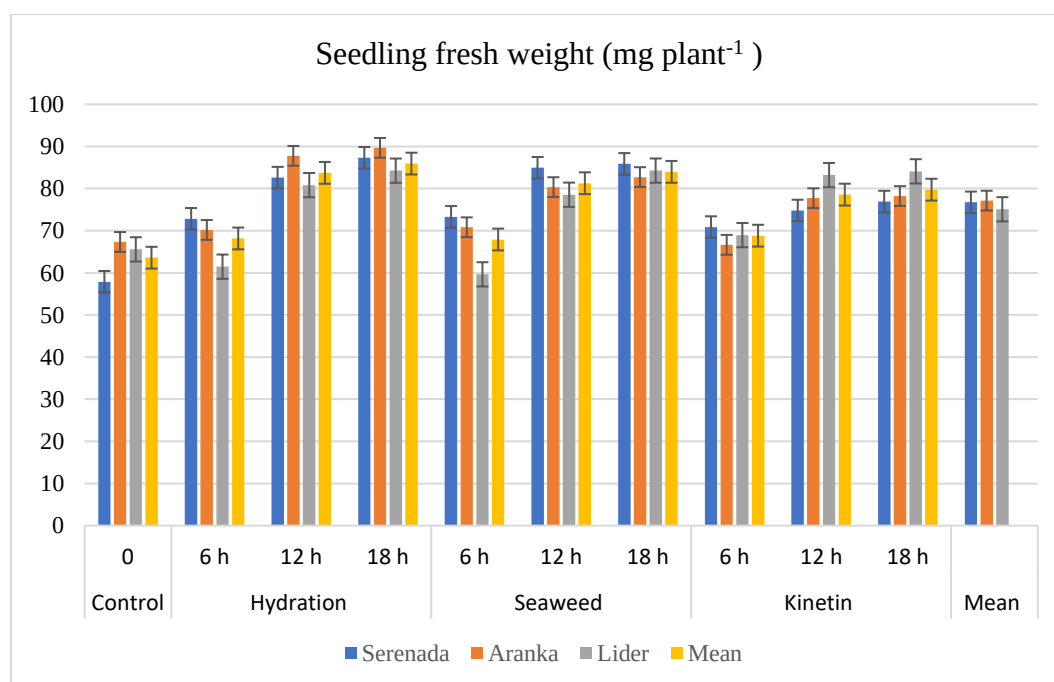
Priming Treatment	Duration (h)	Cultivars		
		Serenada	Aranka	Lider
Control	0	3.72 ^{d**}	5.09 ^{e**}	4.15 ^{g**}
	6	5.55 ^c	7.55 ^{bc}	6.70 ^{c-e}
Hydropriming	12	6.04 ^{bc}	7.87 ^{a-c}	7.61 ^{bc}
	18	6.62 ^b	8.70 ^a	7.99 ^b
Priming with Seaweed extract	6	8.06 ^a	7.04 ^{cd}	6.60 ^{c-e}
	12	8.08 ^a	7.66 ^{a-c}	7.29 ^{b-d}
	18	8.26 ^a	8.37 ^{ab}	9.12 ^a
Priming with Kinetin	6	5.06 ^c	5.30 ^e	5.47 ^f
	12	5.07 ^c	6.16 ^{de}	6.02 ^{ef}
	18	5.14 ^c	7.70 ^{a-c}	6.34 ^{d-f}

**All values shown by small letters in a column are significantly different using Duncan's multiple range test at $p < 0.01$ level of significance

3.5. Seedling fresh and dry weights

Seedling fresh and dry weight (mg plant^{-1}) of sugar beet varieties used in the study showed statistically significant interaction among three different varieties and priming treatments ($p \leq 0.01$). Seedling fresh weight (mg Plant^{-1}) ranged from 86.66 and 57.88 mg plant^{-1} , and seedling dry weight (mg plant^{-1}) ranged from 12.31 - 5.02 mg plant^{-1} (Figures 1-2). Among

the treatments, seedling fresh weight increased by 12 and 18 h duration of hydropriming, seaweed, and kinetin treatments compared to hydration, seaweed, and kinetin treatments ($p \leq 0.01$). However, the maximum dry weight of 11.59 mg plant^{-1} among the treatments was obtained within the seaweed (18 h) treatment, and the minimum value was obtained with 5.83 mg plant^{-1} in the control treatment ($p \leq 0.01$) (Figures 1-2).

**Figure 1.** Effects of differential priming on seedling fresh weight (mg plant^{-1}) in different sugar beet varieties

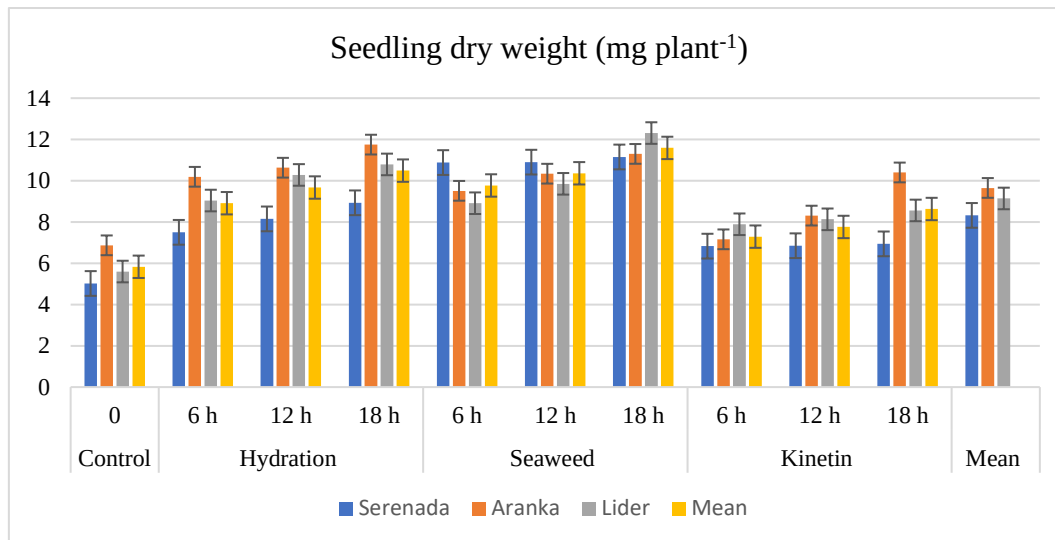


Figure 2. Effects of differential priming on seedling dry weight (mg plant⁻¹) in different sugar beet varieties

The results of the present study showed that the prepared seeds (hydropriming, seaweed, and kinetin) exhibited improved parameters used in the study. Priming treatments showed faster and shorter germination than the control treatments. This had a positive effect on seedling length and root growth. It was noted that priming increased seedling height and root growth compared to the control treatments. Higher fresh and dry seedling weights were also obtained from all primed seeds compared to the control. The major advantage of seed treatment was attributed to faster seed germination, seedling growth and faster emergence, which gave more time for seedlings to develop (Mutlu-Durak and Yildiz Kutman, 2021). Seed priming has been shown to increase germination and seedling quality for many crops (Sağlam et al., 2010; Guo et al. 2022). Researchers have reported that priming has a beneficial effect on different plant seeds, such as sugar beet (Chomontowski et al., 2020), rice (*Oryza sativa*) (Afzal et al., 2021), soybean (*Glycine max*) (Miljaković et al., 2022), and triticale (Guo et al., 2022).

Germination begins when a dry seed absorbs water and is completed when part of the seed emerges from the surrounding structure, known as the emergence phase (Lara-Viveros et al., 2020). Previous studies demonstrated that corn (*Zea mays* L.) seed hydropriming for 12 and 18 h showed an agronomic advantage for mature plants (Lara-

Viveros et al., 2020). Sung and Chiu (1995) claimed that emergence vigor and seedling growth were increased by hydropriming in watermelon (*Citrullus lanatus* (Thunb.)). The findings we obtained from hydropriming are similar. This can be related to faster water uptake and earlier onset of metabolic processes in the hydroprimed seeds compared to the control, resulting in a shorter germination time. (Day, 2022).

Nedumaran (2017), has reported that seeds treated with seaweed germinate faster due to the high mineral content of ocean water and its ability to absorb water at maximum level. The stimulant effect of seaweed (SW) priming may be due to the content of enzymes, phytohormones, minerals, and low molecular weight compounds in the extracts (Stirk and Van Staden, 2020). Thriunavukkarasu et al. (2020) reported that different concentrations of seaweed in red pepper (*Capsicum annum* L.) seeds increased germination by 8 %. Another researcher emphasized the positive effect of seaweed application on germination and other yield elements in bean plants (*Phaseolus vulgaris* L.) (Abou El-Yazied et al., 2012). They reported that seaweed extract applications significantly increased the germination rate compared to the control application (Demirkaya and Arslan, 2021). The impact of seaweed application on germination and seedling strength of corn

seeds was found to be positive (Matysiak et al., 2011).

Application of the plant growth hormone kinetin is known to be effective in improving both germination and growth of various crop species (Ansari et al., 2020). Kinetins have been shown to increase germination percentage and seedling growth, as they were found to play a controlling role in integrating responses expressed by plants, which could be explained by faster water uptake in primed seeds, as germination in primed seeds started after one day (Abeed et al., 2021; Sharma et al., 2022). Barley seeds treated with 60 mg l⁻¹

kinetin gave the highest germination percentage and root length values, while 120 mg l⁻¹ concentration gave the highest germination vigor and shoot length values (Mohammed, 2023). In another study, it was reported that kinetin had a positive effect on emergence percentage, mean germination time, seedling length, and seedling fresh weight in sugar beet seed treatments compared to the control (Kaya and Kulan, 2020). Similar results were obtained for tomato by Zeb et al. (2018), wheat by Jaiswal et al. (2022), rice by Kareem et al. (2023), and papaya by Rani et al. (2024).

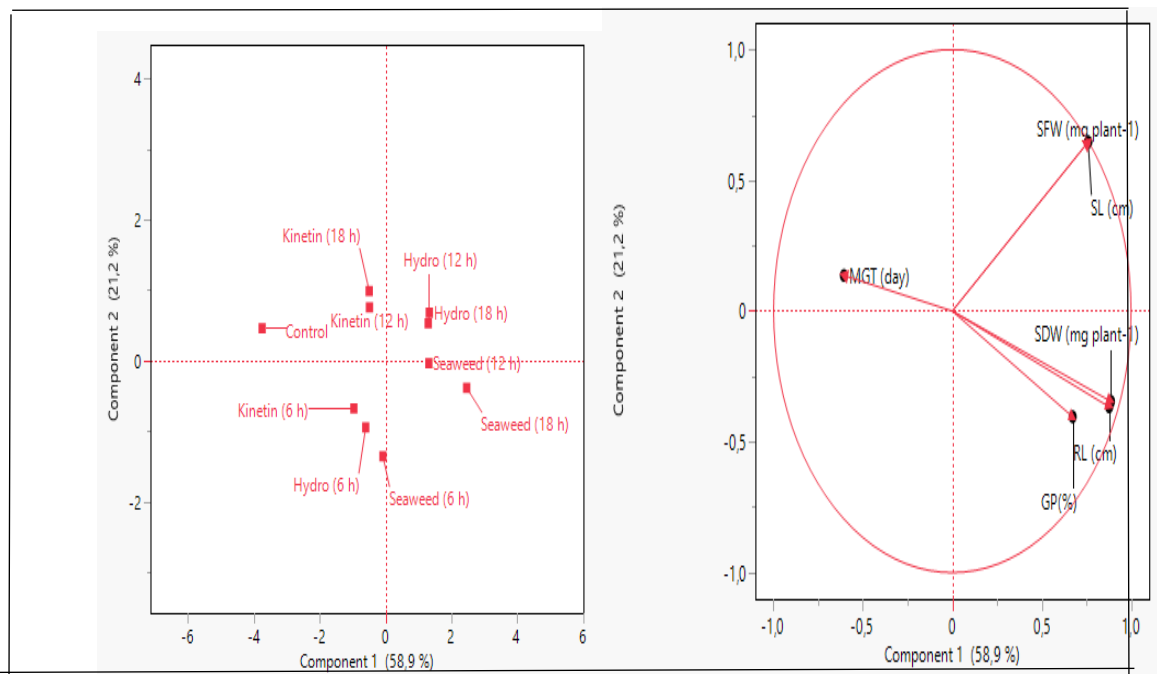


Figure 3. Principal components 1 and 2 were obtained from germination and seedling growth data in sugar beet varieties (GP= germination percentage, MGT=mean germination time, SL= shoot length, RL =root length, SFW= seedling fresh weight and SDW =seedling dry weight)

The gradient of impact on the growth correlation groups for each different time and priming agent used in this study is depicted in a two-dimensional representation known as a principal component analyzing (PCA) (Figure 3). The model including the principal components accounted for 80.1 % of the total variance explained by the different priming treatments applied (58.9 % for PC1 and 21.2 % for PC2). The first principal component (PC1) explained 58.9 % of the variance and was positively correlated with SL (cm), GP (%), RL (cm), and SFW and SDW while they were

negatively correlated with mean germination time (day) (Figure 3). The second principal component (PC2) was positively correlated with MGT (day), SL (cm) and SFW, and was negatively correlated with GP (%), RL (cm), and SDW (mg plant⁻¹), and accounted for 21.2 % of the total variance of the data (Figure 3). By visualizing the data obtained, it was observed that the germination and seedling growth parameters differentiated SL (cm) from other parameters when applying different priming treatments (Figure 3).

4. Conclusions

Sugar beet holds strategic importance for Türkiye, and needs seed priming treatments for uniform seed germination. Three varieties (Serenada, Aranka and Lider) were used in this study to determine the effect of different seed treatments on germination. Seed priming treatments for several durations of time indicated an improvement in germination parameters compared to the control among the varieties. The best germination response was obtained from the Aranka variety, using seaweed and hydropriming extract treatments for 18 h. It is concluded that growth parameters are genotype-dependent. Therefore, the genotypes should be carefully selected depending on the objectives.

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Türkiye’de Karadeniz Bölgesinde Üretilen Balık Unlarının Hayvan Besleme Açısından Mikrobiyolojik Analizleri

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Özet

Balık unu insan tüketimine uygun olmayan balıkların veya balıkçılık endüstrisinden arta kalan atıkların işlenip kurutulması sonucu elde edilmektedir. Balık unları gerek içerdikleri yüksek düzeydeki besin madde içerikleri, gerekse üretim esnasında uygulanan işlemler nedeniyle mikroorganizmalar için uygun üreme ortamı oluşturmaktadır. Karadeniz bölgesinde balık unu üreten dört farklı fabrikadan (A, B, C ve D) temin edilen balık unlarının Aerobik Koloni Sayımı (Kob g⁻¹), sırasıyla 4.9x10⁵, 9.7x10⁴, 2.76x10⁵ ve 9.8x10² olarak tespit edilmiştir. Enterobakteri Koloni Sayımı (Kob g⁻¹) ve Koliform Bakteri Sayımı *E. coli* (Kob g⁻¹) düzeyleri de benzer şekilde <10 olarak bulunmuştur. Balık unlarında hem *Listeria monocytogenes* ve hem de *Salmonella* spp. bakteri varlığına rastlanılmamıştır. *Fusarium* spp. ve *Penicillium* spp. türü funguslar tüm balık unlarında tespit edilmiş iken, *Rhizopus* spp. ve *Trichoderma* spp. funguslar ise sadece bir fabrikadan elde edilen balık unlarında tespit edilmiştir. Toplam Organizma Sayıları ise 7x10³ ile 5x10⁴ Koloni/ml arasında değişmektedir. Makroskobik ve mikroskobik incelemeler sonucunda balık unlarında küf başta olmak üzere hayvan besleme adına yemlerde bulunmaması gereken herhangi bir materyale rastlanılmamıştır. Sonuç olarak, Türkiye’de Karadeniz Bölgesinde üretilen balık unlarının mikrobiyolojik ve makroskobik yönden son derece kaliteli ve hayvan besleme açısından uygun olduğu görülmektedir. Bu nedenle bu çalışmada, ülkemizde balık unlarının önemli bir bölümünün üretildiği Karadeniz Bölgesindeki balık unlarının mikrobiyolojik içeriğine ve kalitesine dikkat çekilmiştir.

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Balık unu
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Microbiological Analysis in Terms of Animal Nutrition Fish of Meal Produced in the Black Sea Region in Türkiye

Abstract

Fish meal is obtained by processing and drying fish that are not suitable for human consumption or waste left over from the fishing industry. Fish meal creates a suitable breeding environment for microorganisms due to both the high level of nutrients they contain and the processes applied during production. Aerobic Colony Count (Cob g⁻¹) of fish meal obtained from four different factories (A, B, C and D) producing fish meal in the Black Sea region were determined as 4.9x10⁵, 9.7x10⁴, 2.76x10⁵ and 9.8x10², respectively. Enterobacteria Colony Count (Cfu g⁻¹) and Coliform Bacteria Count *E. coli* (Cob g⁻¹) levels were similarly found to be <10. Both *Listeria monocytogenes* and *Salmonella* spp. wasn't detected in fish meals. While *Rhizopus* spp. and *Trichoderma* spp. fungi were detected in fish meal obtained from only one factory. *Fusarium* spp. and *Penicillium* spp. fungi were detected in all fish meals. Total Organism Numbers vary between 7x10³ and 5x10⁴ Colonies/ml. According to macroscopic and microscopic examinations, no material that should not be included in feed for animal nutrition, especially mold, was found in fish meal. As a result, it is seen that the fish meal produced in the Black Sea Region of Turkey is of extremely high quality in terms of microbiological and macroscopic and is also suitable for animal nutrition. Therefore, in this study was pointed out to the microbiological content and quality of fish meal in the Black Sea Region, where a significant portion of fish meal is produced in our country.

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1. Giriş

Balık unu, balık artıklarından ve insan gıdası olarak kullanılmayan balıkların pişirilmesi, ön damıtma, presleme, pres sıvısının ayrıştırılması, su buharlaştırma ve kurutma gibi süreçler sonucu elde edilen yüksek sindirilebilir protein ve dengeli esansiyel aminoasitler (özellikle lizin, metiyonin, treonin ve triptofan) bakımından zengin bir içeriğine sahip mükemmel bir yem hammaddesidir (Windsor, 2001; Mohanty ve ark., 2014; Masagounder ve ark., 2016). Balık unu genellikle toz formda satışa sunulmakta, çoğunlukla balık rasyonlarında yer almak üzere kanatlı hayvanların ve domuzların beslenmesinde kullanılmaktadır. Kültür balıkçılığının yaygınlaşması ile balık ununa olan talepte günden güne artış göstermektedir (Keleştemur ve Uslu, 2017). Balık ununa olan talebin fazlalığı, balık ununun fiyatını da artırmaktadır (Ween ve ark., 2017). Hayvansal protein kaynakları içerisinde yer alan balık unu diğer hayvansal protein kaynaklarına göre fiyatı oransal olarak daha yüksek olan bir yem hammaddesidir. Balık unlarının, işlendiği tesis kaynaklı bir takım patojen mikroorganizma taşıma riskleri mevcuttur (Cho ve Kim, 2011). Hayvanlardan yüksek kaliteye sahip ürünler elde edebilmek için, yemlerin içerdiği besin maddesi kadar yemlerin mikrobiyolojik içeriği de önemli bir yere sahiptir (Basmacıoğlu ve Ergül, 2003). Bu konuda yapılan bir çalışmada (Onarbay, 2010), yemlerde mikrobiyolojik ve mikroskobik analizlerin en önemli ve öncelikli avantajının, kimyasal analizlerden daha hızlı ve daha ucuz sonuç alınması olduğunu bildirmiştir. Aynı çalışmada yemlerin depolama süresi ve depolama koşullarının da yemin kalitesi üzerine etkili olduğu bildirilmiştir.

Koliform grubu bakteriler; Enterobacteriaceae familyasında yer alan, fakültatif anaerobik, gram negatif ve spor oluşturmeyen mikroorganizmalardır. Bu grup mikroorganizmalar taze sebzeler, hayvansal gıdalar ve koliform bakımından zengin su ürünlerinde bulunabilmektedir. Gıdalarda bu mikroorganizmaların bulunması kötü sanitasyon, yanlış pişirme ve pastörizasyon

sonrası tekrar bulaşma olduğunun bir göstergesi olarak kabul edilmektedir (Çakır, 2000). İşlenmiş hayvansal kökenli yemlerin 1 (bir) gramında Enterobacteriaceae sayısının en çok 300 olması gerektiği bildirilmektedir (Anonim, 2008). İşlenmiş balık ürünlerindeki Aerobik mikroorganizma sayısının ise kabul edilebilir en üst eşik sınırının 10^6 olması gerektiği ifade edilmiştir (USDA, 2018). Salmonella türleri doğada yaygın olarak bulunmaktadır. Salmonellalar yem ve hayvansal ürünler vasıtasıyla taşınarak, bunları tüketen insan ve hayvanlarda zoonoz bir hastalık olan Salmonellozise sebep olmaktadır. Bu hastalığın, kontamine balık unu ile kıtalar arasında yayıldığı ve insanlarda gıda zehirlenmesine de neden olduğu bildirilmektedir (WHO, 1988; Açılıoğlu, 1998). İşlenmiş hayvansal kökenli yemlerin 25 gramında *salmonella* sayısının hiç olmaması gerektiği bildirilmektedir (Anonim, 2008). Gram pozitif bir bakteri türü olan *Listeria* spp., ilk olarak Almanya'da 1891 yılında izole edilmiştir (Atasever, 2012). *Listeria monocytogenes* çubuk formda, gram pozitif, fakültatif anaerob ve biyofilm oluşturabilme özelliğine sahip bir mikroorganizmadır. Oldukça geniş pH ve sıcaklık aralıklarında gelişebildikleri gibi düşük su aktivitesi değerlerinde de yaşamlarını sürdürebilmektedirler (Gönen, 2021). Listeriozis, *L. monocytogenes* ile kontamine olmuş gıdaların tüketilmesiyle oluşan ciddi bir enfeksiyondur (Embarek, 1994). Listerioziste ciddi klinik tabloların oluşabilmesi, insidensin düşük olmasına karşın mortalitenin yüksek olması, halk sağlığı bakımından gıda kaynaklı önemli bir patojen olarak değerlendirilmektedir (Gönen, 2021). Başlıca tatlı sulardan, endüstriyel, insan ve hayvan kaynaklı kontaminasyona maruz kalmış kıyı deniz sularından avlanan balıklar, *Listeria* spp. ile kontamine olabilirler. Bu nedenle balık ve diğer su ürünleri, hayvan türleri ve insanlar için potansiyel bir risk oluşturmaktadır (Çaklı, 2007). *Listeria* spp., çevrede (toprak, su, lağım suları, atık sular, gübre), gıdalarda (et, süt ve bunlardan elde edilen gıda ürünlerinde) ve hayvan yemlerinde (yem, ot, silaj, sebze, bitki) bulunurlar. Enfekte olmuş insanlarda,

hayvanlarda ve hayvanların atık yavruları, vajinal akıntıları, fetal membranları, süt, idrar ve gaitalarında, çürümüş sebzelerde ve deniz ürünlerinde etkeni izole etmek ve tanımlamak mümkündür (Brackett ve ark., 1988; Aydın ve ark., 2006). Türkiye’de gıdalarda *L. monocytogenes*’in belirlenmesinde sıfır tolerans politikası geçerli olup sayım yerine doğrudan 25 g örnekte var/yok testi uygulanmaktadır (Tunail, 2000). Türk Gıda Kodeksi tarafından 17.03.2001 tarihli 24345 sayılı Resmi Gazete’de yayınlanan Mikrobiyolojik Kriterler Tebliğine göre et ve et ürünlerinin 25 gramında *L. monocytogenes* bulunmaması gerekmektedir (Anonim, 2001a). Küfler, mikotoksinler üreterek gizzard erozyonuna ve *Penicilium lanosum* üreterek civcivlerde yavaş büyümeye neden olabilmektedir. Yapılan bir çalışmada 0.6 veya 3 mg kg⁻¹ aflatoksin, gizzard lezyonlarını arttırmadığı ve büyüme performansında bir olumsuzluğa neden olmadığı, 4 mg kg⁻¹ ve daha yüksek aflatoksin dozlarının ise ekonomik kayıplara ve öldürücü etkiye sebep olabileceği bildirilmiştir. Yine rasyonda % 7-12 Peru balık unu içeren rasyonların etlik piliçlerde gizzard erozyonuna neden olabileceği de bildirilmiştir (Summers ve ark., 2013). Mikrobiyolojik ve hayvan besleme açısından olumsuz bir durum ile karşılaşmamak için, balık ununun su oranı % 10’dan fazla olmamalı ve tuz oranı % 7’yi aşmamalıdır (Anonim, 2023a).

FAO, 2020 verilerine göre Dünya balık unu üretim miktarının yaklaşık 5 milyon ton olduğu, 2030 yılı itibarıyla bu üretimin 6 milyon tona ulaşacağı bildirilmektedir (Lopes, 2022). Türkiye’de balık unu üretimi yıllık yaklaşık 5550 tondur (Anonim, 2023b). Türkiye’de faaliyet gösteren balık unu fabrikalarının çoğunluğu Karadeniz bölgesinde (5 adeti Sinop ilinde, 3’ü Samsun’da, 3 tanesi Trabzon’da, 1 adeti de Rize’de) bulunmakla birlikte (Bayraklı ve ark., 2019) 1 adet fabrikada Çanakkale ilinde bulunmaktadır. Mevcut çalışmada Türkiye’de Karadeniz bölgesinde üretilen balık unlarının mikrobiyolojik açıdan kalitesi değerlendirilmiştir. Burada elde edilen sonuçlar, var olan kaynaklarımızın hak ettiği

değeri görmesine katkıda bulunacağı düşünülmektedir. Ayrıca bu sonuçlar, hayvan beslemesi ve hayvan sağlığı açısından da oldukça önem taşımaktadır.

2. Materyal ve Yöntem

Araştırma materyalini oluşturan balık unları 2022 yılının Mayıs ayında, Karadeniz bölgesinin Samsun ve Trabzon illerinde bulunan dört farklı balık unu üreten fabrikadan bir kerede 3 tekerrürlü olacak şekilde toplamda 12 numune olarak temin edilmiştir. Balık unları, çoğunluk Karadeniz hamsisi olup iz miktarda Çaçı balığından üretilmiştir. Balık unu üreten fabrikaların çalışma prensibi; balık ve kullanılmayan balık atıklarının önce buharda kurutulması sonra değirmenden geçirilip yağı alındıktan sonra ürünün elde edilmesi şeklindedir. Gruplar, balık unlarının elde edildiği fabrikalara bakılmaksızın, A, B, C ve D fabrikası olarak isimlendirilmiştir. Balık unu fabrikasından numune alma işleminde, yem örnekleri bütün yığının çeşitli yerlerinden ve homojen olmasına dikkat edilerek ağzı kilitli steril naylon poşetlere 1 kg olacak şekilde alınmıştır. Naylon poşetlerin üzerine üretim bilgileri kayıt edilerek soğuk hava zinciri ile Malatya’da özel bir firmaya ait olan yem laboratuvarına getirilerek Aerobik Koloni Sayımı, Enterobakteri Koloni Sayımı, Koliform Bakteri sayımı, *Listeria monocytogenes* ve *Salmonella* spp. analizleri yapılmıştır. Arta kalan balık unları -20 °C’de muhafaza edilerek Van Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü laboratuvarına getirilerek *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Mucor* spp., *Fusarium* spp., *Alternaria* spp., *Trichoderma* spp. ve fungus türleri belirlenmiştir.

Araştırmada incelenen balık unu örneklerinde; Aerobik koloni sayımı için TS EN ISO 4833-1 metodu (Anonim, 2014), Enterobakteri sayımı için TS EN ISO 21528-1 metodu (Anonim, 2018), Koliform bakteri sayımı için TS EN ISO 4832 metodu (Anonim, 2010), *Listeria monocytogenes* sayımı için TS EN ISO 21528-1 metodu (Anonim, 2017a) ve *Salmonella* spp., sayımı için TS EN ISO 6579-1 metodu (TSE, 2017b) kullanılmıştır. Balık

unu örneklerinden fungusların izolasyonunda “Toprak Seyreltme Tekniği (Soil Dilutions Technique)” kullanılmıştır (Waksman, 1922). Her bir balık unu örneğinden 10 gram tartılarak erlenmayere konulmuş ve üzerlerine 90 ml steril saf su ilave edilmiştir. Bu şekilde 1/10'luk (seyreltme faktörü 10^1) süspansiyon hazırlanmış ve mekanik çalkalayıcıda 10 dakika çalkalanmıştır. Bu işlemi takiben süspansiyondan steril bir pipetle 1 ml örnek alınmış ve 9 ml steril saf su içeren tüpe aktarılmıştır. Böylece 1/100'lük (seyreltme faktörü 10^2) süspansiyon hazırlanmıştır. Bundan da yine 1 ml örnek alınarak 9 ml steril saf su içeren başka bir tüpe aktarılmıştır. Bu şekilde 1/1.000'lük (seyreltme faktörü 10^3) süspansiyon elde edilmiştir. Bundan da 1ml örnek 9 ml steril saf suya aktararak 1/10.000'lik (seyreltme faktörü 10^4)

süspansiyon elde edilmiştir. Bu yöntemde izolasyon için en iyi oranların seyreltme faktörü 10^3 ve 10^4 olduğu ifade edilmiştir (Hasenekoğlu, 1989). Bu çalışmada, 10^3 seyreltme faktörlü süspansiyonlar kullanılmıştır. Hazırlanan patates dekstroza agar ortamına süspansiyondan 1 ml bırakılmıştır (Hasenekoğlu, 1989). Petriler 25 ± 2 °C' de inkübatörde yaklaşık 10 gün süreyle inkübasyonu takiben, iki gün aralıklarla kontrol edilip gelişen koloniler işaretlenmiştir. Çalışma her örnek için 5 tekerrürlü yürütülmüştür. Yapılan incelemeler sonucunda gelişen koloniler ışık mikroskobu ile mikroskopik incelemeye tabi tutulmuşlar ve tür anahtarlarına göre teşhis edilmişlerdir. Sayılan kolonilerin değerlendirilmesi aşağıdaki formüle göre yapılmıştır (Cansu ve ark., 2021);

$$\text{Toplam Organizma Sayısı (Koloni ml}^{-1}\text{)} = \frac{\text{Petride Gözlenen Koloni Sayısı} \times \text{Dilüsyon Faktörü}}{\text{Petri Kabına Aktarılan Miktar (ml)}}$$

Balık unlarının mikroskopik incelemesi için, preparatlar ilk önce 4x ve ardından sırasıyla 10x, 40x ve 100x büyütme ile binoküler mikroskopta incelenmiştir. Çalışmada Leica ICC50HD mikroskobu kullanılmıştır.

3. Bulgular ve Tartışma

Karadeniz'in farklı bölgelerinden elde edilen balık unlarının mikrobiyolojik analiz sonuçları Tablo 1'de verilmiştir. Karadeniz bölgesinde balık unu üreten dört farklı fabrikadan temin edilen balık unlarının Aerobik Koloni Sayımı (Kob g^{-1}), sırasıyla 4.9×10^5 , 9.7×10^4 , 2.76×10^5 ve 9.8×10^2 olarak tespit edilmiştir (Şekil 1a). Enterobakteri Koloni Sayımı (Kob g^{-1}) ise dört farklı balık unu içinde <10 olarak belirlenmiştir (Şekil 1b). Koliform Bakteri Sayımı ve *E. coli* (Kob g^{-1}) da benzer şekilde <10 olarak bulunmuştur (Şekil 1c). *Listeria monocytogenes* (Kob g^{-1}) (Şekil 1d) ve *Salmonella* spp. (Kob g^{-1}) (Şekil

1e) ise tespit edilmemiştir. Balık unlarının tümünün ortalama Aerobik Koloni Sayımı 2.16×10^1 olarak tespit edilmiştir. Yenilmeye Hazır Gıdalar için Mikrobiyolojik Rehber 2001'e göre kabul edilebilir A sınıf balık unu için Aerobik Koloni Sayımı $<10^5$, kabul edilebilir sınırı da 10^5 - 10^6 olarak belirlenirken (Anonim, 2001b), $\geq 10^6$ değerini ise kabul edilemez C sınıfı olarak tanımlamıştır. Mevcut çalışmada analiz edilen dört farklı balık ununun da Aerobik Koloni Sayımı bakımından kullanılabilir olduğu sonucuna varılmıştır. Dört farklı balık unu fabrikasından temin edilen balık unlarının tümünde de Enterobakteria Koloni Sayımı (Kob g^{-1}), <10 olarak tespit edilmiştir. T.C. Tarım ve Köyişleri Bakanlığı'nın Hayvansal Kökenli Yemlerde Mikrobiyolojik Kriterler Tebliğine (Tebliğ No: 2008/47) göre hayvansal yemlerde Enterobakteria Sayısı (Kob g^{-1}) için maksimum değer 3×10^2 Kob g^{-1} olarak bildirilmiştir.

Tablo 1. Karadeniz bölgesinin farklı bölgelerinden elde edilen balık unlarının mikrobiyolojik analiz sonuçları

SONUÇLAR / İŞLETMELER	A Fabrikası	B Fabrikası	C Fabrikası	D Fabrikası
Aerobik Koloni Sayımı (Kob g ⁻¹) Limit değer Uygunluk	4.9*10 ⁵ 10 ⁶ U	9.7*10 ⁴ 10 ⁶ U	2.76*10 ⁵ 10 ⁶ U	9.8*10 ² 10 ⁶ U
Enterobakteri Koloni Sayımı (Kob g ⁻¹) Limit değer Uygunluk	<10 3*10 ² U	<10 3*10 ² U	<10 3*10 ² U	<10 3*10 ² U
Koliform Bakteri Sayımı ve <i>E. coli</i> (Kob g ⁻¹) Limit değer Uygunluk	<10 1*10 ² U	<10 1*10 ² U	<10 1*10 ² U	<10 1*10 ² U
<i>Listeria monocytogenes</i> (Kob g ⁻¹) Limit değer Uygunluk	Tespit edilmedi - U	Tespit edilmedi - U	Tespit edilmedi - U	Tespit edilmedi - U
<i>Salmonella</i> spp (Kob g ⁻¹) Limit değer Uygunluk	Tespit edilmedi 0/25 U	Tespit edilmedi 0/25 U	Tespit edilmedi 0/25 U	Tespit edilmedi 0/25 U

U: Uygun, UD: Uygun değil, Kob: Koloni oluşturma birimi

Mevcut sonuçlar, Enterobakteria Koloni açısından da balık unlarının kabul edilebilir, uygun olduğunu göstermektedir. Farklı fabrikalardan temin edilen tüm balık unları için Koliform Bakteri Sayımı ve *E. coli* (Kob g⁻¹) düzeyleri de <10 olarak belirlenmiştir. USDA (2018)'e göre pişirilmiş balıklar için en yüksek değer, 1*10² olarak bildirmiştir. Mevcut çalışmada da analiz edilen tüm balık unlarının Koliform Bakteri Sayımı ve *E. coli* (Kob g⁻¹) düzeyleri bu kritik sınırın çok çok altında olması nedeniyle, Koliform Bakteri Sayımı ve *E. coli* açısından da kabul edilebilir ve uygun olduğu sonucuna varılmıştır. Yine mevcut çalışmada analizleri yapılan balık unlarında hem *Listeria monocytogenes* ve hem de *Salmonella* spp. bakteri varlığına rastlanılmamıştır. Türk Gıda Kodeksi Yönetmeliği Mikrobiyolojik Kriterlerine göre (Anonim, 2011), balık ürünlerindeki *Listeria monocytogenes* ve *Salmonella* spp. türleri için üst sınırı 0/25 g ml⁻¹ olarak bildirmişlerdir. Bu sonuçlara göre hem *Listeria monocytogenes* ve hem de *Salmonella* spp. bakteri açısından, tüm balık unları kabul edilebilir uygun sınıf içerisinde yer almaktadır. Yemlerin

mikrobiyolojik içeriklerinin tespiti ile ilgili yapılan bir çalışmada (Açıoğlu, 1998); et-kemik ununda 4.0x10⁵ koliform bakteri g⁻¹, toz yemde 4.6x10⁵ koliform bakteri g⁻¹, pelet yemde 2.3x10¹ koliform bakteri g⁻¹, yumurta tavuğu yem örneğinde 6.7x10³ koliform bakteri g⁻¹ olarak tespit etmiştir. Genel olarak toz yemde, pelet yeme göre daha fazla koliform bakteri olduğu bildirilmiştir. Et-kemik ununda belirtilen koliform düzeyleri mevcut çalışmada kullanılan hayvansal kaynaklı bir yem olan balık unların elde edildiği iki fabrikadan elde edilen değerlere benzer iken, iki fabrikada elde edilen değer ise Açıoğlu (1998)'in belirtmiş olduğu değerden daha düşük olduğu görülmüştür. Şüpheli durumlarda ve hayvansal kaynaklı protein içeriği yüksek yemlerde *Salmonella*'ların varlığı araştırılması gerektiği bildirilmektedir. *Salmonella*'ların izolasyon ve tanımlanması zor olduğundan, yemlerde Enterobakterilerin sayımından yararlanılmaktadır (Açıoğlu, 1998). *Salmonella*, Enterobakteri familyasına ait olup hem insanlar hem de hayvanlar için tıbbi açıdan önemli bir patojendir. *Salmonella*'nın birçok türü olup 2579'dan

fazla serotipi vardır (Andino ve Hanning, 2015). Salmonella cinsine ait bakterilerin neden olduğu Salmonellozis, ekonomik açıdan en önemli zoonoz hastalıklardan biridir. Hastalığın yayılmasında yem, gıda ve suların kontaminasyonu önemli rol oynar (WOAH, 2018). *Escherichia* spp., *Klebsiella* spp. dahil olmak üzere *Enterobacter* spp.'ler koliform grubu mikroorganizmalar olup yemlerde ve sulardaki dışkı kontaminasyonunun bir göstergesi olarak kabul edilmektedir (Sanderson ve ark., 2005). Diyarbakır yöresinde yemlerin mikrobiyolojik özellikleri ile ilgili yapılan bir çalışmada (Baran ve ark., 2008), en düşük toplam mezofilik aerob bakteri sayısı süt yeminde, en yüksek ise kuzu yeminde bulunmuştur. Sığır besi, buzağı ve kuzu yemlerinde *Salmonella* tespit edilmezken, sığır süt yeminde % 13.30 düzeyinde tespit edilmiştir. Sığır süt, sığır besi, buzağı ve kuzu yemlerindeki kontaminasyon oranları sırasıyla *Listeria monocytogenes* için % 13.33, 46.66, 33.33, 13.33 koliform bakteri için % 93.33, 86.66, 93.33, 80.00; *Escherichia coli* için % 40.00, 26.66, 20.00, 40.00; *Staphylococcus* spp. için % 86.66, 80.00, 93.33, 60.00; sulfite indirgeyen anaerob bakteriler için % 73.33, 73.33, 60.00, 46.66; *Bacillus cereus* için % 100.00, 100.00, 86.66, 93.33 olarak tespit etmişlerdir. Lee (2006), tarafından Bursa ilinde ihracatı yapılan su ürünleri üzerine bir çalışma yapmıştır. Yapılan çalışmada; 46 adet işlenmiş deniz salyangozu, 34 adet canlı kerevit, 32 adet dondurulmuş ve taze soğutulmuş balık, 20 adet marine hamsi, 18 adet canlı kurbağa ve dondurulmuş kurbağa bacağı, 11 adet dondurulmuş karides, 7 adet ton balık konservesi, 9 adet dondurulmuş kum midyesi örneğini mikrobiyolojik yönden analiz etmiştir. Toplam 177 örneğin 130 adedi (% 73) Su Ürünleri Yönetmeliği'ne uygun bulunmuştur. Örneklerin hiç birisinde *Salmonella* spp., *Sytophylococcus aureus*, *Vibrio parahaemolyticus* ve *Vibrio cholerae* saptanmamıştır. Toplam 41 adet (% 23) üründe ise Mezofilik aerobik bakteri üremesi gerçekleşmiş bunların 26 adedi (% 63) limitin üzerinde bulunmuştur. Toplam 34 (% 19) üründe fecal koliform bakteri saptanmış, 15 adedinin (% 44) limitin üzerinde olduğu

görülmüştür. Fecal koliform bakteri üremesi canlı örneklerde gerçekleşmiştir. Canlı örneklerde fecal koliform saptanmış olması bunların pirimer kontaminasyona maruz kaldıklarını ve avlandıkları ortamda bir çevresel kirliliğin mevcut olduğunu düşündürmektedir. 9 adet canlı kerevit ve 2 adet taze soğutulmuş balık örneğinden *E. coli* izole edilmiştir. İki adet dondurulmuş kum midyesinden ise *Listeria* spp. izole edilmiştir. Bu araştırmacının bulguları, balık unlarında *Salmonella* spp. türlerinin tespit edilmediği mevcut araştırma bulgularıyla uyusmaktadır. Atasever (2012), Karadeniz Bölgesi Ordu ilinde insan tüketime sunulan balıklarda *Listeria* spp. izolasyonu ve tanımlanması yapmıştır. Sonuç olarak toplam 100 balık örneğinin 10'undan (% 10) izole edilen *Listeria* spp.'nin halk sağlığı açısından potansiyel risk oluşturduğunu bildirmiştir. *Listeria* türleri arasında insanlarda ve hayvanlarda en önemli patojeniteye sahip tür *L. monocytogenes*'dir. Bu konuda yapılmış bir çalışmada taze ve donmuş balıklar ve bunlarla elde edilen ürünlerde *Listeria* türlerinin çoğunlukla bulunabileceği ve *L. monocytogenes* bulunma olasılığı oranlarının % 4 ile % 12 arasında değişebileceği belirtilmiştir (Emberek, 1994). Mevcut çalışmada incelenen balık unlarının hiçbirinde *Listeria monocytogenes* tespit edilmemiş olması, Emberek (1994) ve Atasever (2012), isimli araştırmacıların taze ve donmuş balıklarda elde ettiği *Listeria* değerlerinden de daha düşük bulunmuştur. Mevcut sonuçların, bu araştırmacılardan farklı olması kullanılan balık unlarının yüksek sıcaklıklarda işlenmiş ve kurutulmuş olmasına bağlanılabilir. Lee ve ark. (2017)'de balık ununda yapmış oldukları mikrobiyolojik analizlerde, Laktik asit bakteri ve *Bacillus subtilis*, 7.15 ve 7.70 cfu g⁻¹ olarak belirlemişlerdir. Tüm mikrobiyolojik analiz sonuçları değerlendirildiğinde Türkiye'de Karadeniz Bölgesinde üretilen balık unlarının mikrobiyolojik açıdan son derece kaliteli ve uygun olduğunu göstermektedir.

Hayvanların hastalıklardan korunması, kaliteli ve çok miktarda ürün alınabilmesi yemin mikrobiyolojisi ile yakından ilgilidir. Patojenik mikroorganizmaların yemden

hayvanlara, hayvanlardan da ürün vasıtasıyla insanlara bulaşmasıyla, insanlarda gıda kaynaklı zehirlenmeler görülebilir. Yemlerdeki ekonomik kayıpların, hayvanlarda ve insanlarda da yem kaynaklı sağlık

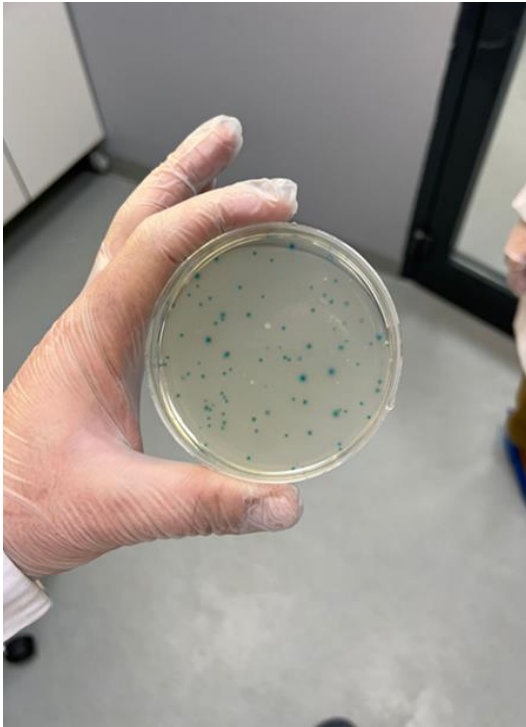
problemlerinin önüne geçilmesi için, yem hammaddelerinde özellikle de hayvansal kaynaklı yem hammaddelerinde mikrobiyolojik kontroller yapılmalıdır.



a) Aerobik Koloni Sayımı



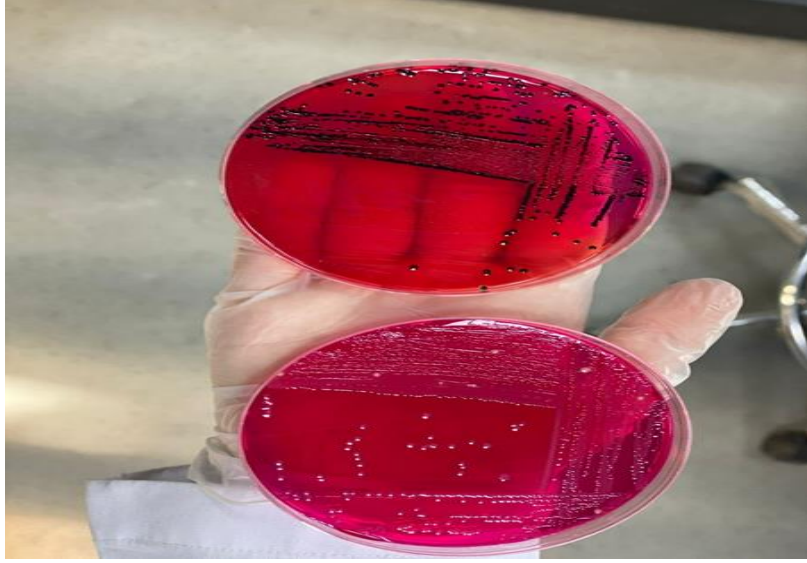
b) Enterobakteri Sayımı



c) Koliform Bakteri Sayımı ve *E. coli*



d) Listeria Sayımı

e) *Salmonella* spp sayımı

Şekil 1. Balık unlarının mikrobiyolojik analiz açısından petri kabında üretilen bakteri görünüşleri

Karadeniz Bölgesinde balık unu üreten dört farklı fabrikadan elde edilen balık unlarının fungus içerikleri ve toplam organizma sayısı Tablo 2’de verilmiştir. Mevcut çalışmada balık unlarının; *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Mucor* spp., *Fusarium* spp., *Alternaria* spp., *Trichoderma* spp., fungus cinsleri ile Toplam Organizma Sayıları test

edilmiştir. *Fusarium* spp. ve *Penicillium* spp., türü funguslar tüm balık unlarında tespit edilmiş iken, *Rhizopus* spp., ve *Trichoderma* spp., funguslar ise sadece bir fabrikadan elde edilen balık unlarında tespit edilmiştir. Toplam Organizma Sayıları ise 7×10^3 ile 5×10^4 Koloni/ml arasında değişmektedir.

Tablo 2. Karadeniz Bölgesinden elde edilen balık unu örneklerinde fungusların durumu ve toplam organizma sayısı (Koloni ml⁻¹)

Fungus cinsleri	Balık unu örnekleri			
	A Fabrikası	B Fabrikası	C Fabrikası	D Fabrikası
<i>Aspergillus</i> spp.	+	+	+	
<i>Penicillium</i> spp.	+	+	+	+
<i>Rhizopus</i> spp.		+		
<i>Mucor</i> spp.			+	
<i>Fusarium</i> spp.	+	+	+	+
<i>Alternaria</i> spp.	+		+	+
<i>Trichoderma</i> spp.			+	
Toplam organizma sayısı (Koloni ml ⁻¹)	2×10^4	7×10^3	11×10^3	5×10^4

Yemlerdeki en önemli mikroorganizmalar bakteriler, küf mantarları ve mayalardan oluşmaktadır. Erdoğan ve Aslantaş (2005), Hatay yöresinde karma yemlerin ve yem hammaddelerinin mikrobiyolojik kalitesini belirlemek amacıyla yaptıkları çalışmada, Örneklerin % 72’sinde *Penicillium* spp., %

54’ünde *Aspergillus* spp., % 34’ünde *Fusarium* spp. ve % 28’inde *Mucor* spp. tespit etmişlerdir. Toplam mantar sayısı ise karma yemlerde, 4.25×10^4 - 1.7×10^6 adet g⁻¹, yem hammaddelerinde ise 4.00×10^3 - 5.90×10^5 adet g⁻¹ olarak tespit etmişlerdir. Diyarbakır yöresinde yemlerin mikrobiyolojik özellikleri

ile ilgili yapılan bir çalışmada (Baran ve ark., 2008), küf miktarlarını ortalama olarak süt ve buzağı yemlerinde 3.3×10^3 , besi yemlerinde 4.1×10^3 , kuzu yemlerinde de 3.0×10^3 ; maya miktarlarını ise süt yemi, besi yemi, buzağı yemi ve kuzu yemi olmak üzere sırasıyla 6.7×10^3 , 1.4×10^4 , 1.6×10^3 ve 9.8×10^3 şeklinde belirlemişlerdir. Yemlerinde küf ve maya sayısı bakımından en yüksek değerler besi yemlerinde tespit edilmiştir. Karma yemlerdeki küf oranı % 90, maya oranı ise % 83.33 olarak tespit edilmiştir. Diyarbakır yöresinde yemlerin mikrobiyolojik özellikleri ile ilgili yapılan çalışmada (Baran ve ark., 2008), küf miktarlarını ortalama olarak süt ve buzağı yemlerinde 3.3×10^3 , besi yemlerinde 4.1×10^3 , kuzu yemlerinde de 3.0×10^3 ; maya miktarlarını ise süt yemi, besi yemi, buzağı yemi ve kuzu yemi olmak üzere sırasıyla 6.7×10^3 , 1.4×10^4 , 1.6×10^3 ve 9.8×10^3 şeklinde belirlemişlerdir. Yemlerinde küf ve maya sayısı bakımından en yüksek değerler besi yemlerinde tespit edilmiştir. Karma yemlerdeki küf oranı % 90, maya oranı ise % 83.33 olarak tespit edilmiştir. Şamlı ve Onarbay (2011), farklı depolama süresi ve sıcaklığın balık unlarının mikrobiyolojik ve mikroskopik özellikleri üzerine etkisini incelemişlerdir. Depolama öncesi ve sonrası balık unlarında küf tespit edilmemiş ancak, depolamanın 1. ayında 26°C 'de 2.216 cfu g^{-1} olan maya sayısı 2. ayda 3.782 cfu g^{-1} olarak tespit edilmiştir. Lee ve ark. (2017)'de balık ununda yapmış oldukları analizlerde, maya içeriğini $7 \times 10^2 \text{ cfu g}^{-1}$ olarak belirlemişlerdir. Mevcut çalışmada belirlenen fungus cinsleri, Erdoğan ve Aslantaş (2005)'in belirlediği fungus cinsleri ile uyushmaktadır. Benzer olarak en fazla *Penicillium* spp., en az ise *Mucor* spp. cinsi funguslar tespit edilmiştir. Ayrıca Erdoğan ve Aslantaş (2005)'in yem hammaddelerinde, Şamlı ve Onarbay (2011)'in balık unlarında belirtmiş olduğu toplam mantar sayıları da, mevcut çalışmada elde ettiğimiz toplam organizma sayıları ile uyushmaktadır. Ancak mevcut çalışmada elde edilen balık unlarının toplam organizma sayısı, Lee ve ark. (2017)'nin bildirdiği $7 \times 10^2 \text{ cfu g}^{-1}$ değer ile uyushmamaktadır. Bu durum Lee ve ark. (2017)'nin çalışmada kullandıkları balık

ununu fermente etmelerine ve üretim aşamasındaki farklılığa bağlanabilir. GMP, 2024'e göre yemlerdeki maya ve küf için üst limit sınırı 10^6 cfu g^{-1} olarak bildirmiştir. Karadeniz'de dört farklı balık unu üreten fabrikadan temin edilen balık unlarının tümünde, maya ve küfler için belirtilen bu üst sınırın altında olması, balık unlarının hayvan besleme açısından son derece uygun ve kaliteli olduğunu göstermektedir.

Balık unlarının mikroskopik incelemesine ait görüntüler Şekil 2'de verilmiştir. Balık unlarına ait görüntüler ilk önce 4x ve ardından sırasıyla 10x, 40x ve 100x büyütme ile binoküler mikroskopta incelenmiştir. Şekil 2'de görüldüğü üzere balık unları parlak, kahverengimsi ve sarı renktedirler. Balık unlarının içermiş olduğu kemik ve kılçıklar şekil üzerinde kırmızı oklarla gösterilmiştir. Özellikle 4. fabrikaya ait olan balık unlarının içermiş olduğu kemik ve kılçıkların daha ince yapıda olduğu dikkat çekmiştir. Yemlerin kalitesini, hile yapıp yapılmadığını ve kontaminasyon derecesini tespit etmek için mikroskopik incelemeye ihtiyaç duyulmaktadır. Mikroskopik analiz, diğer analiz yöntemlerine göre basit, hızlı ve daha az zaman alan bir tekniktir. Özellikle yem hammaddelerinin ön incelemesi için oldukça uygun bir yöntemdir. Yemlerin mikroskopik incelemesi teknik bilginin yanı sıra mesleki deneyim ve tecrübe gerektirmektedir. Kop ve ark. (2006), makroskopik incelemelerde, balık ununun kahverengimsi ve sarı renkte olduğunu, yoğun balık kokusu içerdiğini ve kemiklerin parlak olduğunu bildirmişlerdir. Mikroskopik olarak incelendiğinde de kemiklerin silindirik şeklinde ve benekli olduğunu ifade etmişlerdir. Mikroskopik görüntünün et ununa benzediğini ama daha açık renkte olduğunu belirlemişlerdir. Diş, göz ve otolit ismi verilen taş sıklıkla rastlanılmaktadır. Khajareen ve Khajareen (2008), balık unlarının mikroskop altında incelendiğinde, balık kemikleri ve kılçıklarının inci parlaklığında olduğunu bildirmiştir. Kemik parçalarının şekli balık unlarının elde edildiği balık türüne bağlı olarak çok çeşitli olabilmektedir. Büyük parçalar düzgün yüzeylidir. Yılmaz ve Duru (2023), yem

hammaddeleri için yapmış oldukları mikroskopik incelemede, balık unundaki kemiklerin, kemik unundaki kemiklere göre daha şeffaf olduğunu bildirmişlerdir. Balık pullarının ve kum saatine benzer omurga parçalarının da bulunduğunu bildirmişlerdir. Mevcut çalışmada da mikroskopik görüntüler çok parlak olup, kemik ve kılçıklar silindirik şeklinde ve inci parlaklığındadır. Bu bulgular,

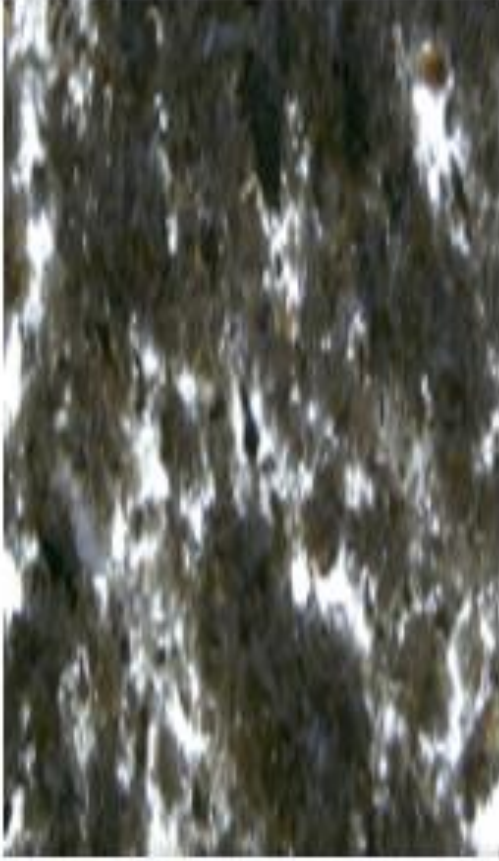
yukarıdaki araştırmacıların balık unlarının mikroskopik analizleriyle uyumlu olarak değerlendirilmiştir. Kop ve ark. (2006), balık unlarının makroskopik incelemelerine ait olan balık ununun kahverengimsi ve sarı renkte oluşu ve yoğun balık kokusu içerdiği şeklindeki bildirişleri de mevcut çalışma ile uyum içerisinde olmuştur.



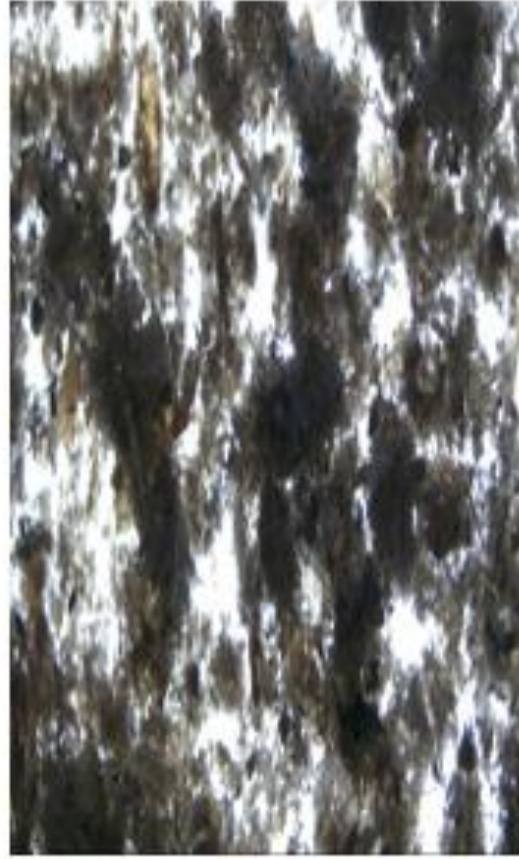
Fabrika 1, 4x



Fabrika 1, 10x



Fabrika 1, 40x



Fabrika 1, 100x



Fabrika 2, 4x



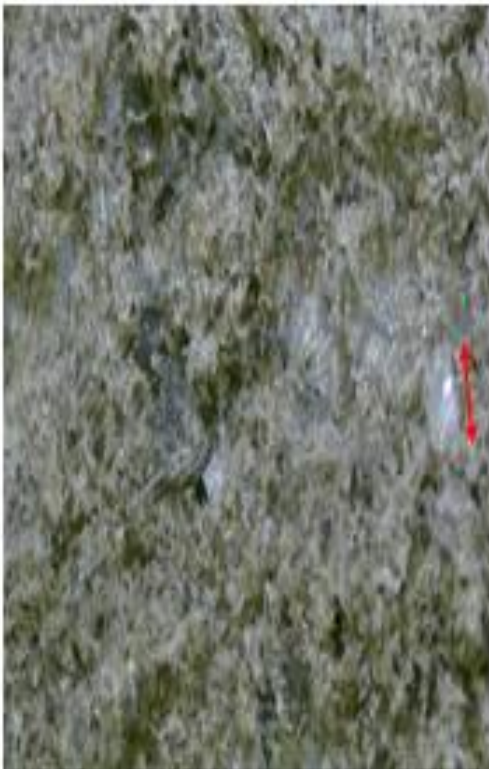
Fabrika 2, 10x



Fabrika 2, 40x



Fabrika 2, 100x



Fabrika 3, 4x



Fabrika 3, 10x



Fabrika 3, 40x



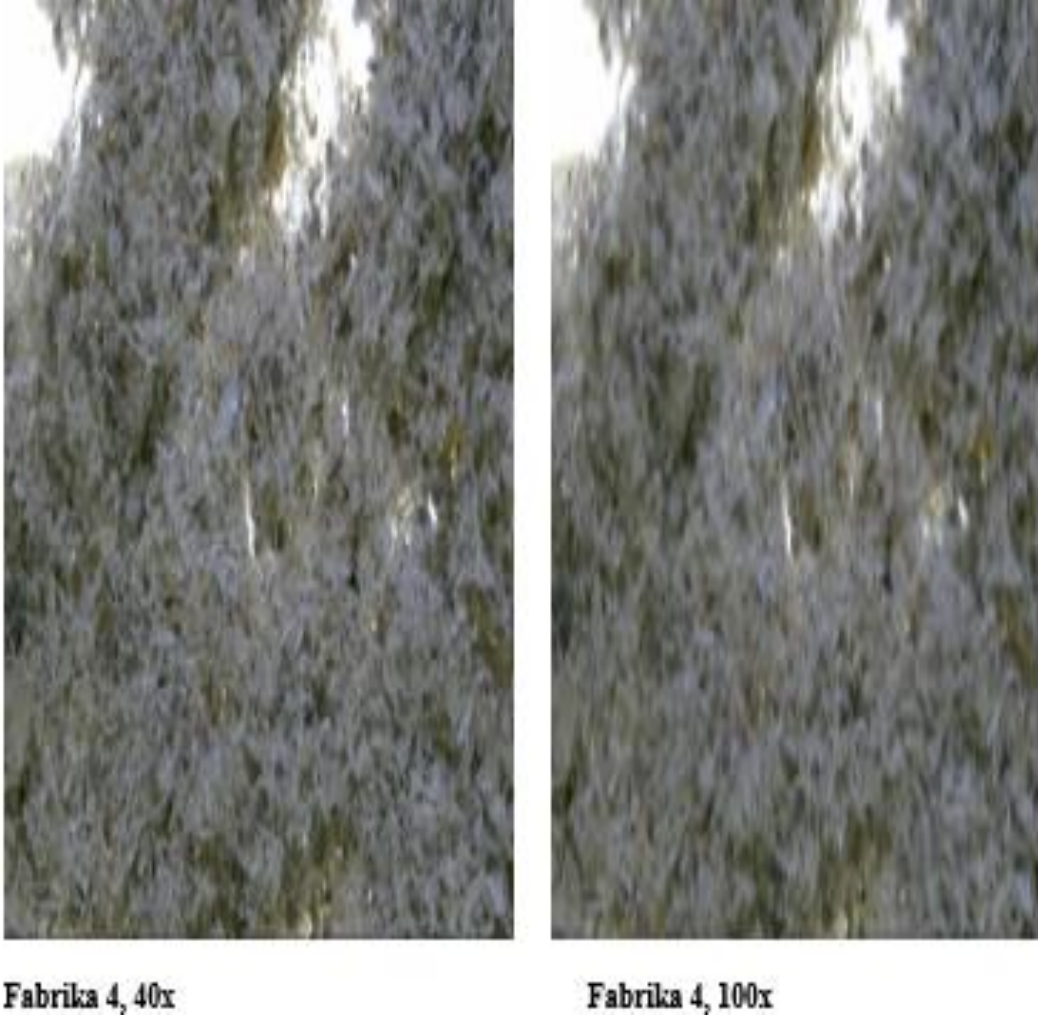
Fabrika 3, 100x



Fabrika 4, 4x



Fabrika 4, 10x



Şekil 2. Balık unlarının mikroskobik incelemesine ait (4x, 10x, 40x ve 100x) görüntüler

4. Sonuç

Yemlerde yüksek oranda mikroorganizma, maya, küf bulunması yemin sadece kalitatif değerini düşürmekle kalmaz aynı zamanda hayvanların sağlık ve verimlerini de olumsuz yönde etkileyebilmektedir. Bu nedenle, bir yem hammaddesinin ya da karma yemin kalitesini belirlerken, fiziksel, kimyasal özelliklerinin yanı sıra mikrobiyolojik özelliklerinin de incelenmesi gerektiği unutulmamalıdır. Balık unlarının, ruminant hayvanların yemine katılmadığı, özellikle kanatlı ve balık rasyonlarına katıldığı için, balık unlarının kalitesi oldukça hassas bir konudur. Balık unları gerek içerdikleri yüksek düzeydeki besin madde içerikleri, gerekse üretim esnasında uygulanan işlemler nedeniyle mikroorganizmalar için uygun üreme ortamı da oluşturmaktadır. Türkiye’de balık unu

deyince ilk akla gelen Karadeniz Bölgesidir. Bu çalışmada, Karadeniz Bölgesinde balık unu üreten dört farklı fabrikadan temin edilen balık unlarının gerek mikrobiyolojik gerekse mikroskobik incelmeleri sonucunda, son derece kaliteli ve uygun olduğu sonucuna varılmıştır. Bu durum gerek hayvan besleme gerekse hayvan sağlığı açısından son derece önemlidir. Bu sonuçlar, var olan kaynaklarımızın ekonomiye katma değer olarak katılması açısından da son derece önem taşımaktadır. Bunun için Karadeniz Bölgesinde üretilen balık unlarının kalitesine gerek ulusal gerekse uluslararası yem borsalarında dikkat çekilmeli ve uluslararası bağlantılar kurulmalıdır. Daha sonraki yapılacak çalışmalarda balık unlarının tazeliğini gösteren parametreler olarak, balık unlarında toplam uçucu bazik nitrojen (TVB-

N) ve histamin içeriğine bakılması önerilmektedir. Yeni açılacak balık unu üretecek fabrikalara da üretim sistemlerinde evaporatör sistemi kullanılması balık ununun kalitesi açısından yararlı olacaktır. Çalışmada elde edilen bulgular ışığında, kanatlı, domuz, balık ve karides formülasyonlarında kullanılan birincil protein kaynağı olan balık ununun, işlenme şekline bağlı olarak kalitesi değişebilmektedir. Ülkemizde Karadeniz bölgesinde yer alan balık unu fabrikalarının mikrobiyolojik ve mikroskobik teknikleri üretim sistemlerinde kullanmaları; ürettikleri son ürünlerin kalitesi ile ilgili çok kısa sürede ve az masrafla fikir sahibi olabilmelerine imkân sağlayacaktır. Bu fabrikaların kaliteli ürün işlemesi, uygun kalite ve istenilen standartta balık unu üretebilmeleri, hammadde alıcılarının da yurtdışı pazarına yönelimini engelleyecek ve balık unu sektöründe çalışan bu fabrikaların gelişmesine yardımcı olacaktır.

Yazarların Katkı Beyanı

Yazarlar makaleye eşit katkıda bulunduklarını, makalenin yayına hazır son halini gördüklerini/okuduklarını ve onayladıklarını beyan ederler.

Çıkar Çatışması Beyanı

Tüm yazarlar, bu çalışma için herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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Opinion of Academicians Regarding the Use of the CRISPR-Cas System in Türkiye

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Abstract

The field of gene control is huge and still much unexplored, and one way to modify and alter genes is called CRISPR. The purpose of this study is to discuss the application of the CRISPR-Cas system in Turkey and find out what genetics professors think about it, as it has been a hot topic lately. The study's findings came from a survey that was given to 26 academicians who agreed to engage in the research and are recognized authorities in their professions. According to 74.1 % of participants, CRISPR-Cas 9 was not well known in the Turkish genetics community and was only known by those in the field. The participants stated that the CRISPR method can be used to treat cancer and many genetic diseases 100 % of the time, to breed animals for high-yield breeds in animal husbandry 100 % of the time, to provide resistance and protection against many infectious diseases 84.6 % of the time, and to improve animal welfare 88.8 % of the time. Although 76.9 % of experts said that there is room for ethical debate over the use of CRISPR, 92.3 % of experts predicted that gene-edited animal farms will be built in the near future. A sizable majority of participants 92.3 % said that Turkey must develop and apply the CRISPR method to stay ahead of developed nations in genetic science, as well as for application in animal breeding and the treatment of genetic illnesses. Therefore, even if CRISPR technology has benefits and drawbacks, it's critical to create legislative guidelines that consider the environment, public health, food safety, global trade, and the moral implications of the CRISPR technique.

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1. Introduction

The system known as CRISPR/CRISPR-associated protein 9 (Cas9) is an adaptive immune system that is made up of numerous bacteria and archaea that carry Cas genes, related proteins, and CRISPR loci (Jiang et al., 2024). With the help of the Cas9 protein's nuclease activity, this system can detect exogenous DNA, split the DNA double helix, and allow base addition or deletion with subsequent DNA repair (Zhang et al., 2019). Gene regulation can be classified into transcriptional regulation and post-transcriptional regulation based on the gene expression process (Sorek et al., 2008). DNA regulation at the genetic level and chromatin regulation at the epigenetic level are two categories of transcriptional regulation (Silas et al., 2016). According to Zakrzewska and Burmistrz (2023), the Cas system is regarded as one of the genome editing methods that is helpful in functional genetic research, biotechnological applications, and medical research.

According to Jinek et al. (2012), genome editing technology allows for the precise targeted change of an organism's endogenous genes. To allow for the insertion, deletion, and replacement of certain target DNA sequences, an endonuclease specific to the task is employed to cleave DNA strands (Mali et al., 2013; Cui et al., 2020). Because of its speed and ease of use, CRISPR is changing the process of gene knockout (Nishimashu et al., 2014). The temporal and geographical modulation of CRISPR-Cas activity is still difficult to achieve, despite numerous advancements (Jinek et al., 2012).

Crop development and animal breeding can both benefit from the adaptable CRISPR-Cas9 approach (Li et al., 2024). This emphasizes how far technology has come in incorporating this into practical uses. Additionally, current uses of CRISPR-Cas-based nucleic acid detection in the livestock sector are

highlighted. These uses include the identification of early embryo sex, specificity and composition of meat and dairy products, and developing infectious illnesses (Zhang, 2022).

Currently, there are numerous issues contributing to the decline in animal and agricultural output. Reduced yields may result in a shortage of nutrient-dense food compared to the world's demand (Shin et al., 2017). According to reports in this context, the use of CRISPR-Cas9 technology for genomic editing offers numerous benefits for product development (Jiang et al., 2013). It has been suggested that worldwide starvation can be avoided, particularly by utilizing transgenic-free CRISPR-Cas9 technology and animal breeding-based methods to increase food productivity (Rao et al., 2022).

Up until now, vaccination and antibiotic use have been the primary methods for controlling disease in cattle. On the other hand, chronic antibiotic usage exacerbates diseases, promotes resistance to dangerous microbes, and pollutes the environment (Tian et al., 2021). Furthermore, it seems that some viral diseases cannot be prevented by effective vaccinations (Aslam et al., 2018).

It has been established that the emergence of diseases is influenced by genetic variables, animal care and nutrition, and the production environment (Wang et al., 2022). Consequently, in an effort to make animals more resistant to illness, several scientists have recently focused on enhancing animal genomes (Van, 2019). The efficiency of livestock production will rise with the application of contemporary genetic and molecular biology techniques to enhance animals' immune systems and resistance to disease (Gonen et al., 2017). In fact, research have shown that CRISPR-Cas9 may be a more effective method for improving livestock's genetic makeup (Makarove and Koonin, 2015).

Prior studies utilizing CRISPR-Cas9 have concentrated on bacterial cells. CRISPR technology started to be applied in fields such as agriculture and medicine after (Zhang et al., 2020) publication. In every area of biology, the usage of genome editing technologies has grown quickly (Pickar and Gersbach, 2019). As a result, the CRISPR-Cas9 system has rapidly evolved and grown over the course of the 12 years between 2012 and 2024 (Liao et al., 2021).

Scientists have been able to quickly alter the genomes of mice, rats, fruit flies, and plants like rice and wheat since CRISPR-Cas9 was demonstrated to function in mammalian cells (Chen et al., 2019). Innovation in applied and technological domains, such as health, agriculture, and aquaculture, has also been significantly influenced by technology (Katti et al., 2022).

This study aims to determine the advantages and disadvantages of the CRISPR-Cas9 based method, especially in animal husbandry, and to determine the opinions of academicians working in the field of genetics regarding its applicability in Turkey.

2. Materials and Methods

Experts in a field provide their thoughts and recommendations on a topic using the Delphi Method (Dalkey and Helmer, 1963). In order to achieve this, a series of inquiries about the CRISPR technique and its relevance in Turkey were developed and distributed via Google Forms to specialists who expressed interest in taking part in the research, after the identification of academicians with relevant expertise in the field of genetics. The study's Cronbach's alpha reliability coefficients were computed. According to Cortina (1993), an acceptable result for the dependability coefficient of the initial survey was 0.94, provided the alpha value was greater than 0.70. The study's data came from a survey that was completed with 26 academicians who operate in the field of genetics throughout Turkey and gave their consent to take part. The data from the surveys were analyzed using the SPSS 16 program. The mean, standard deviation, and percentage values of the data were computed and displayed in tables during statistical analysis.

Table 1. Participant individual characteristics

Parameter		n	%
Gender	Woman	9	25.9
	Man	17	74.1
Degree	Prof. Dr.	1	3.8
	Assoc. Dr	7	26.9
	Dr. Lecturer	8	30.8
	Research Assistant	2	7.7
	Expert Dr.	2	7.7
	Expert	6	23.1

Examining Table 1, we see that the experts who took part in the survey were titled as follows: research assistant (n = 2), expert (n = 6), associate Dr. (n = 7), professor (n = 1), and

research assistant (n = 2). According to 74.1 % of participants, the CRISPR technique is not well known in Turkey and is solely used by those in the genetics field (Figure 1).

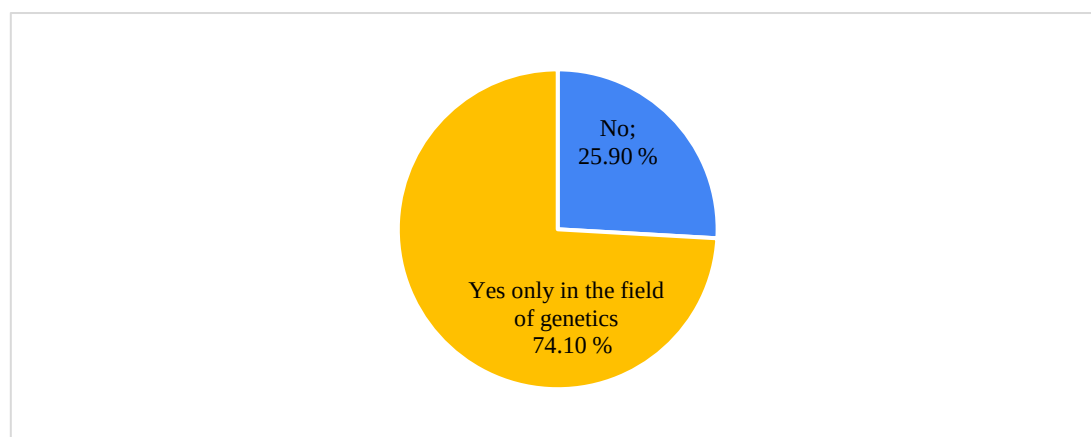


Figure 1. How well-known is CRISPR in Turkey's agricultural and animal husbandry fields?

Table 2. Questions and Answers about Applications of CRISPR

Questions for Surveys	Yes		No	
	n	%	n	%
Is there any promise for treating disorders impacting genetic health and cancer with Crispr cas9?	26	100	0	0
Can improvements in gene editing techniques benefit animal husbandry methods?	26	100	0	0
Can effective gene transfer and long-term stable transgenic expressions made possible by Crispr technology boost animal productivity?	26	100	0	0
Is it possible to prevent diseases in animals using the Crispr method?	22	84.6	4	15.4
Can animal welfare on farms be enhanced by the use of genome editors?	21	80.8	5	19.2

Table 2 provides the responses to the study's inquiries regarding the domains in which CRISPR is used. Table 2 shows that a sizable percentage of experts concur that the CRISPR technique is used in animal breeding for high-yield breeds in livestock (100 %), in the treatment of cancer and many genetic diseases (100 %), in providing resistance and protection

against many infectious diseases (84.6 %), and in animal breeding (100 %). Eighty-eight percent of them said it might be applied to improve animal welfare. Figure 2 displays the participants' perspectives on the usefulness of CRISPR technology in the field of animal husbandry.

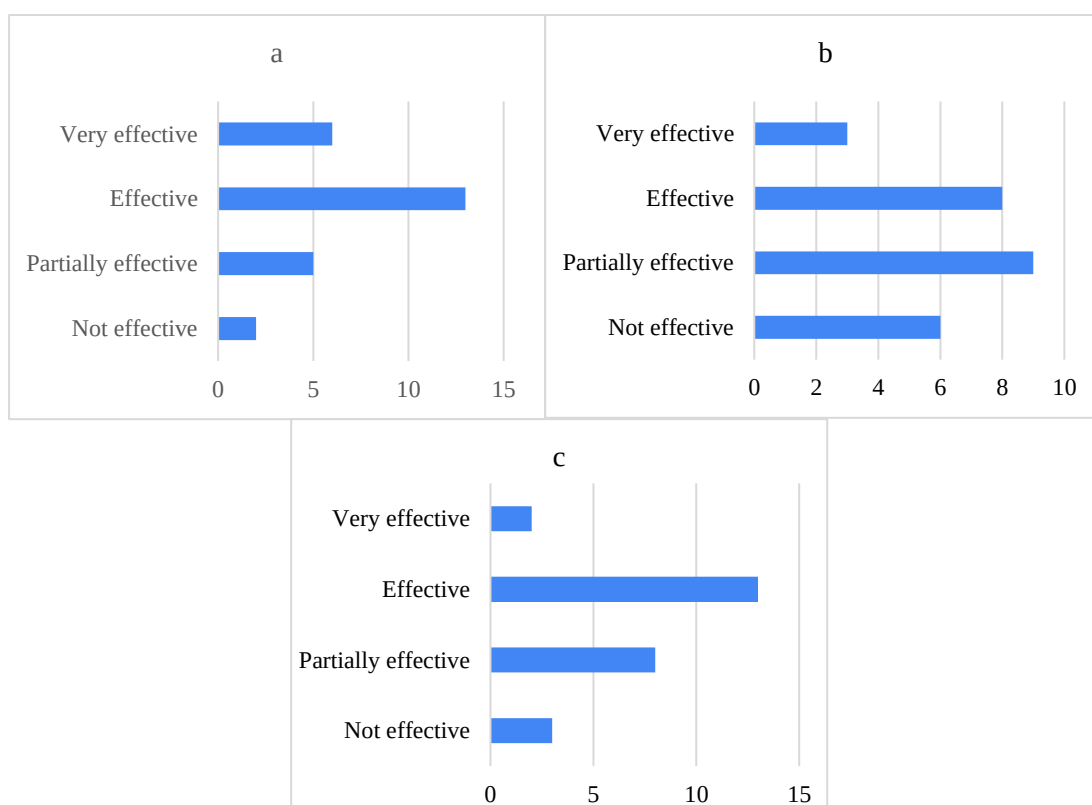


Figure 2. The degree of efficacy of CRISPR technology in animal husbandry applications

Examining Figure 2, one can see that professionals employ the CRISPR technology to produce high-yielding breeds. a- The effect of CRISPR on reaching high-yield breeds, b- The impact of CRISPR in disease control and prevention, c- The impact of CRISPR in improving animal welfare. 7.7 % of respondents say it is ineffective, 19.2 % say it is moderately effective, and 73.1 % say it is

effective. Although 42.3 % of experts believe that the CRISPR approach is efficient in controlling and avoiding diseases, 57.7 % believe that it is an excellent method for enhancing animal welfare. Table 3 presents the views of the professionals who answered the survey with reference to the CRISPR technique.

Table 3. A few viewpoints regarding the CRISPR technique

Questions	yes		no	
	n	%	n	%
Do you believe that farm animals with altered genes will be able to be produced soon?	24	92.3	2	7.7
Could there be problems with food safety with goods derived from genetically engineered animals?	20	76.9	6	23.1
Could there be moral dilemmas with CRISPR/Cas 9?	20	76.9	6	23.1

According to Table 3, 76.9 % of experts stated that goods derived from gene-edited animals are safe for consumption and could potentially raise ethical concerns in the future, despite 92.3 % of experts believing that gene-

edited animal farms will be built soon. Table 4 presents the participants' perspectives regarding the suitability of the CRISPR technique in Turkey.

Table 4. Professional views regarding the suitability of the CRISPR technique in Turkey

Questions	yes		no	
	n	%	n	%
Does Turkey have the necessary infrastructure to use CRISPR technology?	10	38.5	16	61.5
Is it feasible to create and use the CRISPR system in Turkey as a tool for genome editing?	24	92.3	2	7.7
Do you believe Turkey should use the CRISPR method?	24	92.3	2	7.7

Upon examining Table 4, it becomes evident that a mere 38.5 % of the experts believe that Turkey possesses the necessary infrastructure to effectively implement CRISPR technology. However, a substantial majority of responders (92.3 %) stated that

Turkey should develop and apply the CRISPR system. Table 5 lists the benefits and drawbacks of using CRISPR technology in Turkey based on the opinions of the experts that took part in the survey.

Table 5. Lists the benefits and drawbacks of using CRISPR technology

Advantages	n	%	Disadvantages	n	%
Creating breeds with high yields	10	38.5	Using technology in an unethical manner	18	69.2
In the defense against a range of illnesses	7	26.9	genetic illness	4	15.4
prevention of hereditary illnesses for which there is no known cure	6	23.1	Absence of expertise and experience	2	7.7
Increasing the animals' ability to fend off illnesses	3	11.5	Absence of infrastructure	2	7.7
Other	26	100	Other	26	100

The benefits of CRISPR technology, as reported by the participants, when Table 5 is looked at, include producing high-yield breeds through animal breeding, preventing genetic diseases and cancer, making animals more resistant to disease, speeding up and improving the efficiency of production, and boosting the national economy by lowering production costs. The emergence of genetic abnormalities as a result of poor design, inexperience, and lack of infrastructure are among the drawbacks of CRISPR technology.

3. Discussion

To create high-yielding, stress-resistant types, scientists and breeding researchers are putting a lot of effort into their work. When paired with nanotechnology, CRISPR-Cas9 has become a powerful tool for raising the yields and quality of agricultural and animal products (Tsveta et al., 2021). Gene therapy can be used to repair or correct defective or undesirable genes in a cell (Akram et al., 2020). Gene therapy is the best course of treatment, particularly for hereditary illnesses

that are not amenable to medication (Brokowski and Adli, 2019). It is now possible to execute gene therapy on both plants and animals (Akram et al., 2023; Ayanoğlu et al., 2020). The agriculture industry has begun to be the main user of the CRISPR-Cas9 system (Naik et al., 2022). Cas9 technology has been employed in the growth of grains, vegetables, fruits, and nuts with high nutritional value because the production of nutritious goods mostly depends on the fertility of the seed and the condition of the soil (Panahi et al., 2022). In this regard, the CRISPR-Cas9 system, which is presently employed in tobacco, rice and Arabidopsis, offers enormous development potential (Kan et al., 2021). Research on heat-resistant grain production and drought has been moving quickly lately. The study concluded that the livestock industry may employ the CRISPR technology to produce 100 % high-yield breeds of animals through animal breeding. Actually, a lot of study and application of this method has been done recently to enhance animal heredity, reproduction and nutritional levels (Perisse et

al., 2020). Research indicates that improving animal husbandry practices can boost the output of animal products, including meat and milk yield, fertility and egg and wool yield (Zhou et al., 2019). Growth, hairiness and milk-producing qualities have all enhanced as a result of research into changing sheep genomes using gene editing (Zhou et al., 2020). According to a recent article, it is anticipated that lambs resistant to disease would be produced by modifying the *HYAL2* and *PrP* genes using the CRISPR-Cas9 system (Kalds et al., 2019). Expert academicians interviewed claimed that the CRISPR-Cas9 system can be used to increase resistance and protection against a variety of infectious diseases, particularly genetic diseases. In a work carried out concurrently with the results of this investigation, the *NRAMP1* gene was incorporated into the *FA* locus and the bovine homology of the mouse *Rosa26* locus using the CRISPR-Cas9 technology. As a result, it was shown that transgenic cattle had higher resistance to tuberculosis and *M. bovis* infection (Yuan et al., 2021). CRISPR-Cas9 technology has been claimed to have considerable potential, particularly recently, for the development of animal models and cell lines, illness treatment, pharmacological target screening, and targeted therapy (Cox et al., 2015). Recently, the CRISPR gene editing technique has been used to develop sustainable products, eradicate genetic diseases, boost productivity in agriculture and animal husbandry, make living things resistant to environmental stresses, and create individuals who may pass on desired traits to future generations (Zhang et al., 2022). Liu et al. (2013) effectively incorporated the lysostaphin encoding vector using ZFNs in bovine fetal fibroblasts into the native β -casein locus. According to Liu et al. (2013), these genetically altered cows were able to express milk that contained lysostaphin, which relieves mastitis. These gene-edited animals are only commercially available in a few countries due to various laws and restrictions (Gim et al., 2021). In the survey, all participants (100 %) said that high-yield breeds could be produced using the CRISPR approach; 19.2 % said the

method was moderately effective, and 73.1 % said it was highly effective. Concurrent with the findings of this investigation, CRISPR-Cas9 has been effectively employed to enhance the meat production characteristic of diverse cattle through the elimination of the *MSTN* gene (Wang et al., 2015). The stearoyl-CoA desaturase gene was knocked down using CRISPR-Cas9, increasing the nutritious value of milk (Tian et al, 2018; Tian et al., 2022). Goats and sheep using CRISPR-Cas9-mediated fibroblast growth factor 5 gene deletion have better growth and hair qualities (Wang et al., 2015; Zhang et al., 2022). These research demonstrate the enormous potential of CRISPR-Cas9 to enhance contemporary livestock systems. Apart from its high efficiency and focused genome-wide editing methods, the CRISPR approach has been applied recently to control the genetic regulation and function of disease resistance genes in cattle. 42.3 % of participants in the survey said that the CRISPR method was useful in controlling and preventing infections, and 84.6 % of people said that it might be utilized to offer resistance and protection against numerous infectious diseases. In fact, research in the literature has shown that cattle with *NRAMP1* knockouts caused by CRISPR/Cas9 have greater disease resistance (Xu et al., 2020; Burkard et al., 2017; Yuan et al., 2021). A Chinese team created a CRISPR-Cas9 knockout library in 2020. It contained over 85,000 single-stranded RNAs (sgRNAs) that targeted 17,743 protein-coding genes, 11,053 long non-coding RNAs, and 551 microRNAs. The work has led to significant progress in the breeding of disease-resistant animals, and it has been suggested that this breeding technology can help the livestock industry achieve sustainable development (Zhao et al., 2020). In the poultry business, CRISPR-Cas-mediated techniques are employed to get genetic traits that are unachievable through other means, resulting in the development of several genetic variations required in the chicken population (Hellmich et al., 2020). According to reports, CRISPR-Cas technology can be applied to poultry as a different approach to disease prevention (Ishii,

2016). Items derived from animals that have undergone gene editing could potentially jeopardize food safety, according to 76.9 % of survey participants. Government restrictions and public acceptance pose a greater challenge to the promotion of products derived from genetically modified animals than the technology itself (Lander et al., 2019). The introduction of genetically modified items to the consumer market in the past ten years has been met with skepticism by many practitioners. Instead of 92.3 % of the experts in this study predicted that gene-edited animal farms will be built soon, based on the study's findings. The Chinese government has started a number of initiatives to bring genetically modified crops to the public market in accordance with the study's findings (Gao et al., 2017). Predictably, given the rapid advancement of science and the ongoing growth in public awareness, it appears possible that this genetically engineered food derived from animal husbandry may soon be served on regular dinner tables. Indeed, research indicates that as gene editing technology advances, food safety and superiority will rise even higher and people will have access to better, healthier products (Ray et al., 2020; Zhou et al., 2021). The editing of a living organism's genome is one of the most talked-about CRISPR subjects (Schleidgen et al., 2020). Specifically, there is a great deal of controversy in the fields of ethics and legislation around the potential for modifying human DNA (Furtado, 2019). In fact, according to 76.9 % of the researchers surveyed, there may be ethical issues down the road as a result. Many national regulators have restricted or banned human applications due to the assumed risk associated with editing the human genome. For instance, in Germany, it is illegal to modify germline cells artificially; likewise, the National Institutes of Health in the United States has decided not to fund the use of gene editing technologies in human embryos (Locke, 2020). 38.5 % of study participants said that Turkey had the necessary infrastructure in place to use CRISPR technology. According to the literature review, there are eight research centers in Turkey

focused on agricultural biotechnology, and both the R&D teams and the faculty members at universities with relevant departments have access to adequate infrastructure (Bolukbas and Gucukoglu, 2022). It has been asserted, therefore, that additional resources, investments, and unique incentives are required because the current legislative regulations are insufficient in this area (Mali, 2022). The benefits of CRISPR technology, according to this academic study, include producing high-yield breeds through animal breeding, preventing genetic diseases and cancer, boosting animal resistance to illness, speeding up and improving production efficiency, and boosting the national economy by lowering production costs. In fact, Cao (2021) stated the primary benefits of the CRISPR-Cas9 system in parallel with the findings of this investigation, including its excellent specificity, high efficiency, straightforward design, affordable price, and capacity to edit several genes at once. Breeders now have to put in a lot of time and effort to produce plants and/or animals with the right traits. Supplying a growing population with enough food and goods is particularly difficult. It is believed that CRISPR-Cas9 genome editing (GE) using methods based on nanotechnology may be able to overcome these obstacles (Oz et al., 2021). In this case, it is believed that by expanding the CRISPR-Cas9 technology's application space, efficiency may be raised and a greater market demand can be satisfied (Naik et al., 2022). The survey study identified the following as the primary drawbacks of CRISPR technology: inadequate infrastructure, improper design, and the emergence of genetic abnormalities as a result of inexperience. These were classified as ethical issues. Parallel to the results of this study, Tavakoli et al. (2021) noted in their investigation that while there are many positive aspects of this gene technology, there are also drawbacks, such as moral dilemmas involving the disturbance of ecological balance. According to Mao et al. (2019), this approach has certain drawbacks, particularly when it comes to generating mutations with a lot of nucleotides. Although there are

challenges with CRISPR-Cas9 technology, such as the insertion of foreign genes and off-target effects, these challenges are starting to be gradually addressed as a result of advancements achieved by researchers (Gasiunas et al., 2012). In light of the introduction of foreign genes via CRISPR-Cas9 technology, the study discovered that foreign genes can be eliminated by combining sgRNA and Cas9 into a ribonucleoprotein complex, which is the source, or by separating generations to produce offspring who are not transgenic (Rao et al., 2022). It is anticipated that in order to improve it, ever-more-effective optimization techniques will be used (Yang and Wu, 2018). Actually, 92.3 % of respondents to the study stated that Turkey should be the country where the CRISPR system is developed and put into use. Agriculture and animal husbandry may greatly benefit from targeted genome engineering. It is possible to either attain the desired phenotype or inactivate the undesirable trait if one knows the function of a specific gene. Therefore, CRISPR-Cas9 and nanotechnology can be used to easily design goods that can endure biotic and abiotic challenges (Jinek et al., 2012). It takes longer in traditional breeding operations to introduce new features related to quality or disease tolerance. Thus, it can be concluded that genome engineering is moving quickly in the direction of becoming an amazing technology for the production of agricultural and animal goods in the future.

4. Conclusion

CRISPR-Cas is no longer restricted to rupturing DNA strands, since it has led to the creation of a broad range of single-base gene editing, transcriptional regulation, and RNA strand cutting techniques after decades of research and development. Consequently, the majority of human diseases, including cancer, chronic illnesses, and hereditary disorders brought on by a single gene, can be treated using these methods. Indeed, by directly deleting disease-prone genes and pathogen receptor genes or adding disease-resistant genes, breeding using the CRISPR-Cas9 gene editing capability in cells can quickly breed

new animal species with disease-resistant features, according to both the research findings and the literature review. To deliver gene editing tools to sick cells in vivo and employ them in therapeutic applications, a safe, stable, and successful technique must be developed. Additionally, off-target CRISPR-Cas9 impacts have been found to have catastrophic repercussions. In order to better ensure the safety of animal husbandry and breeding, these potential scenarios should be identified and refined prior to clinical applications. Because it is anticipated that gene editing technology will soon produce much more amazing outcomes in animal husbandry and agriculture, providing better, healthier products for all of humanity. As a result, although CRISPR technology has disadvantages as well as advantages, it is of great importance to establish legal regulations that take into account the environment, human health, food safety, international trade and ethical aspects of the CRISPR method for the development and implementation of the CRISPR system in Turkey.

Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Conflict of Interest

All authors declare that there is no conflict of interest related to this article.

Ethical Statement

Ethical approval and permission for this study was obtained from Uşak University non-interventional clinical research ethics committee. (Date: 10.07.2024, Decision No: 410-410-08).

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Aflatoxin Risk in Pepper Crops and Flaked Pepper in Southeastern Anatolia, Turkey

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Abstract

The formation of aflatoxins in agricultural products, especially in peppers used as spices, has been determined that the products are affected by various stages from the field to the spice. These toxins are formed when fungi belonging to the genus *Aspergillus* spp. colonize the plant during the growing stage and produce aflatoxins. Post-harvest processes, especially drying and storage, significantly affect aflatoxin levels. Toxin accumulation can increase when drying processes are not carried out under appropriate conditions, while during storage, factors such as humidity and temperature can promote aflatoxin production. The aim of this study was to determine the risk of aflatoxin in pepper plants and chili peppers used as spices. Various aflatoxin species were detected in the analysis of samples taken from pepper fields and spice shops in Southeastern Anatolia, Turkey. While AFG1 and AFG2 were found in one of the 15 chili pepper samples, AFB2 was detected between 0.0125 and 0.1875 ppb in 11 samples and AFB1 was detected between 0.1125 and 3.95 ppb in 13 samples. Total aflatoxin content ranged from a minimum of 0.15 ppb to a maximum of 4.1375 ppb with an average of 0.9455 ppb. In addition, when the aflatoxin contents of *Aspergillus* spp. fungi obtained from pepper plants taken from the field were analyzed, the presence of AFB1 and AFB2 was detected in some samples. These findings suggest that aflatoxin formation starts at the growing stage of peppers and that harvesting, drying and storage processes can increase toxin levels. Therefore, these processes need to be carefully managed and controlled to minimize aflatoxin risk.

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1. Introduction

Microorganisms can be present in the flora of agricultural products and various foodstuffs, or they can be transmitted to these products in different ways (Bilgili, 2022). Foods undergo changes under favorable conditions by contact with microorganisms in their natural environment or in unhygienic conditions. Fungi constitute a large part of these changes. The presence of fungi in foods and their consumption by humans adversely affects health (Baysal, 1984). These fungi have the ability to produce chemical substances with carcinogenic, teratogenic, mutagenic, and toxic content for humans and animals. These substances are defined as mycotoxins (Viladimir, 1989). Mycotoxins are considered a major problem worldwide public health, agriculture, international trade, and the economy (Özer et al., 2012; Özer et al., 2017b). Approximately 10 % of the world's crops are destroyed each year due to fungal infections (Zhang et al., 2012). Losses due to mycotoxins are only a part of this. Aflatoxins in agricultural products are the most toxic mycotoxins and one of the most important microbiological problems of recent years (Bilgili, 2022).

Aspergillus spp. fungal species are widespread worldwide and can easily contaminate foodstuffs. This is especially a problem in tropical and subtropical climatic zones where environmental conditions such as high humidity and temperature prevail (Adeyeye, 2019). *Aspergillus flavus* fungus can infect field and soil crops such as barley, maize, cottonseed, rice, wheat, peanuts, cocoa, pistachios, hazelnuts, spices, olives, figs, sunflowers and capsicum and can multiply during storage. When environmental conditions are favorable, toxigenic strains of these organisms can produce aflatoxins (Öksüztepe and Erkan, 2016).

Mycotoxins are secondary metabolites of some sub-fungi that grow and multiply in agricultural products at all stages from field to consumption depending on ecological conditions. These toxins are produced by some fungi such as *Aspergillus*, *Penicillium*, and

Fusarium under certain humidity and temperature conditions (Kumar et al., 2008). Aflatoxins are metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* species known as *Aspergillus flavus* group. Aflatoxins, as a type of mycotoxins, are a major concern and pose a serious global health problem due to their high toxicity and widespread availability (Özer et al., 2012; Özer et al., 2017a). The most common mycotoxins are aflatoxin (AF), ochratoxin, trichothecene, zearanol, patulin, cyclopiazonic acid, and fumonisin (Kumar et al., 2008; O'Riordan and Wilkinson, 2008).

The color, taste, and shape of the peppers supplied for the production of red powder and chili peppers, which are consumed as a popular spice in Turkey, broadly (Ateş et al., 1992). The production of pepper products in our country is especially widespread in the Southeastern Anatolia Region, Gaziantep, and its surroundings (Ayda, 2020). In addition, various pepper products are produced from pepper with traditional methods in Şanlıurfa (Altun et al., 2020).

Dried spices are susceptible to fungal growth and toxin formation due to their growing conditions, processing characteristics and sensitivity during storage (Peter and Cotty, 2017). Pepper production is carried out under primitive conditions, increasing the risk of fungal contamination. Peppers are collected from the fields where pepper is grown, cut into small pieces, laid on the soil and dried, then pulverized in the mill and sold in sacks. Peppers produced under these primitive conditions are frequently contaminated with soil-borne fungi with toxicogenic effects and toxin formation (Yıldırım, 1996). However, in developed countries, contamination is kept to a minimum since the drying process is carried out using special devices (Semple et al., 1989). Especially *Aspergillus flavus* can cause problems in many products grown in tropical and subtropical climates. Various studies have indicated that fungi cannot survive in unsuitable conditions after producing toxins, but the toxins they produce can remain in the product (Taydaş, 1993).

Studies have shown that pre-harvest aflatoxin production is caused by climatic stresses such as drought, high temperature and rainfall, climatic conditions that are not suitable for the genotype of the plant and insect damage (Wu and Khlangwiset, 2010). Post-harvest toxin contamination can occur as a result of improper agricultural practices during storage, transportation and in the food processing industry (Halkman, 2013). In red pepper, fungal contamination starts while the fungus is still on the plant and continues until the end of the harvest period and then during the drying process. It has been determined that aflatoxin starts to form when the pepper fruit is on the branch (Ünlütürk and Turantaş, 1998).

Among aflatoxins, aflatoxin B1 is known to have the highest toxic effect (Gürhayta and Çağındı, 2015). Aflatoxins (B1, B2, G1 and G2) have been found to have acute toxic effects on animals used as test subjects. However, aflatoxins have carcinogenic properties rather than acute toxic effects. It was determined that aflatoxin B1 caused liver cancer in test animals. Consumption of aflatoxin-containing foods at certain intervals causes liver tumors in humans and aflatoxins have been found to cause teratogenic and mutagenic effects in a study on animals (Betina, 1989). According to the Turkish Food Codex Regulation on Contaminants (TGK, 2011) and the European Union food legislation (EU, no 165/2010), the permissible limit for aflatoxin B1 is set at 5 ppb and 10 ppb for total aflatoxin (Anonymous, 2010). However, aflatoxin limits vary from country to country, making international trade difficult (Özer et al., 2017b).

This study aims to evaluate the presence of aflatoxins, known for their carcinogenic,

teratogenic, hepatotoxic, and mutagenic effects, in red pepper and to assess their transmission from field to table. Fungal samples collected from the roots of pepper plants in the provinces of the Southeastern Anatolia region of Turkey were analyzed, and soil-borne fungi, including *Aspergillus* spp., *Aspergillus niger*, and *Aspergillus flavus*, were identified (Bilgili, 2017; Bilgili, 2022). Additionally, chili pepper samples were gathered from the spice market in Balıklıgöl, Şanlıurfa. Aflatoxin levels were measured in both the fungal samples taken from the pepper plant roots and in the chili powder samples. The results indicate that fungi originating in the root zone of the pepper plant can be transferred to the fruit through transport and contamination processes. The initial contamination is likely to occur due to conditions in the root zone and can proliferate under favorable environmental conditions. Consequently, this study focuses on the investigation of four types of aflatoxins B1, B2, G1, and G2 which have carcinogenic potential in both pepper plants and chili peppers.

2. Materials and Methods

2.1. Experimental materials and tools

In this study, 9 fungal samples from diseased plants collected from pepper cultivation fields in different provinces of the Southeastern Anatolia region of Turkey were used (Figure 1). In addition, 15 chili pepper samples were taken from spice shops by random sampling method in Balıklı Lake spice shops bazaar in Şanlıurfa province and 50 grams of chili pepper for each sample was used in this research (Figure 2).

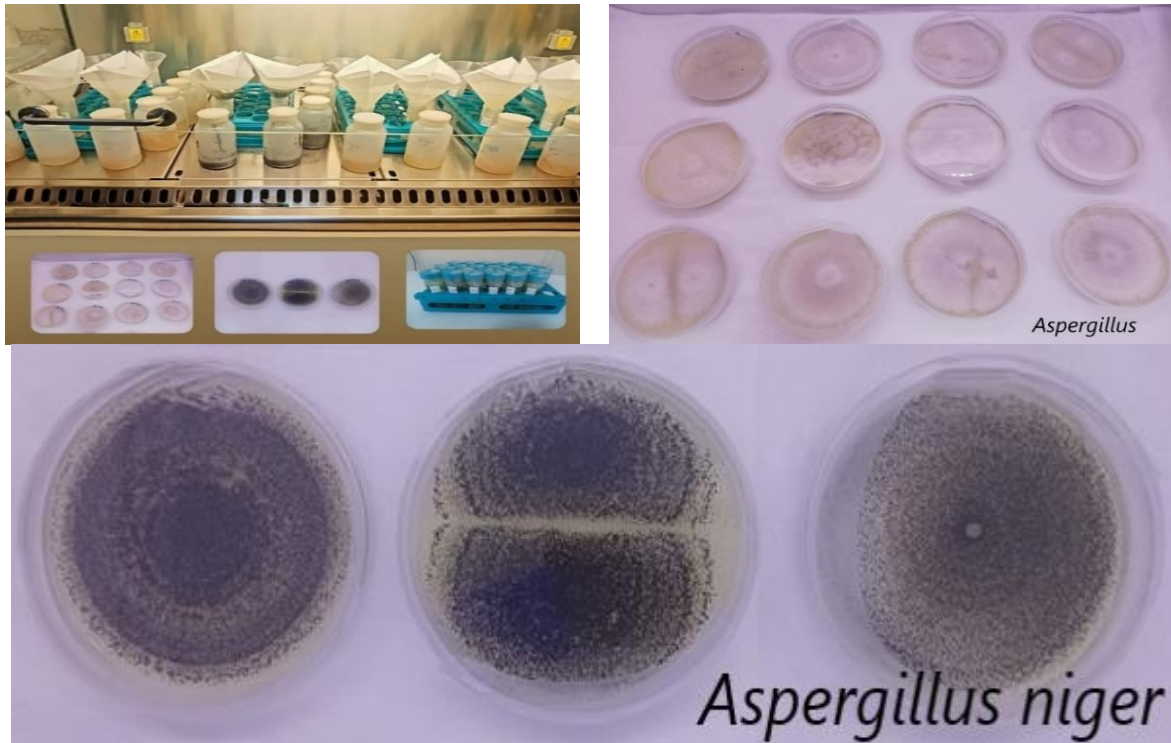


Figure 1. Growth status of fungal isolates 1 week after inoculation on PDA and images from extraction



Figure 2. A sample from the sampling points of chili pepper samples sold in the open and a view from the extraction stage

2.2. Methods

2.2.1. Aflatoxin analysis

Aflatoxin analysis was performed using the HPLC system according to AOAC Official Method 999.07 (Anonymous, 2000). There are three main steps in this analysis: extraction, cleaning, and injection into HPLC.

2.2.2. Preparation of samples for analysis and methods used

The samples taken from Balıklı Lake Spice Bazaar were brought to the laboratory in 100 gram transparent plastic bags and 50 grams of these samples were used for analysis. Fungal isolates were cultured on PDA and incubated at 26 °C for 7 days. In the study, cultivation on PDA, extraction and aflatoxin reading by HPLC method were performed. Sowing of fungal isolates on potato dextrose agar (PDA) was performed according to (Bilgili, 2017). For toxin extraction of the isolates, the method

of Aydın et al. (2008) was used as modified. In this process, *Aspergillus* spp. fungi cultured on agars were transferred to 250 ml tubes. Then 100 ml of 60% (v/v) methanol/water solution was added to the tubes and mixed with a stomacher for 30 min at room temperature. The mixture was filtered using Whatman no.1 filter paper. After filtration, the samples were diluted 1/5 with 60% (v/v) methanol/water and filtered through a 0.45 µm filter attached to the syringe tip. Finally, the samples were submitted to the HPLC-FLD for analysis to determine aflatoxin ratios. Extraction, HPLC

conditions and quantification of aflatoxin in chili peppers were performed according to (Atasoy et al., 2017). The HPLC-FLD used in the analysis is SHIMADZU Nexera XR 20 series and this device measures in the wavelength range of 365-435 nm. *Aspergillus* spp. fungi taken from pepper plants for aflatoxin analysis and the aflatoxin species investigated are given in Table 1. In Table 2, the points where the chili pepper spices sampled for aflatoxin analysis were taken and the aflatoxin species investigated are given.

Table 1. *Aspergillus* spp. and aflatoxin species obtained from the plant for aflatoxin analysis

Sample No	Sample Name	Provinces of Pepper Plant	Aflatoxin Analyzed
1	7 ASPN	Şanlıurfa Center Kephirli Village	B1, B2, G1, G2
2	14 ASP	Şanlıurfa Umut Village	B1, B2, G1, G2
3	30 ASPN	Şanlıurfa Bozova Yaylak	B1, B2, G1, G2
4	33 ASP	Diyarbakır Bismil	B1, B2, G1, G2
5	37 ASPN	Diyarbakır Çınar Yuvacık	B1, B2, G1, G2
6	51 ASP	Diyarbakır Çermik	B1, B2, G1, G2
7	57 ASPN	Batman Kozluk	B1, B2, G1, G2
8	85 ASPF	Gaziantep Yavuzeli, Halilbaş	B1, B2, G1, G2
9	ASP-Urfa	Şanlıurfa	B1, B2, G1, G2

ASP: *Aspergillus* spp., ASPN: *Aspergillus niger*, ASPF: *Aspergillus flavus*

Table 2. Points of collection of chili pepper spices sampled for aflatoxin analysis and aflatoxin species investigated

Sample No	Sample Name	Where chili pepper samples were taken	Aflatoxin Analyzed
1	Sweet chili pepper1	Spice market, workplace number 1	B1, B2, G1, G2
2	Hot chili pepper 2	Spice market, workplace number 2	B1, B2, G1, G2
3	Sweet chili pepper 3	Spice market, workplace number 3	B1, B2, G1, G2
4	Hot chili pepper 4	Spice market, workplace number 4	B1, B2, G1, G2
5	Hot chili pepper 5	Spice market, workplace number 5	B1, B2, G1, G2
6	Sweet chili pepper 6	Spice market, workplace number 6	B1, B2, G1, G2
7	Hot chili pepper 7	Spice market, workplace number 7	B1, B2, G1, G2
8	Hot chili pepper 8	Spice market, workplace number 8	B1, B2, G1, G2
9	Sweet chili pepper 9	Spice market, workplace number 9	B1, B2, G1, G2
10	Hot chili pepper 10	Spice market, workplace number 10	B1, B2, G1, G2
11	Sweet chili pepper 11	Spice market, workplace number 11	B1, B2, G1, G2
12	Hot chili pepper 12	Spice market, workplace number 12	B1, B2, G1, G2
13	Hot chili pepper 13	Spice market, workplace number 13	B1, B2, G1, G2
14	Sweet chili pepper 14	Spice market, workplace number 14	B1, B2, G1, G2
15	Hot chili pepper 15	Spice market, workplace number 15	B1, B2, G1, G2

3. Results and Discussion

The aflatoxin contents of *Aspergillus* spp. fungi obtained from pepper plants are given in Table 3. When the contents of *Aspergillus* spp. fungi from different provinces of the Southeastern Anatolia region were analyzed,

AFB1 and AFB2 were detected in 2 samples. The presence of *Aspergillus* spp. fungi in pepper plants indicate that aflatoxins begin to form at the stage when the peppers grow in the field. Aflatoxin formation may also continue with the harvesting method, drying and storage processes. These stages may cause an increase

in aflatoxin levels. İnanç (2024), aflatoxin formation in spicy red peppers starts while the fruits ripen on the plant due to the effect of fungi transmitted from air or soil. The harvesting method also affects this situation; because for economic reasons, all peppers are

usually harvested at one time. This can cause early ripe fruits to remain on the plant for a long time and increase aflatoxin formation. Furthermore, aflatoxin content is directly related to subsequent processing such as storage, drying, and packaging.

Table 3. Aflatoxin amounts of *Aspergillus* spp. fungi obtained from pepper plants

	AFB1 (ppb)	AFB2 (ppb)	AFG1 (ppb)	AFG2 (ppb)	TOTAL AFs (ppb)
7ASPN	-	-	-	-	-
14 ASP	-	-	-	-	-
30ASPN	-	-	-	-	-
33ASP	-	-	-	-	-
37ASPN	-	-	-	-	-
51ASP	-	-	-	-	-
57ASPN	-	-	-	-	-
85ASPF	1094.38	23.35	-	-	1117.73
ASP-Urfa	2034.43	18.35	-	-	2052.78

ASP: *Aspergillus* spp., ASPN: *Aspergillus niger*, ASPF: *Aspergillus flavus*, -: not detected

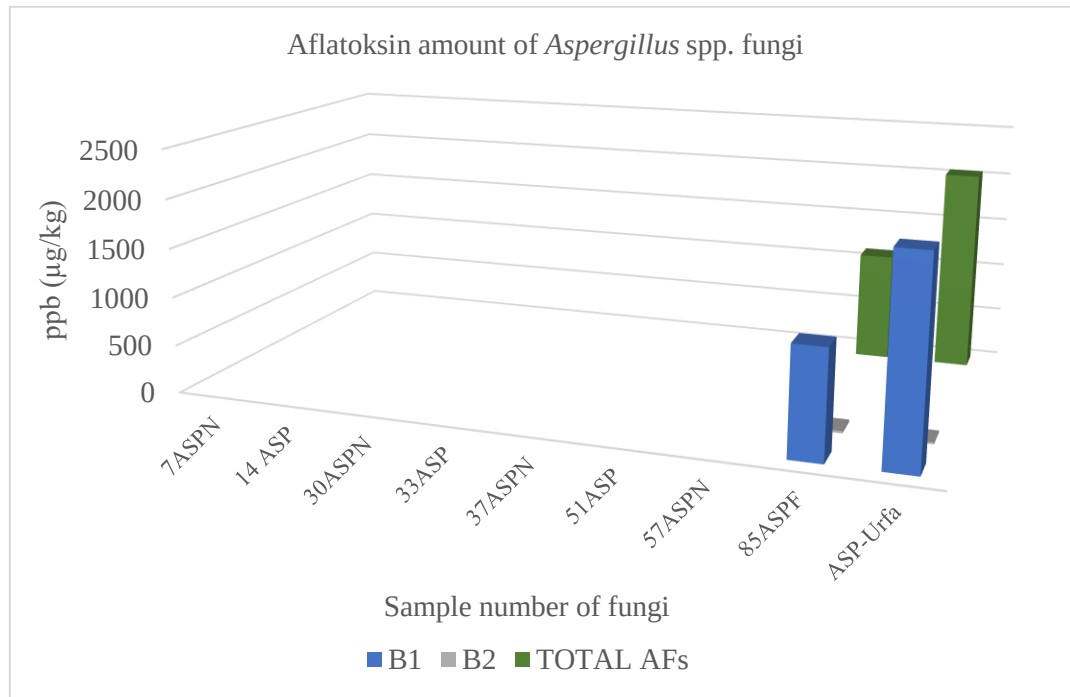


Figure 2. Graph of aflatoxin content of *Aspergillus* spp. fungi obtained from pepper plants

The aflatoxin contents of the chili pepper samples analyzed are given in Table 4. It was determined that 1 of the samples did not contain any aflatoxin. The presence of AFG1 and AFG2 was detected in 1 of the 15 chili pepper samples taken from the spice bazaar. AFB2 was detected in 11 of the analyzed samples and their amount was determined to be between 0.0125 and 0.1875 ppb. AFB1 content of chili pepper samples was found to vary between 0.1125- 3.95 ppb. Total aflatoxin

content of chili pepper spices was found to be at least 0.15 ppb, maximum 4.1375 ppb and average 0.9455 ppb. Ardiç et al. (2008) investigated the presence of aflatoxin in 75 isot samples and found that 72 samples contained AFB1 and these amounts ranged between 0.11 and 24.7 ppb. In a study conducted by Erdoğan (2004) using thin layer chromatography, 20 isot samples were searched for aflatoxin and aflatoxin (B+G) was detected in only 1 sample and its value was reported as 13.8 ppb. Atasoy

et al. (2017), in a study conducted with 20 isot spices, no aflatoxin was detected in 1 sample, and the presence of G1 and G2 was not detected in any of the 20 isot samples. B2 was detected in 6 samples and was determined to

be between 0.02 -1.09 ppb and B1 content in 19 samples ranged between 0.02 - 8.45 ppb. Total aflatoxin content ranged between 0.02 - 9.54 ppb and the average aflatoxin content was 1.52 ppb.

Table 4. Detected aflatoxin amounts of chili pepper spice

	AFB1 (ppb)	AFB2 (ppb)	AFG1 (ppb)	AFG2 (ppb)	TOTAL AFs (ppb)
Pepper1	0.3625	0.0375	-	-	0.4
Pepper2	0.3375	0.025	-	-	0.3625
Pepper3	0.525	0.0375	-	-	0.5625
Pepper4	1.3	0.025	-	-	1.325
Pepper5	3.95	0.1875	-	-	4.1375
Pepper6	0.2125	0.0125	-	-	0.225
Pepper7	0.5125	0.025	-	-	0.5375
Pepper8	0.15	-	-	-	0.15
Pepper9	-	-	-	-	-
Pepper10	0.225	-	-	-	0.225
Pepper11	1.275	0.025	-	-	1.3
Pepper12	0.7625	0.0625	-	-	0.825
Pepper13	0.5875	0.0125	-	-	0.6
Pepper14	1.3625	0.1	0.9625	0.1625	2.5875
Pepper15	0.1125	-	-	-	0.1125
Minimum	0.1125	0.0125	0.9625	0.1625	0.1125
Maximum	3.95	0.1875	0.9625	0.1625	4.1375
Average	0.7783	0.0366	0.9625	0.1625	0.89

-: not detected

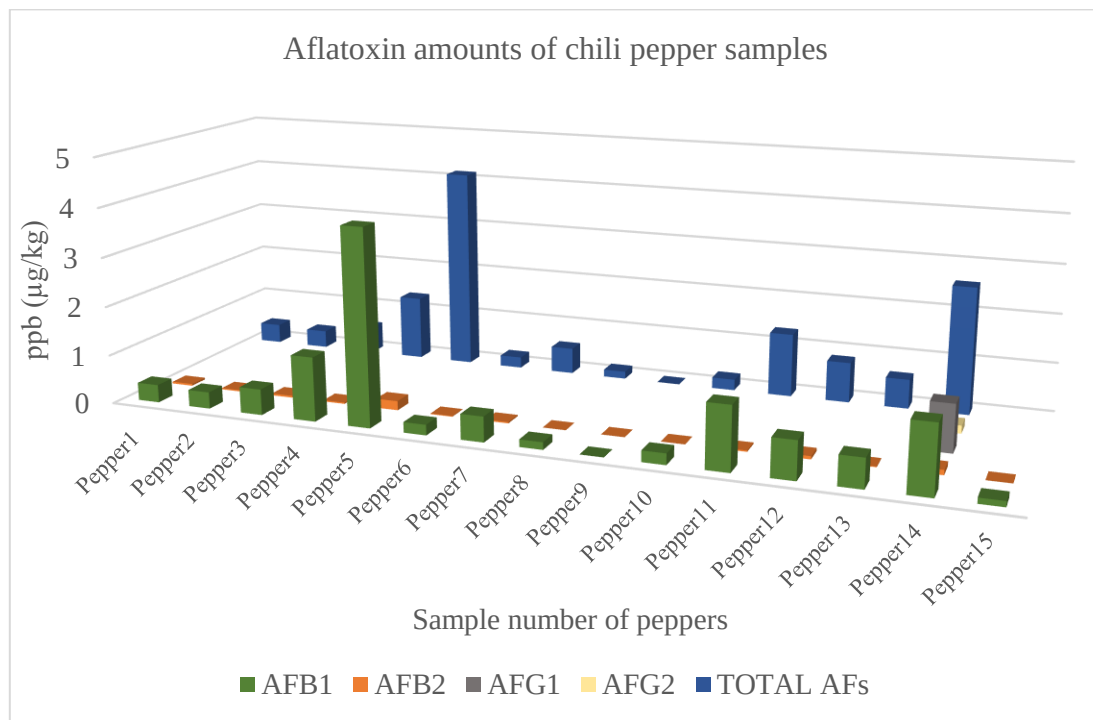


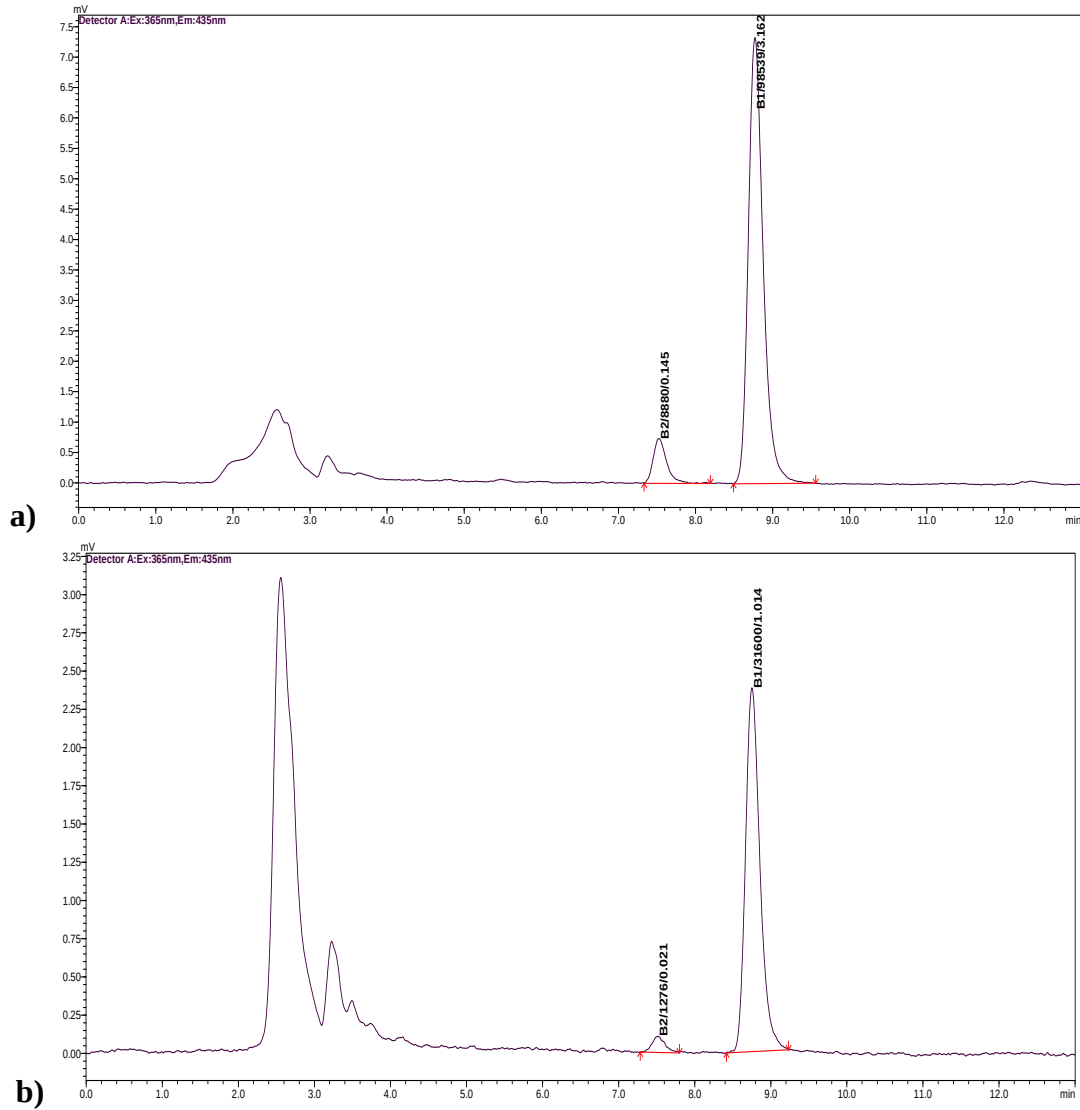
Figure 3. Graph of detected aflatoxin content of chili pepper spice

Graphs showing the aflatoxin types and amounts of fungal samples taken from the root of pepper plant and chili pepper samples are given in Figure 2 and Figure 3. When the

graphs of fungal samples taken from pepper root are analyzed, aflatoxins in 85 ASPF and ASP- Urfa samples were B1: 1094.38 ppb, B2: 23.35 ppb, total AFs: 1117.73 ppb and B1:

2034.43 ppb, B2: 18.35 ppb, total AFs: 2052.78. When the graph of chili pepper samples is analyzed, it is seen that while aflatoxin was detected in all 14 samples, it did not exceed the limit value determined for B1 and total AFs. However, this situation has the potential to change over time depending on the

ambient conditions of aflatoxin. Accordingly, depending on the ambient conditions, aflatoxin content may decrease or increase. Chromatogram graphs showing aflatoxin peaks of some samples are presented in Figure 4.



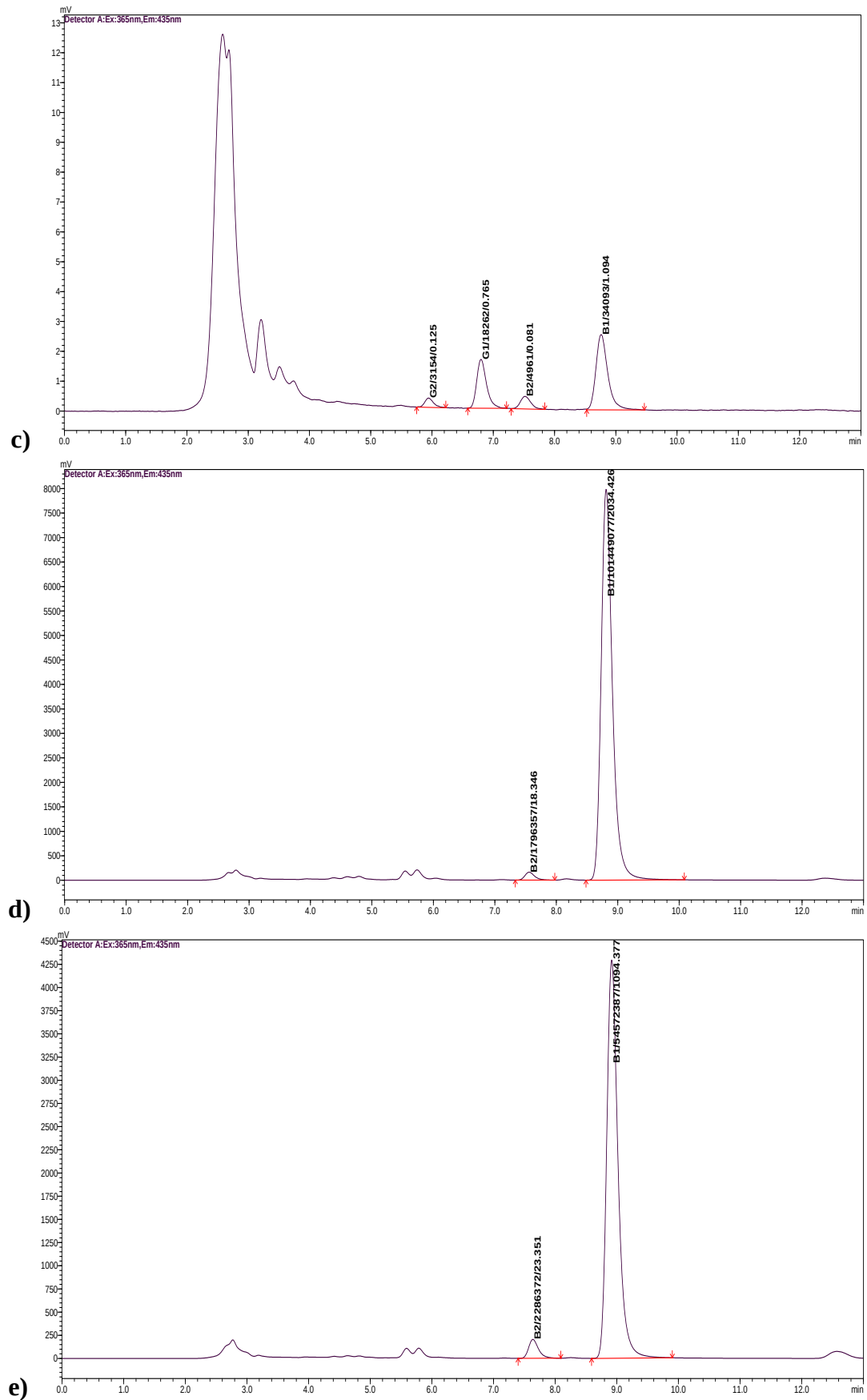


Figure 4. Chromatogram plots showing aflatoxin peaks of some samples (Chromatogram plots a) Pepper 5, b) pepper 11, c) pepper 14, d) ASP-Urfa, e) 85ASPF)

In general, the aflatoxin content of chili peppers is quite low; however, the fact that some samples are close to the limit values may be due to the variety and quality of the fresh pepper used, the place and method of production. In addition, the difference in production times and the duration of production stages may also affect these differences. Atasoy et al. (2016) reported that the aflatoxin content in dry flaked isot spice produced under controlled conditions was very low. According to the Turkish Food Codex Regulation on Contaminants (TGK, 2011) and the European Union food legislation (EU, no 165/2010), the permissible limit for Aflatoxin B1 is set at 5 ppb and for total aflatoxin at 10 ppb (Anonymous, 2010). It was determined that chili pepper samples did not exceed the limit values in terms of AFB1 and total AFs. Atasoy et al. (2017), none of the analyzed samples exceeded the legal limits in terms of total aflatoxin amount. In a study conducted on 75 isot samples, it was found that 14.7% of the samples did not comply with the standards set in terms of AFB1 (Ardıç et al. 2008). Fresh peppers used in the production of chili pepper spice have a high water content and need to be dried for a long time to obtain the original color of isot. However, aflatoxin formation is kept under control as the water activity of the peppers decreases rapidly during the drying process. In addition, the concrete floor used during production accelerates the drying process by increasing the temperature of the peppers due to sunlight. Atasoy et al. (2016), In their study, they supported the current study and stated that aflatoxin content in dry flaked isot seasoning produced under controlled conditions was very low. The amount of aflatoxin in pepper spices can vary depending on many factors. Among these, the type and water activity of the dried pepper (Marin et al., 2009), drying temperature and climatic conditions (Cho et al., 2008) play an important role. It is also stated that prolonged drying time is also effective in aflatoxin formation and the amount of aflatoxin may increase with increasing drying time (Inan et al., 2007). In recent years, extensive research has been conducted on the detoxification of toxins in

foods. Pankaj et al. (2018) reviewed the methods applied to reduce aflatoxin levels in various foods. These methods include conventional heating, microwave, chemical methods (e.g. lactic acid, hydrogen peroxide, ozone or ozonated water), radiation (such as X, ultraviolet or gamma rays), electrolyzed water and cold plasma treatments. Park et al. (2007) reported the complete degradation of AFB1 on glass substrate within 5 seconds using microwave argon plasma at atmospheric pressure. However, these methods are not yet economically efficient and are generally not sufficiently successful. The most effective method to ensure food safety for consumers is government-led inspections. Out-of-bounds levels of aflatoxins in food pose a similar problem not only in Turkey but also in many other countries (Koutsias et al., 2021; Tsehaynesh et al., 2021). There are also studies on similar issues related to red pepper in Turkey (Demir et al., 2019; Coşkun and Ünsal, 2020). Aflatoxin B1 levels were found between 0.20-79.37 µg/kg and total aflatoxin levels were found between 0.22-93.05 µg/kg in red pepper samples taken from spice trading companies in Malatya province (Uğur, 2022). In a systematic review and meta-analysis of the presence of Aflatoxin B1 in red pepper, the lowest AFB1 concentration was 0.14 µg/kg in Korea and the highest concentration was 31.13 µg/kg in Turkey (Sevdin et al., 2021). It was determined that 28% (7 samples) of the red chili pepper samples offered for sale in Çukurova and Eastern Mediterranean Region did not meet the standards for AFB1 levels. In addition, total aflatoxin was detected in 16% (4 samples) of these samples (Hepsağ and Hayoğlu, 2022).

4. Conclusion

In conclusion, AFB1 was detected in 14 chili powder samples at concentrations ranging from 0.1125 to 3.95 ppb, and AFB2 was found in 11 samples at levels between 0.0125 and 0.1875 ppb. In one sample, AFG1 and AFG2 were detected at 0.9625 ppb and 0.1625 ppb, respectively. AFB1 and AFB2 were also detected in the fungal samples taken from the pepper roots, specifically in the 85ASPF and

ASP-Urfa samples. The 85ASPF sample contained 1094.38 ppb of AFB1, 23.35 ppb of AFB2, and a total aflatoxin (AFs) concentration of 1117.73 ppb. Similarly, the ASP-Urfa sample contained 2034.43 ppb of AFB1, 18.35 ppb of AFB2, and a total of 2052.78 ppb AFs. Based on these findings, the chili powder samples did not exceed legal aflatoxin limits. However, the high levels of AFB1 and AFB2 detected in the fungal samples from the pepper roots indicate the presence of aflatoxins originating in the field and persisting under favorable conditions. The formation of aflatoxins in agricultural products, especially in peppers used as spices, is affected by various stages in the process from field to spice. Aflatoxins are formed as a result of molds belonging to the genus *Aspergillus* spp. colonizing on the plant during the growing phase of the plants and producing aflatoxins as metabolic products. Post-harvest processes also significantly affect the levels of these toxins; in particular, drying processes are a determining factor in the presence and concentration of aflatoxins, and drying under inappropriate conditions can increase the accumulation of toxins. In the storage process, factors such as humidity and temperature can promote aflatoxin production. Although the methods applied in the processing stages have the potential to reduce the presence of aflatoxins, their effectiveness depends on the processing conditions. Therefore, the presence of aflatoxins in food poses a serious risk to both public health and food safety. To prevent the formation of aflatoxins, all processes from agricultural production to final consumption must be carefully managed and controlled. At this point, it is recommended that studies on factors such as production and storage conditions should be carried out and planned in the future.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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Kadmiyum Toksisitesinin Adaçayı (*Salvia officinalis* L.) Bitkisinin Bazı Fizyolojik ve Biyokimyasal Özelliklerine Etkisi

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Özet

Yetiştirme ortamındaki yüksek seviyede bulunan kadmiyum (Cd) bitkilerde fizyolojik bozulmalara neden olup bitki gelişimini olumsuz etkilemektedir. Bu çalışmada, farklı Cd dozlarında (0 (kontrol), 100 ve 200 ppm) yetiştirilen Adaçayı (*Salvia officinalis* L.) bitkisinin fizyolojik, oksidatif stres belirteçleri ile antioksidan enzim aktiviteleri incelenerek bitkinin Cd stresine karşı gösterdiği tepki ve tolerans belirlenmiştir. Cd uygulaması yapılan bitkilerin gelişimi incelendiğinde kontrole kıyasla gövde ve kök yaş ve kuru ağırlıkları önemli ölçüde azalmıştır. Özellikle 200 ppm Cd uygulaması, bitki kuru ağırlığında % 79, kök kuru ağırlığında ise % 58 oranında azalmaya neden oldu. Yüksek seviyede Cd uygulaması, kontrole kıyasla klorofil a, klorofil b ve karotenoid içeriğini sırasıyla % 44, % 65 ve % 54 oranında düşürdü. Ayrıca yaprak bağlı su içeriği kontrole kıyaslandığında 100 ppm Cd ile % 15 ve 200 ppm Cd uygulaması ile % 33 oranında azaldı. Oksidatif stresin önemli göstergeleri olan hidrojen peroksit, malondialdehit ve elektrolit sızıntısı seviyeleri, Cd uygulamaları ile önemli artış gösterdi ve bu parametrelerde maksimum değerler 200 ppm Cd uygulamasında elde edildi. 200 ppm Cd antosiyanin içeriğini % 115, fenolik madde içeriğini ise % 47 oranında arttırdı. Ayrıca bitkilerin Cd ile muamele edilmesi antioksidan enzim aktivitelerinde önemli değişikliklere neden oldu. 200 ppm Cd ile muamele edilen bitkilerin, peroksidaz (1.8 kat) ve katalaz (3.1 kat) aktivitelerinde artış gözlemlenirken, askorbat peroksidaz aktivitesinde önemli bir azalma kaydedilmiştir. Bu çalışma, adaçayı bitkisinin Cd toksisitesine karşı gösterdiği fizyolojik ve biyokimyasal tepkilerini ortaya koymuş ve sonuçlar adaçayı bitkisinin 100 ppm düzeyindeki Cd'a karşı direnç gösterdiğine işaret etmektedir. Çalışma Cd ile kirlenmiş topraklarda adaçayı bitkisinin ıslah materyali olarak kullanılmasında önemli bilgiler sunmaktadır.

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Anahtar Kelimeler

Cd toksisitesi
adaçayı
oksidatif stres
antioksidan enzimler

Effect of Cadmium Toxicity on Some Physiological and Biochemical Properties of the Sage Plant (*Salvia officinalis* L.)

Abstract

High cadmium (Cd) levels in the growing environment cause physiological deterioration in plants and negatively affect plant development. In this study, physiological, oxidative stress indicators and antioxidant enzyme activities of Sage (*Salvia officinalis* L.) plants grown at different Cd doses (0 (control), 100, and 200 ppm) were examined to determine the plant's response and tolerance to Cd stress. When the development of plants treated with Cd was discussed, the fresh and dry weights of stems and roots were significantly reduced compared to the control. Specifically, applying 200 ppm Cd resulted in a 79 % reduction in plant dry weight and a 58 % reduction in root dry weight. Application of high Cd concentrations decreased chlorophyll a, chlorophyll b, and carotenoid contents by 44 %, 65 %, and 54 %, respectively, compared to the control. Furthermore, the relative water content of leaves decreased by 15 % at 100 ppm Cd and 33 % at 200 ppm Cd compared to the control. The levels of hydrogen peroxide, malondialdehyde, and electrolyte leakage, which are important indicators of oxidative stress, showed a significant increase with Cd applications. The maximum values of these parameters were achieved with a Cd dose of 200 ppm. 200 ppm Cd increased anthocyanin content by 115 % and phenolic content by 47 %. Furthermore, Cd treatment of plants resulted in significant changes in the activities of antioxidant enzymes. While an increase in peroxidase (1.8-fold) and catalase activities (3.1-fold) was observed in plants treated with 200 ppm Cd, a significant decrease in ascorbate peroxidase activity was observed. This study revealed the physiological and biochemical responses of sage to Cd toxicity and the results suggest that sage is resistant to 100 ppm Cd. The study provides important insights into the use of sage as a remediation material in Cd-contaminated soils.

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antioxidant enzymes

1. Giriş

Tarım alanlarındaki topraklar hatalı gübreleme, yoğun pestisit kullanımı, kirli sulama suları ve endüstriyel atıklar nedeniyle ağır metallerle kirlenmektedir (Abd Elnabi ve ark., 2023). Bitkilerin optimum büyüme ve gelişmesi için gerekli bir besin elementi olmayan ağır metallerin yüksek konsantrasyonları bitkilerde fitotoksisteye neden olmaktadır. Ağır metallerin bitkiler üzerindeki toksik etkileri, metalin cins ve miktarına, bitkinin türüne, toleransına, maruz kalma süresine bağlı olarak değişmektedir (Yadav, 2010). Bitki dokularında yüksek oranda biriken ağır metaller bitkide, fotosentez ve klorofil sentezini, enzim aktivitesini ve iyon dengesini olumsuz etkilenmektedir. Ağır metaller bitkide membran hasarına, su ve hormon dengesinin bozulmasına neden olur; hücre büyümesi durur, kök ve sürgün gelişimi azalır. Stoma hareketleri ve yaprak alanı etkilenir, klorofil sentezi azalır, genç yapraklarda kıvrılma ve sararma gözlenir. Kök gelişimi azaldığından, bitkinin iyon alımı ve beslenmesi olumsuz etkilenir (Goyal ve ark., 2020; Karakas ve ark., 2021b).

Ağır metallerden biri olan kadmiyum (Cd), doğada saf halde bulunmayan, topraklarda hareketliliği nedeniyle bitkiler tarafından kolayca alınan, uzun yarılanma ömrü olup ve çok düşük konsantrasyonlarda bile son derece toksik olan önemli bir kirleticidir (Beduk ve ark., 2022; Li ve ark., 2023). Cd, özellikle fosforlu gübreler veya antropojenik kaynaklar yoluyla topraklara bulaşmaktadır. Yetiştirme ortamında artan Cd konsantrasyonu, bitki klorofil içeriğini, besin elementi alımını olumsuz etkileyerek bitki büyüme ve gelişimini sınırlandırmaktadır (Shafiq ve ark., 2019; Shiyu ve ark., 2020; Haider ve ark., 2021). Ayrıca gelişme ortamındaki Cd'un hafif bir artışı azot metabolizması (Shahid ve ark., 2019) ve karbonhidrat metabolizmasını da olumsuz etkilemektedir (Sabir ve ark., 2019).

Yetiştirme ortamında bulunan Cd bitkilerde oksidatif strese neden olan reaktif oksijen türlerinin oluşumuna (ROT) yol açar (Kanu ve ark., 2019; Jawad Hassan ve ark., 2020; Wu ve ark., 2020). Stres altında üretilen bu ROT'lar,

çekirdek, proteinler, zarlar ve metabolitler gibi farklı hücresel yapıların zarar görmesine neden olur ve sonuç olarak bitkilerde hücre ölümüne yol açar (Hussain ve ark., 2019; Xie ve ark., 2019). Bitkiler ROT'ların olumsuz etkileri ile başa çıkmak için katalaz, peroksidaz, süperoksit dismutaz ve askorbat peroksidaz gibi enzimatik antioksidanlar ile antosiyaninler, askorbik asit, karotenoidler, a-tokoferoller, indirgenmiş glutatyon (GSH), flavonoidler ve fenolik bileşikler gibi enzimatik olmayan antioksidanlar içeren kompleks bir savunma mekanizmasına sahiptir. Bitkilerde stres toleransı bu antioksidanları yüksek aktivite/seviyesi ile yakından ilişkilidir.

Adaçayı (*Salvia officinalis* L.) tıbbi ve aromatik bitkiler grubunda yer alan, çok yıllık, yaprak dökmeyen ve çalimsı bir bitkidir. Doğada kendiliğinden de yetişebilen bu bitki, günümüzde ülkemizin yanı sıra orta Avrupa, Arnavutluk ve Amerika'da yetişmektedir. Bu çalışmada; farklı dozlarda Cd (0, 100 ve 200 ppm) uygulamasının Adaçayı bitkisi üzerindeki fizyolojik ve biyokimyasal etkiler incelenerek bitkinin Cd stresine göstermiş olduğu toleransı bitki büyüme parametreleri, fotosentetik pigmentler, oksidatif stres belirteçleri ve antioksidan enzim aktiviteleri ile ortaya konmuş, böylece yüksek dozdaki Cd stresine karşı bitkinin tolerans durumu belirlenmiştir.

2. Materyal ve Yöntem

Bu çalışma Harran Üniversitesi, Ziraat Fakültesinde yarı kontrollü cam serasında yürütülmüştür. Adaçayı bitki (*Salvia officinalis* L.) tohumları Torf ve Perlit karışımı ortamda çimlendirilmiştir. Harran Üniversitesi Ziraat Fakültesi AR-GE alanından alınan topraklar kullanılmıştır. Araştırma toprağının özellikleri Tablo 1'de belirtilmiştir. Alınan topraklar kurutulup elendikten sonra 1.3 kg tartılarak 1.5 L kapasiteli plastik saksılara aktarılmıştır. Çimlenen bitkiler 4-5 yapraklı fide durumuna geldiğinde her bir saksıya bir fide aktarılmıştır. Temel gübreleme için 100 ppm N (Üre) ve 50 ppm P (KH₂PO₄) uygulanmıştır. Farklı dozlardaki Cd uygulamaları için CdCl₂ kaynağından

hazırlanan 0 ppm (kontrol), 100 ppm ve 200 ppm Cd içeren sulama suları hazırlanmış ve ilgili saksılara haftada 3 kez sulanmıştır. Bitkilerde uygulamadan 4 hafta sonra

sonlandırılmıştır. Cd etkisi ve toleransını belirlemek için bitkide bazı fizyolojik ve biyokimyasal analizler yapılmıştır.

Tablo 1. Araştırma toprağının genel özellikleri ve Cd içeriği

Derinlik	EC (dS m ⁻¹)	pH	Bünye	Kireç (%)	OM (%)	Cd (mg kg ⁻¹)
0-30	1.18	7.75	Kil	23.45	0.73	0.035

2.1. Bitki yaş ve kuru ağırlığı

Hasat edilen Adaçayı bitki üst aksamı (dal yaprak) ve köklerin taze ağırlıkları hassas terazide belirlenmiştir. Bitkilerden eşit miktarda alınan taze örnekler biyokimyasal özellikleri incelenmek üzere (-20 °C) muhafaza edilmiştir. Geri kalan bitki kısımları 70 °C’de etüvde 72 saat bekletildikten sonra kuru ağırlıkları kaydedilmiştir (Karakas, 2013).

2.1. Yaprak bağıl su içeriği

Taze bitki yapraklarının taze ağırlığı (TA) alındıktan sonra aynı bitki yaprakları 6 saat süresince petri kaplarında deiyonize su içinde bekletilmiş ve yaprakların turgorlu ağırlıkları (TA) alınmıştır. Daha sonra aynı yapraklar 70 °C’ de 72 saat kurutulduktan sonra kuru ağırlıkları (KA) alınmış ve yaprak bağıl su içeriği (RWC) denklem 1 ile hesaplanmıştır (Barrs ve Weatherley, 1962).

$$\%RWC = \frac{TA - KA}{TA - KA} \times 100 \quad \text{Denklem 1}$$

2.2. Bitkilerde klorofil ve karotenoid analizi

Adaçayı bitkisi klorofil a, klorofil b içerikleri Arnon (1949), karotenoid Rajput ve Patil (2017) tarafından önerilen yöntemler dikkate alınarak belirlendi. Taze Adaçayı yaprağı (0.5 g) 5 ml % 80 aseton:su (v/v) karışımında homojenize edilen örnekler filtreden geçirildikten sonra karışımın Klorofil a içeriği 663.5 nm, klorofil b içeriği 645 nm, karotenoid içeriği 480 nm ve 510 nm dalga boyunda % 80 aseton kontrolüne karşı UV mikropola te spektrometrede (Epoch, SN: 1611187, ABD) absorbans değerleri belirlenmiştir. Sonuçlar mg g⁻¹ taze ağırlık olarak ifade edilmiştir.

2.3. Oksidatif stres ile ilişkili parametrelerin belirlenmesi

Taze bitki yaprakları % 0.1 trichloroasetik asit (TCA) ile homojenize edilmiş ve 10.000 g’de 5 dakika süreyle santrifüj edilmiştir. Lipid peroksidasyonu (MDA) için homojenat’dan (1000 µl) alınarak yeni bir tüpe aktarılmış ve üzerine 4 ml % 20 TCA ile hazırlanmış % 5’lik tiyobarbiturik asit eklenmiştir. Karışım 30

dakika 95 °C sıcak su banyosunda bekletilmiş ve ardından buz banyosunda soğutulmuştur. Karışım 532 nm ve 600 nm’deki dalga boylarında UV mikropate spektrometre ile absorbansları belirlenmiştir. Sonuçlar MDA nmol g⁻¹ taze ağırlık olarak belirtilmiştir (Karakas ve ark., 2021a; Weisany ve ark., 2012). Bitki Hidrojen peroksit (H₂O₂) seviyesi Loreto ve Velikova (2000) yöntemi ile küçük değişiklikler yapılan Karakas ve ark. (2021a) ile belirlenmiştir. Yaprak örnekleri (0.5 g) 5 mL % 0.1 TCA ile ekstre edildi. Ekstraktan 0.5 ml alınarak, üzerine 0.5 ml 10 mmol L⁻¹ K-fosfat tampon (pH 7.0) ve 1.5 ml 1 M KI ilave edilerek karışımın absorbans değerleri 390 nm’ de UV mikropate spektrofotometresinde belirlendi. Sonuçlar µmol g⁻¹ taze ağırlık olarak ifade edildi. Elektrolit sızıntısı (% ES) için 0,1 g yaprak 10 ml deiyonize su içeren tüplere aktarıldı. Tüpler 32 °C 2 saat inkübasyona bırakıldıktan sonra EC1 ölçüldü. Bu aşamadan sonra 121 °C 20 dk. Bekletildikten sonra EC2 ölçüldü ve sonuçlar EC/EC2*100 formülü ile hesaplandı (Dionisio-Sese ve Tobita, 1998).

2.4. Antioksidan enzim aktivitelerinin tayini

Peroksidaz (POD) enzim aktivitesi (E.C.1.11.1.7), bazı küçük düzenlemeler ile (Karakas ve ark., 2021b) Cvikrova ve ark. (1994) yöntemi kullanılarak belirlendi. Taze yaprak örnekleri (0.5 g) 10 mL 50 mmol L⁻¹ Na-fosfat tampon çözeltisinde (pH 7.0) homojenize edildi. Daha sonra, 10 µL süpernatant (50 mmol L⁻¹ Na-fosfat, 13 mmol L⁻¹ guaiacol ve 5 mmol L⁻¹ H₂O₂) içeren 290 µL reaksiyon karışımına eklendi ve bir dakikalık aralıklarla 470 nm'de UV mikroplate spektrofotometrede absorbansları belirlendi. Sonuçlar ünite mg⁻¹ T.A. olarak belirtildi. Katalaz (CAT) enzim aktivitesi (EC 1.11.1.6), Aebi (1984) yönteminde yapılan küçük değişiklikler (Karakas ve ark., 2021b) ile belirlendi. Analiz için, yukarıda elde edilen homojenat 5 µL alınarak 50 mmol L⁻¹ Na-fosfat tamponu, 10 mmol L⁻¹ H₂O₂ ve 4 mmol L⁻¹ Na₂EDTA içeren 295 µl reaksiyon karışımına eklendikten sonra 30 saniye süresince 240 nm'de UV mikroplate spektrofotometrede absorbansları belirlendi. Sonuçlar ünite mg⁻¹ T.A. olarak belirtildi. Askorbat peroksidaz (APX) enzim aktivitesinin belirlenmesinde Nakano ve Asada (1981) tarafından bildirilen prosedür uygulanmıştır. Reaksiyon karışımının (10 µl süpernatant, 50 mmol L⁻¹ K-fosfat, 0,5 mmol L⁻¹ H₂O₂, 500 µmol L⁻¹ askorbik asit ve 1 mmol L⁻¹ EDTA) 290 nm'deki absorbans değişimi 3 dakika boyunca ölçüldü. Enzim aktivitesi askorbatın ekstinksiyon katsayısı kullanılarak hesaplandı (2.8 mM⁻¹ cm⁻¹).

2.5. Antosiyanin ve fenolik madde içeriğinin belirlenmesi

Antosiyaninin belirlenmesi için 0.3 g taze bitki yaprağı üzerine 10 ml metanol (% 1 HCl içerir) ilave edilerek +4 °C 24 saat inkübasyona bırakıldı. Inkübasyon sonunda 10000 rpm'de santrifüj edilen örnekler 530 ve 657 nm spektrofotometrede absorbansları

belirlendi. Toplam antosiyanin miktarı (A530-0.25)*A657 ile hesaplanarak mg g⁻¹ taze ağırlık olarak hesaplandı (Mita ve ark., 1997). Toplam fenolik madde tayini ise Shetty ve ark. (1995) tarafından bildirilen yöntem ile belirlendi. Taze bitki yaprağına (0.5 g) 5 ml % 80 metanol (% 1 HCl içerir) ilave edilerek 95 °C 30 dakika süreyle inkübasyona bırakıldı. Daha sonra örnekler santrifüj edilip filtre edildi. Elde edilen ekstraktan 300 µl alınıp üzerine 1.5 ml fenol (% 10) ve 1.2 ml Na₂CO₃ ilave edilip 15 sn çalkalandı. Karışım 40 °C 30 dakika bekletildikten sonra absorbansları 760 nm'de spektrofotometrede okundu. Sonuçlar gallik asit ile oluşturulan bir standart kurve ile hesaplandı ve mg gallik asit g⁻¹ TA olarak ifade edildi.

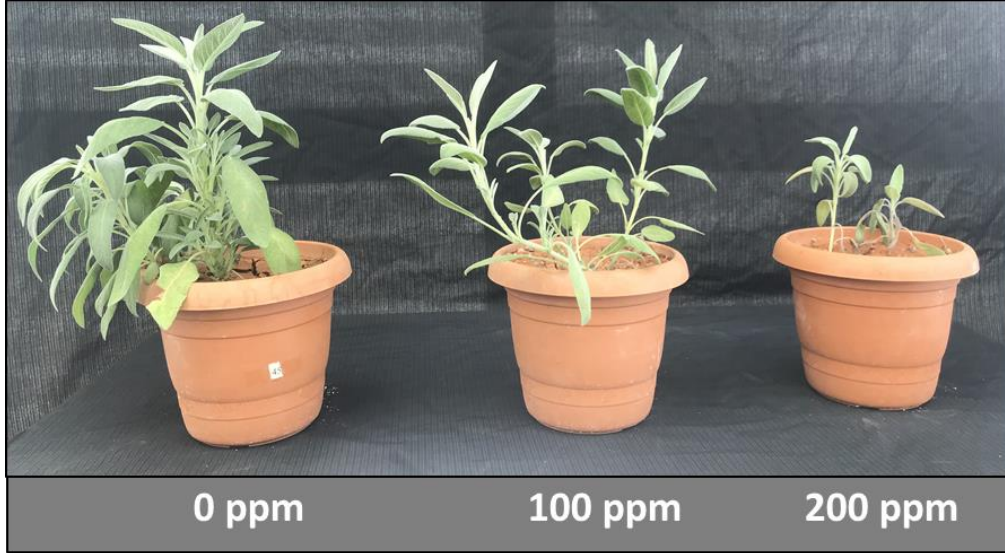
2.6. Verilerin değerlendirilmesi

Elde edilen tüm veriler SPSS (Version 22.0) programı kullanılarak varyans analizleri (ANOVA) ve Duncan Çoklu Karşılaştırma Testi ile P<0.05 önem düzeyinde analiz edilmiş ve grafikler halinde sunulmuştur.

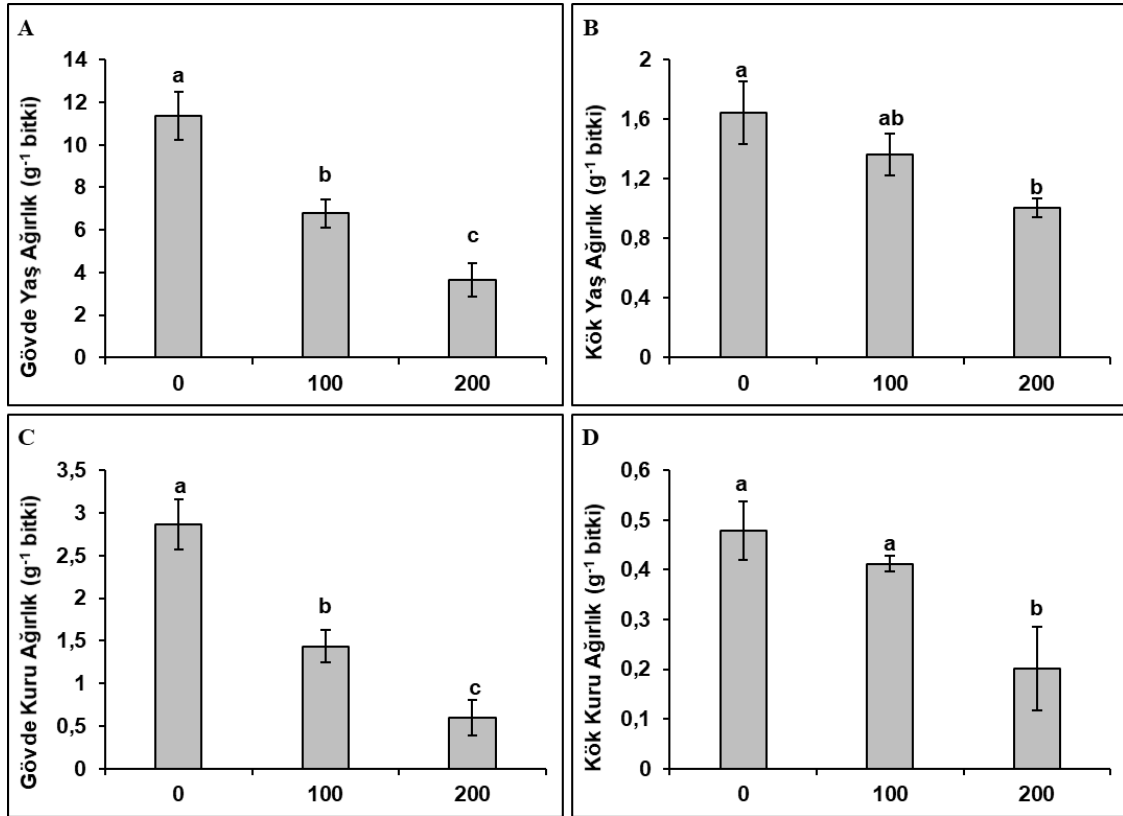
3. Bulgular ve Tartışma

3.1. Bitki fizyolojik özellikleri

Adaçayı bitkisinin fizyolojik özellikleri üzerine artan dozlarda Cd uygulamasının etkileri istatistiksel olarak önemli bulunmuştur (P<0.05). Artan dozda Cd maruz kalan Adaçayı bitkisinde büyüme ve gelişme kontrole kıyasla önemli derecede azalmıştır (Şekil 1). Gövde yaş ağırlığı kontrole kıyasla 100 ppm Cd uygulaması ile % 40 ve 200 ppm Cd uygulaması ile % 68 oranında, kök yaş ağırlığı ise sırasıyla % 17 ve % 39 oranında azaldı. Artan dozlarda Cd muamalesi, bitki gövde kuru ağırlığının sırasıyla % 50 ve 79 oranında, kök kuru ağırlığının ise sırasıyla % 14 ve 58 oranında azalmasına neden oldu. En düşük bitki yaş ve kuru ağırlıklar 200 ppm Cd uygulamasında belirlendi (Şekil 2A, 2B, 2C ve 2D).



Şekil 1. Artan Cd (Cd; 0, 100 ve 200 ppm) uygulamalarına maruz kalan Adaçayı bitkisinin fizyolojik görünümünden bir kesit



Şekil 2. Artan Cd (Cd; 0, 100 ve 200 ppm) uygulamalarına maruz kalan Adaçayı bitkisinin gövde yaş ağırlığı (A), kök yaş ağırlığı (B), gövde kuru ağırlığı (C) ve kök kuru ağırlığı (D). Hata çubukları her bir uygulamanın standart hatasını göstermektedir (n=3). Harfler uygulamalar arasındaki istatistiksel farklılıkları ($p < 0.05$) belirtmektedir.

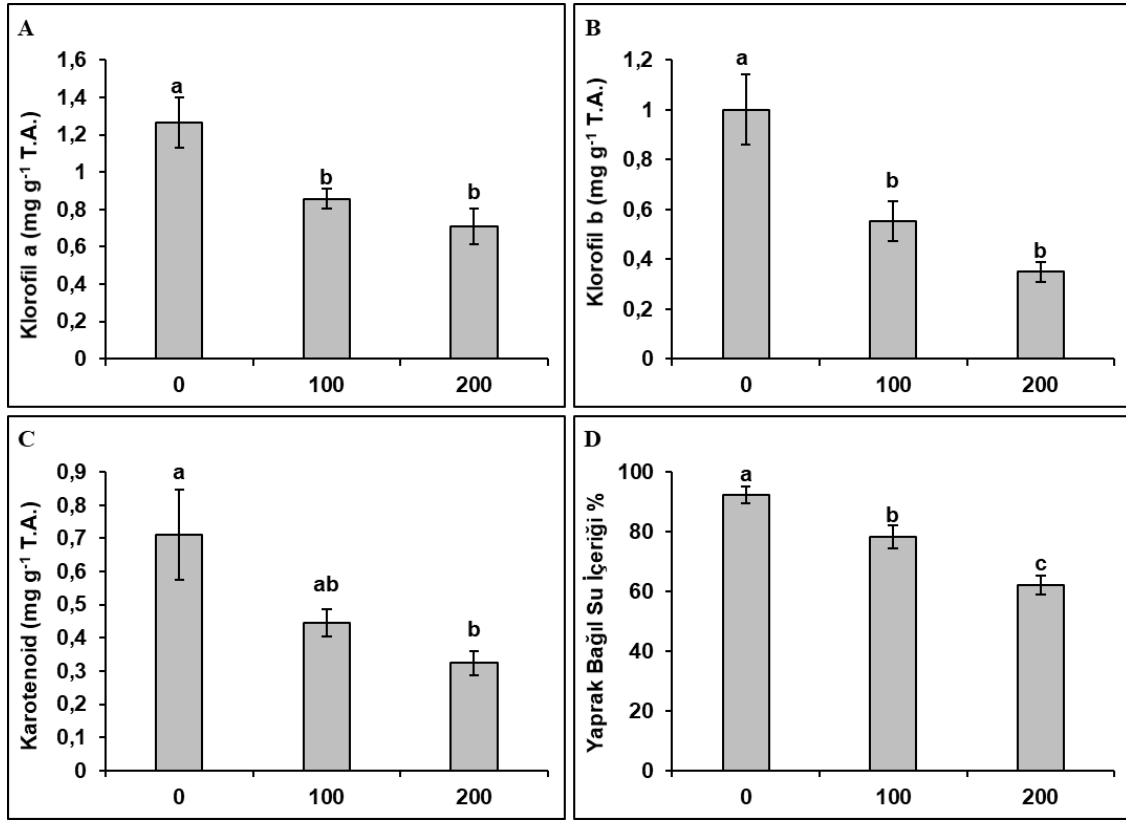
3.2. Fotosentetik pigment, karotenoid ve yaprak bağıl su içeriği

Adaçayı bitkisinin Cd ile muamele edilmesi bitki klorofil a ve klorofil b içeriğinin kontrole oranla önemli düzeyde azalmasına neden olmuş, ancak 100 ppm ve 200 ppm Cd muameleleri arasında istatistiksel açıdan farklılık bulunmamıştır ($P < 0.05$). Farklı

düzeyde Cd uygulamalarının adaçayı bitkisinin karotenoid ve yaprak bağıl su içeriğine etkisi istatistiksel olarak önemli bulunmuştur ($P < 0.05$). Cd uygulaması kontrole kıyasla klorofil a içeriğinin sırasıyla % 32 ve % 44, klorofil b içeriğinin sırasıyla % 45 ve % 65, karotenoid içeriğinin ise sırasıyla % 37 ve % 54 oranında azalmasına neden

olmuştur (Şekil 3A, 3B ve 3C). Cd uygulamaları yaprak bağıl su içeriğinin önemli düzeyde azalmasına neden oldu ve en düşük

yaprak bağıl su içeriği 200 ppm Cd doz uygulamasında belirlendi (Şekil 3D).

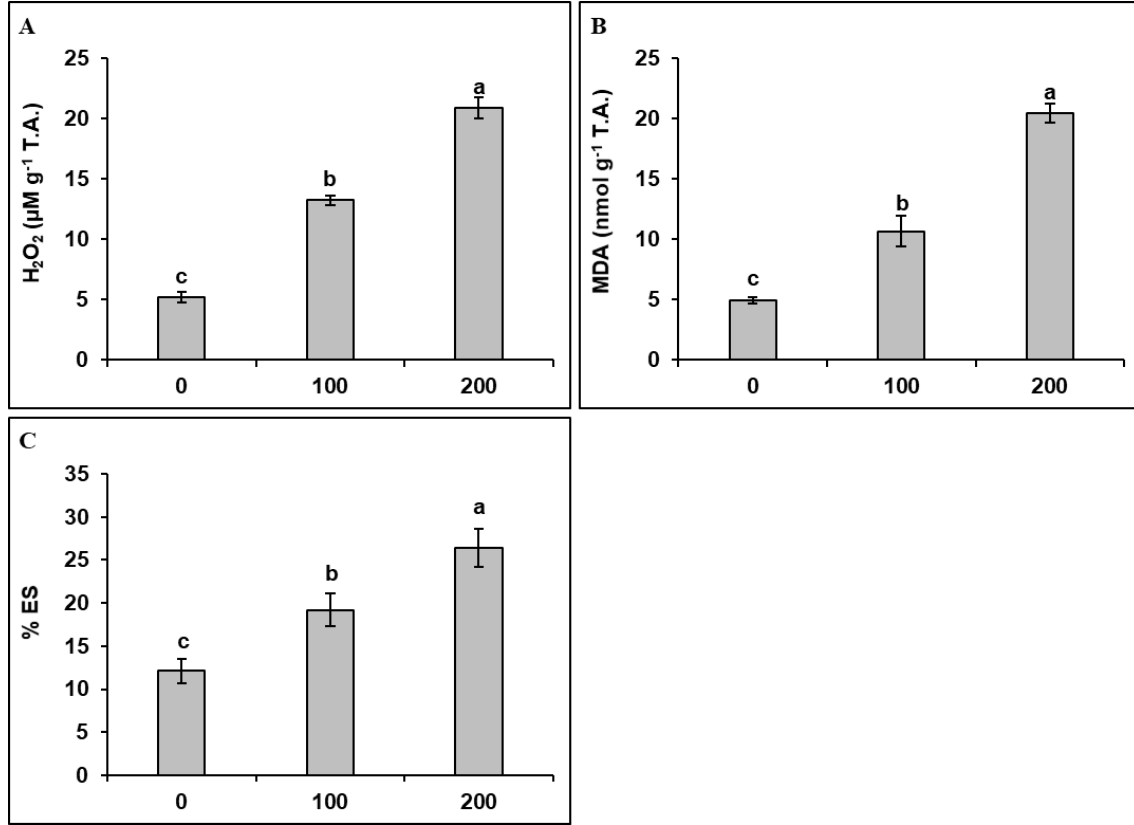


Şekil 3. Artan Cd (Cd; 0, 100 ve 200 ppm) uygulamalarına maruz kalan Adaçayı bitkisinin klorofil a (A), klorofil b (B), karotenoid (C) ve yaprak bağıl su içeriği (D). Hata çubukları her bir uygulamanın standart hatasını göstermektedir (n = 3). Harfler uygulamalar arasındaki istatistiksel farklılıkları (P<0.05) belirtmektedir.

3.3. Oksidatif stres ile ilişkili parametreler

Bitkide önemli oksidatif stres göstergeleri olana hidrojen peroksit (H₂O₂), malondialdehit (MDA) ve elektrolit sızıntısı (% ES) içerikleri Adaçayı bitkisine uygulanan Cd ile kontrole kıyasla önemli düzeyde artış göstermiş, Muameleler arasındaki farklılıklar istatistiksel olarak önemli bulunmuştur (P<0.05). (Şekil

4A, 4B, 4C). Adaçayı bitkisinde 100 ve 200 ppm Cd dozları kontrole kıyasla, H₂O₂ içeriğinin sırasıyla 2.5 ve 4 kat, MDA içeriğini ise sırasıyla 2.2 ve 4 kat, % ES içeriğinin yaklaşık olarak sırasıyla 1.5 ve 2.2 kat artmasına neden olmuştur. Yukarıda belirtilen oksidatif stres ile ilişkili bu parametrelerde en yüksek değer 200 ppm Cd uygulamasında belirlenmiştir.

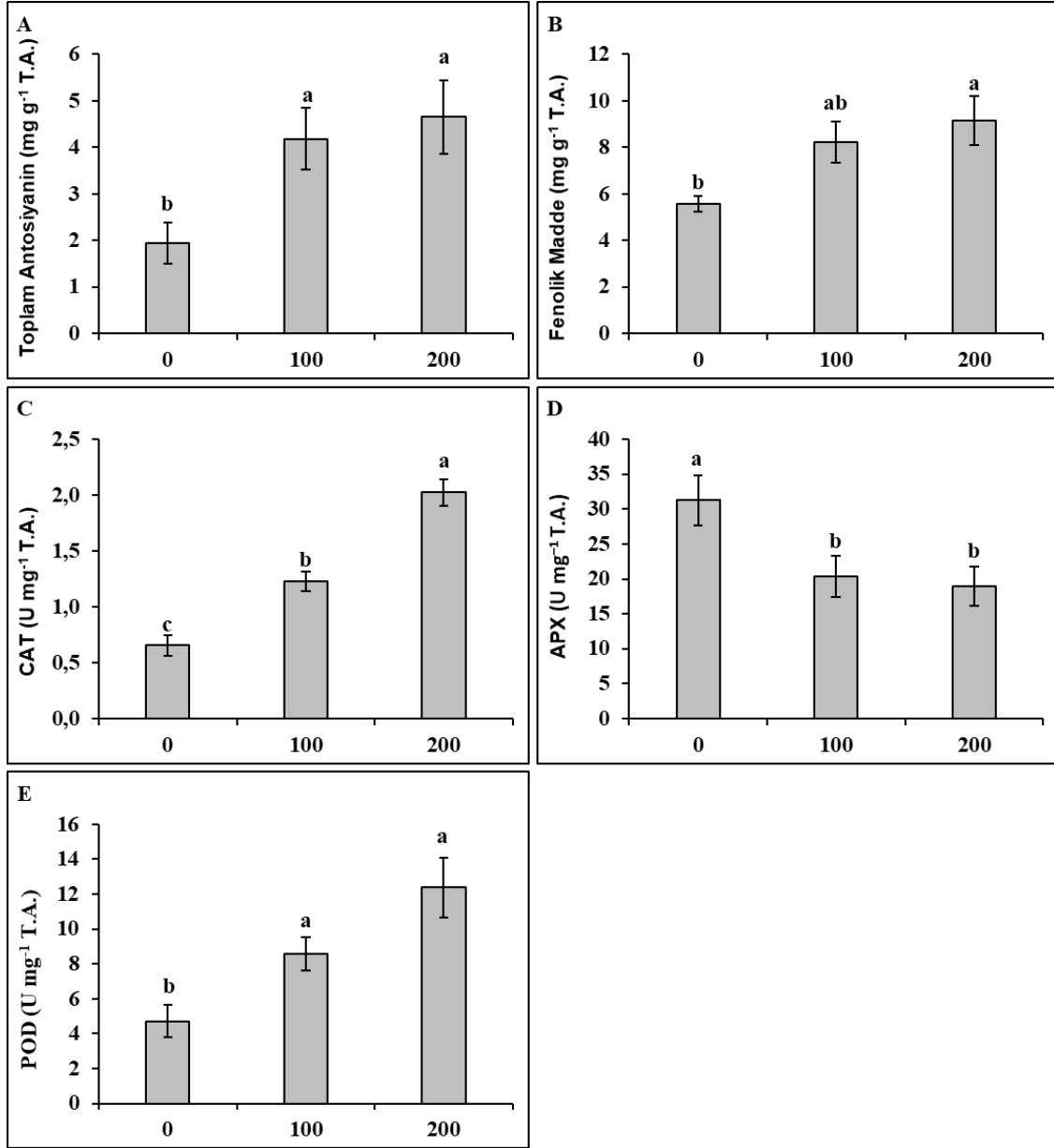


Şekil 4. Artan Cd (Cd; 0, 100 ve 200 ppm) uygulamalarına maruz kalan Adaçayı bitkisinin H₂O₂ (A), MDA (B), ES (C) değerleri. Hata çubukları her bir uygulamanın standart hatasını göstermektedir (n=3). Harfler uygulamalar arasındaki istatistiksel farklılıkları (P<0.05) belirtmektedir.

3.4. Antosiyanin ve fenolik madde içeriği

Farklı düzeyde Cd uygulamalarının adaçayı bitkisinin fenolik madde içeriğine etkisi istatistiksel olarak önemli bulunmuştur (P<0.05). Cd uygulaması ile kontrole kıyasla artan antosiyanin içeriği 100 ppm ile 200 ppm Cd muameleleri arasında istatistiksel açıdan fark görülmemiştir (P<0.05). Antosiyanin içeriği 100 ppm Cd uygulaması ile % 115 ve 200 ppm Cd uygulaması ile % 140 oranında, fenolik madde içeriği ise sırasıyla % 47 ve % 64 oranında kontrole kıyasla artmıştır (Şekil 5A, 5B). Stres altındaki adaçayı bitkilerinin antioksidan enzim aktivitelerinde önemli değişimlere yol açtı. Adaçayı

bitkilerinin 100 ve 200 ppm Cd ile muamelesi POD enzimi aktivitesini sırasıyla 1.8 ve 2.6 kat, CAT enzimi aktivitesini sırasıyla 1.9 ve 3 kat arttırdı, ancak askorbat peroksidaz (APX) aktivitesinin sırasıyla 1.5 ve 1.7 kat azalmasına neden oldu. Farklı düzeyde Cd uygulamalarının adaçayı bitkisinin POD ve CAT enzim aktivitelerine etkisi istatistiksel olarak önemli bulunmuştur (P<0.05). Adaçayı bitkisinin Cd ile muamele edilmesi APX enzim aktivitesinin kontrole kıyasla önemli düzeyde azalmasına neden olmuş, ancak 100 ppm ve 200 ppm Cd muameleleri arasında istatistiksel açıdan farklılık bulunmamıştır (P<0.05, (Şekil 5C, 5D ve 5E).



Şekil 5. Artan Cd (Cd; 0, 100 ve 200 ppm) uygulamalarına maruz kalan Adaçayı bitkisinin antosiyanin (A) ve fenolik madde (B) içeriği, CAT (C), APX (D) ve POD (E) enzim aktiviteleri. Hata çubukları her bir uygulamanın standart hatasını göstermektedir (n = 3). Harfler uygulamalar arasındaki istatistiksel farklılıkları (P<0.05) belirtmektedir.

Cd, bitki metabolizmasında yararlı rolü olmayan bir ağır metaldir. Bitkiler Cd stresine maruz kaldığında büyüme ve gelişimde gerileme, bodurlaşma en yaygın görülen belirtilerendir. Cd toksisitesi bitkilerde bitki büyümesinin ve biyokütle veriminin azalmasına neden olur (Anlı ve ark., 2022). Çalışmamızda, Chen ve ark. (2021) ayçiçeği, Çelim ve Gülser (2020) fasulye, Hasan ve ark. (2019) domates ve Kaur ve ark. (2022) hardal bitkisinde bildirdiğine benzer bulgular gözlemlenmiş, Cd stresi adaçayı bitkisi fizyolojik gelişiminde azalmalara neden olmuştur. Bitki büyümesinin azalmasının

muhtemel nedeni klorofil sentezinin azalması ve fotosentezin inhibe olmasının yanı sıra Cd'a maruz kalan köklerdeki büyümenin engellenmesi ve bitkilerin bulunduğu ortamdan su ve besin element alımındaki sınırlama ile ilişkilendirilebilmektedir (Gonçalves ve ark., 2009; Ismael ve ark., 2019). Cd stresi, klorofil içeriğini ve fotosentetik aktiviteyi olumsuz etkiler ve sonuçta bitki büyümesini engeller (Karakas ve ark., 2021b). Araştırmamızda da Cd toksisitesi bitkide klorofil ve karotenoid sentezini etkilemiş ve klorofil a, klorofil b ve karotenoid içeriğinin azalmasına yol açmıştır. Benzer

sonuçlar diğer araştırmacılar tarafından da bildirilmiştir (Aamer ve ark., 2018; Ali ve ark., 2018; Li ve ark., 2019; Faraz ve ark., 2020; Karakas ve ark., 2021b). Yaprak bağıl su içeriği bitki su durumunun değerlendirilmesinde önemli bir parametredir ve stres koşullarında olumsuz etkilenir (Elsayed ve ark., 2011). Cd toksisitesinin yaprak bağıl su içeriği üzerindeki olumsuz etkisi birçok araştırmacılar tarafından da rapor edilmiştir (Li ve ark., 2019; Sadeghipour, 2020; Ozfidan-Konakçı ve ark., 2022). Benzer sonuçlar bu çalışmada da belirlenmiş olup Cd stresi ile birlikte yaprak bağıl su içeriğinin azaldığı görülmüştür. Kadmiyum toksisitesi, oksidatif stresin önemli göstergeleri olan H_2O_2 ve MDA seviyelerinin artışına ve hücre zarı bozulmasının bir sonucu olan elektron sızıntısına (ES) yol açar (Kaya ve ark., 2019; Jawad Hassan ve ark., 2020). Araştırmamızda artan Cd toksisitesine bağlı olarak H_2O_2 , MDA ve % ES değerleri önemli düzeyde artmıştır. Yüksek Cd varlığı nedeniyle meydana gelen oksidatif stres, bitkilerdeki normal fizyolojik ve moleküler mekanizmaların bozulmasına yol açarak bitkilerin büyümesini engellediği bildirilmiştir (Bashri ve ark., 2016). Araştırma sonuçlarımızda, Cd toksisitesine maruz kalan adaçayı bitkilerinde, oksidatif stres ile ilişkili parametrelerdeki artış ve azalan biyokütle verimi belirgindi. Bitkiler, stresin azaltılması veya tolere edilmesi için reaktif oksijen türlerinin detoksifikasyonunu sağlayan çeşitli antioksidanlara sahiptirler. Antosiyaninlerin, mezofil hücrelerinin vakuollerinde ROS temizleyicileri olarak işlev gördükleri bildirilmiştir (Moustaka ve ark., 2020). Ayrıca antosiyanin-şelatlı metal kompleksleri oluşturarak, bitkilerde ağır metal stresine karşı daha yüksek bitki toleransını sağlayan koruyucu bir mekanizma olduğu da rapor edilmiştir (Chadzinikolau ve ark., 2017). Araştırma sonuçlarımız, Cd stresi altındaki adaçayı bitkilerinin antosiyaninler ve fenolikler gibi enzimatik olmayan antioksidanların birikiminin arttığı görülmüştür. Benzer sonuçlar diğer araştırmacılar tarafından da rapor edilmiştir (Khanna ve ark., 2019; Thind ve ark., 2020; Dobrikova ve ark., 2021). Artan Cd

konsantrasyonuna bağlı olarak antosiyanin ve fenolik madde içeriğindeki artış, Adaçayı bitkisinin Cd stresine karşı toleransını artırmak için bu bileşikler biriktirdiğini göstermektedir. Antosiyaninler ve fenolikler, oksidatif strese karşı koruyucu rol oynayarak ve metal şelatörü olarak işlev görerek adaçayı bitkisinin Cd stresine karşı toleransını artırabilir. Stres koşullarında artan APX, CAT ve POD enzim aktiviteleri H_2O_2 'i parçalayarak zararsız bileşiklere dönüştürürler ve oksidatif stresin olası etkilerinden bitkilerin korunmasını sağlamada önemli rol alır (Dikilitaş ve ark., 2016). Araştırmamızda, artan Cd konsantrasyonuna bağlı olarak CAT ve POD enzim aktivitelerinin arttığı bulunmuştur ve bu sonuçlar Sardar ve ark. (2022) kişniş, Karakas ve ark. (2021b) *Carpobrotus acinaciformis*, Fu ve ark. (2019) arpa ve Guo ve ark. (2019) buğday bitkisinde bildirdiği sonuçlarla benzerdir. Buna karşın Cd toksisitesinin adaçayı bitkisinde APX aktivitesini büyük ölçüde baskıladığı görülmüştür. Benzer sonuçlar diğer araştırmacılar tarafından da bildirilmiştir (Wu ve ark., 2017; Singh ve ark., 2019; de Anicésio ve Monteiro, 2022). Cd toksisitesine bağlı olarak APX aktivitesinde meydana gelen azalma, bu enzimin bulunduğu kloroplastlardaki bozukluklardan kaynaklanabilir (Lou ve ark., 2017). Ayrıca, Cd stresi altında APX aktivitesindeki azalma, enzimler arasındaki dengesizliklerden kaynaklanabilir (Soares ve ark., 2016). Kesin mekanizma bilinmemektedir, ancak enzim dengesindeki değişiklikler APX aktivitesindeki azalmaya katkıda bulunabilir.

4. Sonuçlar

Çalışmamızda, farklı dozlarda Cd uygulanan adaçayı (*Salvia officinalis* L.) bitkisinin Cd stresine karşı gösterdiği bazı fizyolojik ve biyokimyasal tepkileri incelenmiştir. Sonuçlar Cd stresinin bitkinin gövde ve kök aksamalarında azalmalara neden olduğunu göstermiştir. Ayrıca yüksek dozda uygulanan Cd, bitkinin klorofil pigment içeriği ve yaprak bağıl su içeriğinin önemli düzeyde azalmasına yol açmıştır. Cd stresi oksidatif stresin önemli göstergeleri olan H_2O_2 , MDA ve ES'de önemli artışlara neden olmuştur. En

yüksek oksidatif stres göstergeleri 200 ppm Cd uygulamasında belirlenmiştir. Bitkilerin ağır metal stresi ile başa çıkmasında kritik rollere sahip olan antosiyanin ve fenolik madde içerikleri 100 ppm ile önemli artış göstermiş, ancak Cd dozunun artırılması bu değerleri daha da yükseltmemiştir. Bitkilerin stresle mücadelesinin değerlendirilmesinde önemli bir gösterge olan CAT, APX ve POD enzim aktivitelerinden CAT ve POD artan Cd ile artış göstermiş, APX ise artan Cd ile azalma göstermiştir. Adaçayı bitkisinin Cd toksisitesine karşı gösterdiği fizyolojik ve biyokimyasal tepkiler bitkinin Cd stresine toleransını ortaya koymuş ve özellikle 100 ppm seviyesindeki Cd'a toleransının yüksek olduğu yapılan bu çalışma ile belirlenmiştir. Çalışmamız adaçayı bitkisinin 100 ppm düzeyine kadar Cd ile kirlenmiş ortamlarda yetiştirilebileceğini öne sürmüştür. Ayrıca bu bitkinin Cd ile kirlenmiş topraklarda ıslah materyali olarak kullanılmasında önemli bir kaynak oluşturmuştur.

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An Empirical Analysis of the Relationship Between Agricultural Revenue and Tax Burden

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Abstract

The concept of total tax burden, derived by calculating the ratio of all taxes collected within a specific period to the Gross National Product (GNP), serves as a key indicator of a country's level of development and its fiscal policies. Agricultural revenue holds a significant position within the GNP. Another critical point is the scale of taxes generated from agricultural production. This study aims to empirically analyze the relationship between the tax burden and agricultural revenue. For this purpose, data from the Turkish economy covering the period from 1965 to 2022 has been utilized. The empirical analysis reveals a long-term cointegrated relationship between the series. According to the coefficient estimators, it was found that, for the period covered by the study, there is an inverse relationship between the tax burden and agricultural revenue. In other words, increases in the tax burden have been shown to decrease agricultural revenue.

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agricultural revenue

1. Introduction

For taxpayers, taxes represent a non-reciprocal payment and place a burden on those who pay them. Thus, the tax burden is defined as the proportional relationship between taxes paid and income earned (Egeli and Karakoyun, 2019). The concept of tax burden can be divided into objective and subjective tax burden. The objective tax burden is expressed as the ratio of taxes and similar payments made within a given time to the income earned during that same period. However, it is often challenging to calculate both the exact amount of taxes paid and the exact income earned. Among the reasons for this are the complexities involved in accounting for the share received from public services and the difficulties associated with calculation methods. For these reasons, the issue of tax burden has become a topic of debate (Herekman, 1989). Individuals experience psychological pressure resulting from the taxes they pay, which is known as the subjective tax burden. In the literature, terms like tax pressure and tax strain are also used to refer to this concept (Egeli and Karakoyun, 2019). Calculating the subjective tax burden is even more challenging than calculating the objective tax burden. This is due to varying perceptions of tax among taxpayers, as well as differences in how taxpayers at similar economic levels experience the impact of taxation (Çiçek, 2006).

T: Tax

I: Income

TB: Tax Burden

The tax burden can be formulated as $TB = T / Y$, but since individual income is not the sole source of tax-paying capacity, it would be more accurate to express the tax burden as:

- I: Income

- W: Wealth

- S: Special Circumstances of the Taxpayer

- T: Tax

- TB: Tax Burden

Thus, $TB = T / (W + I + S)$ is a more precise formulation (Özbilen, 2010).

In each country, the total tax burden is defined as the ratio of all taxes collected by the

state over a specified period to the gross national product (GNP) (Pehlivan, 2018). The concept of total tax burden, a key indicator in cross-country comparisons, reflects how much of a country's output is paid to the state in taxes (Özbilen, 2010). The type and amount of tax paid to the state are critical for numerous sectors and transactions. The tax burden impacts several areas, including the agricultural, industrial, and services sectors, as well as foreign trade activities such as imports and exports (Ömür, 2021).

The agricultural sector has been a field of considerable focus since the establishment of the republic, serving as a platform for the implementation of various ideas. While some initiatives aimed at enhancing productivity in agriculture have positively impacted agricultural production and efficiency, it has also been observed that certain policies have not yielded the desired outcomes.

Investments in the agricultural sector in Türkiye are made by both the public and private sectors. Fixed capital investments by the government and those by the private sector are of great significance for the agricultural industry (Olgun et al., 2018). The gross agricultural revenue is defined as the monetary equivalent of the physical value of products produced as a result of agricultural activities. The contribution of the agricultural sector to the economy is calculated using the production method for Gross Domestic Product (GDP) estimations by the Turkish Statistical Institute, reflecting the sector's economic impact (Ege, 2011). According to 2018 data, the agricultural gross output in Türkiye amounted to 7 billion TL, contributing 5.8% to GDP. By 2020, agricultural GDP reached approximately 44 billion USD. Examining the tax relationship with the agricultural sector reveals that only 0.4% of individuals engaged in agricultural activities are taxed under the real method, while the remaining 99.6% are taxed via withholding. In terms of tax revenue for 2018, the proportion of income taxes derived from the agricultural sector was approximately 3.3% of total tax revenues (Yılmaz and Doğan, 2024).

Numerous studies have been conducted on the tax burden in both national and international literature. Some of these studies compare the tax burdens of OECD countries with that of Türkiye, while others primarily examine the relationship between tax burden and economic growth.

Marsden (1984) conducted a regression analysis of 20 countries between 1970 and 1979. Their findings indicated that a 1% decrease in the share of tax revenues within national income resulted in a negative effect of -0.36 on economic growth.

Widmalm (2001) investigated 23 OECD countries from 1965 to 1990. In this study, boundary analysis was employed, revealing that an increase in personal income tax negatively affects economic growth.

Lee and Gordon (2005) examined the relationship between tax burden and economic growth across 70 countries. Utilizing panel and regression analysis economic models over the period from 1970 to 1997, they concluded that a 10% reduction in corporate tax rates could increase economic growth by between 1% and 2%.

Öztürk and Ozansoy (2011) analyzed the tax burden on wage income and noted that, while the tax burden in Türkiye has been increasing rapidly, it remains lower than that of European Union countries. Their study found that the heavy tax burden based on production and employment, along with tax exemptions and exclusions, leads to disparities in the tax burdens experienced by public sector employees and those in the private sector.

In a comparative study of OECD countries and Türkiye, Kılıçaslan and Yavan (2017) concluded that Türkiye's tax burden is below the OECD average. They also highlighted concerns regarding the distribution of the tax burden, indicating issues related to tax equity within the system.

Organ and Ergen (2017) explored the relationship between tax burden and economic growth in Türkiye from 1980 to 2015, employing a boundary-testing approach. The study concluded that there is a negative

relationship between the two variables in the long term.

In their study examining OECD countries, Tosun (2018) noted that Türkiye's tax burden remains below the OECD average. Furthermore, he concluded that the share of indirect taxes in total tax revenues exceeds that of direct taxes in Türkiye, which hinders the equitable distribution of the tax burden.

Koç (2019) examined the relationship between economic growth and tax burden from 1980 to 2017. Utilizing Johansen's cointegration analysis and an error correction model, the study concluded that an increase in the tax burden also leads to economic growth in the long term.

Çelikay (2020) studied OECD countries and found that variables such as per capita income, trade volume, employment capacity, unemployment, and the industrial sector's economic share positively influence the tax burden. It was noted that a positive relationship exists between unemployment and tax burden due to the principles of the welfare state.

Karaş and Selen (2021) analyzed the relationships between taxes and public borrowing among 36 OECD countries from 1995 to 2018. According to their causality analysis, a long-term relationship between tax burden and debt burden was identified.

Püren (2023) conducted a literature review on tax burden, analyzing 47 studies. It was noted that most of the research focused on objective measures of tax burden, while there is a limited number of studies addressing subjective perceptions of tax burden.

2. Materials and Methods

2.1. Data and variables

This study utilizes time series data covering the period from 1965 to 2022. Data on the variables were obtained from the online databases of the OECD and the World Bank. The E-Views 9.0 software package was employed for estimating the empirical model. Descriptive information regarding the variables is presented in Table 1.

Table 1. Variables used in the model

Variable	Description	Period	Source
AGR	Agricultural revenue (% of GDP)	1965-2022 (Annual)	World Bank (WB)
TXR	Tax burden (% of GDP)	1965-2022 (Annual)	OECD

The mathematical expression of the model is shown in equations (3.1) and (3.2):

$$AGR = f(TXR) \quad (3.1)$$

$$\text{Model: } AGR_t = \beta_0 - \beta_1 TXR_t + \varepsilon_t \quad (3.2)$$

2.2. Empirical findings

The empirical results obtained from the unit root, cointegration, and coefficient estimators used in the analysis are summarized below.

2.2.1. Unit root test results

Unit root tests play a crucial role in assessing the variability and stationarity levels of time series data and are frequently employed in analyses. The primary reason for this significance is the uncertainty surrounding the presence of unit roots, which can lead to

inconsistent test results and potentially biased interpretations (Çelik et al., 2022: 93-94). When a time series exhibits stationarity, the changes within the series tend to stabilize around a constant mean over time, indicating that the variations are not random. Conversely, if a time series contains a unit root, it suggests that the changes in the series either increase or decrease over time, indicating a lack of stationarity. Consequently, the study utilized one of the most employed unit root tests, the Augmented Dickey-Fuller (ADF) test (Çelik et al., 2020: 94).

Table 2. Unit root test results

Level	Variables		ADF	First Differences	Variables		ADF
	Constant	AGR	-3.240 (0.02)**		AGR	-6.474 (0.00)***	
		TXR	-1.120 (0.70)			TXR	-7.331 (0.00)***
	Constant + Trend	AGR	-1.070 (0.92)		AGR	-7.837 (0.00)***	
		TXR	-0.969 (0.93)			TXR	-7.350 (0.00)***

Note: Series are seasonally adjusted. P<0.01*** indicates the stationarity of the series.

Table 2 presents the results of the Augmented Dickey-Fuller (ADF) unit root test for both the constant term and the constant plus trend specifications. At the level values, all variables exhibit the presence of a unit root. However, when the first difference of the variables is taken, it is observed that they become stationary at all significance levels in both the constant and constant plus trend equations.

2.2.2. ARDL bounds test results

The ARDL (Autoregressive Distributed Lag) bounds test is a statistical test used to assess the suitability of the ARDL model. As a regression model, it examines the effects of dependent and independent variables on each

other. This bounds test is utilized not only to select the highest-order lags but also to determine the existence of a long-term relationship among the independent variables. The F-test is employed to identify the most appropriate model, incorporating the estimated autoregressive (AR) and moving average (MA) terms for stationarity evaluations. This test was developed by Pesaran et al. (2001) (Pesaran et al., 2001: 290). The ARDL test interprets long-term significance levels at 1%, 5%, and 10% using the F-statistic and conducts a comparison with critical values. If the F-statistic exceeds the upper bound critical value, it indicates the presence of cointegration. Conversely, if the F-statistic is below the lower

bound critical value, it suggests that cointegration does not exist (Narayan and Smyth, 2006: 337).

The ARDL bounds test was employed to identify the presence of cointegration among the series, and the results of the ARDL bounds test are presented in Table 3.

Table 3. ARDL bounds test results

		F Statistic
		10.348
Critical Value	I(0)	I(1)
10%	3.02	3.51
5%	3.62	4.16
2.5%	4.18	4.79
1%	4.94	5.58

The F-statistic, developed by Pesaran et al. (2001), indicates the presence of a cointegrating relationship between variables when it exceeds the upper critical value at the 5% significance level. According to the results in Table 3, the calculated F-statistic value is 10.348. Since this value surpasses the upper critical value of 5.58, it is concluded that a cointegrating relationship exists among the series.

2.2.3. Parameter estimation results

Accurate estimation of long-term coefficients among the series is crucial, as these coefficients determine the magnitude and direction of the relationships between the variables. Therefore, correctly estimated parameter coefficients are vital in the construction and analysis of the model.

Table 4. Parameter estimation results

Variables	OLS		FMOLS		CCR	
	Coefficient	Probability	Coefficient	Probability	Coefficient	Probability
TXR	-1.876	0.00***	-1.873	0.00***	-1.874	0.00***
Constant Term	51.840	0.00***	51.663	0.00***	51.677	0.00***

Note: $p < 0.01$ *** indicates that the variables are statistically significant.

When evaluating the results presented in Table 4, the coefficients for the constant term and the independent variable TXR are statistically significant across the OLS, FMOLS, and CCR estimators, with the TXR coefficient exhibiting a negative sign. In terms of interpreting the parameter estimation methods:

- According to the OLS method, a one-unit increase in the tax burden reduces agricultural revenue by 1.876 units.
- According to the FMOLS method, a one-unit increase in the tax burden reduces agricultural revenue by 1.873 units.
- According to the CCR method, a one-unit increase in the tax burden reduces agricultural revenue by 1.874 units.

3. Conclusion

The agricultural sector and high productivity in agricultural production are crucial for national economies. Due to the impact of agriculture-related economic activities on other sectors, countries' agricultural economic practices remain a topic of ongoing discussion. After taxes are paid, individuals often feel the urge to see a return on their tax contributions, which influences their tax payment behavior in subsequent periods. Additionally, understanding how taxes are collected from various sectors and how similar practices are implemented globally is of great importance to taxpayers. Since the Neolithic era, agricultural production and its taxation have influenced agricultural revenue. Changes in agricultural revenue naturally affect the tax burden and reveal the

sectoral impacts of taxation. There is an important situation to know about agricultural revenue. Agricultural revenue does not provide information about the economic situation or social welfare of individuals in the agricultural sector. It only represents a size indicator. Evaluating agricultural revenue together with tax burden allows an idea to be formed about the welfare of individuals in the agricultural sector. Therefore, it is correct to examine the tax burden on a sectoral basis. Data from the OECD and the World Bank from 1965 to 2022 were analyzed using the E-Views 9.0 software for estimating the empirical model. The findings indicate that, according to the OLS method, a one-unit increase in the tax burden reduces agricultural revenue by 1.876 units. In the FMOLS method, a one-unit increase in the tax burden results in a decrease of 1.873 units in agricultural revenue, while the CCR method shows that a one-unit increase in the tax burden leads to a reduction of 1.874 units in agricultural revenue.

Tax rates, along with the equitable distribution and collection of taxes among sectors, are significant. Furthermore, tax practices globally affect not only the agricultural sector but also other sectors. In Türkiye, the reliance on indirect taxes for a substantial portion of tax revenues raises questions about the concept of tax fairness, the existence of a shadow economy, and individuals' inclination toward informality, leading to negative economic consequences. It is essential to examine the sectoral impact of the tax burden, which is a crucial indicator for developed countries, and to seek solutions based on these findings.

In order to increase agricultural revenue, policies that will not negatively affect public finances should be developed in incentives for production. In addition, the correct operation of the control mechanism should be ensured. The sectors that contribute the most to the national income should have higher tax burdens. However, it should not be forgotten that some important sectors can directly affect the basic production and exports in the country. It should be investigated how

agricultural-focused incomes and taxes have affected social life and welfare levels throughout the historical process, and these studies should be evaluated together with tax policy.

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